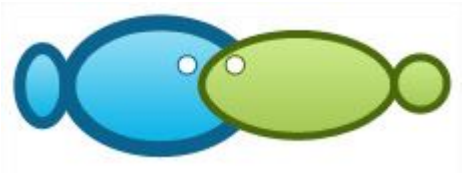

The enhancement of growth performance and feed efficiency of Asian catfish, *Pangasianodon hypophthalmus* fed on *Cinnamomum burmannii* leaf powder and extract as nutritional supplementation

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Abstract. This research was performed to evaluate and compare the growth performance of Asian catfish (*Pangasianodon hypophthalmus*) fed diets containing *Cinnamomum burmannii* leaf powder and extract. Catfish with an initial body weight of 319±36 g fish^{-1} was reared in 9 different floating cages (2 x 1 x 1.5 m) with a density of 15 fish per cage for 60 days period. The fish were fed with diets containing cinnamon leaf either in powder or extract form at different doses: 0% cinnamon leaf, 0.1% cinnamon leaf extract, 1% cinnamon leaf powder. Fish were fed twice a day with a feeding rate of 3% of the average body weight. The results showed that the addition of cinnamon leaf extract and powder significantly increased the fish specific growth rate, feed efficiency, protein retention as compared to the control ($p < 0.05$). Biochemical analysis of blood showed that the cinnamon leaf extract and powder considerably lowered cholesterol, triglycerides, and increase high-density lipoprotein (HDL0 ($p < 0.05$). However, cinnamon leaf extract more effectively increased HDL levels and decreased fat in the liver by 41% and 38% respectively, than cinnamon leaf powder did ($p < 0.05$). Supplementation of 0.1% extract and 1% powder of cinnamon leaf in diet was efficient and applicable in improving the growth performance of catfish.

Key Words: blood biochemistry, *Cinnamomum burmannii,* growth, *Pangasianodon hypophthalmus.*

Introduction. Asian catfish, *Pangasianodon hypophthalmus,* is one of the freshwater fish that is rich in nutrient such as protein (18%), eicosapentaenoic acid (EPA) (0.4%), and docosahexaenoic acid (2%) (Kunnath et al 2015). To support industrialization of the catfish, evaluation of the catfish culture especially related to slow growth and low feed efficiency is required. Catfish with weight approximately 7 g requires 8-9 months to achieve ideal weight for consumption (600-700 g) (Widodo et al 2010). Feed enrichment using cinnamon (*Cinnamomun burmannii*) leaf promotes desirable effects on increasing fish growth (Setiawati et al 2014; Jusadi et al 2016).

Cinnamon is a herbal plant that contains polyphenol and cinnamaldehyde compound that serve as antioxidants and improve the metabolism of blood glucose and fatty acids (Gruenwald et al 2010). Bioactive compounds in the cinnamon such as tannins, saponins, flavonoids can lower cholesterol, triglycerides and increase high density lipoprotein (HDL) (Azima et al 2004). Additionally, the polyphenols serve as mimetic insulin to stimulate glucose metabolism (Jarvill-Taylor et al 2001), while the cinnamaldehyde can increase fat metabolism and shows antioxidant activity (Jayaprakasha & Rao 2011). Cinnamaldehyde is able to activate the insulin-like growth factor (IGF-1) which enhances the biosynthesis of protein and collagen in the tissues of the body, thereby increasing the deposition of proteins in the body to build muscle (Takasao et al 2012).

The cinnamon leaf use for fish has been previously reported by some researchers. Rattanachaikunsopon & Phumkhachorn (2010) have studied the cinnamon oil potential to control a *Streptococcus iniae* infection on Nile tilapia (*Oreochromis niloticus*). Ahmad et al (2011) reported that administration of 1% cinnamon *(C. zeylanicum)* powder in feed could increase the specific growth rate, feed efficiency, protein and energy utilization efficiency respectively by 19.41%, 13.67%, 12.19 % and 10.19%, in Nile tilapia. Sivagurunathan & Innocent (2014) reported that administration of 1% cinnamon flour on tilapia feed could increase the specific growth rate of up to 50% compared to the control. Rolin et al (2015) showed that administration of 1% cinnamon extract in catfish (*Pangasianodon hypophthalmus*) feed could increase protein retention by 24% compared to control. Setiawati et al (2016) also informed that the addition of cinnamon leaf (1%) in the diet of catfish *P. hypophthalmus* could improve flesh fat content, cholesterol, and triglycerides deposition.

