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Effect of Traditional Fish Bio-extracts Feeding Regimen on Growth, Feed Utilization, and Disease Resistance of Red Tilapia (*Oreochromis niloticus* x *O. mossambicus*) Against *Streptococcus agalactiae*

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Abstract

Fish bio-extracts (FBE) is fermented water from the decomposition of remaining dead fish by microorganisms of semi-anaerobic conditions derived from the Nile tilapia philosopher technique. There are few studies on dietary FBE for practical FBE utilization. Hence, the effect of FBE and feeding duration on growth performance, innate immunity, and disease resistance was investigated for red tilapia. Fish were divided into 5 groups with three replications. The control group was fed the basal commercial diet which was no FBE. The basal commercial diet was incorporated with FBE at 5 ml/kg feeding regimens were evaluated, including i) feeding every day, ii) feeding every other day, iii) feeding every week, and iv) every other week. After 8 weeks of the trial, red tilapia fed with FBE exhibited significantly (p<0.05) better overall parameters, including growth, feed utilization, and innate immunity, compared to the control group. Moreover, the addition of FBE into diets reduced fish mortality after *S. agalactiae* infection. The highest values were displayed in fish fed with FBE every day. Thus, the administration of FBE at least 5 mL/kg once a day can be used as a growth promoter and a practical disease prophylactic strategy for cultural management in red tilapia farming.

Introduction

Aquaculture industry is the fastest-expanding food production sector for human consumption in the world. Due to the high-quality and inexpensive protein of fish, it is appropriate for the increasing human population (FAO, 2021). Nowadays, tilapia is the most demanding freshwater fish, making it the highest aquaculture volume worldwide (DOF, 2022). Recently, one of the most effective and promising ways to supply the demand for tilapia has seen an expansion of an intensive aquaculture system. Efforts to intensify aquaculture can lead to increased stress that weakens fish immune systems and limited growth performance in farmed fish. Typically, several chemotherapeutics and antibiotics have often been utilized both as growth promoters and as a means of preventing and/or controlling infectious diseases caused by bacterial pathogens (Dawood et al., 2018). However, public awareness regarding the prophylactic use of chemotherapeutics and antibiotics in aquafeeds, which may lead to residual problems in the surrounding environment affecting animals and human health, has led to their ban on aquaculture (Vijayaram et al., 2022). Therefore, the concept of the potential substitute for chemotherapeutics and antibiotics has been interested considerably by the utilization of novel functional dietary supplements as natural growth promoters and immunostimulants that improve growth and health in the tilapia aquaculture industry.

Bio-extracts or bio-fermented or bio-decomposed matter is fermented water derived from the decomposition of remaining materials from various types of agricultural by-products including plants, vegetables, fruits, or animals by the fermenting procedure of semi-anaerobic or/and anaerobic conditions. This fermentation process creates a large number of beneficial microorganisms, especially lactic acid bacteria. These microbes help to decompose nutrients in various substances to be released including proteins, amino acids, and organic acids which are good for plant growth (Wangkheirakpam et al., 2019; Wongsrisakulkaew, 2017). Currently, bio-extracts play a significant role in the promotion of sustainable agriculture and aquaculture due to their low cost, reduced chemical input, and low impact on soil and water. In addition, bio-extracts are an alternative for plant growth promoters, eliminating and preventing pests, soil and water quality management, and disease control in aquaculture without the need for chemical substances (Chauhan & Singh, 2019; Hoseinifar et al., 2019; Sarakham et al., 2017; Wang et al., 2019; Zorriehzahra et al., 2016).

Fish bio-extract (FBE) is fermented water derived from the decomposition of remaining dead fish. This bio-fermented water is one of the local wisdoms especially since it's derived from the Nile tilapia philosopher technique that has been typically used for water quality management in aquaculture. (Karaket et al., 2021; Loh, 2017). In our previous study, it was found that the main beneficial composition of FBE water was various beneficial microorganisms, especially lactic acid bacteria, and the nutrient sources including proteins, amino acids, enzymes, and elements (Rana et al., 2023; Shi et al., 2018; Sharma et al., 2020). Those beneficial microbes potentially are administered either as a feed supplement or to the rearing water and help the host by enhancing disease resistance, growth, health status, immunity, feed utilization/conversion, microbial balance, and rearing water quality (Sharifuzzaman & Austin, 2017; Wang et al., 2019; Zorriehzahra et al., 2016). Nevertheless, there are few studies on feeding FBE that correlate to growth and health performance and are important considerations for practical FBE utilization in tilapia farms.

