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Original Article

Evaluation of pineapple waste crude extract in improving growth performance and resistance to *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*)

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Abstract: The study on the antibacterial activity of fruit, peel, stem, and core of the pineapple, *Ananas comosus* crude extract against *Aeromonas hydrophila* and the effect of supplementing the diet with pineapple waste crude extract on growth performance and resistance against *A. hydrophila* in Nile tilapia, *Oreochromis niloticus* were conducted. The best result of antibacterial screening was performed by disc method and the minimum inhibitory concentration was found in pineapple fruit extract with an inhibition zone of 7.96±0.20 mm and the minimum inhibitory concentration value was 125 mm per liter. The minimum bactericidal concentration value was not observed in all parts of the pineapple extract at any concentration. Furthermore, Nile tilapia (2.34±0.82 g) were stocked in 12 aquaria and fed a diet supplemented with 4 different doses of pineapple peel extract (0, 1, 2 and 3%) for 8 weeks. The results revealed that the best value of weight gain, average daily weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and feed utilization efficiency was observed in fish fed 1% pineapple peel extract and additionally, the mortality of fish challenged by *Aeromonas hydrophila* was recorded for 10 days after the challenge. The result showed that fish-fed diet containing 1% pineapple peel extract had significantly the lowest mortality and the highest relative survival percentage.

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Introduction

The total aquatic animal production is 82.1 million tons, of which 54.3 million tons of finfish (FAO, 2020). Forecasts of the total aquaculture production in Thailand have been gradually growing from 0.6 to 0.9 million tonnes over the past 20 years (Tiptiwa et al., 2020). In Thailand, the most popular economic freshwater fish is Nile tilapia, *Oreochromis niloticus*. The highest tilapia production in 2019 was 228982 tons, or 53.58% of the total output, with a market value of 1143446 million baht (Department of Fisheries, 2021). Nile tilapia is one of the species of global aquaculture because they tolerate the environment well and tolerance to low dissolved oxygen. Due to the widespread adoption of intensive aquaculture systems in tilapia farming, which requires higher stock densities to achieve higher yields per unit area, the feed requirement in tilapia farming has continued to increase.

The quality and quantity of aquaculture feed are important in the aquaculture business. Nutritious aquaculture feeds and appropriate daily feeding rates are important factors in increasing fish growth and survival rates (Asma et al., 2016). Over the past three decades, fishmeal production has been limited, and the price has continued to rise in line with demand, negatively affecting commercial aquaculture's profitability (Naylor et al., 2000). Therefore, several studies are being conducted to find alternative protein sources, including plant, single-cell, and animal proteins (Hardy, 2010). The potential for replacing fishmeal with plant-based proteins is great, and it may eventually take precedence in aquaculture feed (Olsen and Hasan, 2012). It has anti-nutritional properties that decrease fish's nutrient utilization capacity and growth rate (Francis et al., 2001). In

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addition, an exogenous enzymatic supplementary diet can improve nutrient utilization (Kolkovski, 2011), and increase protein digestibility (Yigit et al., 2018), and an appropriate nutritional value is an alternative solution that is necessary to be pursued for fish.

Additionally, health performance improvement and disease resistance of Nile tilapia/ are significant challenges faced by aquarists. Moreover, the factor affecting tilapia culture is *A. hydrophila* causes the death of freshwater fishes. Management of aquatic animal health in aquaculture is essential for a successful production. The prevention and control of bacterial diseases in antibiotic-used aquaculture has increased antibiotic-resistant bacteria. It can also lead to human complications due to the consumption of antibiotic-treated fish. Plant extracts are currently used in aquaculture for treating and controlling bacterial diseases instead of antibiotics, causing resistance to specific pathogenic bacteria (Seyfried et al., 2010). The use of plant extracts is an alternative option for prophylaxis by increasing the immunity of fish, allowing it to control the spread of the infection. In addition, the plant extracts are environmentally friendly and do not cause any side effects.