The use of the cinnamon leaf extract and powder is the effort to improve previous studies. Former researches were performed in laboratory scale; therefore the application of cinnamon leaf in the feed to improve the growth performance of catfish on a field scale is needed. This study was conducted to assess and contrast the growth performance of Asian catfish (*P. hypophthalmus*) fed diets containing *Cinnamomum burmannii* leaf in powder and extract form.

Material and Method

Preparation of cinnamon leaf powder and extract. The cinnamon *(C. burmannii)* leaves used were obtained from Jambi province, then dried in an oven at 40°C. Dried leaves were grinded and sieved to produce cinnamon leaf powder. For extraction, cinnamon leaf powder was extracted using ethanol 96% based on Prasad et al (2009) with slight modification. Cinnamon leaf powder was extracted with a ratio of 1:10 (sample:solvent). Extraction was performed using maceration for 18 h under constant stirring using orbital shaker. The filtrate was further filtered, and then evaporated with a rotary evaporator at 40°C, which produced the condensed extract of cinnamon leaves. Proximate and phytochemical result analysis of cinnamon leaf extract and powder were presented in Table 1 and Table 2.

Table 1

The proximate composition of cinnamon leaf powder and extract

Composition	*Leaf powder*	*Leaf extract*
Protein (%)	9.36	7.43
Lipid (%)	2.31	24.61
Crude fiber (%)	36.84	0.84
Ash (%)	4.19	2.61
Nitrogen free extract (%)	41.91	47.04
Moisture (%)	5.40	17.47

Table 2

Bioactive compounds of cinnamon leaf powder and extract

Bioactive compounds	*Leaf powder*	*Leaf extract*
Flavonoid (%)	1.80	5.05
Tanin (%)	2.74	1.30
Saponin (%)	2.32	3.65
Cinnamaldehyde (%)*	59.46	1.62

*Essential oil.

Feed preparation. The feed used was commercial feed that was treated by the addition of cinnamon leaf 0%, 0.1% cinnamon leaf extract (Setiawati et al 2016) and 1% cinnamon leaf powder (Setiawati et al 2014). The feeds were treated as follows:
Control : Feed + 0% cinnamon leaf (control)
Extract : Feed + 0.1% cinnamon leaf extract
Powder : Feed + 1% cinnamon leaf powder
Testing feed was prepared by mixing commercial feed, extract and powder of cinnamon leaf. All components were evenly mixed, formed, and dried at 40°C. The dried feed was analyzed for its proximate components. The result was shown in Table 3.

Table 3

Nutritional composition of dried testing feed

Components	*Treatments*		
	Control	*Extract 0.1%*	*Powder 1%*
Protein (%)	28.39	28.44	28.61
Lipid (%)	6.77	6.71	6.65
Crude fiber (%)	4.13	5.23	5.69
Ash (%)	13.95	12.45	13.57
Free nitrogen extract (%)	46.73	47.14	45.46
Gross energy (kcal 100 g^{-1})[1]	414.35	415.74	409.17
C/P ratio (kcal g^{-1} $protein^{-1}$)	14.59	14.61	14.30

[1)]1 g protein = 5.6 kcal, 1 g carbohydrate = 4.1 kcal, 1 g fat = 9.4 kcal.

