

The use of ambon banana (*Musa paradisiaca* var. *sapientum*) stems flour in grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) floating net cage nursery

Pemberian tepung batang pisang ambon (*Musa paradisiaca* var. *sapientum*) pada pendederan ikan kerapu cantang (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) dalam karamba jaring apung di laut

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ABSTRACT

The study aimed to test the effectiveness of ambon banana stems flour through feeding on the health status and performance of the groupers reared in the sea floating net cages. The groupers (8.27 ± 0.20 cm length and 11.69 ± 0.50 g weight) were reared in 1 m × 1 m × 1.5 m floating net cages with a stocking density of 112 fish /m³ for 42 days. The feed was commercial feed with 46% and 48% protein, which was added with 30 g/kg of ambon banana stems flour with a coating method. This study consists of three treatments and three replications, i.e. feeding at the beginning of reared for 7 days (A), 14 days (B), and feeding without ambon banana stems flour (control). The observed parameters were production performance, morphometric, hematological test, and water quality. The results showed that the production performance between treatments was not significantly different (P>0.05). Based on the morphometric test the total length for treatment A increased significantly (P<0.05) compared to the control and B. On the 14th day of the hematological test, it was known that the highest total erythrocyte, hemoglobin, hematocrit, and total leukocyte was found in treatment B, however, the differential leucocyte did not differ significantly (P>0.05). The addition of Ambon banana stems flour in feed in the first 14 days (B) can improve health status but has not been able to increase production performance and morphometric grouper.

Keywords: cantang grouper fish, production performance, health status, Ambon banana stems flour

ABSTRAK

Tujuan penelitian ini adalah menguji efektivitas tepung batang pisang ambon dalam pakan terhadap status kesehatan dan kinerja produksi ikan kerapu cantang yang didederkan dalam KJA di laut. Ikan kerapu cantang berukuran 8.27 ± 0.20 cm dengan bobot rata-rata 11.69 ± 0.50 g dipelihara dalam KJA berukuran 1 m × 1 m × 1.5 m (kedalaman air 2,5 m) dengan kepadatan 112 ekor/m³ dan dipelihara selama 42 hari. Pakan yang digunakan adalah pakan komersil dengan protein 46% dan 48%, yang selanjutnya ditambahkan tepung batang pisang ambon sebanyak 30 g/kg pakan dengan metode coating. Penelitian ini menggunakan tiga perlakuan dan tiga ulangan yakni pemberian pakan perlakuan di awal pemeliharaan selama 7 hari (A), 14 hari (B) dan tanpa pemberian pakan dengan tepung batang pisang ambon (kontrol). Parameter yang diamati adalah kinerja produksi, morfometrik, uji hematologi dan pengukuran kualitas air. Hasil penelitian menunjukkan kinerja produksi antar perlakuan tidak berbeda signifikan (P>0.05). Berdasarkan pada uji morfometrik pada bagian panjang total untuk perlakuan A ditemukan peningkatan signifikan (P<0.05) dibandingkan perlakuan kontrol dan B. Pada uji hematologi hari ke-14 diketahui total eritrosit, hemoglobin, hematokrit dan total leukosit tertinggi terdapat pada perlakuan B, sedangkan nilai diferensial leukosit antar perlakuan tidak berbeda signifikan (P>0.05). Penambahan tepung batang pisang ambon dalam pakan pada 14 hari pertama pemeliharaan (B) dapat meningkatkan status kesehatan, namun belum mampu meningkatkan kinerja produksi dan morfometrik ikan kerapu.

