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RESEARCH PAPER

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Potential Benefits of Ripe Cultivated Banana (*Musa sapientum* Linn.) in Practical Diet on Growth Performance, Feed Utilization and Disease Resistance of Hybrid Tilapia (*Oreochromis niloticus* x *O. mossambicus*)

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Abstract

The potential benefits of ripe cultivated banana CV. Kluai Namwa (*Musa sapientum* Linn.) as a natural feed supplementation in practical diet on growth performance, feed utilization, innate immunity and disease resistance against *Streptococcus agalactiae* infection in hybrid tilapia (*Oreochromis niloticus* x *O. mossambicus*) were evaluated. Fish were fed with the test diet containing different levels of ripe cultivated banana (RCB) supplements (10, 30 and 50 g/kg diet) compared with the negative control and positive control diet for 60 days. Results showed that growth parameters, feed efficiency and lysozyme activity values in the positive control group (vitamins and minerals premix added) were significantly higher than the negative control group (without any premix supplements) but differences among the RCB supplementary diet group and the positive control group were not statistically significant. After challenged with *Streptococcus agalactiae*, the cumulative mortality of fish in the positive control group was lowest (0%), the percent survivals were 62.22%, 66.67%, 83.33% and 88.89% in the negative control, 10RCB, 30RCB, and 50RCB treatments, respectively. These results showed that the RCB supplementary diets had unaffected on growth performances and could be potential as an immunostimulant to improve fish health for red tilapia aquaculture.

Introduction

Nile tilapia, including hybrid tilapia, is one of an economically important cultured freshwater fish in the world because they are easily cultured and highly adaptable to a wide range of environmental conditions and has made a contribution to the production of good-quality protein food for human (FAO, 2018a; Mjoun et al., 2010). From past to present, aquaculture operations have encountered many challenges, especially, disease outbreak caused by various pathogens (Vaseeharan & Thaya, 2014). Reduction in mortality rates would have direct positive effects on the profitability of the modern intensive aquaculture industry.

Recently, the concept of functional feed has been interested considerably by utilizing a new staple locally available to develop a diet formula that meets adequate basic nutritional requirements and also enhancing the immunity of the cultured fish. To control disease outbreaks, several chemotherapeutics and antibiotics were used indiscriminately, which, in turn, results in excessively deposit within the surrounding environment, finally affecting animal and human health (Vaseeharan & Thaya, 2014). The use of plant polysaccharides (PPS) as immunostimulants are considered as an alternative for antibiotics and chemical drugs. Various PPS had positive effect on the growth rate, survival and immune responses in crucian carp (Wang et al., 2016), Nile tilapia (Zahran et al., 2014), Rohu (Giri et al., 2015), gilthead seabream and European seabass (Carbone & Faggio, 2016). Also, the beneficial effect of PPS, its cost-effectively and eco-friendly, will be a suitable approach for feed supplementation in fish farming.

Bananas are tropical fruit of the Musaceae family and are economically important food crops in the world (Ashokkumar et al., 2018). Banana can be used as a good natural source of vitamins and minerals (Englberger et al., 2003; Sulaiman et al., 2011; Wall, 2006). Besides, the main bioactive compounds (phenolics and carotenoids, etc.) and polysaccharides of those chemical compositions may contribute to the beneficial health effects (Bird et al., 2010; Liu et al., 2015; Singh et al., 2016).

In Thailand, cultivated banana CV. Kluai Namwa (*Musa sapientum* Linn.) is widely consumed as a food staple and has been traditionally used for gastrointestinal tract disorders such as diarrhea and gastritis. From the previous study of Palintorn et al. (2019), Kluai Namwa consists of numerous vitamins and minerals especially vitamin C, calcium, magnesium, phosphorus and potassium, which are adequate replacements to commercial vitamin premix. Additionally, they demonstrated that supplementation of flesh ripe fruit of Kluai Namwa in commercial feed for rearing Nile Tilapia could stimulate their growth performance. Considering these advantages, Kluai Namwa can be regarded as an important fruit due to its potential as functional and nutraceutical food (Pereira & Maraschin, 2015).

