Growth and immunity of African catfish (*Clarias gariepinus*) with dietary inclusion of ginger (*Zingiber officinalis*) and turmeric (*Curcuma domestica*)

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Abstract. Ginger and turmeric herbs represent a traditional medicine used in different treatments, profilaxy and as immunostimulants. In aquaculture industries, some antibiotics and chemical compound are commonly used as treatments and for prevention of diseases but can cause bacterial resistance and environment pollution. The purpose of this study was to evaluate the growth, hematocrit level and leucocyte differential of lymphocyte and monocyte by dietary inclusion of ginger and turmeric herbs for catfish (*Clarias gariepinus*) fingerlings. This study used a completely randomized design, with ginger (*Zingiber officinalis*) and turmeric (*Curcuma domestica*). A dietary inclusion of ginger powder (control, 2.5, 5 and 7.5 g kg^{-1} of feed) and tumeric powder (control, 25, 50 and 75 g kg^{-1} of feed) were applied to catfish fingerlings with an initial length of 6-7 cm. The experiment lasted 40 days. Fish was fed at a daily rate of 3% of body weight at 7:00 and 17.00. Measurement of the weight growth, length and feed consumption was conducted at the start and end of the experiment, while the hematological parameters were determined in the last day. The results showed that dietary inclusions of ginger and turmeric increase the weight growth (WG) and feed conversion ratio (FCR), hematocrit (%) and the leucocyte differential count of lymphocyte (%). The dietary inclusion of ginger increases the percentage of monocyte, but this was not observed in the case of turmeric. The dietary inclusion of turmeric provided better results than ginger for WG, FCR, hematocrit level and lymphocyte. Dietary inclusions of ginger and turmeric can be used in catfish hatcheries to increase its immunity.
Key Words: FCR, growth response, haematology, supplement.

Introduction. Increased growth and immunity in fish can be obtained by providing synthetic supplements combined with antibiotics. However, the use of synthetic supplements and antibiotics results in unfavorable effects for the organism, consumers and the environment. Therefore, natural materials or ingredients that are easily biodegradable are needed, such as ginger (*Zingiber officinalis*) and turmeric (*Curcuma domestica*). Some advantages in the use of natural ingredients are safety, ease of procurement, low prices, and do not endanger the surrounding environment (Purwanti et al 2012). Ginger and turmeric are widely used herbal medicines because they have advantages for health, supporting growth and immunity (El-Sayed et al 2014; Abdel-Tawwab & Abbass 2017).

Ginger is known to increase the immunity and growth of tilapia (*Oreochromis niloticus*) (Payung & Manoppo 2015). Dietary inclusion of ginger showed a significant effect ($p<0.05$) on the increase in total fish leucocytes after being administered for 4 weeks. The study proved that the dietary inclusion of ginger at a dose of 2.5-7.5 g kg^{-1} of feed increased the total leucocytes of tilapia (Payung & Manoppo 2015). Similar results were reported by Nya & Austin (2009), where dietary ginger at a dose of 5 g kg^{-1} of feed resulted in an increase in the number of leucocytes (neutrophils, macrophages and lymphocytes) in *Oncorhynchus mykiss*. In addition to ginger, another plant that has also been used as an immunostimulant and for improving growth in fish is turmeric.

Turmeric contains curcumin and essensial oils that can increase appetite in fish and function as antioxidants (Prabowo et al 2017). Curcumin compounds are present in turmeric and ginger, both being rhizome plants belonging to the family Zingiberaceae. Several studies have shown that diets containing curcumin increase growth, feed utilization, digestibility, and inhibit bacterial growth in carp (*Cyprinus carpio*) (Abdel-Tawwab & Abbas 2017). Prasad & Aggarwal (2011) report that curcumin from turmeric is a digestive stimulant and it favorably enhanced the activities of digestive enzymes such as lipase, chymotrypsin, and amylase. The obtained results agree with those obtained by Sahu et al (2008), who reported that turmeric powder supplemented diets containing curcumin significantly improved the growth performance of rohu (*Labeo rohita*) and their optimal growth was obtained at a dose of 1 g kg^{-1} of feed. This study aimed to evaluate the effect dietary of ginger and turmeric powder separately on the growth performance and innate immunity of African catfish (*Clarias gariepinus*) juveniles.

Material and Method

Preparation of ginger and turmeric. The experiment was conducted in a laboratory of the University of Muhammadiyah Purwokerto, Indonesia, from Augus to September 2019. Ginger and turmeric were bought from traditional markets in Purwokerto, Central Java, Indonesia. Ginger and turmeric were washed and cut into small pieces. The small pieces of ginger and turmeric were dried in the sun indirectly for 24 hours and blended into powder. Ginger and turmeric powders were filtered using a soft cloth into fine flour.

