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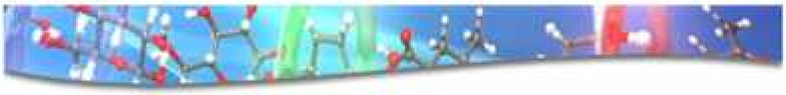

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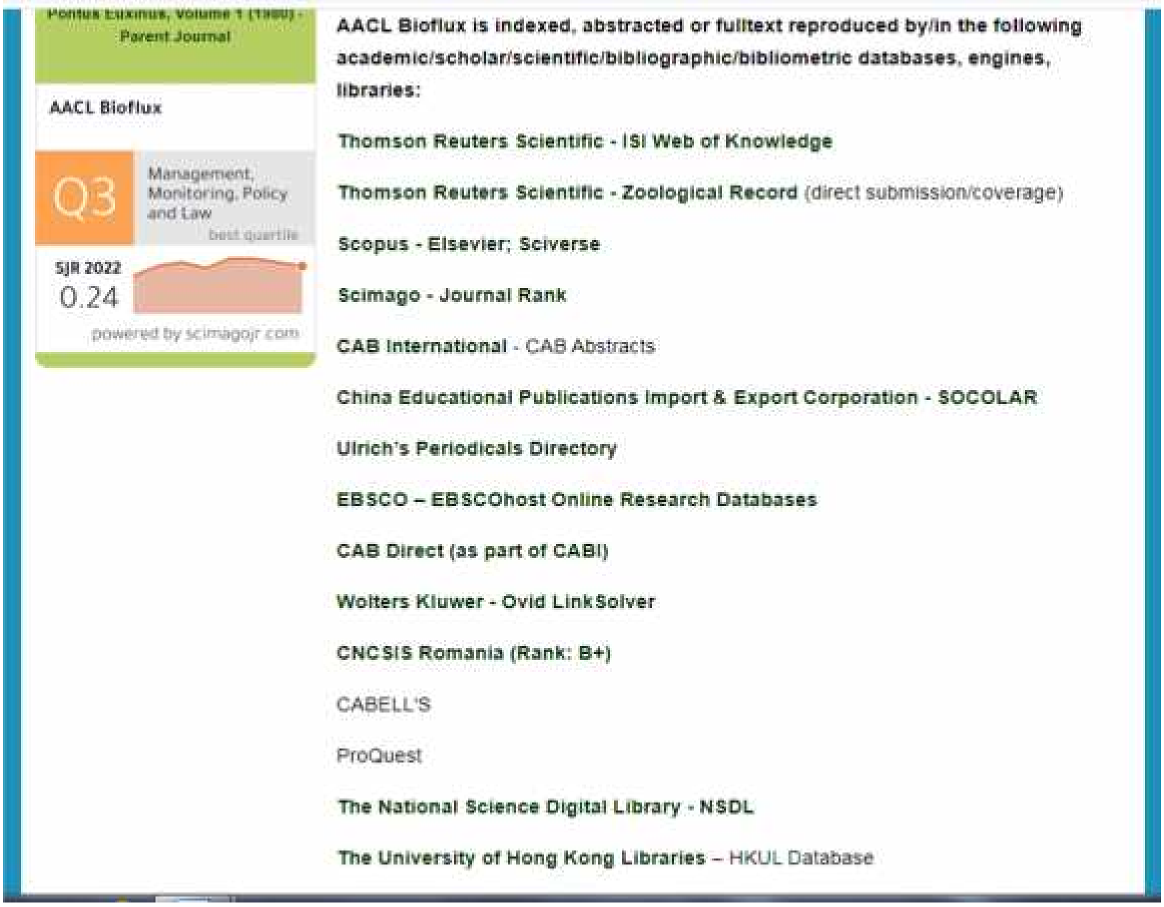

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Effects of ameliorant on the growth of snakehead fish (*Channa striata*) juvenile reared in acid sulfate water medium located in tidal land

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Abstract. The aim of this study was to evaluate the effects of the use of an ameliorant produced from banana stems to increase water pH and improve the growth of the snakehead fish (*Channa striata*). The experiment applied a completely randomized design. The treatments applied in this study consisted of three treatments including ameliorant (300 kg ha^{-1}), lime ($CaCO_3$) (180 kg ha^{-1}), and control (without ameliorant and lime). Each treatment applied four replicates. *C. striata* juveniles with an average initial length of 2.5±0.2 cm and an average initial weight of 0.27±0.03 g were reared in 12 units of hapa net cages measuring 2 x 3 x 1.5 m with a stocking density of 2 fish per L, for 60 days. The fish were fed commercial feed with a protein content of 40%, twice a day (morning and afternoon) until to apparent satiation. The use of an ameliorant in the experimental medium significantly gave better results shown by higher survival (70.55%), specific growth rate (6.42% day^{-1}), feed efficiency (89.07%), protein retention (53.41%), fat retention (89.41%), and albumin content (3.12 g per 100 mL) than those of control.
Key Words: Air-breathing fish, banana stems, dolomite, tidal area.