Since, the composition of the FBE which is a source of beneficial microbes and various necessary nutrients, FBE shows the potential as dietary supplements, despite some concerns about their safety and efficacy. Thereby, the objectives of this study were to investigate the effect of FBE and feeding duration on growth performance, innate immunity, and disease resistance was investigated to establish an optimal feeding regimen of FBE for red tilapia (*Oreochromis niloticus* x *O. mossambicus*). Furthermore, this study might utilize FBE as a new feed additive and a practical disease prophylactic strategy for cultural management in tilapia and other fish farming.

Materials and Methods

Fish Bio-extracts Preparation

The fish bio-extracts (FBE) method was adapted from the Nile tilapia philosopher technique (Karaket et al., 2021). Briefly, the 10 kg dead red tilapia were obtained from the GAP-certificated farm. Freshly dead fish weighing 500-600 g with a normal external appearance were collected within one day and placed in an ice box for transportation to the laboratory. The dead fish were then cleaned with 1,000 ppm chlorine to disinfect contaminated bacteria. The 10 kg pineapple was purchased from the local market. Both were chopped into 2.5 cm pieces. Then, they were mixed well with 10 L molasses, 10 L water, and 1 pack of Microbial Activator Super LDD 2 (PD.2) in the 150 L plastic tank. PD.2 is a product developed by the Department of Land Development**,** Ministry of Agriculture and Cooperatives, Bangkok, Thailand. It is a group of spores of various microorganisms that help decompose organic matter with high moisture content, consisting of yeast, lactic acid bacteria, *Lactobacillus* spp., and *Bacillus* spp. etc. (LDD, 2023). Besides the fermentation process, the tank was opened once a week to release the gas, and the admixture was mixed well within 10 minutes. After 30 days, fermented dark water was filtered and used for preparing experimental diets.

Some types of probiotics bacteria in fermented water were determined using the spread plate technique on specific selective media including total plate count, lactic acid bacteria (LAB), *Bacillus cereus*, *B. subtilis*, *Escherichia coli,* coliform bacteria, *Staphylococcus aureus, Streptococcus agalactiae*, and *Aeromonas hydrophila* by following the bacteriological analytical manual (BAM) (AOAC, 2000).

Feed Preparation

The basal commercial feed was additionally sprayed with the fish bio-extract at 5 ml/kg, the recommended dose derived from the Nile tilapia philosopher technique (Karaket et al., 2021). The diet was air-dried for 24 hours and thoroughly sprayed with 2% fish oil. It was used as an experimental diet. The controlled diet received only the same quantity of fish oil. All experimental diets were prepared weekly and then stored in a refrigerator at 4°C for daily use.

Proximate composition analysis including crude protein, crude lipid, fiber, and ash for each diet and FBE was performed using AOAC methods (AOAC, 2000). Nitrogen-free extracts were calculated using the equations: NFE=dry matter - (crude lipid+crude ash+crude protein) and gross energy calculated according to 23.6 MJ/kg protein, 39.5 MJ/kg lipid, and 17.0 MJ/kg NFE (Molina-Poveda, 2016).

Experimental Fish

One thousand healthy juvenile red tilapia weighing approximately 10.15±0.58 g each were purchased from Phitsanulok Inland Fisheries Research and Development Center, Phitsanulok Province, Thailand. Fish were acclimated in two 500-L fiberglass tanks containing fresh water and full aeration via air stone for 4 weeks before the trial. During this period, fish were fed daily with the basal diet at 8% body weight, and approximately 50% of the water volume was exchanged every other day or more to maintain good water quality (5-6 mg/L DO, pH 7-8, Ammonia<0.05 mg/L, Nitrite<0.01 mg/L).

Experimental Design and Feeding Trial

After acclimatization, two hundred fish were randomly separated into five groups (40 fish for each) with three replicates. One was the control group fed with the basal diet. Others were treatment groups fed with the 5 ml/kg FBE-supplemented diet following four different feeding regimens (Figure 1) including i) feeding every day (ED), ii) feeding every other day (EOD), iii) feeding every week (EW), and iv) every other week (EOW). Fish were fed at 9:00 am and 4:00 pm with a feeding rate of 8% biomass per day for 8 weeks. Water in 150-L experimental tanks was monitored at least three times a week. Water was changed twice a week or more to maintain good water quality (5-6 mg/L DO, pH 7-8, Ammonia<0.05 mg/L, Nitrite<0.01 mg/L) during the trial.