The purpose of this study was to evaluate the potential effect of ripe cultivated banana CV. Kluai Namwa (*Musa sapientum* Linn.) as a natural feed supplementation on growth performance, feed utilization, innate immune response and disease resistance against *Streptococcus agalactiae* of hybrid tilapia (*Oreochromis niloticus* x *O. mossambicus*) in the intensive culture system.

Materials and Methods

Ripe Cultivated Banana, Fish and Bacteria Strain

Ripe cultivated banana, *Musa sapientum* Linn., was obtained from a local market. Banana was peeled and diced into small pieces and grinded thoroughly with a blender until becoming homogeneously and sticky. Then, it was stored in a refrigerator at 4°C until use. The healthy male red tilapia (2 months old) were purchased from the GAP-certificated farm in Phitsanulok province, Thailand. Fish were acclimatized in 500-L fiberglass tanks for 2 weeks before experimental manipulation. The average initial weight of tilapia at the start of the experiment was 7.67±0.16 g. The bacterium *Streptococcus agalactiae* was received from the Laboratory of Aquatic Animal Health Management, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand and stored in our laboratory.

Experimental Diets

The five isonitrogenous (35% crude protein) and isoenergetic (450 kcal/100g) experimental diets were formulated (Table 1). For the preparation of the three test diets, the homogenate ripe cultivated banana (RCB) was added at the level of 10, 30, and 50 g/kg diet, respectively. The two control diets without RCB supplements were prepared including 1) the positive control diet contained vitamin and mineral premix, 2) the negative control diet did not add any premix. The ingredients were mixed in a blender, the feeds were pressed through a 2-mm sieve in a pelleting machine, and the pellets were dried in a drying cabinet (40°C) for 12 hr. The experimental diets were prepared biweekly and then stored in a refrigerator at 4°C for daily use. Proximate composition analysis for each diet was performed using AOAC (2000) methods. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method after acid digestion using an auto Kjeldahl system; lipid content was determined by ether extraction using the Soxhlet method; ash composition was determined by combustion at 550°C; nitrogen-free

Table 1. Formulation of the experimental diets.

Composition (g)	Diets				
	Negative control	Positive control	10RCB	30RCB	50RCB
Fish meal	300	300	300	300	300
Soybean meal	300	300	300	300	300
Broken-milled rice	290	290	290	290	290
Rice bran	90	90	90	90	90
Fish oil	20	20	20	20	20
Ripe cultivated banana	0	0	10	30	50
Vitamin & Mineral Premix(1)	0	15	0	0	0
Vitamin C	0	0.5	0	0	0

(1) Vitamin & minerals premix: each 2.5 kg contain vitamin A 12 MIU, D3 2 MIU, E 10 g; K 2 g, B1 1 g, B2 4 g, B6 1.5 g, B12 10 mg, Pantothenic acid 10 g, Nicotinic acid 20 g, Folic acid 1 g, Biotin 50 mg, Choline chloride 500 mg, copper 10 g, iodine 1 g, iron 30 g, manganese 55 g, zinc 55 g and selenium 0.1 g.

extracts were calculated using the equations: NFE = dry matter - (lipid + ash + crude protein + fiber) and gross energy calculated according to 5.64, 9.44 and 4.11 kcal/g for protein, lipid and NFE, respectively (NRC, 2011).

Experimental Design and Feeding Trial

The experiment was conducted at the Department of Agricultural Science, Naresuan University, Phitsanulok Province, Thailand. After acclimatization for two weeks, 45 fish were randomly distributed into each group (150-L aquaria) with three replicates. Water quality was maintained as 4-6 mg/L DO, pH 7-8, total ammonia below 0.5 mg/L, nitrite below 0.1 mg/L and temperature 28-30 °C under natural photoperiod and continuous aeration system. The aquaria were supplied with 50% water exchange with de-chlorinated freshwater supply weekly. The fish were fed with the experimental diets at the rate of 3-5% of body weight twice daily for 8 weeks. The fish from each aquarium were randomly weighed for growth determination and feeding adjustment biweekly. Total feed consumed by fish was recorded.