Introduction. Indonesia has about 20.11 million ha of tidal swamp land consisting of 2.07 million ha of tidal potential land, 6.71 million ha of acid sulfate land, 10.89 million ha of peatland, and 0.44 million ha of saline land. The tidal land is spread in several regions, including Sumatera, Kalimantan, Papua, and Sulawesi (Arsyad et al 2014). Tidal swamp land area of West Kalimantan is around 1.12 million ha, which is spread in several districts (BPS Kalbar 2012) and 20,532 ha of it is acid sulfate land (Alwi 2014). Most of these lands have been used for fish farming, but in general, the productivity is still relatively low. The low productivity of fisheries sectors in this area is due to various problems, especially poor water quality. In a low water quality condition, there are only certain fish species that can be cultivated, one of those species is the snakehead fish (*Channa striata*).

C. striata, is a freshwater fish species, classified in Channidae family and Perciformes order (Nakkrasae et al 2015). This fish is a potential and important species to be developed as an aquaculture commodity (Mollah 1985; Marimuthu et al 2009; Mollah et al 2009; Rahman et al 2013), and has high economic value with prices ranging from 3.78 to 4.47 USD kg^{-1}. Moreover, this fish flesh is used as a treatment of post-surgical therapy and can increase body endurance (Gam et al 2006; Marimuthu et al 2009; Mohd & Abdul 2012).

C. striata usually lives in rivers, rice fields and ponds. *C. striata* relatively prefers calm water (without agitation). Another advantage possessed by *C. striata* is its ability to live in low oxygen environment. But to grow optimally, this species needs to live in a medium with optimal water quality ranges. One of limiting factors that influences the *C. striata* cultivation in acid sulfate land is pH. Tidal land has a pH range of 2.53–3.39 (Purnamawati et al 2017a), sulfate concentration at 6.91–8.7 mg L^{-1}, dissolved oxygen less than 5 mg L^{-1} (Purnamawati et al 2017b). This water quality condition can be improved by increasing the pH of the culture medium.

To increase the water pH in fish ponds can be done by adding a certain amount of lime into fish ponds (de Queiroz et al 2004; Cavalcante et al 2012). However, the required lime is not always available, especially in some locations that are far from the centers of agricultural production facilities. To overcome this issue, some local based plant materials can be used as ameliorants to substitute the utilization of lime.

Banana stem is a waste that can be used as an ameliorant. This is caused by banana stem has some advantages, including low cost, abundant availability, easy to be processed, and it is not harmful for the cultivated organisms. This material is expected to be a solution in aquaculture practices conducted in acid sulfate medium, especially in tidal land. This study aimed to evaluate the effectiveness of the use of banana stems as an ameliorant in acid sulfate medium in order to promote the growth of *C. striata* juveniles.

Material and Method. The present study was conducted on July-September 2018. The rearing of *C. striata* was conducted in a tidal pond located in Kubu Raya District, West Kalimantan, Indonesia. The measurement of plasma glucose levels, albumin levels, the proximate analysis of the fish feed and the experimental fish were conducted at Environmental and Food Technology Laboratory, Tanjungpura University, Pontianak, Indonesia.

Experimental design. This study was conducted through a completely randomized design (CRD) in a tidal pond using hapa net cages. The treatments applied in this study consisted of three treatments including ameliorant using banana stems (300 kg ha^{-1}), lime ($CaCO_3$) (180 kg ha^{-1}), and control (without ameliorant and lime). Each treatment applied four replicates.

Experimental fish. The experimental fish used in this study were *C. striata* juveniles. The experimental fish had an average initial length of 2.5±0.2 cm and an average initial weight of 0.27±0.03 g.

Experimental mediums. The experimental mediums used in this study were 12 units of hapa net cage with a size of 2 x 3 x 1.5 m. The hapa net cages were set in a pond, the top sides were covered with nets to avoid fish jumping out of the rearing cages.

