

Effect of *Durio zibethinus* Murr. cv. Monthong Rind as a Dietary Ingredient in Feed on the Growth Performance and Disease Resistance Against *Aeromonas hydrophila* in Red Tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*)

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Abstract: Monthong durian is a durian species mainly cultivated in Thailand. The rind makes up about 60-75% of whole fruit and is often discarded. Here, we evaluated the effect of durian rind, specifically the inner white peel, as a supplement for a fish diet on the growth performance and disease resistance against *Aeromonas hydrophila* in Red Tilapia. Fish with an initial average weight of 42.68 ± 0.11 g were fed diets supplemented with durian rind at 0, 10, 15, and 20% for 140 days. Subsequent results showed that growth performance in terms of weight gain, average daily gain, length gain, and specific growth rates were not significant among treatments ($P > 0.05$). In addition, the feed conversion ratio and survival rate of fish fed with diets supplemented with durian rind were also not significantly different from the control ($P > 0.05$). After the 140-day feeding trial, fish were injected intraperitoneally with *A. hydrophila*, and the cumulative mortality was recorded for 14 days. The survival rate of fish-fed durian rind-supplemented diets at all levels was higher than that of the control. The highest survival rate and relative survival percentage were fish fed with 10% and 15% durian rind in the diet. Taken together, the white peel of the durian rind can be a potential fish feedstuff and can act as a natural antibiotic to improve fish resistance against *A. hydrophila*. Recommended level of durian rind supplementation is 10%-15% in the diet which will not affect growth but can enhance disease resistance to *A. hydrophila*.

Keywords: *Durio zibethinus* Murr. cv. Monthong; *Oreochromis niloticus* x *Oreochromis mossambicus*; growth performance; disease resistance

1. Introduction

Tilapia is often called the "Aquatic chicken" due to its high growth performance, low cost, and palatability [1]. It has become one of the most popular cultured aquatic species worldwide and is farmed under semi-intensive and intensive

systems. Unfortunately, intensive culture practices for tilapia resulted in low water quality and a high incidence of diseases, especially bacterial infections, which subsequently led to reduced production. "Motile Aeromonas Septicemia" caused by *Aeromonas hydrophila*, a Gram-negative bacilliform, is one of the most concerning diseases in farmed fish. This disease affects various freshwater fish species, including Channel catfish (*Ictalurus punctatus*) [2], Nile tilapia (*Oreochromis niloticus*) [3], and Red tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) [4]. In tilapia farms, *Aeromonas* infections have been reported to occur frequently [5]. The symptoms of this disease include skin ulcerations and fin erosion [6]. To curb the devastating effects of this disease, stimulating fish immunity has been postulated as a solution. In the past decade, there has been an increased interest in developing fish feed additives that can serve as immunostimulants [7].

Durian (*Durio zibethinus* Murr.) is a popular fruit in many countries such as Malaysia, Philippines, Indonesia, China, and Thailand [8-10]. It has a thick rind that constitutes about 60-75% of the whole fruit [11], which are discarded and form part of agricultural waste. Previous studies have shown that polysaccharides (PG) can be isolated from durian-rind waste [12-13]. Bioactivities of PG extracted from durian rind have been reported to have antibacterial [8, 14] and immunomodulating properties [15-17]. These studies, however, used the extracted form, which may be laborious and costly for fish farmers. Moreover, there is very little information on durian rind-supplemented diets for fish. Hence, the present study aimed to determine the effect of dried and ground durian rind supplemented in fish diets on the growth performance and disease resistance against *A. hydrophila* in Red Tilapia (*O. niloticus* × *O. mossambicus*).

2. Materials and Methods

2.1 Experimental design

All experiments were approved by the Ethics Committee of King Mongkut's Institute of Technology Ladkrabang (Approval no. ACUC-KMITL-RES/2021/034). The Fingerlings of Red tilapia were sourced from the Aquaculture Genetics Research and Development Center, Department of Fisheries, Chumphon Province, Thailand. These fish were cultured in the pond of King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon Province, for two months. Only fish with an average weight of 40-45 g were used in this study. After initial acclimatization, 400 Red tilapia were randomly distributed to 16, 500-L plastic tanks, each holding 25 fish. The tanks were again randomly assigned to feed diets supplemented with durian rind at 0, 10, 15, and 20%. Each treatment has four replicates. The Red tilapia were trained to feed on sinker pellet feed using a control diet for 14 days before the start of the experiment.