Experimental design. African catfish fingerlings were purchased from the Central Public Hatchery in the village of Dukuhwaluh, Banyumas Regency, Central Java, Indonesia. The fish were placed in plastic tanks (2 m^3) and acclimatized at room temperature (27-29^{o}C) for 1 week. During acclimatization the fish were fed the control feed.

After acclimatization, the fish were transferred to 16 plastic tanks (45 L each). Each tank was stocked with 15 fish with an average initial length of 7.31±1.51 cm and weight of 9.63±0.74 g. A completely randomized design was applied for ginger powder (control, 2.5, 5, and 7.5 g kg^{-1} of feed) and turmeric powder (control, 25, 50, and 75 g kg^{-1} of feed), separately. Ginger and turmeric powders were diluted with aquadest (100 mL kg^{-1} of feed) and sprayed throughout the commercial feed (PT Matahari Sakti, Sidoardjo, East Java) with the following composition, according to the producer label: crude protein 39-41%, crude fat 5%, crude fiber 6%, ash 16%, and moisture content 10%. Fish were fed at a daily rate of 3% of body weight at 7:00 and 17.00, during 40 days of experiment.

Evaluation of growth performance. The parameters measured in this study were growth and non-specific immunity. Growth parameters measured were weight gain and feed conversion ratio, while non-specific immunity measured was hematology in the form of differential leukocytes of lymphocyte and monocyte percentages. Growth and non-specific immune data were taken on day 0 (before starting the study) and on day 40 (end of the study).

In order to measure the growth of the catfish, all fish from each tank were weighted using a digital analytic scale with a precision of 0.01 g at the start and end of the experiment. At the end of the feeding trial (day 40), the weight gain (WG) and feed conversion ratio (FCR) were calculated according to the following formulas (Abdel-Tawwab & Abbas 2017):

$$WG=(Wt-Wi)$$

$$FCR=F\ (g)/(Wt-Wi)$$

Where: Wt and Wi are the final and initial body weights, respectively; F is feed intake and t is time in days.

Hematological assay. At the end of the trial (day 40), the hematological parameters were measured by taking 1 mL of blood samples from the caudal vein of the 5 fishes (out of 15 fish, 33.3%) from each tank. The blood samples were suspended in heparinized tubes, and the values of hematocrit and leucocytes differential count for lymphocytes and monocytes were calculated. Leucocyte differential counts were determined using the Giemsa (10%; w/v)) staining method and were detected in blood smears under a light microscope (Isnansetyo et al 2016).

Statistical analysis. Growth performance and hematological parameters were tested using one-way ANOVA and Duncan's multiple range test (5% level of significance), if differences were detected, using SPSS software (version 21.0).

Results and Discussion

Growth performance. WG of fish fed the ginger diet at 7.5 g kg^{-1} (T3) was significant different ($p<0.05$) from the WG of fish fed the control diet. WG of fish fed turmeric diets in all treatments (25, 50 and 75 g kg^{-1}) were significantly different ($p<0.05$) compared to controls (Table 1).

Table 1

Growth parameters of African catfish (*Clarias gariepinus*) juveniles fed with ginger and turmeric extract (% feed) for 40 days

Ginger			*Turmeric*		
Treatment (g kg^{-1} of feed)	*Weight gain (g)*	*Feed Conversion Ratio*	*Treatment (g kg^{-1} of feed)*	*Weight gain (g)*	*Feed Conversion Ratio*
Control	$9.51±0.71^{a}$	$4.93±0.40^{a}$	Control	$8.28±0.24^{c}$	$3.21±0.16^{a}$
2.5	$9.57±0.71^{ab}$	$4.35±0.64^{a}$	25	$11.09±0.90^{b}$	$2.86±0.65^{b}$
5	$9.56±1.06^{ab}$	$3.62±0.66^{ab}$	50	$12.24±0.56^{a}$	$2.60±0.21^{bc}$
7.5	$9.60±0.87^{b}$	$3.36±0.20^{b}$	75	$12.47±0.24^{a}$	$2.52±0.11^{c}$

Note: data are presented as mean ± SEM; different superscripts show significant differences ($p<0.05$).

The FCR of the ginger and turmeric diets of African catfish were significant different ($p<0.05$) compared to the control. The FCR of the ginger diet at 7.5 g kg^{-1} and of turmeric at all treatments (25, 50 and 75 g kg^{-1}) were significantly different compared to controls ($p<0.05$) (Table 1). The FCR of the turmeric diet at dose of 75 g kg^{-1} and of the ginger diet at 7.5 g kg^{-1} showed the best performances.