Pineapple, *Ananas comosus*, native to Central and South America. It is grown in tropical and tropical countries such as Thailand, the Philippines, Malaysia, Indonesia, China, Kenya, and India. In 2020, Thailand was reported as the seventh pineapple producer worldwide, with 1.5 million tons. The export volume of canned pineapple and pineapple juice amounted to 290123 and 41914 tons, respectively (Shahbandeh, 2022). During pineapple processing, approximately 60% of the waste is produced, including peel, core, stem, and crown (Ketnawa et al., 2012). Disposing of large amounts of waste from processing is an ongoing problem due to its tendency to spoilage by microorganisms and cause serious environmental problems. Therefore, innovations leading to the utilization of this waste are useful in managing the large amount of waste generated by processing.

According to the composition of physicochemical and nutritional values such as bromelain, organic acids, antioxidants and phenolic compounds, pineapple can be considered one of the most useful fruits in producing value-added substances (Ali et al., 2020). Pineapple by-products are an abundant source of bromelain (proteolytic sulfhydryl enzymes) belonging to the family of protein-digesting enzymes that can be extracted from pineapple in several native cultures (Gregory and Kelly, 2016). Bromelain is a pricey combination of proteolytic enzymes with various biological properties that are utilized in the pharmaceutical, culinary, and cosmetic industries (Ramli et al., 2017). Moreover, pineapple has been used as a medicinal plant is attributed to bromelain. The pineapple extract also contains cytotoxic, antidiabetic, hyperlipidemia, antioxidant properties (Das et al., 2019), anti-edematous, various closely related proteinases, fibrinolytic, anti-inflammatory activities, antithrombotic and anti-cancer activity and for a wide range of therapeutic benefits (Pavan et al., 2012). Pineapples are important in the medical, nutritional supplement to promote health, leather and textile industries, folk medicine and digestive assistance. It is appropriate to consider the pineapple as a feed additive for aquaculture. Therefore, this research aimed to evaluate the utilization of pineapple properties to increase the growth efficiency and disease resistance of Nile tilapia.

Material and Methods

Pineapple crude extract preparation: The pineapples were bought from the market in Si Chiang Mai district, Nong Khai province, Thailand. The fruits were cleaned, air-dried, and its part was separated (fruit, peel, stem, and core) and then chopped into small pieces before being blended with cold distilled water in a 1: 1 ratio for 3 minutes. The resulting mixture was filtered through a cheesecloth and centrifuged at 10,000×g for 20 minutes and collected the obtained supernatant (crude enzyme extract).

Bacteria preparation: The bacteria isolated from

Table 1. Ingredients and chemical composition of the experimental diet.

Ingredients (%)	Basal diet
Fishmeal (60%)1	33
Soybean meal (45%)2	10
Rice bran	36
Corn meal	9
Soybean oil	2.5
Fish oil	2.5
α-starch	5
Dicalcium phosphate	1
Premix[3]	1
Total	100
Proximate composition by analysis (% dry weight on basis)	
Protein	30.28
Ash	11.39
Fat	12.86
Moisture	8.72
Fiber	1.31

[1]Fish meal and [2]Soybean meal obtained from Faculty of Agriculture, Khon Kaen University, Thailand. [3]Vitamin and mineral mixture provided the following per 1 kg diet: vitamin A 4,00,000 IU, vitamin D3 450,000 IU, vitamin E 6,500 IU, vitamin K3 1,000 mg, vitamin B1 3,900 mg, vitamin B2 2,400 mg, vitamin B6 2,250 mg, vitamin C 15,000 mg, vitamin B12 5 mg, biotin 120 mg, and niacin 5,520 mg.

the liver and spleen of the diseased fish and crossed steak on a nutrient agar plate (Himedia) and incubated at 37°C for 18 hours. The predominant bacterial colony was selected and grown on NA plates to obtain a pure culture. The species of bacteria were identified by morphology using Gram's stain and biochemical properties using API 20E strip (Biomerieux). The isolated colonies were cultivated in nutrient broth (Himedia) and incubated at 37°C for 18 hours. The bacteria's turbidity was adjusted to 0.5 McFarland turbidity standards No.0.5 (1.5x108 CFU/ml). This bacterial suspension was used for the antibacterial activity and challenge test.