Parameters of Fish Growth, Survival Rate and Feed Utilization

After 8 weeks of feed trial, all survival fish from each replicate were counted and weighed in bulk. Parameters of fish growth, survival rate and feed utilization were calculated using the following equations:

Weight gain (WG, g)=Final body weight - Initial body weight;

Feed conversion ratio (FCR) = Total feed intake / weight gain;

Survival rate (SR, %) = 100 x (Total number of fish at the end / Total number of fish at the end);

Average daily gain (ADG, g/fish/day) = (Final body weight - Initial body weight) / experimental time (days);

Specific growth rate (SGR, % BW/day) = 100 x (Ln final weight - Ln initial weight) / experimental time (days).

Digestibility Coefficients

After 8 weeks of feed trial, the apparent digestibility coefficient (ADC) was carried out by the indirect method using chromic oxide (Cr_2O_3) as the non-absorbed reference substance. In brief, the experimental diets as shown in Table 2 including 0.5% chromic oxide in each formula were fed to the fish for two weeks. Then, faeces 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. Then, dried feces and feed was digested and determined for chromic oxide and crude protein (CP) by the colorimetric method as described in Lupatsch et al. (1997). The apparent digestibility coefficients were computed according to the formula:

$$\text{ADC dry matter} = 1 – [\%Cr_2O_{3Food} / \%Cr_2O_{3Faeces}]$$

$$\text{ADC protein} = 1 – [(\%Cr_2O_{3Food} / \%Cr_2O_{3Faeces}) \times (\%CP_{Faeces} / \%CP_{Food})]$$

Blood Sampling

Ten fish were randomly taken from each replicate to collect blood samples (0.5 ml) after 8 weeks of feed trial. Blood was withdrawn from the caudal vein using 1-ml syringes and 26-gauge needles that were rinsed with 3%EDTA (anticoagulant) for determining the hematocrit (HCT, %) values. A further 0.5 ml blood sample (non-anticoagulant) was centrifuged at 2,500 rpm at 25 °C for 15 min to separate the plasma (Abbass et al., 2012). The serum was stored at -20 °C until use for lysozyme activity test.

Hematological Analysis and Immunological Test

Blood Cell Count

Whole blood samples were diluted and then applied on a haemacytometer to determine the total number of red blood cell and white blood cell under a compound microscope.

Table 2. Proximate analysis of the experimental diets and ripe cultivated banana.

Proximate composition	Ripe cultivate banana	Diets				
		Negative control	Positive control	10RCB	30RCB	50RCB
Moisture (%)	68.01	6.31	6.19	6.93	8.11	9.62
Crude protein (%DM)	1.1	35.36	35.01	34.99	34.36	33.37
Crude lipid (%DM)	0.2	8.79	8.66	8.73	8.60	8.48
Fiber (%DM)	1.07	1.82	1.82	1.82	1.82	1.83
Ash (%DM)	3.77	7.69	7.61	7.65	7.57	7.50
NFE (%DM)	25.85	40.03	40.71	39.88	39.54	39.20
GE (kcal/100g)	110	454.3	454.20	451.20	445.60	440.17
Vitamin C (mg/100g)	10.83	ND	49	0.17	0.33	0.55

Haematocrit (HCT) Values

Haematocrit capillary tubes were filled with whole blood to ¾ of the tube and centrifuged using hematocrit centrifuge machine at 12,000 rpm 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 according to Zahran et al. (2014). Briefly, *Micrococcus lusodeikticus* at a concentration of 0.2 mg/ml in 0.04 M Sodium phosphate buffer (pH 6.2) was used as a 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 min 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 min.