Kata kunci: ikan kerapu cantang, kinerja produksi, status kesehatan, tepung batang pisang ambon

INTRODUCTION

Grouper culture is one of the most economically essential aquaculture commodities in Asia, especially Indonesia, and it is striving to boost its productivity (Rimmer & Glamuzina, 2019). Supporting that objection, the aquaculture nursery section should be developed to obtain qualified seeds (Effendi, 2010). An intensive grouper nursery in a sea floating net cage is currently developing as an alternative besides a concrete or glass fiber nursery (Effendi, 2019; Mahasri *et al.*, 2020, Effendi *et al.*, 2021). A cantang hybrid grouper (*Ephinephelus fuscoguttatus* × *Ephinephelus lanceolatus*) is one of the grouper species that is highly prospective to rise aquaculture production (Fan *et al.*, 2020) the present study examined the protective effect of dimethyl sulphoxide (Me₂SO; 8-12%, v/v. Cantang hybrid grouper grows swiftly (Ebi *et al.*, 2018) *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus* for its optimum growth, survival, and normal skeletal development. Eight experimental diets containing graded levels of ascorbic acid (4.8, 11.2, 24.1, 47.2, 75.6, 95.4, 156.2, and 303.0 mg/kg, is highly tolerated towards environmental changes, and is disease resistant (Kim *et al.*, 2020).

Sea nursery frequently faces challenges, e.g. water current and dynamic wave (Effendi, 2019; Effendi *et al.*, 2021). It potentially causes stress, is easily exposed to a certain disease, even mortality, harvest failure, and decrease production (Kim *et al.*, 2017). Grouper is easily exposed to vibriosis caused by *Vibrio alginolyticus*, *V. parahaemolyticus*, and *V. harveyi* (Zhu *et al.*, 2018; He *et al.*, 2019; Kuo *et al.*, 2020). Cantang hybrid grouper exposed by vibriosis often shows clinical symptoms, such as ulcers, pale skin, torn tail, low appetite, and red patches appearance, especially on mouth and fin area (Dahlia *et al.*, 2017). The disease can be avoided by doing some prevention.

One of the disease prevention steps in aquaculture is using natural ingredients. Natural remedies for disease prevention in aquaculture often use several parts of the whole part of ingredients (seed, leaves, roots, or fruit) (Reverter *et al.*, 2017). Natural ingredients as natural remedies for an animal is considered safe and potential as antibacterial agents and immunostimulant (Tan *et al.*, 2017). The advantages of using natural remedies in animal disease prevention are easy to get, simple processing procedures, and environment friendly

(Wahjuningrum *et al.*, 2013). The ability of natural remedies as immunostimulants in aquaculture was massively studied (Caruso *et al.*, 2013). This kind of healing approach is considered to reduce chemical material abundance in food production (Srivastava *et al.*, 2014). Banana stem is one of the natural ingredients that is mainly used as natural remedy because of its high immunostimulant active compound (Latanza *et al.*, 2020).

Several former studies showed that banana stem could boost immune response and growth of tested fish. Ramadhan *et al.* (2017) reported that banana stem extract addition could increase the growth and nonspecific immune response of white shrimp. Juvenile catfish submersion in squeezed banana stem water strengthened the immune system towards *Aeromonas hydrophila* (Astria *et al.*, 2017). Study about the banana stem powder through feeding to induce health status of cantang hybrid grouper reared in the sea floating net cages has not existed yet. It motivated authors to held this study to evaluate the effectiveness of banana stem powder towards cantang hybrid grouper health status and production performance.

MATERIALS AND METHODS

Banana stem powder production

Banana stem powder production methods were referred to Pattah *et al.* (2020). Ambon banana *Musa paradisiaca* var. *sapientum* in this study was the one that already had fruit. The banana stem was chopped until 1 cm size then it was airdried at room temperature for three days. The dry and chopped banana stem were put in the oven at 60°C for two hours afterward. The material was put in a grinder machine until became powder then filtered using a 150 micro strainer. Furthermore, it was stored in an air-tight container.

Experimental design and tested feed production

This study applied a completed randomized design with three treatments and three replications. The commercial feed was combined with the banana stem powder and set according to each treatment, i.e. seven days of tested feed feeding (A), 14 days of tested feed feeding (B), and without banana stem powder addition (control). The feed used in this study was the commercial feed specialized for grouper and the feeds were coded EP2 and EP3 with 46% and 48% protein content, respectively.

The dosage of banana stem powder was 30 g/kg feed. The banana stem powder was added to the commercial feed using the coating method

(Pattah *et al.*, 2020). One kilo of feed required one egg containing the whole white part 2 g of egg yolk and 100 mL of water. Banana stem powder, egg, and water were mixed using a mixer. The mixture was added to the commercial feed then stirred using a coating machine thoroughly. After that, it was air-dried for 24 hours then stored in an air-tight container (Pattah *et al.*, 2020).