Hematological parameters. African catfish treated with ginger and turmeric extracts in diets had a significantly higher percentage of hematocrit compared to the control ($p<0.05$), after 40 days of experiment. The hematocrit of fish administered ginger diets was significantly different in all treatments (2.5; 5 and 7.5 g kg^{-1}) compared to that of control. The hematocrit of fish fed the turmeric diet at 50 g kg^{-1} was significantly different ($p<0.05$) compared to that of the control (Table 2). The fish fed the ginger diet at a dose of 2.5 g kg^{-1} and turmeric diet at 50 g kg^{-1} had the best profile of hematological parameters compared to those of others treatments.

Table 2

The hematocrit of African catfish (*Clarias gariepinus*) juveniles fed with ginger and turmeric extracts in diets after 40 days

Ginger		*Turmeric*	
Treatment (g kg^{-1} of feed)	*Hematocrit value (%)*	*Treatment (g kg^{-1} of feed)*	*Hematocrit value (%)*
Control	29.55±0.51^{a}	Control	48.61±0.94^{b}
2.5	47.31±7.65^{b}	25	54.39±4.64^{ab}
5	51.27±7.56^{b}	50	57.22±2.49^{a}
7.5	45.22±9.96^{b}	75	55.64±7.07^{ab}

Note: data are presented as mean ± SEM; different superscripts show significant differences ($p<0.05$).

Leucocyte differential. Supplementation of ginger and turmeric extracts in African catfish diets increased the percentage of lymphocytes significantly ($p<0.05$) in all treatments after the 40 days of experiment. The ginger extract increased the percentage of monocytes significantly ($p<0.05$) at 7.5 g kg^{-1} compared to controls and other treatments. The turmeric extract did not produce significant differences ($p>0.05$). Ginger extract had a greater potential for increasing the percentage of lymphocytes and monocytes of African catfish than turmeric extract.

Table 3

Leucocyte differential count of African catfish (*Clarias gariepinus*) juveniles fed with ginger and turmeric diets for 40 days

Ginger			*Turmeric*		
Treatment (g kg^{-1} of feed)	*Lymphocyte (%)*	*Monocyte (%)*	*Treatment (g kg^{-1} of feed)*	*Lymphocyte (%)*	*Monocyte (%)*
Control	54.75±3.86^{a}	3.95±0.91^{a}	Control	62.00±1.41^{a}	2.48±0.05^{a}
2.5	63.75±1.71^{b}	4.30±0.59^{a}	25	71.50±1.29^{b}	2.52±0.16^{a}
5	70.00±2.16^{c}	4.88±1.63^{a}	50	73.50±1.29^{b}	2.53±0.07^{a}
7.5	69.25±2.22^{c}	6.47±0.18^{b}	75	76.25±1.25^{c}	2.47±0.07^{a}

Note: data are presented as mean ± SEM; different superscripts show significant differences ($p<0.05$).

The WG of fish fed ginger diet at 7.5 g kg^{-1} and turmeric diet at 50 g kg^{-1} showed the best performance when compared to other treatments.

Prabowo et al (2017) reported that turmeric could increase growth, has bactericidal properties, lyses toxins attached to the intestinal wall and increases the absorption of nutrients in milkfish (*Chanos chanos*). Turmeric can also increase appetite and growth because it contains curcumin compounds (Purwati et al 2015). As stated by Alipin et al (2016), curcuminoids in *Curcuma zanthorrhiza* have kolagoga activity, which increases the production and secretion of bile, and stimulates the release of pancreatic enzymes.

The administration of ethanolic extract and powdered ginger increases the growth performance and mucosal immune responses in rainbow trout (*Oncorhynchus mykiss*) (Shaluei et al 2016), *Lates calcarifer* (Talpur et al 2013), and *Macrobrachium rosenbergii* (El-Desouky et al 2012). Increment of WG of fish administered ginger diets could be due to the role of ginger as an appetizer. The WG rate could be due to the enhancement of digestibility and metabolic processes. Dietary ginger can improve the growth performance by decreasing FCR (Jafarinejad et al 2020).

The results of this study are in accordance with Payung & Mannopo (2015), where an addition of ginger (7.5 g kg^{-1} of feed) stimulated the growth of *O. niloticus*, because ginger has potential as an immonustimulant, improving non-specific immune responses. Further supported by Robiansyah et al (2018), supplementation of ginger extract in feed significantly decreased FCR compared to controls in *Barbonymus schwanenfeldii*. However, the functional mechanisms of dietary ginger regarding the growth of fish are not entirely understood yet, and need to be further studied.

Generally, hematological parameters are indicators of fish health, as well as the physiological status of an organism (Jafarinejad et al 2020). In this study, hematocrit of fish fed ginger and turmeric diets increased significantly ($p<0.05$) compared to control, which demonstrates the stimulatory properties of ginger and turmeric. The increase of red blood cell (RBC) count in fish fed with ginger diets implies the suitable health effect due to the bioactive components in ginger. Talpur et al (2013) noted that the RBC count and hemoglobin content increased significantly in *Lates calcarifer* fed on diets containing all doses of ginger (3, 6, 9, 12 and 15 g kg^{-1} of feed). Haghighi & Rohani (2013) revealed that feeding rainbow trout with a dose of powdered ginger rhizome in the diet for 12 weeks significantly increased hematocrit, hemoglobin, and erythrocyte.