Antibacterial activity of pineapple extracts by disc diffusion method: The antibacterial activity of different parts of pineapple extracts was conducted by the disc diffusion method (Bauer et al., 1966). Briefly, the prepared bacteria were inoculated onto Mueller Hinton Agar (MHA; Himedia) surface and distributed evenly with a sterile L-shaped glass rod. The sensitivity test was performed after the plates were dried for 3 to 5 minutes. The different pineapple extracts (fruit, core, stem, and peel) in a volume of 20 µl were added to the filter paper discs (5 mm in diameter) and allowed to dry. Then the paper discs were impregnated on the agar plate and incubated at 37°C for 24 hours. The diameter of the inhibitory zone surrounding the discs was measured to assess the antibacterial activity. All tests were carried out in triplicates and expressed in millimeters.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC): MIC values were determined by the two-fold serial dilution method. Briefly, one ml of MHB and one ml of pineapple extract were added to each test tube. After that, one ml of suspended *A. hydrophila* was inoculated into these test tubes, followed by incubation at 37°C for 24 h. The tube with the lowest concentration and no visible bacteria growth was considered as MIC. Two test tubes were used as negative and positive controls, including the extract with no bacteria and bacteria without the extract. All tubes with no visible growth were used to examine the presence or absence of growth by plating onto an agar plate and incubating at 37°C 24 h. The MBC was recorded as the lowest concentration with no detectable growth.

Experimental diets and experimental design: The experimental diet was prepared using a fish meal, soybean meal, rice bran, corn meal, soybean oil, fish oil, α-starch, dicalcium phosphate, and premix as ingredients which contain 30.28% protein, 12.86% lipid and 11.39% ash estimated by AOAC (2010) (Table 1). In the experimental diet, pineapple extract was sprayed on top of the experimental diet and varnished with fish oil by giving four different concentrations of 0, 1, 2 and 3% (v/w feed) pineapple peel extract (PPE). Group I received 0% of PPE (control), group II 1%, group III 2% and group IIII 3% of PPE, respectively.

The experimental design used a complete randomized design (CRD), which consisted of 4 treatments. Twelve aquaria containers (0.45×0.90×0.45 m, water volume 150 liters) were each stocked with 15 fish. Each experimental diet was fed to fish in three aquaria. The fish were fed the experimental diet at 3% of their body weight twice daily at 09:00 and 15:00 for 8 weeks. The feed consumption was recorded weekly, and every two weeks was weighed to measure the growth of the fish in each tank. The amount of feeding was changed every two weeks at the end of the experiment. At the final weighing and growth performance was calculated.

Growth performance: Fish from all groups were selected randomly every two weeks and the growth performance was assessed by calculating the following growth parameters (Silva and Anderson, 1995; Tacon, 1987; Bake et al., 2014).

Weight gain (WG) = final body weight - initial body weight

Average daily gain (ADG) = ((final body weight-initial body weight))/ (total duration of experiment (days))

Specific growth rate (SGR) = ((ln final weight-ln initial weight))/ (total duration of experiment (days)) x 100

Relative growth rate (RGR) = [(final body weight-initial body weight) / (initial body weight x total duration of experiment (day)] x 100

Feed conversion ratio (FCR) = (total feed intake (g))/ (weight gain (g))

Survival rate (SR) = (final fish number)/ (initial stocked number) x 100

Protein Efficiency ratio (PER) = [(final body weight (g) - initial body weight (g)) / protein consumed (g)] x 100

Feed utilization efficiency (FUE) = [(final body weight (g) - initial body weight (g)) /total amount of feed consumed (g)] x 100

ISI (%) = [wet weight of intestine (g)/wet weight to fish (g)] × 100

Fish proximate composition: At the end of the 8-week, fish were taken from each tank. Proximate constituents, including protein, moisture, ash, and lipids, were evaluated based on AOAC (2010). The moisture was determined by drying the sample in an oven at 105°C. Crude lipid was determined by chloroform-methanol extraction (2:1 v/v). Crude protein was determined (Kjeldahl procedure: N x 6.25) using an automatic Kjeldahl system. Ash was measured by incineration in a muffle furnace at 500°C for 6 hours.