Fish Growth Performances and Feed Utilization

At the end of the experiment, fish per aquarium were harvested, counted, and weighed in bulk. Fish growth and feed utilization variables were calculated as follows

Survival rate (SR, %)=(final fish number/initial fish number)×100

Weight gain (g)=final body weight–initial body weight;

Specific growth rate (SGR; %/day)=100x(Ln final body weight–Ln initial body weight)/days;

Average daily growth rate (ADG; g/day)=weight gained by fish/time interval;

Feed conversion ratio (FCR)=dry weight of feed given/weight gained by fish;

Feed conversion efficiency (FCE, %)=100x(weight gained by the fish/feed given);

Protein efficiency ratio (PER)=weight gained by the fish/protein intake.

Digestibility Test

After eight weeks of the feed trial, chromic oxide (Cr_2O_3) was supplemented into the experimental diets as an inert indicator for the apparent digestibility coefficient (ADC). In brief, the basal commercial diet was mixed well with 0.5% chromic oxide and then fed to the fish for two weeks. Then, feces from each tank were collected once daily after the final meal of the day by siphoning. Immediately after the collection, feces from each tank were centrifuged, pooled, and stored at -20°C until analysis. The chromium oxide content of the diet and dried feces was analyzed by the method described by Dos Santos Cardoso et al. (2021). The ADC of the experimental diets was calculated by the formula:

ADC of dry matter (ADCdm; %)=100–100×(% Cr_2O_3 in diet/% Cr_2O_3 in feces);

ADC of protein (ADCcp; %)=100–100×(% Cr_2O_3 in diet/% Cr_2O_3 in feces) × (% protein in feces/% protein in diet).

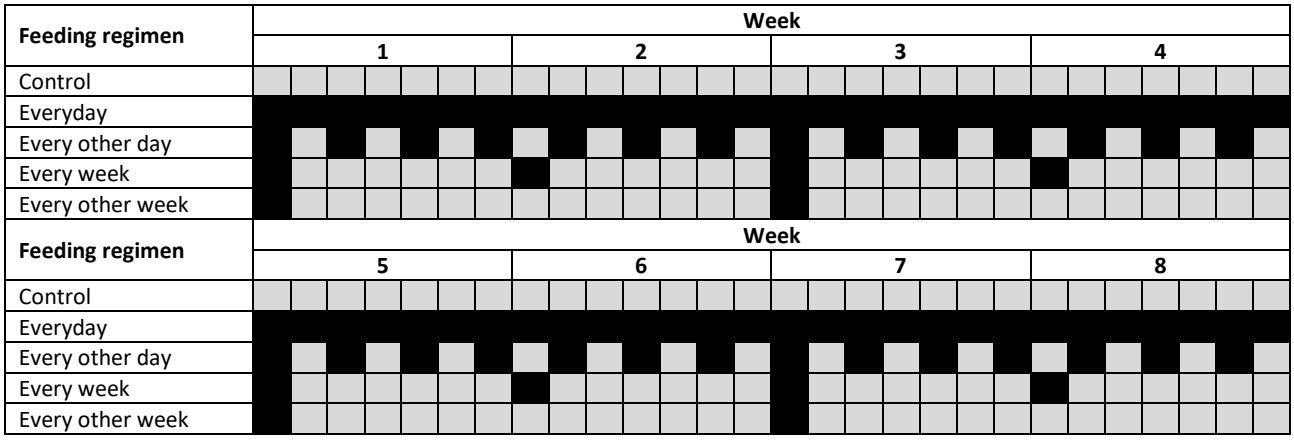

Figure 1. Feeding regimen and experimental design during eight weeks of the trial. Feeding regimen: □ fed basal diet and ■ fed fish bio-extracts.

Hematological Analysis and Immunological Test

Blood Sampling

At the end of the trial, three fish were selected randomly from each replicate, and therefore, were used to analyze the selected immune parameters. Blood was sampled after fish were anesthetized with 50 ppm clove oil (Priborsky & Velisek, 2018). Blood samples were drawn at the caudal peduncle by using sterilized 1-ml syringes and 26-gauge needles that were rinsed with EDTA (anticoagulant) for determining the hematocrit (HCT, %) values. A further 0.5 ml blood sample (non-anticoagulant) was centrifuged at 10,000 x g for 10 minutes at 4°C to separate the plasma. The serum was stored at -20°C until use for the lysozyme activity analysis.