Challenge Test with *S. agalactiae*

At the end of the feeding trial, ten fish were randomly collected from each replicate for *S. agalactiae* challenge test. Fish were allocated according to treatments in glass aquarium supplied with oxygenation and temperature maintained at 25 °C. Control feed (Table 1) was fed to each aquarium twice daily throughout the test. Then each fish was subjected to bacterial challenge. Bacteria were inoculated in 3 ml TSB medium prior to growing overnight at 37 °C. Cultures were centrifuged at 8,000 rpm at 4 °C for 5 min. The supernatant was discarded and the pelleted bacteria were washed twice in sterile normal saline (0.85% NaCl). Then, the concentration of bacteria was adjusted to 10^9 CFU/ml with the normal saline. Fish were challenged with *S. agalactiae* by intraperitoneal injection of 0.1 ml of the bacteria and fish were fed with the control diet for 14 days. The number of dead fish was recorded daily 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 by modifying the protocol of Sookchaiyaporn et al. (2020).

Statistical Analysis

Data were analyzed using the R statistical analysis system 3.5.1 (R Core Team, 2016) as a completely randomized design. For the mean comparison of growth parameters, survival rate, feed utilization, hematological and immunological data were analyzed by Duncan's New Multiple Range Test (DMRT). Percent survival of fish after the bacterial challenge was performed using Kaplan−Meier plots. All data that were presented in the tables are means and SD derived from the ANOVA. $P<0.05$ was considered to be significant.

Results

The effects of ripe cultivated banana (RCB) supplementing diet on growth performance including the feed efficiency and survival rate was shown in Table 3. Better value on all parameters of fish in the positive control group was displayed. Growth parameter and feed efficiency values in the positive control group showed significantly higher than the negative control group ($P<0.05$) but the differences among the RCB supplementary diet group (10, 30, and 50 g/kg diet) and the positive control group were not statistically significant ($P>0.05$). The feed conversion ratio was non-significant different ($P>0.05$) among groups. Similarity, no significant differences in red blood cell, white blood cell and hematocrit values were found ($P>0.05$) among all treatments. The significant difference of lysozyme activity value between the RCB supplement group and the positive control group was not found ($P>0.05$). However, this value was significantly higher in both of the positive control group and the RCB supplement group than the negative control group ($P<0.05$) (Table 4).

Table 3. Growth performances, survival rate and feed utilization of red tilapia fed different levels of ripe cultivated banana for 8 weeks (mean±SD).

Parameters	Diets				
	Negative control	Positive control	10RCB	30RCB	50RCB
Initial weight (g)	7.57±0.21	7.67±0.06	7.90±0.20	7.63±0.12	7.67±0.21
Initial length (cm)	7.50±0.76	7.60±1.00	7.90±0.58	7.50±0.38	7.90±0.15
Final weight (g)	57.39±2.21^{bc}	62.23±2.62^{a}	61.13±4.69^{ab}	58.93±3.11^{ab}	58.03±7.60^{ab}
Final length (cm)	13.06±0.33^{c}	15.73±0.59^{a}	13.90±0.47^{b}	14.06±0.15^{b}	13.96±0.47^{b}
Weight gain (g/fish)	49.82±2.01^{bc}	54.57±2.57^{a}	53.23±4.50^{ab}	51.27±3.07^{ab}	50.40±7.46^{ab}
ADG (g/fish/day)	0.86±0.03^{bc}	0.94±0.04^{a}	0.92±0.08^{ab}	0.88±0.05^{ab}	0.87±0.13^{ab}
SGR (%/day)	3.49±0.05^{bc}	3.61±0.06^{a}	3.52±0.09^{ab}	3.50±0.08^{ab}	3.49±0.18^{ab}
Survival rate (%)	78.03±3.65^{b}	89.33±7.63^{a}	85.83±3.82^{ab}	82.50±9.02^{ab}	82.50±9.01^{ab}
FCR	1.61±0.26	1.42±0.07	1.47±0.17	1.57±0.25	1.58±0.18
ADC dry matter (%)	71.89±1.24^{b}	77.25±1.08^{a}	76.49±0.54^{a}	76.52±1.43^{a}	76.48±0.94^{a}
ADC protein (%)	80.63±0.39^{b}	86.21±0.64^{a}	86.49±0.35^{a}	86.79±0.78^{a}	86.59±0.36^{a}

Data with different superscript letters were significant different ($P<0.05$).