Challenge study with *A. hydrophila*: The challenge test with *A. hydrophila* was performed based on a modified method of Dong et al. (2017). The fish from the four groups was selected randomly at 10 per group. They were challenged with 0.1 mL of intraperitoneal *A. hydrophila* containing 1.5x108 CFU/ml. The fish was then kept for another 10 days. Mortality was recorded every day to day 10 of treatment. The data were calculated for mortality rate percentage and relative percentage of survival (RPS) (Ellis, 1988) as the following:

RPS = 1- ((% mortality in treatment / % mortality in control)) × 100

Statistical analysis: All data were expressed as mean ± standard deviation (SD). The treatments were compared using one-way analysis of variance (ANOVA), followed by Duncan's New Multiple Range Test (DMRT) in SPSS 27.0 software. The level of significant difference of $P<0.05$.

Results

Antibacterial activity: The results of the antimicrobial activity assay of all crude extracts against *A. hydrophila* are presented in Table 2. The

Table 2. Antimicrobial activity of different part of pineapple crude extract against *Aeromonas hydrophila*.

Pineapple Part	Inhibition zone (mm.)	MIC (mg/ml)	MBC (mg/ml)
Peel	7.87±0.20[a]	125	NA
Stem	7.10±0.10[b]	125	NA
Fruit	7.97±0.20[a]	125	NA
Core	6.97±0.15[b]	250	NA
P-value	0.00	-	-

NA :No activity; the different superscript in column as significance difference ($P<0.05$).

Table 3. Growth parameters of Nile tilapia fed diets containing different levels of pineapple pulp extract (PPE) for 8 weeks.

Parameters	Different levels of PPE (%)				*P*-Value
	0	1	2	3	
Initial wt (g)	2.77±0.05	2.76±0.05	2.85±0.11	2.84±0.09	0.430
Final wt (g)	7.44±0.19[a]	8.26±0.33[b]	8.09±0.16[b]	7.87±0.18[b]	0.011
WG (g)	4.67±0.23[a]	5.49±0.39[b]	5.24±0.13[b]	5.03±0.25[ab]	0.029
ADG (g)	0.083±0.004[a]	0.098±0.007[b]	0.094±0.002[b]	0.090±0.004[ab]	0.036
SGR (%)	1.76±0.074	1.95±0.109	1.86±0.053	1.81±0.091	0.122
RGR (%/day)	3.01±0.20[a]	3.76±0.48[b]	3.39±0.32[ab]	3.47±0.21[b]	0.019
FCR	1.66±0.11	1.41±0.13	1.53±0.07	1.59±0.13	0.127
SR (%)	84.44±7.70	91.11±10.18	86.67±6.67	93.33±6.67	0.535
PER (%)	6.85±0.34[a]	8.09±0.28[b]	8.08±0.47[b]	7.91±0.47[b]	0.015
FUE (%)	2.06±0.10[a]	2.43±0.08[b]	2.42±0.14[b]	2.37±0.14[b]	0.015
ISI (%)	16.22±1.10	16.73±2.54	15.61±0.95	14.90±0.99	0.538

Within a row, means with the difference letters are significantly different ($P<0.05$).

Table 4. Carcass proximate composition of Nile tilapia fed supplementary diets with different levels of peel pineapple extract (PPE) for 8 weeks . Data are presented as mean±SD.

Proximate composition	Different levels of PPE (%)				*P*-value
	0	1	2	3	
Moisture (%)	4.96±2.18	6.13±1.08	4.67±0.36	4.88±0.13	0.663
Crude lipid (%)	13.24±0.12[a]	13.62±0.32[a]	15.99±0.13[b]	13.57±0.34[a]	>0.001
Crude protein (%)	7.03±0.28[c]	6.07±0.67[ab]	5.75±0.33[a]	6.56±0.18[bc]	0.021
Ash (%)	12.07±0.18[c]	12.38±0.31[a]	12.59±0.12[c]	12.38±0.21[b]	0.009

Values in each row with different superscripts show a significant difference ($P<0.05$).

highest antibacterial activity was observed in pineapple fruit extract with an inhibition zone of 7.96±0.20 mm and significantly different from extracts from other parts ($P<0.05$). The highest MIC value as 125 mg/ml was found in pineapple peel, fruit, and stem extract. The determination of MBC was not observed in all pineapple part extracts.