Experimental procedures. The production of ameliorant from banana stems used a bio-activator (EM4: effective microorganism 4) to accelerate the composting process. The composting process occurred aerobically. After the raw material became a compost, it was then dried under sunlight. The chemical composition of a compost from banana stems consisted of Ca (1.54%), Mg (0.86%), C-organic (43.39%), N (2.48%), C/N (17.50), pH (8.8), KTK^{+} (41.07 $cmoL^{+}$ kg^{-1}), humic acid (2.50 ppm), fulvic acid (0.80 ppm), and moisture (68.78%) (Purnamawati 2017).

Prior to the experiment, the experimental fish were acclimatized to experimental conditions. This step was carried out for 7 days in three net cages with a size of 4 x 3 x 1.5 m. The snakehead fish were then reared in a tidal pond using hapa net cages with a stocking density of 2 fish per L (Vivekanandan 1977; Hidayatullah et al 2015). This study was conducted for 60 days. The experimental fish were fed commercial feed with a protein content of ±40%, twice a day to apparent satiation.

Experimental parameters. The measurements of water quality parameters were performed following the procedures described by APHA (1989). The water quality parameters measured were temperature, pH, dissolved oxygen, sulfate (SO_4^{2-}), alkalinity, and ammonia. The measurements of temperature, pH and dissolved oxygen level were carried out every 15 days, while the measurements of sulfate, alkalinity and ammonia were performed at the beginning, middle and the end of the experiment.

The measurement of albumin levels followed the method described by Infusino & Panteghini (2013), while the proximate analysis of the fish feed and the experimental fish were carried out according to the procedure by Takeuchi (1988). The measurement of plasma glucose levels was performed using a liquicolor glucose commercial kit GOD-PAP with calorimetric method and the results of the measurement were read with a spectrophotometer at a wavelength of 500 nm following the procedure constructed by Wedemeyer & Yasutake (1977).

The daily mortality was recorded to obtain the survival data. Survival was calculated using a formula according to Kang'ombe & Brown (2008):

$$SR = (N_t \times N_0^{-1}) \times 100$$

Note:

SR = Survival (%)

N_t = The number of the fish at the end of the study (individual)

N_o = The number of the fish at the beginning of the study (individual)

The fish weight samplings were done every 15 days to obtain the growth data (specific growth rate). Specific growth rate was calculated using a formula stated by Weatherley & Gill (1989).

$$SGR = [(\ln W_2 - \ln W_1)/(t_2 - t_1)] \times 100$$

Note:

SGR = Specific growth rate (% day^{-1})

W_1 = The average weight of the fish at the beginning of the study (g)

W_2 = The average weight of the fish at time t_2 (g)

$t_2 - t_1$ = Experimental duration

Feed intake (F) was obtained by summing the daily amount of the feed consumed during the study. The feed efficiency was calculated using a formula constructed by Kang'ombe & Brown (2008).

$$e = [((Wt + D) - Wo) / F] \times 100$$

Note:

e = Feed efficiency

W_o = The weight of the fish at the beginning of the study (g)

W_t = The weight of the fish at the end of the study (g)

D = The weight of the dead fish during the study (g)

F = The weight of total feed given during the study (g dry weight)

Statistical analysis. The water quality parameters were analyzed through descriptive statistic. Survival, specific growth rate, albumin content, feed efficiency, protein retention, and fat retention were analyzed through ANOVA with a help of SPSS version 22 to see the differences among treatments .

Results and Discussion. The water quality ranges in the experimental mediums are shown in Table 1. Water quality ranges varied among one treatment and other treatments. Water temperature ranges in the experimental mediums were not relatively different among treatments and those were an optimal range for the fish growth. The pH, total alkalinity, and sulfate showed differences between ameliorant added and lime added treatments to control (without the addition of ameliorant and lime). This could be seen from the high pH and total alkalinity, as well as the low sulfate levels dissolved in water. There was no difference in dissolved oxygen (DO) and ammonia levels among ameliorant, lime and control treatments.

Table 1
The values of physical-chemical parameters of the water in each treatment during the study

Parameters	Treatments			Tolerance range* and optimum range**
	Ameliorant	Lime (Dolomit)	Control	
pH	6.8–7.2	6.9–7.4	5.3–5.8	4.25–9.4*[3)]
Sulfate (SO_4^{2-}) (mg L^{-1})	10.2–12.6	10.7–29.6	20.1–48.2	5–150*[1)]
Alkalinity (mg L^{-1})	85.1–115.8	71.0–110.4	22.1–26.6	100–150*[1)]
Ammonia (NH_3) (mg L^{-1})	0.01–0.05	0.01–0.07	0.03–0.05	0.54–1.57*[2)]
DO (mg L^{-1})	4.12–5.80	4.15–5.47	4.79–5.12	> 5[3)]
Temperature (°C)	29.32–30.12	29.42–30.29	28.32–30.02	26–32**[3)]

[1)]Wedemeyer (1996), [2] Qin et al (1997), [3)]Courtenay, Jr. & Williams (2004), [*)]tolerance range, [**)]optimum range.