Experimental design. The catfish (average initial weight 319.64±35.99 g, length 15 cm) was maintained for 60 days at a fish pond (20 x 10 m^2) with water level of 100 cm. The pond was split using 9 nets with a size 2x1x1.5 m^3 with a density of 15 fish $cage^{-1}$. Feeding rate was 3% of the average weight of the fish (two times a day at 8 am and 4 pm). Adaptation process was performed for a week. During adaptation, the catfish were fed by commercial feed without containing cinnamon leaves. After adaptation, the fish were not fed for 24 h to eliminate the influence of previous feeding. The fish was then weighed to determine the initial weight. The experiment was conducted in July to December 2015.

Cleaning the pond was conducted once a week, and water replacement was 20% of the pond water volume. Water quality was measured including temperature range (29-34°C), pH (7.04-7.51), dissolved oxygen (1.10-7.60 mg L^{-1}) and total ammonia nitrogen (0.21-0.80 mg L^{-1}). Sampling was done each 20 days to determine the weight of the fish. At the end, the fish was fasted for 24 h, then counted and weighed to determine the amount and the final weight. Three fishes were randomly taken for each analysis (body and liver proximate compositions, blood biochemistry).

Proximate analysis. The proximate parameters were water, protein, fat and water content, liver water content, fat, and liver glycogen. Water content was measured by heating in the oven at 110°C for 6 h, and protein content was determined by the Kjeldahl method, while wet fat was measured by the method of Folch (Takeuchi 1988). Analysis of glycogen was done by dissolving the samples using 30% KOH, saturated Na_2SO_4, and 95% alcohol and then heated in a water bath (110°C). The deposite obtained from previous stage was titrated with NaOH 0.5 M, added O-tolueidin + CH_3COOH, subsequently measured using a spectrophotometer with a wavelength of 635 nm.

Biochemistry analysis of blood. Prior to analysis, the fish was anesthetized using tricane methane sulphonate (MS-222) at a dose of 200 mg L^{-1}. The fish blood (1 mL) was drawn at the base of the tail using a 3 mL syringe with an anticoagulant (3.8% sodium citrate). The blood sample was transferred in the microtube, then centrifuged (10,000 rpm, 5 min). The supernatant was taken and put in a new microtube and stored in a freezer (-20°C) for analysis of cholesterol, triglycerides, and high density lipoprotein.

Data collection and statistical analysis. Growth performance was indicated by feed intake that was calculated based on the 3% feeding rate of the average weight of biomass (g per day), specific growth rate (Guo et al 2012), fat retention, protein retention, feed efficiency (Takeuchi 1988), and the survival rate. The fish liver was analyzed in early and end of the stage, including the fat content, liver glycogen, and hepatosomatic index according to Wedemeyer & Yasutake (1977).

Biochemical measurements of blood included cholesterol, triglyceride, HDL. Cholesterol measurement was performed using the CHOD-PAP method (enzymatic colorimetric test for cholesterol with lipid clearing factor) based on Trinder (1969) with the kit CHOLESTEROL liquicolor Human mbH, Germany. Triglyceride was measured using the CHOD-PAP method (enzymatic colorimetric test for triglycerides with lipid clearing factor) according to Jacobs & Vandemark (1960) with triglyceride kit liquicolor-mono Human mbH, Germany. HDL was determined using Friedewald et al (1972) method with a kit HUMAN CHOLESTEROL liquicolor Precipitant and Standard Human mbH, Germany.

The completely randomized design (CRD) was used with three treatments and three replications. The parameters (the growth performance, blood biochemistry, liver analysis) were statistically tested. Data were tabulated with Microsoft Office Excel 2013 and analyzed by ANOVA using SPSS version 22. Significant different treatment was further tested using Duncan. Water quality was tested using descriptive observation.

Results

Growth performance. Table 4 shows the growth performance of the catfish during 60 days of culture. Feed intake and fat retention were not statistically different among treatments ($p > 0.05$), but presence of the cinnamon leaf powder and extract could improve the final body weight, specific growth rate, feed efficiency, and protein retention, respectively by 13-16%, 24-29%, 40-46%, 56-67%, significantly different compared to control ($p < 0.05$). Survival rate for all treatments during 60 days of maintenance was 100%.