Dietary turmeric increases the percentage of hematocrit in *A. testudineus*, accompanied by an increased number of RBC, the amount of hemoglobin (Hb) and the percentage Hb in RBC (Manju et al 2013). Literature on the effect of turmeric on fish is scarce. The increase in RBC count and Hb content in the curcumin treated fish may help in the efficient transport of oxygen to the tissues. Curcumin is known to protect hemoglobin from oxidation. Results obtained by Mooraki et al (2019) show that fish fed with diet containing 0.3% turmeric powder increased RBC, packed cell volume (PCV), hemoglobin, and mean corpuscular haemoglobin concentration (MCHC), but not significantly. Al-Faragi & Hassan (2017) proved that dietary turmeric can significantly increase hematocrit and Hb levels in all treatments compared to control diets for *Cyprinus carpio*.

According to Van Hai (2015), white blood cells are indicators of health status in fish and play an important role in the regulation of immunomodulatory. Many studies demonstrated the effect of immunostimulant plants on blood cells to improve immunity in aquatic animals (Talpur et al 2013; Hwang et al 2013; Van Hai 2015). The present study showed that dietary ginger increases the percentage of lymphocytes and monocytes. This was also reported by Aysel et al (2016), where dietary ginger at concentrations of 3, 6, 9, 12 and 15 g kg^{-1} of feed significantly increased the percentage of lymphocytes and monocytes compared to the control diet for *O. niloticus*. The same was noted by Talpur et al (2013), where dietary ginger induced beneficial effects such as disease prevention due to improved immunity, with a higher survival of the treated groups of *L. calcarifer*. Dietary ginger at certain levels improves the non-specific immunity of fish and displays encouraging health benefits in terms of a reduction in mortalities (El-Sayed et al 2014). According to Payung & Manoppo (2015), the action mechanism of ginger is to stimulate the immune system (immunomodulatory), because it contains gingerol, which can increase IL-6 activity.

Similar to ginger, dietary inclusion of turmeric in this study increased the percentage of lymphocytes compared to the control diet. The increase in the lymphocyte percentage is due to the natural content of turmeric in flavonoids and curcumin, which can increase lymphocyte levels in the fish body. The increased lymphocyte counts are a sign of fish immunity system success in developing cellular (non-specific) immunity responses as a trigger of the immune response (Lestari et al 2019). Matofani et al (2013) explain that the increased activity of leucocytes is caused by infections that trigger cell division activities. This is in accordance with Riauwaty et al (2019), who noted that an increase in the number of circulating lymphocytes is observed in fish infected by *A. hydrophila*. An increased number of lymphocytes is an integral part of the defense mechanism against infections.

Dietary turmeric did not increase the percentage of monocytes; however, the 25 g kg^{-1} feed and 50 g kg^{-1} feed treatments caused a higher percentage of monocytes than the control. Riauwaty et al (2019) reported that *Pangasius hypophthalmus* infected with *A. hydrophila* fed with a turmeric supplemented diet had an increased percentage of leucocytes, but the increase was insignificant. However, the percentage of monocytes decreased significantly compared to the control. In early infection, neutrophils are the first line of innate immunity and activated neutrophils provide signals for the activation and maturation of monocytes (Rosales et al 2016). In such conditions, monocytes in the blood penetrate blood vessels into infected tissue and change in macrophages. The movement of monocytes into the infected tissue causes the number of monocytes in the blood circulation to decrease (Shi & Pamer 2011). However, in this study, the diets with ginger and turmeric for African catfish were not complemented by an infection, and, therefore, the number of monocytes in the circulation was relatively stable.

During the trial period, the temperature of the water ranged 26.5-27.8°C, pH 7.1-7.3, and dissolved oxygen concentration (DO) 5.3-5.8. The water quality in this study still meets the requirements for African catfish cultivation. This is in accordance with what was reported by Boyd (1982), who stated if these physico-chemical parameters of the water are at adequate levels, they do not influence food ingestion and growth. The pH should be between 6.5-9 (Najiyati 2007), and the dissolved oxygen content should exceed 3 ppm (Najiyati 2007).

Conclusions. Dietary inclusion of ginger (*Zingiber officinalis*) and turmeric (*Curcuma domestica*) in African catfish diets can significantly increase body weight gain (WG), feed conversion rate (FCR), hematocrit (%) and lymphocyte percentage significantly compared to controls. In addition to monocytes, dietary ginger increased the percentage of monocytes, but this did not happen in the case of the turmeric diet.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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