The growth performance (survival, SGR and feed efficiency) and physiological characteristics (protein retention, energy retention, and albumin content) of *C. striata* in 60-day-experiment can be seen in Table 2. The addition of an ameliorant into ponds located in an acid sulfate land could produce the highest specific growth rate (SGR) and albumin level of *C. striata* (6.42% day^{-1} and 3.12 g per 100 mL, respectively), followed by lime added and control treatments ($p<0.05$). The survival (SR) of *C. striata* juveniles ranged from 54.45 to 70.55%. The highest SR was obtained in ameliorant treatment, but it was not significantly different from the lime added treatment. The same results were also found in feed efficiency and protein retention (PR), while fat retention (FR) does not show a significant difference between among treatments tested in this study.

Table 2
Survival (SR), specific growth rate (SGR), albumin content, feed efficiency, protein retention (PR), and fat retention (FR) of *Channa striata* reared in the different mediums during the study

Parameters	Treatments		
	Ameliorant	Lime (Dolomit)	Control
SR (%)	70.55±7.21^a	62.58±3.60^a	54.45±3.13^b
SGR (% day^{-1})	6.42±0.33^a	5.54±0.21^b	4.77±0.39^c
Albumin content (g per 100 mL)	3.12±0.06^a	2.88±0.10^b	2.69±0.08^c
Feed efficiency (%)	89.07±6,33^a	79.66±6.98^a	67.61±6.36^b
PR (%)	53.41±4.70^a	47.98±4.48^a	40.79±3.14^b
FR (%)	89.41±13.17^a	88.04±14.67^a	76.79±8.03^a

Different superscript letters in the same row indicate significant different results ($p<0.05$).

C. striata is classified as a fish that is able to live in marginal medium, but it is sensitive to extreme environmental changes as well as majority of organisms. In relation to environmental conditions, the survival of the fish will be high, if the living medium is in a good condition, but when the living medium is not in a good condition, it will lead to the increasing of the fish mortality. The survivals of *C. striata* juveniles in the current results using ameliorant and lime were higher than the previous studies conducted in the laboratory using acidic sulfate water medium that resulted SR at a value of 54.44% (Purnamawati et al 2017a), but those were lower than the nursery phase in soil ponds with an SR of 57.33–76.67% (Rahman et al 2013) and the rearing in acidic sulfate water medium without aeration (SR 92%) (Purnamawati et al 2017b).

The administration of ameliorant is able to produce water quality conditions that are appropriate to the habitat of *C. striata*. The improvement of water quality in acidic sulfate water through the addition of an ameliorant allows the fish to utilize feed more optimal. The pH range of 6.8-7.4 in the ameliorant and lime treatments is still good enough for the growth of *C. striata* juveniles, because it is still in tolerance range of pH for the *C. striata* life (4.25-9.4) (Courtenay & Williams 2004), while the optimal pH for the intensive fish farming is 7.0–8.0 (Wedemeyer 1996). In this pH range, gills can maximally bind oxygen and ions required by the body, so that the physiological activities of the fish will be more optimal.

The use of ameliorant is related to the increase in Ca^{2+} needed to bind sulfate (SO_4^{2-}) (a source of the water acidity) to form gypsum deposits (Fitzpatrick et al 1998). Humic acid, fulvic acid and humin acid contained in an ameliorant cause an increase in pH (H^+ decreases). The sulfate level in ameliorant treatment was 10.2–12.6 mg L^{-1}, this range was still within the tolerance range for the fish life. In all treatments, the sulfate levels in the experimental mediums were low and still within the tolerance range for fish life according to Boyd (1988).

The improvement of water quality in ponds located in acid sulfate land through the administration of an ameliorant produced from banana stems in the *C. striata* farming provided the best results compared to the control and a treatment using lime, because the water's toxicity potential became reduced. This was shown by a lower ammonia (NH_3) level (0.01–0.05 mg L^{-1}) in ameliorant treatment compared to other treatments. This level can still be tolerated by the *C. striata*, because at ammonia level of 1.57 mg L^{-1}, the *C. striata* juvenile can still grow (Qin et al 1997).

Dissolved oxygen (DO) level range during the study was 4.12-5.80 mg L^{-1}. This range still meets the range recommended for the life and the growth of the *C. striata* (Boyd 1988). The *C. striata* is categorized in air breathing fish that has diverticula as the additional respiratory organ, so the fish can live in poor-oxygen-medium and can tolerate hypoxia by using oxygen derived from the air (Huang et al 2011; Song et al 2013).