2.2 Preparation of durian rind and experimental diet

Ripe durian fruits from the organic garden in Chumphon Province were selected, and only the white inner rind was used. The rind was cleaned with tap water. After that, the rind was cut into 1-2 cm thick pieces, dried at 60°C for 48 h, and ground and sifted through a 0.5 mm sieve. The chemical composition of the processed rind was analyzed following earlier methods [18], and the minerals present were evaluated using ICP-OES (Perkin Elmer version AVIO500); samples were prepared following Allan [19] before it was mixed with the other components of the diet. The composition of each diet is shown in Table 1. The ingredients of each diet were homogeneously mixed using a Hobart mincer (model HL200) for 10 min and pelleted to make a diet at 3.0 mm in size. All diets were dried in a hot air oven at 60°C for 12-24 h. The experimental diets were kept in plastic bags and refrigerated at 4°C until use. The proximate analysis of all experimental diets was also analyzed following AOAC [18].

2.3 Feeding management

Red tilapia were fed with experimental diets twice daily, at 8:30 a.m. and 4:00 p.m., at 3% of their body weight for 140 days. Water quality, including temperature, pH, dissolved oxygen, ammonia, and nitrites, was checked every 2 weeks. The tanks were cleaned daily, and water exchange at about 50% was done every 2 days.

Table 1. Composition of experimental diets

Diet ingredients (g)	Durian rind (%)			
	0	10	15	20
Fish meal	35	35	35	35
Soybean meal	17	17	17	17
Rice bran	20	13.76	8.87	3
Durian rind	0	10	15	20
Broken rice	12.04	11	11.75	15.15
Yellow corn	6	6	5.8	3
Rice hulls	4.7	1.4	0.3	0
Soybean oil	2.04	2.62	3.06	3.63
Tuna oil	1	1	1	1
Vitamins and minerals mix	2	2	2	2
Butylated hydroxyl toluene (BHT)	0.02	0.02	0.02	0.02
Calcium propionate	0.2	0.2	0.2	0.2
Total (g)	100	100	100	100

Vitamins and minerals mix/kg: vitamin A 10.00 MIU; vitamin D3 2.50 MIU; vitamin E 5,000 IU; vitamin K 1.60 g; vitamin B1 1.20 g; vitamin B2 3.20 g; vitamin B6 1.20 g; niacin 5.00 g; pantothenic acid 4.00 g; folic acid 5.00 g; biotin 0.12 g; vitamin C 30.00 g

2.4 Growth performance

The weight, length, feed intake, and survival rate were noted at the end of the experiment. All the data collected were used to calculate growth efficiency, including weight gain, average daily gain, length gain, specific growth rate, feed conversion ratio, and survival rate.

2.5 Challenge test

The *Aeromonas hydrophila* strains were kindly provided by the Aquatic Animal Health Research and Development Division, Department of Fisheries, Bangkok Province, Thailand. The protocol was modified by Julie et al. [20]. In brief, one loop of *A. hydrophila* was cultured in 100 mL of tryptic soy broth (Difco™, France) at 37°C for 24 h. The bacterial cultures were centrifuged at 5000 rpm for 10 min (Eppendorf 5920 R, Germany), and then the supernatant was discarded. The pellets were washed once and adjusted to the optical density of 1.0 at 540 nm (Thermo Scientific-Evolution 201, America) using sterile 0.85% NaCl corresponding to a concentration of 4.1×10^7 CFU/ml. After the 140 days feeding trial, 10 fish from each tank were randomly collected and placed in 200-L plastic tanks. The fish were injected intraperitoneally with 0.3 ml of the *A. hydrophila* suspension (4.1×10^7 CFU/ml). The mortality pattern was examined for 14 days. The formulas calculated the cumulative mortality rate and relative percentage survival of fish [21-22].

Cumulative mortality rate (%) = (total of dead fish/number of injected fish) × 100

Cumulative survival rate (%) = (total of live fish/number of injected fish) × 100

Relative percentage survival (%) = [1- (% mortality of experimental group / % mortality of control group)] × 100

2.6 Data analysis

The data were analyzed using Analysis of Variance (ANOVA). Duncan's multiple range test compared the mean difference in each experiment with a statistical program at a 95 percent confidence level. Statistical differences in mineral contents in rind were analyzed using a t-test at a significance level of 0.05.