Fish rearing

Cantang hybrid grouper was reared in floating cage nets (1 m × 1 m × 1.5 m) and placed in a bigger floating cage (3 m × 3 m × 3 m) to prevent wild fish attack outside the cage. The initial average weight and length were 8.27 ± 0.20 cm and 11.69 ± 0.50 g, respectively. The fish was transported from Brackishwater Aquaculture Development Center Situbondo and adapted in the floating cage net for two weeks before treatment. During the adaptation, it was fed every two hours. It was also soaked in freshwater every week for one minute to discard any parasites attached on it. After the adaptation, the cantang hybrid grouper was distributed with 112 fish/m³ of stocking density. The experimental fish was reared for 42 days and fed three times a day (08.00, 12.00, and 16.00) using at satiation method. The feed was noted every day. During the rearing period, the floating cage nets were replaced and the experimental grouper was soaked in fresh water for one minute every week.

Production performance parameters

The production performance was calculated at the end of the study (day 42) and the parameters were consists of:

Survival rate (SR)

The survival rate was calculated using the following formula (Yan *et al.*, 2016).

$$SR (\%) = (N_t / N_0) \times 100$$

Note:

N_t = Final population (individual)

N_0 = Initial population (individual)

Daily growth rate

The specific growth rate was calculated using the following formula (Gabriel *et al.*, 2019) 0.5, 1.0, 2.0, and 4.0%/kg diet

$$\text{Daily growth rate (g/day)} = (W_t - W_0)/t$$

Note :

W_t = Average final growth (g)

W_0 = Average initial growth (g)

t = Rearing period (day)

Specific growth rate (SGR)

The specific growth rate is the daily growth percentage and it was calculated using the formula by Giri *et al.* (2016):

$$SGR (\%/day) = 100 ((\ln W_t - \ln W_0)/t)$$

Note :

W_t = Average final growth (g)

W_0 = Average initial growth (g)

t = Rearing period (day)

Feed conversion ratio (FCR)

The feed conversion ratio was calculated at the end of the study using the following formula (Giri *et al.*, 2016).

$$FCR = FI / (W_t - W_0).$$

Note :

FI = Consumed feed (g)

W_t = Final biomass (g)

W_0 = Initial biomass (g).

Feed efficiency (EP)

Feed efficiency was measured to determine the feeding efficiency of the experimental grouper. It was measured using the formula by Watanabe (1988):

$$EP (\%) = (\Delta \text{ biomass} / \sum \text{ feed}) \times 100$$

Note :

Δ biomass = Total feed during the study (g)

\sum feed = The margin between the final and initial fish biomass (g)

Morphometric measurement was conducted at the end of the study and the measured aspects were final weight, total length, standard length, flesh length, and flesh height.

Hematology parameters

Hematology observation was done every two weeks, i.e. day 0, day 14, day 28, and day 42. The observed parameters were total erythrocyte (Blaxhall & Daisley, 1973), hemoglobin (Wedemeyer & Yasutake, 1977), hematocrit (Anderson & Siwicki, 1993), total leucocyte

(Blaxhall & Daisley,1973), and differential leucocyte (Amlacher, 1970).

Water quality

Water quality measurement was managed every week. The parameters consisted of temperature (°C), pH, dissolved oxygen (mg/L), total ammonia nitrogen (TAN) (mg/L), and salinity (g/L). Temperature and dissolved oxygen were measured using a DO meter, while a pH meter was used to measure pH level. TAN and salinity were measured using a spectrophotometer and refractometer, relatively.

Data analysis

Data were analyzed statistically using Ms. Excel 2013 and SPSS 16 through analysis of variance with a 95% of confidence level. Any significant differences would be analyzed further using the Duncan test.