Table 4

The growth performance of catfish for 60 days of culture

Parameters	*Treatments*		
	Control	*Extract 0.1%*	*Powder 1%*
W0 (g fish^{-1})	321.17±6.09 ^{a}	319.49±3.85 ^{a}	318.27±2.74 ^{a}
WF (g fish^{-1})	554.27±6.41 ^{a}	628.51±26.22 ^{b}	643.31±16.62 ^{b}
FI (g fish^{-1})	778.73±23.83 ^{a}	738.67±15.24 ^{a}	741.98±29.32 ^{a}
SGR (% day^{-1})	0.91±0.02 ^{a}	1.13±0.09 ^{b}	1.18±0.05 ^{b}
FE (%)	29.95±1.23 ^{a}	41.89±4.78 ^{b}	43.85±2.93 ^{b}
PR (%)	21.24±0.82 ^{a}	35.58±3.42 ^{b}	33.14±2.01 ^{b}
LR (%)	71.30±6.55 ^{a}	75.92±11.92 ^{a}	83.00±8.21 ^{a}
SR (%)	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}

Mean values in same row with different superscripts vary significantly ($p < 0.05$). W0 - initial weight, WF - final weight, FI - feed intake, SGR - specific growth rate, FE - feed efficiency, PR - protein retention, LR - lipid retention, SR - survival rate.

Biochemistry analysis of blood. Analysis of total cholesterol, triglycerides and HDL are presented in Table 5. The result exhibited that cinnamon leaf powder and extract could reduce level of total cholesterol, triglycerides and increase HDL levels in the blood, 11%, 25- 29%, 31-104%, respectively, which showed significant different compared to controls ($p < 0.05$).

Table 5

Blood biochemical data of catfish for 60 days of culture

Parameters	Treatments		
	Control	Extract 0.1%	Powder 1%
Cholesterol (mg dL^{-1})	164.01±10.09 [b]	145.02±3.54 [a]	144.56±4.02 [a]
Triglycerides (mg dL^{-1})	900.63±74.05 [b]	636.82±17.33 [a]	673.41±30.31 [a]
HDL (mg dL^{-1})	58.81±2.81 [a]	120.00±6.13 [c]	77.28±10.18 [b]

Mean values in same row with different superscripts vary significantly ($p < 0.05$). HDL = high density lipoprotein.

Liver analysis. Table 6 presents the result of catfish liver analysis. Addition of cinnamon leaf extract promoted reduction of fat content (38-41%) in comparison with cinnamon leaf powder and control ($p < 0.05$). Additionally, cinnamon leaf powder resulted in lower hepatosomatic index (HIS) compared to extract and control ($p < 0.05$). Liver glycogen levels were not significantly different among treatments ($p > 0.05$).

Table 6

Initial and end parameter of catfish liver

Parameters	Initial value	Treatments		
		Control	Extract 0.1%	Powder 1%
Moisture (%)	75.18±0.00	74.39±0.62 [a]	80.33±0.89 [b]	75.98±1.07 [a]
Lipid (%)	4.58±0.00	4.96±0.16 [b]	3.50±0.21 [a]	4.83±0.43 [b]
Glicogen (mg 100 mL^{-1})	0.07±0.00	0.05±0.02 [a]	0.05±0.02 [a]	0.05±0.03 [a]
Hepatosomatic index (%)	-	1.12±0.01 [b]	1.17±0.11 [b]	0.98±0.03 [a]

Mean values in same row with different superscripts vary significantly ($p < 0.05$). (-): not being measured.