3. Results and Discussion

3.1 Composition of durian rind and experimental diets

Our results show that the proximate composition of Monthong durian processed white inner rind (Table 2) appear to be similar to the white inner rind of Chanee durian (*Durio zibethinus* L. cultivar Chanee), showing high levels of carbohydrate except for the fiber. In Monthong, dietary fiber ($22.10 \pm 0.58\%$) is lower than in Chanee durian rind, which was reported to be as high as $50.18 \pm 0.43\%$ [23]. Wanlapa et al. [24] reported that the dietary fiber of whole Monthong durian shells, the white inner plus green thorny outer, was $79.18 \pm 3.46\%$ higher than only the white inner rind. This information suggests that using durian rind, either the whole shell or the inner part only, as a dietary ingredient in feeds for other fish species is possible. Feed efficiency for diets high in fiber in herbivorous fish is better than in omnivorous fish and predatory fish because of the differences in the structure of the digestive tracts, especially the length of the intestines [25-27].

The mineral contents of the durian's white inner and green outer rind were also analyzed, as shown in Table 3. Comparing the minerals in the two types of rind, potassium, magnesium, phosphorus, iron, manganese, zinc, copper, and boron significantly differed between the white inner and green outer rind of durian. Focusing on a mineral related to immune response, it was found that the white inner rind contained radically higher magnesium, manganese, and copper than the green outer rind. Magnesium is a mineral that is very important in regulating vital biological functions such as cofactor for more than 300 enzymes, regulating mechanisms of the heart and neurons, muscle contraction [28], and the immune system [29-30]. Manganese had been reported to be involved in the defense against infectious agents and mentioned as well in nutritional immunity [31]. Wu et al. [32] described that manganese could increase the immune response in many species of vertebrates, for instance, humans, mice, birds, and fish. Copper is also reportedly associated with immune functions [33]. Several research reviewed the effects of copper deficiency, which include higher susceptibility to bacterial infections [34], reduced antibody production by spleen cells [35], impaired neutrophil function [36], and decreased bactericidal activity [37].

As mentioned earlier, we chose only the white inner rind of the Monthong durian to produce the experimental diets used in this study. The proximate composition of the experimental diets shows that all treatments are isonitrogenous and isocaloric. It should be noted, however, that crude fiber increases along with the amount of durian rind included in the feed ration (Table 2).

Table 2. Proximate composition of the white inner rind of Monthong durian and experimental diets

Proximate composition	White inner rind	Durian rind (%)			
		0	10	15	20
Crude protein (%)	5.25 ± 0.20	23.15 ± 0.04	24.10 ± 0.11	23.80 ± 0.17	23.38 ± 0.07
Crude lipid (%)	0.66 ± 0.03	6.45 ± 0.07	6.79 ± 0.04	7.53 ± 0.07	7.19 ± 0.01
Crude fiber (%)	22.10 ± 0.58	5.12 ± 0.08	5.73 ± 0.06	5.80 ± 0.02	6.17 ± 0.06
Moisture (%)	6.69 ± 0.03	4.55 ± 0.10	4.68 ± 0.04	4.77 ± 0.04	4.59 ± 0.21
Ash (%)	6.88 ± 0.05	9.54 ± 0.07	10.09 ± 0.23	10.52 ± 0.21	11.54 ± 0.14
Gross energy (Kcal/100g)	3473.53 ± 7.36	3994.30 ± 2.49	4030.70 ± 6.65	3985.10 ± 1.94	3977.80 ± 7.91

Table 3. Mineral contents of the white inner rind and green outer rind of Monthong durian rind.

Parameters	White inner rind	Green outer rind	p-values	Parameters	White inner rind	Green outer rind	p-values
Macro mineral (%)				Micromineral (ppm)			
K	2.33 ± 0.05	1.98 ± 0.05	0.0011*	Fe	13.77 ± 1.08	18.59 ± 1.98	0.0208*
Ca	0.16 ± 0.00	0.15 ± 0.01	0.1583	Mn	22.14 ± 0.20	2.42 ± 0.20	0.0000*
Mg	0.41 ± 0.00	0.22 ± 0.00	0.0000*	Zn	6.93 ± 0.19	5.34 ± 0.14	0.0003*
Na	0.02 ± 0.00	0.02 ± 0.00	0.3739	Cu	7.57 ± 0.22	4.40 ± 0.11	0.0000*
P	0.18 ± 0.00	0.21 ± 0.00	0.0022*	B	18.64 ± 0.06	22.73 ± 0.05	0.0000*
S	0.08 ± 0.00	0.08 ± 0.00	0.3739	Al	5.81 ± 1.79	7.99 ± 0.87	0.1309