Growth performance: When Nile tilapia were fed pineapple peel extract (PPE) for eight weeks, their growth response significantly improved (Table 3). All fish groups fed the PPE-supplemented diet did not exhibit any growth response differences ($P>0.05$). The growth parameters and survival rate of all fish groups fed PPE were higher than those of the control group. All fish groups fed PPE showed significantly higher in weight gain (WG), average daily gain (ADG), relative growth rate (RGR), protein efficiency ratio (PER) and feed utilization efficiency (FUE) than the control group ($P<0.05$). The best value of WG, ADG, PER, FUE, specific growth rate (SGR) and feed conversion ratio (FCR) was observed in fish fed 1% PPE and had a significant difference with control ($P<0.05$).

Fish proximate composition: Fish carcass composition are shown in Table 4. There were significant differences in crude lipid, crude protein and ash between the experimental groups ($P<0.05$), but no differences were found in moisture between all experimental groups ($P>0.05$).

Challenge study: After the challenge test, the lowest mortality rate was found in fish fed pineapple pulp extract (PPE) in the diet at 1%, with an average of

Table 5. Mortality rate and relative percent survival) RPS (of Nile tilapia after infected with *Aeromonas hydrophila* for 10 days.

Different levels of PPE (%)	Mortality (%)	Relative Percentage of Survival (RPS)
0	53.33±11.55	-
1	26.67±11.55	50.00
2	33.33±11.55	37.5
3	46.67±11.55	12.5
P-value	0.077	

Remark :Within a column, means with the difference letters are significantly different ($P<0.05$).

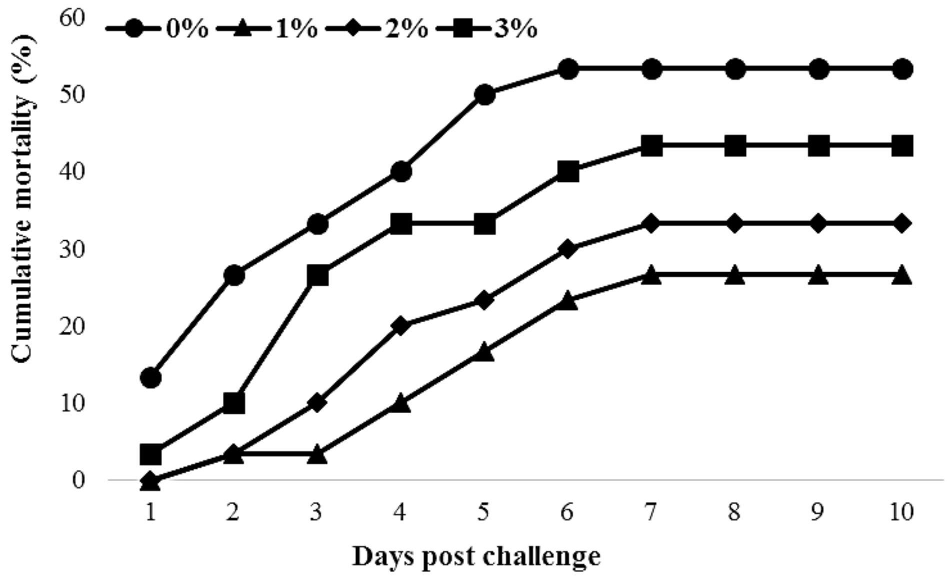

Figure 1. Mortality rate of Nile tilapia fed feeds incorporated with PPE and challenge with *Aeromonas hydrophila* for 10 days.