Discussion

Growth performance. Table 4 indicated that feed intake and survival rate showed no significant different among treatments ($p > 0.05$). However, addition of cinnamon leaf powder and extract in the feed could increase PR 56-67%, which was higher than control ($p < 0.05$), and significantly resulted in higher final weight (628-643 g $fish^{-1}$) compared to control (554 g $fish^{-1}$). These results were in agreement with Setiawati et al (2014) that addition of 1% cinnamon leaf powder in the feed increased protein retention by 53% compared to control ($p < 0.05$). Rolin et al (2015) showed an increase of protein retention (24%) compared to control ($p < 0.05$) as a result of administration of 0.1% cinnamon leaf extract. Increased protein retention due to the treatments may be contributed by polyphenol compounds that effectively improved metabolism of blood glucose and fatty acids, as well as improved the fish health, therefore the protein was usable for fish growth. Jayaprakasha & Rao (2011) stated that cinnamon was an herbal plant that had polyphenol compounds, cinnamaldehyde, and flavonoids that showed antioxidant activity and metabolism-improving effects. Sabitha et al (2014) suggested that antioxidant activity may increase the immune system and maintain the cell physiology, and reduce cell damage caused by free radicals and oxidative stress. Lukacinova et al (2008) showed that flavonoid administration at a dose of 0.05-0.1% was responsible for higher absorption of blood glucose in rats. Lopes et al (2015) argued that the cinnamon could improve the metabolism of fatty acids in adipose tissue. Cinnamaldehyde was able to activate the insulin-like growth factor (IGF-1) (Takasao et al 2012) which enhanced the biosynthesis of protein and collagen in the body tissues thereby increasing the deposition of protein that played key role in increasing body weight (biomass) in fish (NRC 2011; Vinasyiam et al 2016).

Table 4 exhibited that the cinnamon treatments also contributed to higher specific growth rate (24-29%) and feed efficiency (40-46%) compared to control ($p < 0.05$). The results were in line with Ahmad et al (2011), that administration of 1% cinnamon flour on tilapia feed accounted for higher specific growth rate (12%) and feed efficiency (13%)

compared to controls. Sivagurunathan & Innocent (2014) also found that addition of 1% cinnamon flour to tilapia feed could increase specific growth rate (50%) compared to control. These results may correlate with polyphenols which improved metabolism in the body tissues. Lukacinova et al (2008) showed that addition of 0.05-0.1% flavonoid in rats was responsible for higher blood glucose absorption. Azima et al (2004) suggested that flavonoids, saponins and tannins could improve nutrient absorption in rabbits. However, Rolin et al (2015) found that administration of 0.05 to 0.4% cinnamon leaf extract to the diet showed lower specific growth rate and weight biomass of catfish in comparison with controls. This suggested that the bioactive compounds in cinnamon leaf extract used in this study were low, including flavonoid 5.05%, 1.30% tannins, saponins 3.65%. Low content of bioactive compounds may increase the metabolism in the body tissues. Furthermore, Setiawati et al (2016) used higher levels of bioactive compounds such as tannins (9.11%), flavonoids (9.14%), and smaller fish size (±7.43 g), thus the catfish was not able to tolerate the high content of bioactive compounds and inhibit the nutrient absorption due to the limited ability of the fish digestive enzymes. The bioactive compound may be an anti-nutrient substance. NRC (2011) showed that tannin could inhibit the action of digestive enzymes while the saponin was is a limiting factor of protein digestibility and absorption of vitamin.