Alkalinity in water is caused by bicarbonate (HCO_3), carbonate (CO_3^-), and hydroxide (OH^-) ions. At the ameliorant treatment, the alkalinity level was 85.1–115.8 mg L^{-1} $CaCO_3$. This level was slightly higher than those of the lime treatment and control, and it was not far from the optimal range (100-150 mg L^{-1} $CaCO_3$) (Wedemeyer 1996). In the optimal range, the ability of alkalinity to sustain the pH of the water becomes better, but it does not negatively affect the fish growth (Wedemeyer 1996; Boyd 1988).

The results of statistical analysis showed that the administration of an ameliorant and lime significantly affected the survival (SR) of the *C. striata*. The administration of an ameliorant at a dose of 300 kg per ha pond and lime at a dose of 180 kg per ha pond produced a higher SR compared to control ($p<0.05$). This was due to the better water quality in both treatment mediums compared to control. This caused the stress level of the fish decreasing and the fish became healthier. As a consequence, SR in ameliorant and lime treatments was also higher.

Growth is a complex biological process. Growth can occur when there is excess energy and materials derived from the feed. The *C. striata* juveniles which were reared in the ameliorant treated pond produced the highest specific growth rate (SGR) (6.42%), followed by the lime and the control groups with SGR values of 5.54% and 4.77%, respectively ($p<0.05$) (Table 2). The difference in growth rates showed that the *C. striata* which were reared in the ameliorant treated pond were better in utilizing the energy sourced by the feed.

The high SGR in the *C. striata* juveniles reared in the ameliorant treated pond was supported by a finding showing the highest albumin content in the fish blood (3.12 g per 100 mL) found in ameliorant treatment compared to other treatments ($p<0.05$). A better water quality in the ameliorant treated pond will improve the ability of the fish to absorb nutrient from the feed. It is reflected in the high albumin content in the fish reared in the ameliorant treated pond. By maintaining albumin in the blood plasma, the fish can also maintain blood volume. Albumin is one of blood plasma proteins synthesized in the liver (Infusino & Panteghini 2013). Albumin plays a role in transporting small molecules through plasma and extracellular fluid (Suprayitno 2014), maintaining plasma balance in blood vessels with fluid in the interstitial cavity and the formation of cells and tissues (Nugroho 2012).

The *C. striata* juveniles which were reared in the medium treated with an ameliorant had higher feed intake and feed efficiency than those of control, but those were not significantly different from the lime treatment. This showed that the feed given could be properly utilized by the fish, so that nutrients contained in the feed could be retained in the fish body efficiently.

The feed efficiency is determined by several factors such as genetic, nutritional content, farming management, and environment (Robinson & Li 2015; Hasan & Soto 2017). In normal conditions, physiological processes in the fish's body will run well, so that the feed can be used entirely by the fish and can be converted into the fish flesh (Nelson & Chabot 2011). Daily feed intake is one of factors that influence the potential of the fish to grow optimally and feed consumption rate is closely related to the capacity and the emptying of the fish gut (Abowei & Ekubo 2011).

The high SGR in the ameliorant treatment was not only reflected in the albumin content and feed efficiency, but also reflected in protein retention (PR). The ameliorant and lime treatments resulted in higher PR (53.41% and 47.98%) than that of control (Table 2). Protein retention is affected by the type of the feed, feed quality and environmental conditions (Samantaray & Mohanty 1997; Purnamawati et al 2017a). The better is the feed quality and the environment, higher is the protein retention. The administration of an ameliorant and lime into the pond has a major role in improving water quality, so that the utilization of the feed energy becomes more optimal. This causes the protein derived from the feed will be retained more in the body and decomposed only in a few amount or it will be utilized as energy to maintain homeostasis. Jobling et al (1991) state that the high energy for body activities will reduce the energy budget for the growth. According to Nelson & Chabot (2011), energy in the feed is physiologically used for activities and metabolism, while the residue will be deposited as body tissue through the growth process and the synthesis of reproductive tissue.

The difference in water quality conditions as a result of different treatments did not significantly influence fat retention ($p>0.05$). This described that the utilization of fat by the fish in three experimental groups was not much different. This is thought to occur because the energy derived from the feed, especially derived from protein, is widely released by the body for the adaptation to poor environmental conditions.

Conclusions. An ameliorant from banana stems can be used as a substitute for lime in improving water quality of a pond located in acid sulfate land. The use of an ameliorant and dolomite resulted in higher survivals of the *C. striata* than that of without using an ameliorant and dolomite, but the best growth was obtained in the ameliorant group.

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