Blood Cell Count

Whole blood samples were diluted and then applied on a hemocytometer to determine the concentration of red blood cells and white blood cells, under a compound microscope.

Hematocrit (HCT) Values

Hematocrit capillary tubes were filled with whole blood to ¾ of the tube and centrifuged using a hematocrit centrifuge machine at 12,000 x g for 5 min. The percentage of packed cell volume was measured by the hematocrit tube reader.

Lysozyme Activity

The serum lysozyme activity was determined using the turbidimetric method. Briefly, *Micrococcus lusodeikticus* at a concentration of 0.2 mg/ml in 0.04 M Sodium phosphate buffer (pH 6.2) was used as substrate. A total of 10 µl of serum samples were added to 250 µl of bacterial suspension in a 96-well microtiter plate. Then, it was quickly mixed, and the UV absorption value at 540 nm was assayed after 0.5 and 6.5 minutes of reaction time at room temperature using a microplate reader machine (Synergy™ H1, BioTek® Instrument, Inc.). One unit of lysozyme activity (unit) was defined as a reduction in absorbance of 0.001 per minute.

Challenge Test with *Streptococcus agalactiae*

At the end of the feeding trial, ten fish were randomly collected from each replicate group for *S. agalactiae* challenge test. Fish were allocated according to treatments in a glass aquarium supplied with oxygenation and room temperature maintained. Control feed was fed to each aquarium twice daily throughout the test. Fish were challenged with *S. agalactiae* by intraperitoneal injection of 0.1 ml (10^9 cfu/ml) of the bacteria suspension (the result of the pathogenicity test determined earlier). The number of survival fish was recorded daily throughout 14 days for estimating cumulative mortalities and the cause of death was confirmed by re-isolating the bacteria from the liver of dead fish using conventional methods.

Statistical Analysis

The statistical analysis of all data obtained from the experiment was analyzed by one-way analysis of variance (ANOVA) and the means were compared using Duncan's New Multiple Range Test (DMRT). Percent survival of fish after the bacterial challenge was performed using Kaplan−Meier plots. All data in this study were analyzed at a 95% confidence level by using the R program (R Core Team, 2019).

Results

Growth Performances and Survival Rate

The growth performances and survival rate of fish in this study are displayed in Table 1 The final weight, weight gain, and ADG of all FBE feeding groups were not significantly different (P>0.05) but were significantly different from the control group (P<0.05). The survival rate was greater than 80% with no significant difference between all treatments (P>0.05).

Table 1. Growth performance, feed utilization, and survival rate of red tilapia with fish bio-extracts (FBE) feeding regimens for 8 weeks (mean ± SD)

Parameters	Feeding regimens				
	Control	Everyday	Every other day	Every week	Every other week
Initial weight (g)	12.47±1.71	13.50±0.70	13.73±1.36	12.87±1.30	13.76±1.31
Final weight (g)	87.10±8.36^{b}	116.66±3.64^{a}	100.13±17.61^{ab}	99.70±8.21^{ab}	98.46±6.23^{ab}
Weight gain (g)	74.63±9.56^{b}	103.16±4.26^{a}	86.40±19.23^{ab}	86.83±9.21^{ab}	84.70±5.60^{ab}
ADG (g/day)	1.33±0.17^{b}	1.84±0.07^{a}	1.54±0.34^{ab}	1.55±0.16^{ab}	1.51±0.10^{ab}
SGR (%/day)	3.47±0.37	3.85±0.14	3.55±0.30	3.65±0.15	3.51±0.55
FCR	1.36±0.02^{b}	1.11±0.09^{a}	1.31±0.04^{b}	1.28±0.02^{b}	1.32±0.02^{b}
FCE (%)	73.53±1.43^{c}	90.09±2.37^{a}	76.34±1.67^{bc}	78.13±1.54^{b}	75.76±1.34^{bc}
PER	2.44±0.09^{b}	2.97±0.06^{a}	2.52±0.04^{b}	2.58±0.05^{b}	2.50±0.03^{b}
ADCdm	72.88±1.07^{d}	78.86±0.87^{a}	76.54±0.77^{b}	74.87±0.19^{c}	74.66±0.42^{c}
ADCcp	78.75±1.19^{c}	88.87±0.51^{a}	87.20±0.42^{b}	86.71±0.36^{b}	86.08±1.09^{b}
Survival rate (%)	81.17±5.20	88.33±11.27	83.33±10.90	80.33±9.46	80.83±6.29