After 8 weeks of feeding, the challenge test with *S. agalactiae* was conducted to investigate the level of disease resistance over 14 days. Results exhibited that the cumulative mortality of fish in the 30RCB and 50RCB groups (16.67% and 11.11%, respectively) were not significantly different (P>0.05) compared with the positive control group (0% mortality), while the 10RCB group and the negative control group (33.33% and 37.78%, respectively) were significantly lower (P<0.05) than other groups (Table 4). Therefore, the percent survival was 62.22%, 66.67%, 83.33% and 88.89% in the negative control group, 10RCB group, 30RCB group, and 50RCB group, respectively (Table 4 and Figure 1).

Discussion

The current study demonstrated that inclusion of ripe cultivated banana CV. Kluai Namwa (RCB) in the red tilapia diet markedly affects growth performance, feed utilization and resistance against *S. agalactiae* as well as vitamins and minerals supplementation in the positive control group. From the results (Table 3), fish fed dietary RCB had significantly high growth performance and feed utilization values when compared to RCB unsupplemented fish. Various plant polysaccharides from the fruit have been reported to have growth-stimulating effect when consumed by fish. Improved growth performance was recorded in Nile tilapia fed with flesh ripe banana (Palintorn et al., 2019). Das and Biswas (2020) reported an improvement in the growth performance of banded gourami (*Trichogaster fasciata*) fed with ripe papaya supplemented diet as same as the control group. Similarly, feeding African catfish fingerlings with mangosteen extracts as a feed additive has no adverse effect on growth but enhanced the hematological parameters (Soosean et al., 2010). Furthermore, this result also agrees with that of Sittigool and Kaewkong (2016) used ripe banana, papaya, mango and pineapple as a growth-enhancing agent in weaned piglets. The wide range of immuno-nutritional ingredients such as vitamins, minerals, bioactive compounds and polysaccharides in the RCB supplemented diet could have enhanced the growth performance of the fish. Additionally, results on growth

Table 4. Hematological and immunological parameter of red tilapia fed with different levels of ripe cultivated banana for 8 weeks, percent survival and cumulative mortality of red tilapia challenged with *S. agalactiae.* after 14 days (mean±SD).

Parameters	Diets				
	Negative control	Positive control	10RCB	30RCB	50RCB
Red blood cell (x 10^5 cell/mm^3)	20.0 ± 4.65	21.5 ± 5.01	22.6 ± 3.13	22.4 ± 5.65	22.4 ± 7.59
White blood cell (x 10^4 cell/mm^3)	12.8 ± 5.35	17.4 ± 6.55	13.3 ± 5.95	13.6 ± 6.83	14.8 ± 6.20
Hematocrit (%)	30.67 ± 2.35	31.56 ± 2.51	31.78 ± 3.15	31.11 ± 2.62	31.22 ± 3.38
Lysozyme activity (Unit/ml)	13.07 ± 4.26^b	21.65 ± 4.05^a	16.20 ± 6.54^{ab}	16.76 ± 4.07^{ab}	18.33 ± 5.53^{ab}
Cumulative mortality (%)	37.38 ± 9.62^a	0.00^b	33.33 ± 16.67^a	16.67 ± 16.67^b	11.11 ± 9.62^b
% Survival	62.22 ± 9.62^b	100 ± 0.00^a	66.67 ± 16.67^b	83.33 ± 16.67^a	88.89 ± 9.62^a

Data with different superscript letters were significant different (P<0.05).