RESULTS AND DISCUSSION

Results

Hematology

Erythrocyte, hemoglobin, hematocrit, and total leucocyte did not show any significant difference amongst treatments ($P>0.05$) on day 0, day 28, and day 42. On the contrary, erythrocyte in treatment B ($2.45 \pm 0.46 \times 10^6$ cells/mm³) increased significantly on day 14 compared to treatment K ($1.39 \pm 0.39 \times 10^6$ cells/mm³) and A ($1.39 \pm 0.48 \times 10^6$ cells/mm³) (Figure 1A). Hemoglobin in treatment B (9.0 ± 1.0 g %) increased significantly ($P<0.05$) compared to treatment A (6.7 ± 0.99 g %) and K (6.9 ± 0.23 g %) (Figure 1B). Hematocrit levels in treatment A and B were $41.1 \pm 1.33\%$ and $42.6 \pm 2.85\%$, respectively, and it did not differ significantly,

but it was different significantly with treatment K ($36.7 \pm 1.0\%$) ($P<0.05$). The highest leucocyte was noticed in treatment B ($3.25 \pm 0.46 \times 10^5$ cells/mm³) and it was significantly different with treatment A ($2.80 \pm 0.44 \times 10^5$ cells/mm³) and K ($2.42 \pm 0.11 \times 10^5$ cells/mm³). The results of hematocrit and leucocyte can be viewed in Figures 1C and 1D.

Lymphocyte, monocyte, and neutrophil percentage in all treatments were 74.0–80.33%, 12.0–17.67%, and 7.0–10.33% (Figure 1E). The results of lymphocyte and neutrophil measurement in cantang hybrid grouper did not present significant results ($P>0.05$) amongst treatments. On the other hand, the monocyte percentage of treatment K, A, and B did not differ significantly ($P>0.05$) on day 0, day 28, day 42. However, there was a significant difference in monocyte percentage in treatment K ($15.0 \pm 1.0\%$) compared to treatment B ($12.0 \pm 1.0\%$) and A ($14.3 \pm 1.53\%$).

Production performance

Banana stem addition to cantang hybrid grouper feed showed no significant difference ($P>0.05$) amongst treatment in terms of production performance, i.e. survival rate, growth rate, specific growth rate, feed conversion ratio, feed amount, feed efficiency, and total production. The production performance of cantang hybrid grouper is presented in Table 1.

Morphometric parameter

Morphometric measurement was exhibited in Table 2. The results did not differ significantly amongst treatments in all tested parameters, except for the total length. In treatment A, the highest total length was noted and it differed significantly amongst treatments.

Table 1. Production performance of cantang hybrid grouper reared for 42 days using different duration of banana stems flour feeding.

Tested parameters	Treatments		
	K	A	B
Survival rate (%)	94.67 ± 4.16 ^a	92.67 ± 2.52 ^a	93.67 ± 2.31 ^a
Growth rate (g/day)	0.73 ± 0.04 ^a	0.73 ± 0.04 ^a	0.73 ± 0.02 ^a
Specific growth rate (%)	3.07 ± 0.13 ^a	3.02 ± 0.13 ^a	3.13 ± 0.08 ^a
Feed consumption (g)	2700 ± 65.00 ^a	2711.67 ± 68.07 ^a	2723.33 ± 48.56 ^a
Feed conversion ratio	0.94 ± 0.02 ^a	0.94 ± 0.09 ^a	0.92 ± 0.04 ^a
Feed efficiency (%)	106.75 ± 2.09 ^a	106.87 ± 10.12 ^a	109.33 ± 5.30 ^a
Production (g/m ³)	2679.32 ± 51.64 ^a	2653.66 ± 141.23 ^a	2620.74 ± 119.20 ^a

^aDifferent superscript in the same column indicates a significant difference.

Table 2. Morphometric parameters of cantang hybrid grouper reared for 42 days using different duration of banana stems flour feeding.