Means in the same row followed by the different letters did differ statistically according to DMRT's test (P<0.05)

Feed Utilization

The FCR, FE, PER, ADCdm, and ADCcp of fish fed with FBE every day exhibited the best values, especially the FCR was decreased to 1.11±0.09 (Table 1). Overall, those parameters were significantly different among all treatments (P<0.05).

Hematological and Innate Immune Parameters

White blood cells and hematocrit of fish fed with FBE every day group had significantly higher (p<0.05) than other groups. Although, the result did not show a significant (p>0.05) difference between the lysozyme activity of four FBE feeding regimens and the control group. However, the FBE feeding groups showed a slight increase in lysozyme activity compared to the control group (Table 2).

Challenge Test

After 8 weeks of feeding, the challenge test with *S. agalactiae* was carried out to investigate the cumulative mortality and survival over 14 days. Fish-fed FBE could significantly (P<0.05) reduce the percent mortality challenged by *S. agalactiae* compared to fish-fed only basal diet (control group) (Table 2). Accumulation mortality revealed high mortality of red tilapia infected with *S. agalactiae* highly increased since two days of the challenge and all dead fish were observed in a week for the control group. There was a low increase in deaths from the 4th day of the challenge for the FBE feeding groups. The percent survival was the highest (86.67%) in the FBE feeding everyday group (Figure 2).

Discussion

Tilapia has a significant role as a low-cost, high-quality source of protein for human consumption. Due to the increasing demand for this fish, the intensive cultivation to increase the productivity of this species has grown accordingly. Intensive fish culturing systems depend on providing various optimal conditions. Hence, farmers have to encounter many challenges including disease prevention, improving resistance to various bacterial pathogens, increasing growth performance, and immunomodulation to prevent ultimately economic loss (Sookchaiyaporn et al., 2020; Srisapoome &

Table 2. Hematological and innate immune parameters of red tilapia with fish bio-extracts (FBE) feeding regimens for 8 weeks (mean ± SD)

Parameters	Feeding regimens				
	Control	Everyday	Every other day	Every week	Every other week
Red blood cell (x10^6 cell/ml)	2.2±0.7	3.6±0.4	2.8±0.8	2.4±0.3	2.2±0.3
White blood cell (x10^6 cell/ml)	7.2±1.5^b	29.2±7.4^a	16.6±8.1^b	11.4±4.2^b	8.2±0.7^b
Hematocrit (%)	29.16±1.30^b	35.16±3.01^a	28.33±1.00^b	30.22±2.87^b	28.77±1.01^b
Lysozyme activity (Unit/ml)	15.37 ±1.62	18.24± 9.59	18.05±1.54	17.31± 4.97	15.55± 6.84
Cumulative mortality (%)	100.00±0.00^a	13.34±11.54^c	26.67±11.54^{bc}	46.67±23.09^b	100.00±0.00^a
% Survival	0.00±0.00^c	86.66±11.54^a	73.33±11.54^{ab}	53.33±23.09^b	0.00±0.00^c

Means in the same row followed by the different letters did differ statistically according to DMRT's test (P<0.05)