Biochemical analysis of blood. Lipid retention was not different between treatments ($p > 0.05$), but the cinnamon treatments could attenuate cholesterol and triglyceride level, 11%, 25-29%, respectively, and increase levels of HDL by 31-104% compared to control ($p < 0.05$) as shown in Table 5. This was in line with Al Jamal (2009) and Allen et al (2013), that administration of cinnamon in diabetics lowered cholesterol, triglycerides and increased HDL levels in the blood. Khan et al (2003) stated that cinnamon treatment in diabetics may increase the absorption of blood glucose. Li et al (2012) showed that the content of cinnamaldehyde in cinnamon increased HDL levels in mice. These results may be the role of polyphenolic compounds and cinnamaldehyde contained in the cinnamon leaf that is effective in increasing the metabolism. Talpur et al (2005) showed that polyphenol compounds in cinnamon had same activity as insulin (insulin memetic), while cinnameldehyde activated peroxisome proliferator-activated receptors (PPAR) to increase the absorption of blood glucose and cholesterol in the blood (Li et al 2015). In vitro studies showed that cinnamon had the dual function of activating peroxisome proliferator-activated receptors - PPARα and PPARγ - that worked to stimulate the differentiation of cells 3T3-L1 in pre-adipocytes and adipocytes, increasing the levels of mRNA PPARα/γ and mRNA target cells CD36, lipoprotein lipase (LPL), fatty acid synthase (FAS), GLUT4, and increasing the acyl-CoA oxidase (ACO) during 3T3-L1 cell differentiation 6.32-34. In vivo studies indicated that administration of 0.04% cinnamaldehyde in rats improved AMP-activated protein kinase (AMPK). AMPK was responsible for the maintenance of fat, cholesterol balance, stimulation of β-oxidation of fatty acids in the mitochondria for energy. AMPK was an inhibiting factor of acetyl CoA carboxylase (ACC) through phosphorylation that caused a decrease of malonyl-CoA thus decreasing the synthesis of fatty acids and increase fatty acid oxidation through regulating carnitine palmitoyl transferase-1 (CPT-1)35.

Liver analysis. Table 6 showed that liver fat content in the catfish treated by cinnamon leaf extract decreased 41% compared to control, and decreased 38% compared to cinnamon leaf powder ($p < 0.05$). The fat-lowering effect was attributed to the presence of bioactive compound in the cinnamon leaf which contributed to the increasing oxidation of fatty acids. It was observed that HDL with treatment of cinnamon leaf extract increased 31% compared to cinnamon leaf powder and 104% compared to control ($p < 0.05$) as presented in Table 5. Talpur et al (2005) reported that bioactive compounds in the cinnamon leaf extract have similar activity to insulin that effectively worked in the liver. Azima et al (2004) showed that administration of cinnamon extract on rabbit feed can reduce fatty in the liver, indicating that the cinnamon increased fat metabolism. In rats, decreased level of fat in adipose tissue and liver was found after administration of feed containing cinnamon extract (Chen et al 2012). Cinnamon contains methyl hydroxy

chalcone polymer (MHCP), which increased insulin activity. MHCP induced changes in the tyrosine phosphorylation of IRS-1 that activated PI3-kinase thus enhanced the absorption of glucose and fatty acids in the body (Qin et al 2003).

Table 6 presented that HSI of catfish was statistically different between treatments ($p < 0.05$). Treatment of cinnamon leaf powder significantly showed the lowest HSI value (0.98%), compared to control (1.12%) and extract (1.17%). However, the HSI value with powder treatment was still acceptable because no symptom of physiological abnormalities occurred in the catfish with observable weight changes (643.31 g $fish^{-1}$), same as extract treatment (628.51 g $fish^{-1}$), but significantly higher than control (554.27 g $fish^{-1}$) as shown in Table 4. The results were in agreement with Ahmad et al (2011), that addition of 1% cinnamon powder in the tilapia feed resulted in HSI values from 1.2 to 1.6% and no physiological abnormalities were observed in tilapia. The liver was the center of nutrient metabolism in the body (NRC 2011). Halver & Hardy (2002) stated that hepatic portal vein transported fish nutrients from the digestive tract into the liver for further processing and distributed to the body tissues. Li et al (2015) stated that cinnamon may activate PPAR and AMPK (Huang et al 2011). PPAR and AMPK showed an important role in the utilization of blood glucose, fatty acid oxidation and gene expression in tissues (Kramer et al 2007).

Conclusions. Feed enrichment with 0.1% extract and 1% powder of cinnamon leaf was efficient and applicable in improving the growth performance of catfish with increasing specific growth rate (24-29%), feed efficiency (40-46%), protein retention (56-67%). Cinnamon leaf in both extract and powder form did not influence the fish appetite but improved the feed efficiency at range of 40-46%.

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