26.67±11.55%. Although there were no significant differences across the treatments, all of them differed from the control significantly ($P<0.05$). Fish fed diet containing PPE at 1% showed the highest relative percentage of survival (RPS) at 50% compared to the other group supplemented with PPE (37.50% and 12.55%, in PPE 2% and PPE 3%, respectively) (Table 5). While fish fed on PPE in the diet started to die after 48 hours, the control group developed clinical illness and died 24 hours after the challenge (Fig. 1).

Discussion

Sustainable and ecologically friendly aquaculture is one of the most important aspects of any aquaculture business. One approach is to use agricultural by-products as a component in aquaculture feed, encouraging high-quality aquatic feed and minimizing processing waste. Therefore, the purposes of the current investigation were to determine how pineapple peel extract affected growth performance, feed utilization, and disease resistance against *A. hydrophila* in Nile tilapia

The growth performance showed that growth response was better among fish fed PPE mixes, especially with 1% PPE fed fish. Fish-fed PPE supplemented increased WG, ADG, RGR PER and FUR more than those of the control. These findings were in accordance with the finding of Yuangsoi et al. (2018), who reported that Nile tilapia fed 1% pineapple waste extract supplementation improved growth, protein digestibility and feed utilization were higher than those on the control diet. Van Doan et al. (2021) found that 10 g/kg pineapple peel powder was incorporated into the diets is clearly related to growth rate and feed efficiency in Nile tilapia culture under biofloc system. Sukri et al. (2022) reported that the addition of 30% pineapple waste powder in formulated diet can increase the growth rate of Nile tilapia. Furthermore, Singh et al.

(2011) found that the common carp fingerlings that were supplemented with 1% pineapple waste extract had the best fish growth with the lowest FCR value. Sharma et al. (2019) also revealed that common carp (*Cyprinus carpio*) fingerlings fed pineapple peel extract-supplemented diet (ratio of 1:2) showed improved growth performances. Subandiyono et al. (2018) showed that 1.5% pineapple extract in diet significantly affected the growth of Java barb (*Puntius javanicus*). Deka et al. (2003) found that rohu (*Labeo rohita*) fed a diet containing 25% pineapple waste powder showed significantly higher growth performance.

Tongsiri and Pimpimol (2019) revealed that supplementation of pineapple crude extract in diet at 0.5 ml/kg was able to significantly increase growth rate and the average daily weight gain of the climbing perch (*Anabas testudineus*). Yusni et al. (2021) reported that the combination of 1.5% pineapple extract with commercial diet showed a significant increase in weight and length of catfish (*Clarias batrachus*).

The effect of exogenous protease supplementary in low fish meal (30 g/kg) pellets diet and extruded diets may improve growth, nutrient retention, and digestibility of the gibel carp (*Carassius gibelio*) (Shi et al., 2016), whereas supplementation of protease in diets with low fishmeal content can promote the growth of shrimp and tilapia like a diet with a high content of fishmeal (Li et al., 2016). Nilamsari et al. (2021) reported that a highly significant effect on feed utilization efficiency (EPP), protein efficiency ratio (PER) and relative growth rate (RGR) of milkfish (*Chanos chanos*) was seen when fish were fed pineapple extract supplementation. The maximum dose of pineapple extract is 4.50, 4.26 and 4.39 ml can be produced at about 52.73%, PER about 1.55% and RGR about 3.22%, respectively.

Pineapple peels contains a high concentration of bromelain followed by core, crown and stem (Misran et al., 2019). The active ingredients in bromelain are the combination of cysteine proteases which would be hydrolyzing protein into amino acids thereby speeding up the feeding process. When protein in feed is easier to digest, fish will be able to use protein better that will make the growth better. So, fish protein utilization can be increased by supplementing the diet with the enzyme such as bromelain. This will result in increased fish growth and digestibility. Improving growth efficiency is important by increasing protein digestibility and rapid absorption (Wulandhari et al., 2017). As shown in the results, fish-fed a diet supplemented with PPE had a lower FCR than fish-fed a control diet, with the lowest FCR value occurring at 1% PPE. These findings could be attributed to the fish fed a diet supplemented with PPE having a quick metabolism, which enhances FCR. The protease enzymes can help in complete protein digestion. According to reports, 20-25% of proteins in animal feed are indigestible, however adding protease enzymes to the diet improves protein digestion (Shi et al., 2016). Protease enzymes are crucial for the digestive system's ability to break down proteins. It acts to hydrolyze proteins to release short-chain peptides in diet. This plays a key role in boosting growth factors, protein digestibility, and quick absorption. (Rungruangsak-Torrissen and Sundby 2000).