Potassium (K); Calcium (Ca); Magnesium (Mg); Sodium (Na); Phosphorus (P); Sulphur (S); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron(B); Aluminium (Al); *significant ($p < 0.05$) difference between the white inner rind and green outer rind in a row

3.2 Growth performance

Growth-related parameters of red tilapia reared with a diet supplemented with durian rind at 0, 10, 15, and 20% for 140 days are shown in Table 4. The results revealed that growth-related parameters in terms of final weight, weight gain, average daily gain, final length, length gain, and specific growth rate of fish fed with durian rind supplemented diet at 10, 15 and 20% were not significantly different ($P > 0.05$) from that of the control. Moreover, we analyzed the whole-body composition of Red tilapia fed with the experimental diet for 140 days, shown in Table 5. The crude protein, moisture, and ash of fish were not significantly different ($P > 0.05$) among treatments. Crude lipids of fish fed with durian rind supplemented diets at 10, 15, and 20% seem lower than the control. This may be due to the increased levels of fibers in the feed, which may affect the ability of tilapia to utilize feed [38]. Furthermore, feeds with high fiber consumption enhance the movement passing rate in the digestive tract, decreasing digestion and absorption [38-39] and increasing fat in the fecal matter [40]. However, there was no significant difference in growth performance among the fish-fed diets supplemented with durian rind, and the survival rate of these treatments was still more than 90%. Additionally, the feed conversion ratio of fish-fed diets with different concentrations of durian rind was also not significantly different from the control ($P > 0.05$) (Table 4). This is similar to the results conducted earlier in common carp (*Cyprinus carpio* Linn.), an omnivorous fish, [41] in which they were fed a diet substitution of broken rice with ground dried durian peel and durian seed at 0, 25, 50, 75 and 100% for 16 weeks. The growth of common carp fed with 25% ground-dried durian peel substitution was not different from the control. In another study by Lommetta et al. [42], which used the same treatment diets but conducted on another fish, silver barb (*Barbonymus gonionotus*), an herbivorous fish, found that the growth of silver barb fed with 50% ground dried durian peel substitution was not different from control. It can be concluded from these findings that omnivorous and herbivorous fish can be fed diets supplemented with durian rind at 20% and 50%, respectively, with no effect on growth performance.

Water quality throughout the culture period measured in the morning, including temperature, pH, dissolved oxygen, ammonia, and nitrites, were 26.55-29.83°C, 6.36-7.35, 3.55-5.70 mg/L, 0.01-0.56 mg/L and 0.04-0.25 mg/L, respectively. Boyd and Lichkoppler [43] recommended that the range of temperature and pH were suitable for aquaculture, while the range of dissolved oxygen was enough for fish to survive. Still, the effect on growth is slow if under prolonged culture. The values of ammonia and nitrite, especially in the last 6 weeks of rearing, tend to increase due to the fish growth. They produce more feces and waste. Ali et al. [44], who worked on the effects of stocking density and ammonia excretion on Nile tilapia (*O. niloticus*), suggested raising the stocking density from 2.81 g fish/L to 14.07 g fish/L increased the ammonia level from 1.48 to 26.44 mg/L and yielded lower growth rates. Considering the increasing mass of fish per 1 liter of water, it can be

concluded that as the fish grows, the ammonia content will also increase. From our result, the slow growth may be because of water quality. However, the Department of Fisheries [45] previously reported that Nile tilapia cultured in cages for 6 months should be 200 g of body weight. In comparison, our fish cultured in 500-L plastic tanks for 140 days (about 5 months) was 154.36 ± 9.51 g, which is relatively comparable with the above-growth rate.

Table 4. Growth performance of Red tilapia fed with diets supplemented with durian rind at different concentrations for 140 days.