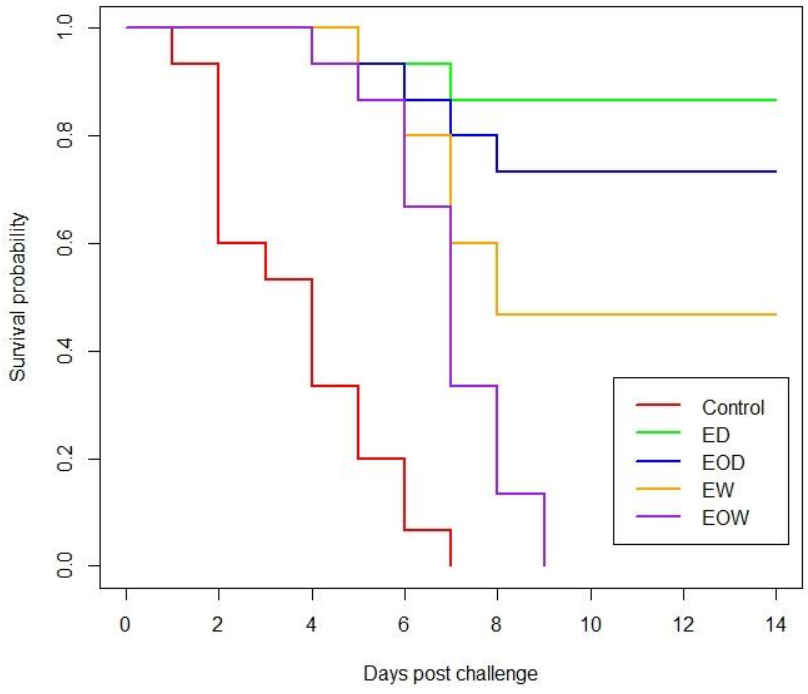

Figure 2. Kaplan-Meier plots of survival probability of red tilapia challenged with *S. agalactiae* (ED=every day, EOD=every other day, EW=every week, and EOW=every other week.

Areechon, 2017). During the last decades, applications of functional feed additives were used as both growth promoters and immune enhancement for the reduction of culture period and facing time with pathogens (Encarnação, 2016; Mustafa & Al-Faragi, 2021). In this study, we focused on a feeding regimen for fish bio-extract (FBE) application that can be implemented at the farm scale in consideration of such factors as FBE usage and amount of FBE of red tilapia culture. The effect of FBE and the feeding regimen was assessed from the perspectives of growth performance, feed utilization, innate immune activity, and disease resistance.

The growth performance of tilapia is positively correlated with feed efficiency and feed digestibility (De Verdal et al., 2017; Yossa et al., 2022). The present study exhibited clearly that FBE supplementation significantly improved nutrient digestibility to promote better growth. Fermented FBE has a variety of benefits that could play a different role in increasing growth performance. Firstly, the low pH of FBE as an acidifier agent reduced the pH of the gastrointestinal content and this might lead to improved digestibility of nutrients, enhanced reproduction of beneficial bacteria, and subsequently an improved microecosystem in various fish (Fabay et al., 2022). This finding agreed with Reda et al. (2016) on Nile tilapia and Huan et al. (2018) on red tilapia who indicated that final body weight, weight gain, SGR, and FCR of fish fed on a diet supplemented with acidifier were significantly improved.

In addition, among the beneficial bacteria in this study such as lactic acid bacteria (LAB), *Bacillus* sp., and *Staphylococcus* sp. (Table 3) might participate directly in the digestion processes of fish. These microbiotas could contribute to the host's nutrition by producing extracellular enzymes to stimulate gut epithelial differentiation and proliferation, gut motility, protein uptake, and nutrient metabolism as well as providing necessary growth factors (Loh, 2017; Rohani et al., 2022). Similarly, various enzymes in FBE produced from beneficial bacteria could break down the diet into small digestible molecules that can be easily digested and absorbed (Swain et al., 2014). Moreover, the nutrient sources including proteins, amino acids, lipids, and elements that are released from fermented-substate (dead fish) in FBE (Table 4) could play a different role in increasing feed quality (Sarakham et al., 2017; Vijayaram et al., 2023; Wongsrisakulkaew, 2017).

These findings indicated clearly that FBE supplementation in diets has unexerted harmful effects on the fish's health. Despite overall bacteria investigation in FBE was not completed in this finding results. However, the beneficial bacteria, especially LAB, were mostly found in about 80-90% of the total bacteria count. Since the low pH or acidic conditions in the FBE fermenting procedure, LAB growth has been promoted but pathogenic bacteria have been suppressed (Ivan, 2003). Moreover, LAB can produce various types of antimicrobial compounds, the most important being bacteriocins which can inhibit the growth of bacterial pathogens (Abbasiliasi et al., 2017; Juturu & Wu, 2016).