Tested parameters	Treatments		
	K	A	B
Weight (g)	42.49 ± 1.39 ^a	42.95 ± 1.69 ^a	41.95 ± 0.89 ^a
Total length (cm)	13.28 ± 0.13 ^{ab}	13.43 ± 0.05 ^b	13.23 ± 0.05 ^a
Standard length (cm)	11.58 ± 0.05 ^a	11.71 ± 0.18 ^a	11.51 ± 0.21 ^a
Flesh length (cm)	7.64 ± 0.18 ^a	7.81 ± 0.06 ^a	7.72 ± 0.01 ^a
Flesh width (cm)	3.55 ± 0.04 ^a	3.61 ± 0.17 ^a	3.73 ± 0.11 ^a

^aDifferent superscript in the same column indicates a significant difference.

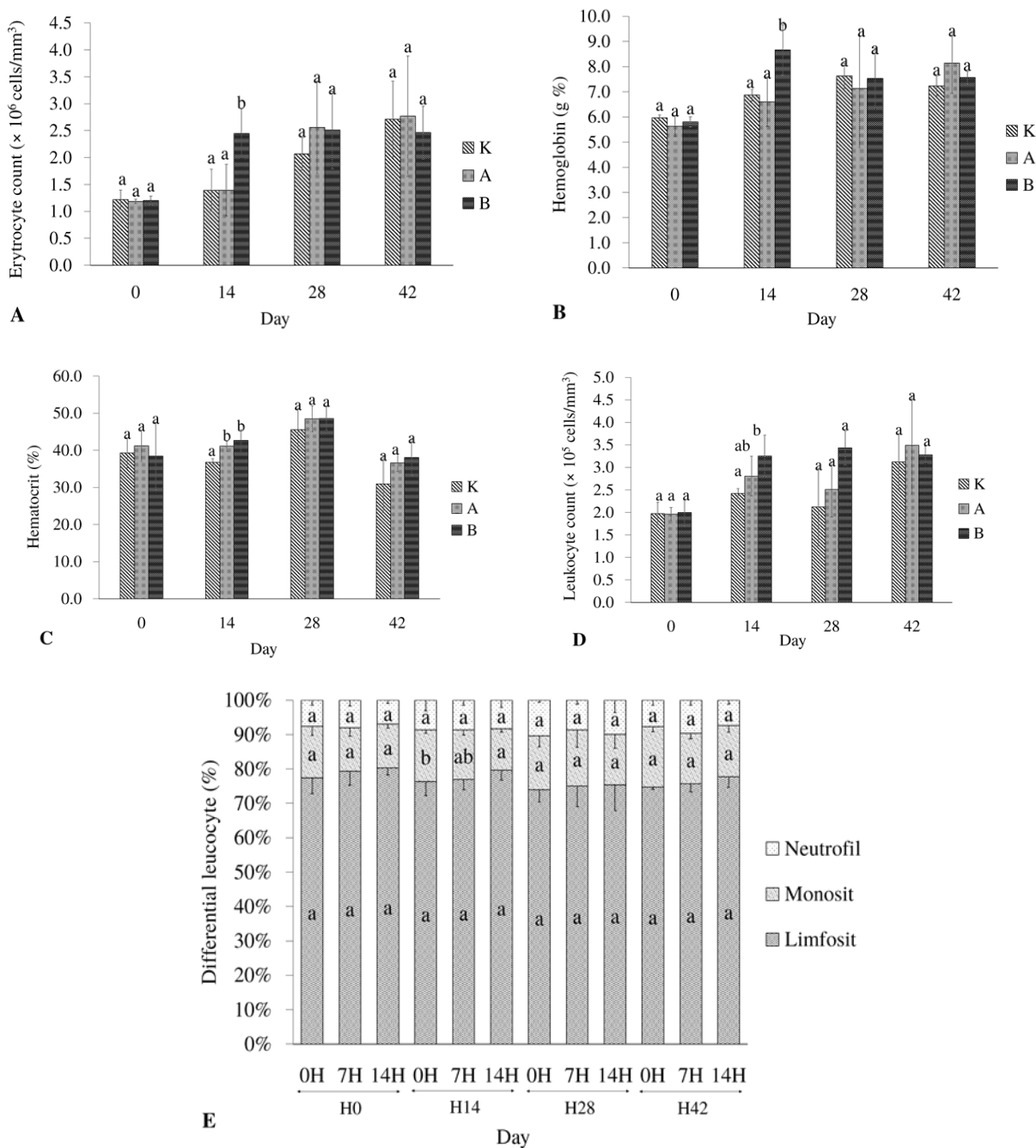


Figure 1. Total erythrocyte (TE) (A), hemoglobin (Hb) (B), hematocrit (Ht) (C), leucocyte count (TL) (D), differential of leucocyte (DL) (E) of cantang hybrid grouper. The different letter above the bars (average ± standard error) indicates significance. K is the control treatment, A and B are banana stem dosages for seven and 14 days.