Growth performance	Durian rind (%)				p-values
	0	10	15	20	
Initial weight (g/fish) ^{ns}	42.62 ± 0.73	42.82 ± 0.47	42.70 ± 0.35	42.58 ± 0.38	0.5340
Final weight (g/fish) ^{ns}	157.81 ± 12.96	163.55 ± 17.67	141.15 ± 16.84	154.92 ± 10.23	0.0690
Weight gain (g/fish) ^{ns}	111.19 ± 12.43	120.73 ± 17.42	98.45 ± 16.99	112.34 ± 15.54	0.0670
ADG (g/fish/day) ^{ns}	0.82 ± 0.09	0.86 ± 0.12	0.70 ± 0.12	0.80 ± 0.07	0.0670
Initial length (cm) ^{ns}	13.65 ± 0.11	13.98 ± 0.25	13.65 ± 0.42	13.88 ± 0.28	0.1500
Final length (cm) ^{ns}	21.80 ± 0.55	22.09 ± 0.63	21.44 ± 0.65	21.64 ± 0.44	0.1660
Length gain (cm) ^{ns}	8.14 ± 0.63	8.10 ± 0.68	7.79 ± 0.31	7.76 ± 0.54	0.3850
SGR (%/day) ^{ns}	0.86 ± 0.18	1.00 ± 0.06	0.91 ± 0.18	0.92 ± 0.05	0.2180
FCR ^{ns}	1.16 ± 0.11	1.16 ± 0.08	1.19 ± 0.13	1.18 ± 0.12	0.7240
Survival rate (%) ^{ns}	94.00 ± 9.52	93.00 ± 6.00	95.00 ± 10.00	94.00 ± 8.00	0.7730

Non statistically significant difference at $P > 0.05$ (ns); Average daily gain (ADG); Specific growth rate (SGR); Feed conversion ratio (FCR)

Table 5. Composition of whole-body fish fed the experimental diet for 140 days.

Proximate composition	Durian rind (%)				p-values
	0	10	15	20	
Crude protein (%) ^{ns}	58.61 ± 0.25	62.03 ± 2.32	60.25 ± 2.49	61.39 ± 2.92	0.0680
Crude lipid (%)	12.33 ± 0.09^a	8.08 ± 0.36^b	11.76 ± 2.26^a	9.32 ± 0.15^b	0.0006
Moisture (%) ^{ns}	4.54 ± 3.02	5.67 ± 0.50	4.68 ± 0.41	5.00 ± 0.36	0.3600
Ash (%) ^{ns}	22.60 ± 1.64	23.89 ± 2.51	22.71 ± 1.40	23.14 ± 0.35	0.3270

Non statistically significant difference at $P > 0.05$ (ns); Different letters in the row indicate a statistically significant difference at $P < 0.05$.

3.3 Antibacterial property

Girddit et al. [46] reported that polysaccharide gel isolated from durian peel contained antimicrobial compounds. Hence, at the end of the growth performance study, fish were intraperitoneally injected with *A. hydrophila*. The mortality pattern was recorded for 14 days (Fig. 1). The cumulative mortality increased after one-day post *A. hydrophila* injection until the 4th day. After that, the cumulative mortality was stable until the 14th day. The lowest mortality rate and the highest relative percentage survival were observed in fish fed with supplemented durian rind diets at 10% and 15%, followed by 20%. In addition, the survival rate of a fish-fed diet supplemented with durian rind at 10% and 15% was statistically significantly different from the control group ($P < 0.05$) (Table 6). The results agreed with the study of Mohammadi et al. [47], which reported resistance against *A. hydrophila* of Nile tilapia-fed diets added with polysaccharide derived from *Pistacia vera* hulls at 2.5, 5, and 10 g/kg were higher than fish fed a diet without polysaccharide. Priya et al. [16] also evaluated the

efficacy of a polysaccharide extracted from durian rind on disease resistance against *Vibrio anguillarum* in Zebrafish (*Danio rerio*). The fish fed with the polysaccharide encapsulated with *Bacillus subtilis* had a higher survival rate than the non-polysaccharide encapsulated group after 5 days of the *Vibrio* immersion challenge. Their result also revealed a significant upregulation of lysozyme in fish fed with polysaccharide-encapsulated probiotics. Lysozyme is an enzyme produced from leucocytes involved in the phagocytosis of Gram-negative bacteria [48]. Previous studies have also reported that polysaccharides extracted from durian peel can inhibit the growth of Gram-positive bacteria such as *Staphylococcus aureus* [8, 14]. This study and these previous reports collectively indicate that polysaccharides from durian rind have broad-spectrum antibacterial properties.