Taqwdasbriliani et al. (2013) also mentioned that bromelain can be activated collagen conversion into gelatin by hydrolyzing the gelatin molecule. Gelatin is a type of protein from the skin's collagen, which is the connective tissue of the bones or ligaments of fish. It acts as a component of bone. Therefore, it may benefit bone growth.

Additionally, Wiszniewski et al. (2018) reported that supplementation of bromelain enzyme improved the length of the villi and folds of the mucous membrane, activating lipase and pepsin and improving intestinal tissue and intestinal adsorbent cells, resulting in better intestinal absorption and digestion. On the contrary, if the amount of enzyme passes through the appropriate point, it may respond to negative effects and may inhibit the growth of fish (Amalia et al., 2013). As shown in the present result, fish fed 3% PPE-supplemented diet decreased growth performance. This occurs because an excess of amino acids will affect how easily fish protein is digested, causing the amino acid-rich protein to be used for energy instead of growth (Delima et al., 2017). Wulandhari et al. (2017) also mentioned that an appropriate increase in the amount of pineapple affects the growth of the fish.

Based on the results of the antibacterial activity of crude pineapple extract in this study, all part of pineapple extracts showed antibacterial efficacy against A. hydrophila. Pineapple peel extract (PPE) is a highly antibacterial waste product from pineapple processing and has no significance difference with pineapple pulp. From the previous results, pineapple peel extract can be further applied in aquatic feed for bacterial resistance. Loon et al. (2018) studied different concentrations of pineapple extract as potential for an antibacterial agent against Staphylococcus aureus, Escherichia coli, Corynebacterium spp., Proteus spp., Bacillus subtilis and S. pyogenes (Ali et al., 2015), Lubaina et al. (2019) reported that the pineapple peel with ethyl acetate extraction demonstrated significant antimicrobial activity against pathogens; *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, and *Klebsiella pneumonia* and the results were comparable with the synthetic antibiotic tetracycline as same in report of Okoh et al. (2019). Nahid Hasan et al. (2021) also revealed that the ethanol and methanol extracts peel of pineapple showed antibacterial activity and the minimum inhibitory concentration (MIC) values against *P. aeruginosa* and *E. coli*. Additionally, the MIC values of extracts of pineapple peel against *S. aureus*, *P. aeruginosa*, and *Salmonella typhi* (Okoh et al., 2019) and the MIC of bromelain from pineapple against *Streptococcus mutans*, *Enterococcus fecalis*, *Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis* were reported by Praveen et al. (2014).

Phytochemicals, also known as phytonutrients, are naturally occurring secondary substances in plants that function as antioxidants, anti-inflammatory and antimicrobial agents. They play an important role in the detoxification of damaging and deleterious chemicals from the body (Jayaraman et al., 2008). The medicinal properties of a crude extract from pineapple are mainly derived from bromelain and phytochemical factors such as vitamin C and flavonoid and various combinations of thiolendopeptidases and other compounds such as carbohydrate, cellulase, phosphatase, glucosidase, glycoprotein, peroxidase, and many protease inhibitors (Pavan et al., 2012). The antibacterial activity of the pineapple peel extracts may be due to the presence of polyphenols, saponins, flavonoids and other secondary metabolites in the extract. Flavonoids play an important role in antimicrobial activity (Namarata et al., 2017) and have antimicrobial potential by binding to adhesion or complexes to the cell wall and inhibiting enzyme activity (Akinpelu et al., 2008). Phenols can denature proteins and its lipophilic property allows them to attract lipid molecules present in cell membranes and destroy bacterial cell membranes (Maurer, 2001). Phenol alters membrane permeability that could result in oxidative phosphorylation uncoupling, active transport inhibition and metabolites losing due to membrane damage (Lubaina et al., 2019). The ability to act as a non-ionic surface agent of phenolic compounds is the primary mechanism of antimicrobial activity, which can disrupt the lipid-protein interfaces or cause proteins deterioration and inhibition of the enzyme activity of pathogens.