Water quality

The temperature during the rearing period was 32–36°C and the pH level ranged from 7.6–8.3. The dissolved oxygen, total ammonia nitrogen (TAN), and salinity were 4.2–11 mg/L, 0.0029–0.0816 mg/L, and 27.8–32.6 g/L, respectively.

Discussion

Hematology is an essential indicator to determine health status. Physiological and pathological changes can be observed through hematology tests (Fazio, 2019). Other than that, a hematology test is frequently used to evaluate the functional condition and blood ability to carry oxygen, nutrient, and metabolic waste ((Fazio *et al.*, 2013a; Burgos-Aceves *et al.*, 2019). The particular hematology parameters that were measured in this study were erythrocyte, hemoglobin, hematocrit, leucocyte, and leucocyte differential count (Figure 1).

The erythrocyte count of treated cantang grouper increased during the study. Erythrocyte is frequently used as an initial diagnosis of anemia (Fazio *et al.*, 2015). The result of erythrocyte count in the tested cantang hybrid grouper ranged from 1–4 × 10⁶ cells/mm³. Irianto (2005) stated that the erythrocyte count of Teleostei commonly ranges from 1.05–3 × 10⁶ cells/mm³. It indicated that ambon banana stems flour did not negatively affect the erythrocyte count of cantang hybrid grouper. The increased erythrocyte count was presumably caused by flavonoids contained in the banana stems flour as an antioxidant to prevent erythrocyte deficiency caused by pathogen infection (Agung *et al.*, 2013). Flavonoid is a polyphenol compound that is commonly found in plants (Bhaigyabati, 2016). Erythrocyte count is in line with hemoglobin content. Hemoglobin increase will be followed by erythrocyte count (De *et al.*, 2019) (Figure 1A; 1B).

Hemoglobin showed a relation with oxygen transportation as a fish metabolism index (Walker *et al.*, 2020). The hemoglobin content during the study ranged from 5.1–8.3 g %. A healthy marine species usually has around 5.1–8.3 g % of hemoglobin content (Fazio *et al.*, 2013b). Huang *et al.* (2018) stated that hemoglobin showed homeostasis in cantang hybrid grouper. The flavonoid in banana stem extract acts as a strong antioxidant that neutralizes free radicals (Pietta, 2000). Hemoglobin fluctuation could be affected by various factors, such as fish sex (Karimi *et al.*, 2014) and environment (Kim *et al.*, 2019). Hematocrit levels in this study ranged from 30%–

49% (Figure 1C). De *et al.* (2019) stated that a normal hematocrit level of grouper is usually between 20%–35% and it barely hit 50% (Huang *et al.*, 2018). Ambon banana stems flour supply to increase hematocrit level of cantang hybrid grouper. A high level of hematocrit causes fish to move actively (Dal’Bó *et al.*, 2015). The flavonoid in the banana stem extracts reduced erythrocyte lysis through biological erythrocyte membrane or erythrocyte cell protection (Wahjuningrum *et al.*, 2021) curative, and controlling treatments. Except negative control, all groups were challenged by *A. hydrophila* at a density of 10⁷ CFU/mL–1 (0.1 mL per fish).

Leucocyte takes an essential role in the nonspecific immune system or innate immunity and the number of leucocyte cells often act as fish health status indicator. The leucocyte of cantang hybrid grouper elevated, especially in the banana stems flour treatment compared to the control (P<0.05) (Figure 1D). The banana stem contains various bioactive compounds, such as antioxidant, antibacterial, and antiviral to boost nonspecific immune responses (Naikwade *et al.*, 2018). This plant particularly contains several antibacterial activities because of the secondary metabolite existence, i.e. alkaloids, tannin, flavonoids, terpenoids, and saponin (Venkatesh *et al.*, 2013). It showed that ambon banana stems flour could increase the innate immune system that would deliver long live protection against diseases. Rattanavichai and Cheng (2014) reported that banana stem extract that delivered through feed, could boost the immune response of giant freshwater prawn *Macrobrachium rosenbergii* and its defense against disease.