Moreover, polysaccharide also has immunomodulating properties. For instance, polysaccharide gel (PG) extracted from *D. zibethinus* could improve prophenoloxidase activity in black tiger shrimp (*Penaeus monodon*) [49], increasing immune-related genes such as interleukin 1 beta (IL1b), lysozyme (lyz), tumor necrosis factor-alpha (TNF- α), superoxide dismutase (SOD) genes in Zebrafish (*D. rerio*) [16]. In addition, the minerals, including magnesium, manganese, and copper, were elevated in fish-fed diets supplemented with durian rind and may improve immune response, resulting in higher survival rates than the control. On the other hand, too many of these minerals may cause detrimental effects. For instance, excess manganese can cause neurotoxicity [32], and excess manganese and copper can be toxic to vertebrates by inducing cell death [50-51]. This could be why fish fed a diet supplemented with durian rind at 20% have a survival rate lower than those fed with 10 and 15%.

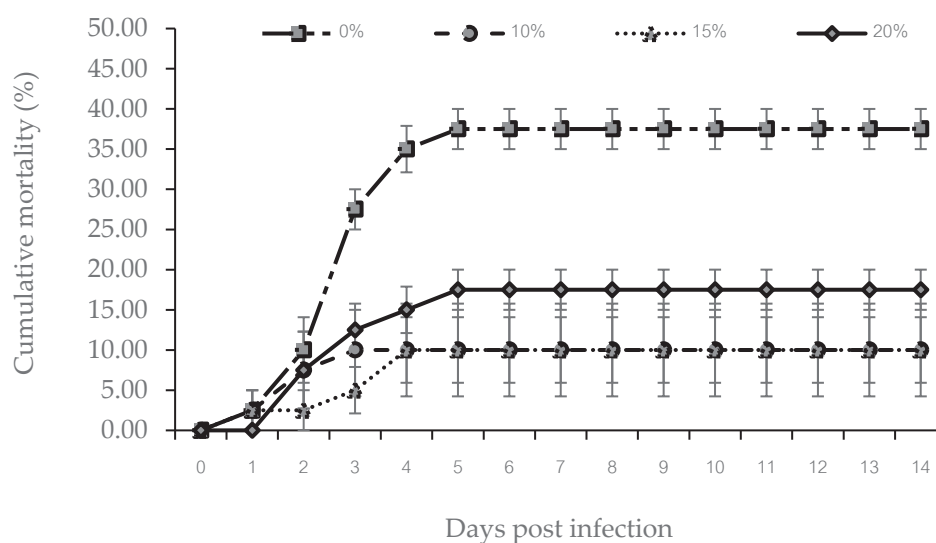


Figure 1. Cumulative mortality rate (%) of Red tilapia after being challenged with *A. hydrophilla*. Values are shown as means $\pm$ S.E. (n=4)

Table 6. Relative percentage survival of Red tilapia fed different levels of durian rind supplementation for 140 days and infected with *A. hydrophilla*

Durian rind (%)	Mortality rate (%)	Survival rate (%)	Relative percentage survival
0	37.50 $\pm$ 5.00	62.50 $\pm$ 5.00 ^b	-
10	10.00 $\pm$ 11.55	90.00 $\pm$ 11.55 ^a	73.33
15	10.00 $\pm$ 8.16	90.00 $\pm$ 8.16 ^a	73.33
20	17.50 $\pm$ 5.00	82.50 $\pm$ 5.00 ^{ab}	53.33

Different letter in the same column indicates a statistically significant difference at $P < 0.05$.

4. Conclusions

Our results revealed that fish-fed diets supplemented with white durian rind at 20% maximum concentration for 140 days still displayed growth performance (final weight, weight gain, average daily gain, final length, length gain, and specific growth rate), feed conversion ratio and survival rate that were not significantly different ($P > 0.05$) from that of the control. However, the challenge test with *A. hydrophila* showed that fish fed a diet supplemented with durian rind at 10% and 15% have the highest survival rate, significantly different from the control ($P < 0.05$). Taken together, our results suggest that the white durian rind can be used as feedstuff and a natural antibacterial ingredient in diets of Red tilapia. The recommended level of supplementation is at 10%-15%, which can elicit improved disease resistance against *A. hydrophila* but has no effect on the growth performance of Red tilapia. Furthermore, we show here that durian rind with minimal processing methods, dried and ground, which is not as laborious and costly as extraction methods and, therefore, ideal for small-scale fish farming, can be used as a diet supplement.

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