The use of various dietary supplements as an environmentally friendly substitute for antibiotics has increased as the aquaculture industry has expanded considerably globally over the past few years. The utilization of substances as feed additives that could treat diseases without causing any negative impact on the environment and humans has been a popular

Table 3. Counts (cfu/ml) of different bacteria isolated from fermented water

Type	Colony
Total Plate Count	$1.35\pm0.07 \times 10^4$
Lactic acid bacteria	$9.35\pm0.15 \times 10^3$
Bacillus cereus	$1.20\pm0.05 \times 10^3$
Bacillus subtilis	$1.25\pm0.15 \times 10^3$
Staphylococcus aureus	$1.10\pm0.10 \times 10^3$
Streptococcus agalactiae	ND
Aeromonas hydrophila	ND
Salmonella spp.	ND
Coliform bacteria	ND
Escherichia coli	ND

ND=Not Detected

Table 4. Proximate analysis of the experimental diets and fish bio-extracts

Proximate Analysis (g/100g DM)	Basal diet	Experimental diet	Fish bio-extracts
Moisture	14.13±0.79	15.41±0.58	79.64±0.88
Crude protein	30.12±0.45	30.29±0.42	6.70±0.25
Crude fat	7.39±0.37	7.32±0.43	3.24±0.71
Crude fiber	2.08±0.71	2.10±0.44	0.01±0.18
Ash	10.82±0.05	9.90±0.11	2.93±0.03
Nitrogen-free extract (NFE)	35.46±0.0.18	34.98±0.0.42	7.50±0.0.49
Gross energy (MJ/kg)	3,859±0.35	3,842±0.0.46	1,197±0.42
pH	-	-	4.0±0.02

interest. In the present study, the low pH or acidic conditions of FBE improved the growth performance and feed utilization efficiency and aided in stimulating the immunity of cultured fish. Likewise, the immune and disease resistance of red tilapia (Koh et al., 2016) and Nile tilapia (Libanori et al., 2021) fed on a diet supplemented with organic acids were significantly improved.

Moreover, numerous microbiomes in the FBE of this study were used as the mixed probiotics which act in multiple ways to support increases in aquaculture production, by promoting enhanced growth, improved feed utilization and nutrition, reducing diseases, and developing immune responses (El-Saadony et al., 2021; Hai, 2015; Iwashita et al., 2015; Ringø et al., 2020; Thy et al., 2017). The results showed positive effects in fishes fed with FBE supplementation in diets throughout the 60-day culture period. Total white blood cells, red blood cells, and lysozyme activity were significantly higher in fish-fed diets supplemented with FBE than in the control group. Similarly, the significant enhancement of resistance to *S. agalactiae* infection in the present study was associated with a significant enhancement of the red tilapia pathogenic resistance.

In the present findings, the application of the FBE every day was found optimum in the enhancement of the fish immunological responses. The number of beneficial bacteria or probiotics correlated with this feeding regimen. Due to only 10^3 cfu/ml of total bacteria in FBE water, indeed, they were not sufficient for various mechanisms in other treatments. The optimal concentration of probiotics in the various reports was 10^7-10^9 cfu per g diet or ml for tilapia (El-Saadony et al., 2021; Ringø et al., 2020; Rohani et al., 2022; Van Hai, 2015). So, FBE feeding every day resulted in close to the optimal bacteria for red tilapia. Although FBE exhibited high potential benefits for fish, the administration of FBE in more than 5 ml/kg could increase the cumulative fungus and other pathogens in a high moisture diet.

Conclusion

The incorporation of FBE at least 5 ml/kg once a day would be a more suitable supplement to enhance growth performance, feed utilization, innate immunity, and disease resistance, indicating that FBE could act as a growth-promoting and pathogenic resistance agent for red tilapia aquaculture. Indeed, FBE is a mass of beneficial bacteria in the water which was been easily produced from aquaculture waste products through the traditional fermentation method. Hence, FBE could be used to replace antibiotics in aquaculture farms due to its environmental-friendly, unharmful effects on fish and human health.

Ethical Statement

The study was conducted according to the Ethical Principles in Animal Research of the Institute of Animals for Scientific Purposes Development and approved by the Center for Animal Research at Naresuan University (Approval Number: NUAACUC. 0013/2564).

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The authors did not receive any full budget for this research work.

Author Contribution

Thuchapol Karaket: Conceptualization, Project administration, Methodology, Data Curation, Formal analysis, Visualization, Writing - Original draft preparation; Jinwara Larnjun: Investigation, Resources, Data curation; Piyapong Suksumpan: Investigation, Resources, Data curation; Pattareeya Ponza: Resources; Nonthawit Areechon: Resources; Mintra Seel-audom: Resources, Writing - Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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