Leucocyte differential showed leucocyte cells (lymphocyte, neutrophile, and monocyte) performance. All the experimental treatments did not affect leucocyte cells (P>0.05) (Figure 1F). Lymphocyte increase was caused by the cell proliferation became lymphocyte T and B. Most of the antigens were expressed on the membrane of lymphocytes T and B (Kelly & Salinas, 2017). Neutrophile and monocyte had the main role in the phagocytic activity, digesting, destroying the bacteria, and being responsible for the initial reaction when inflammation occurred (Akhter *et al.*, 2015). Phagocytic activity increased because of the pathogen existence in the host cell.

The survival rate of cantang hybrid grouper did not differ significantly amongst treatments (P<0.05). It showed that ambon banana stems flour treatment did not affect survival negatively.

The latter statement was supported by Giri *et al.* (2016) banana stems flour feeding had no impact on rohu carp *Labeo rohita* survival rate. A similar nonsignificant result was also encountered in the growth parameters ($P>0.05$). It was in line with Nwana *et al.* (2014) that banana stems flour feeding did not influence the growth rate and feed conversion ratio of African catfish.

The fish growth rate is closely affected by the digestibility rate. Digestibility is also influenced by fiber content and nutrition profile in the feed (Bhuyain *et al.*, 2019). Generally, banana contains anti-nutrition compounds such as fiber, phenolic acids, and flavonoids (Felix e Silva *et al.*, 2020). The existence of the anti-nutritional factors (ANFs), which are relatively high, presumably will influence the fish digestive system and metabolism. Several ANFs that are frequently found in the plant are phytate acids, phenolic compounds (e.g. tannin), and saponin (Nikmaram *et al.*, 2017). Saponin and tannin are ANFs that at the same time function as antibacterial compounds in a banana plant (Sivasamugham *et al.*, 2021).

Ambon banana stems flour addition to fish feed with different duration did not affect significantly ($P>0.05$) the consumed feed, feed conversion, and feed efficiency. It could be assumed that banana stems flour feeding had no impact on fish production parameters of cantang hybrid grouper. Feed conversion ratio and feed amount are closely related to certain species, size, feed nutrition profile, and fish digestibility (Xie *et al.*, 2021). Additional herbs in fish feed can increase fish response and digestibility towards the feed (Reverter *et al.*, 2017). The distinct smell and taste from a certain herb potentially elevate consumed feed (Pu *et al.*, 2017). Pattah *et al.* (2021) stated that the addition of banana stems flour to fish feed boosted the appetite of Asian sea bass *Lates calcarifer*. Similar results have also encountered the study by Gabriel *et al.* (2019) that presented that garlic extract addition uplifted the consumed feed-in African catfish.

Final weight, total length, standard length, flesh length, and flesh width in this study were not different significantly amongst treatments ($P>0.05$). It was shown that banana stems flour feeding did not affect the nutrition absorption in transforming fish bodies. Morphometric measurements were done to determine fish growth patterns and eating ability (Suryana *et al.*, 2015). Fish's ability to utilize carbohydrates and fats was limited and depended on proteins as

the main source of growth. Feed lipid increasing will also elevate the overall body fat. However, fat accumulation in the liver and muscle tissues will be unbalanced (Yue & Li, 2020). The major factor in fish growth is protein. The higher the protein absorbs, the higher the fish grow followed by solid muscle tissue (Munisa *et al.*, 2015). In terms of water quality, each parameter was in an optimal range according to the Indonesian National Standard (SNI) 8036.2:2014 about water quality reference for cantang hybrid grouper.

CONCLUSION

Ambon banana stems flour feeding (30 g/kg) for 14 days elevated cantang hybrid grouper health status significantly compared to the control treatment.

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