Additionally, both bromelain and saponins act on bacterial cell walls and membranes. Bromelain plays an important role in bacterial protein degradation. It is one of the key components in bacterial membranes that causes cell injury and death (Praveen et al., 2014). Bromelain may hydrolyze some of the peptide bonds present in the cell wall of bacteria. However, the mechanism by which bacterial growth is inhibited is still unknown (Bhattacharyya, 2008).

According to the results of the *A. hydrophila* challenge test, the mortality rates in all PPE diets decreased to 26.67% (1% PPE extract), 33.33% (2% PPE extract) and 46.67% (3% PPE extract) compared with the control (53.33%). Furthermore, all fish groups fed a diet containing PPE had better relative percentages of survival (RPS) than the control group, which is one of the most obvious criteria of immunological assessment in the challenge test. The RPS of Nile tilapia in diets containing 1-3% were 50.0, 37.5 and 12.5%, respectively. Fish-fed diet supplemented with 1% PPE produced the highest RPS and highest resistance towards *A. hydrophila*. From our findings, fish-fed diets supplemented with PPE showed an improvement in disease resistance, with the highest value observed in fish- feddiets supplemented with 1% PPE. Dietary PPE supplementation could better support Nile tilapia immune and pathogen resistance, increasing the fish's resistance to bacterial invasion. This was observed as an increase in RPS values against *A. hydrophila* in the fish fed the PPE supplementation compared to the control group in this study. These results might be explained by bromelain's capabilities, which include a range of fibrinolytic, antiedematous, antithrombotic, anti-inflammatory actions and a decrease in inflammatory response in the body. It may also relate to bromelain's interaction with the 3′ :5′ -cyclic monophosphatase adenosine, 3′ :5′ -cyclic monophosphatase guanosine and calcium-dependent signaling cascadesm, which are involved in gastrointestinal secretion signaling pathways. Additionally, bromelain can prevent bacterial adherence. As a result, the bacteria cannot connect to different glycoprotein receptors in the gastrointestinal tract (Praveen et al. 2014). Similar findings were noticed by Van Doan et al. (2021) who reported that improvement disease resistance to *S. agalactiae* was found in fish-fed pineapple peel powder, with the highest value in fish-fed 10 g kg-1 pineapple peel powder. After *A. hydrophila* injection, the mortality of fish fed PPE decreased that was comparable with earlier investigations of other herbs for example, Nile tilapia fed a diet that included *Morus alba* Linn (Mapanao et al., 2019) and *Moringa oleifera* (Mapanao et al., 2021), *L. rohita* fed with a diet containing *Achyranthes aspera* (Kumar et al., 2019) and *C. auratus* fed with a diet containing Phyllanthus niruri and Aloe vera (Ahilan et al., 2010), *Lates calcarifer* fed Citrus depressa Hayata leaf meal (Shiu et al., 2016). Our findings suggested that using pineapple peel extract as a crucial dietary component for fish species to boost immunity against common fish diseases.

Conclusion

The findings of the current research on the effectiveness of pineapple extract in inhibiting the bacterial *A. hydrophila* in Nile tilapia demonstrated that the highest inhibition zones were pineapple extract from fruit at 7.96±0.20 mm. and the MIC was 125 mg/ml. Although it was not bactericidal, the pineapple extract was antibacterial. The result of growth performance showed that WG, ADG, SGR, FCR, PER, and FUE were significantly higher in the fish group fed 1% pineapple peel extract (PPE) supplemented in diet. Additionally, the significantly lowest mortality and the highest relative percentage survival (RPS) were also observed in the fish group fed 1% PPE. Based on Nile tilapia performance, pineapple extract can be used as a natural ingredient in fish feed to promote growth and improve health.

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