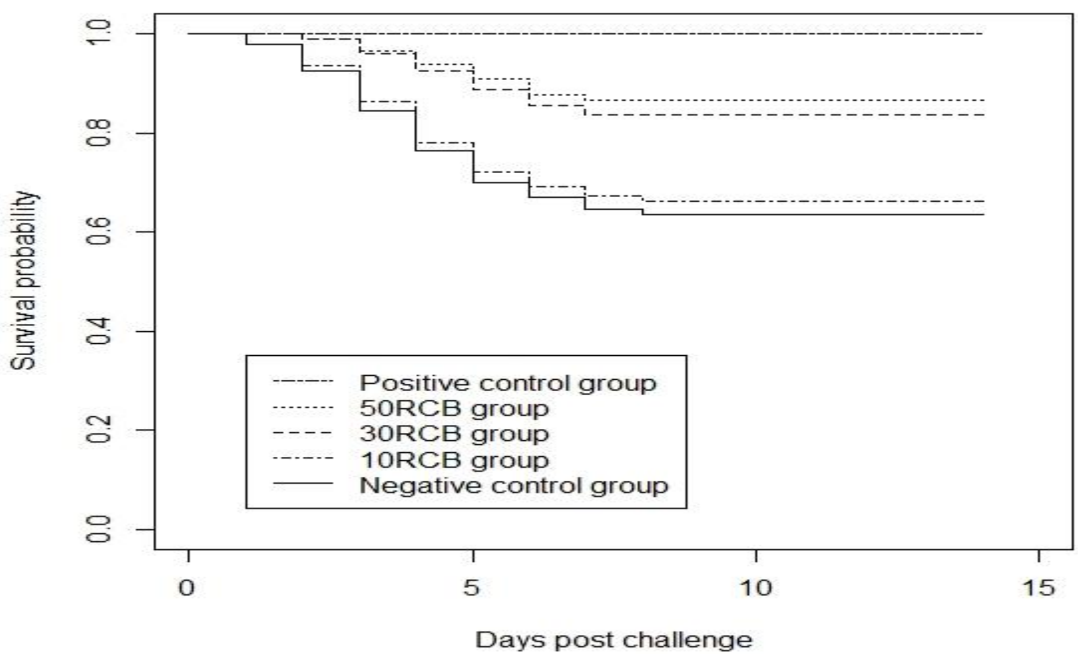

Figure 1. Kaplan–Meier plots of percent survivals of hybrid tilapia challenged with *S. agalactiae.*

and feed efficiency of fish fed with 10-50 g RCB/kg were unaffected as well as the positive control group implying that this fish could digest the high-fiber ingredients from the plant feedstuffs due to the abundant enzymes in RCB. Besides, the polysaccharides molecules such as resistant starch (RS) in RCB have enhanced the food digestibility and absorption of nutrients from feedstuffs in fish (Fuentes-Zaragoza et al., 2010; Gabriel et al., 2015).

Due to poor water quality, high stocking densities and rapid alterations of the environment in culture conditions of intensive aquaculture, these factors can result in stress that can lead to the negative effects on fish growth and health (Nakharuthai et al., 2016). According to requirement for normal fish metabolism, optimum growth and health, vitamins and minerals must be supplied in the diet because these trace substances cannot be synthesized by fish and limited availability in feed ingredients. (Mjoun et al., 2010; NRC, 2011; Craig et al., 2017; FAO, 2018b). This study clearly displayed the necessity of the vitamins and minerals in the negative control group which had no vitamin and mineral premix supplementation in which significantly lower growth performance and survival rate were detected.

Vitamin C is the one of vitamins supplementation that affects growth performance and health in fish and shellfish (NRC, 2011). Vitamin C amount in RCB detected in this experiment exhibited similar concentration as previous reports (Wall, 2006; Palintorn et al., 2019). Although, vitamin C concentration in all test diets (10-50 g RCB/kg) showed an inadequate level (Table 2) for Nile tilapia (Dawood & Koshio, 2018), however, the normal growth pattern of fish in the test diets was observed. These results suggested that the test diets may have an adequate nutritional value confirmed by the insignificant growth rates compared with the positive control group (P>0.05) but significantly higher than the negative control group (P<0.05). These indicated that besides vitamin C, other essential chemical compositions in RCB encouraged fish growth and health as vitamins and minerals supplementation in the positive control group.

Although this study did not evaluate all vitamins and minerals concentration, but the previous study demonstrated that RCB contained natural sources of vitamin and minerals such including 1) vitamins; β-carotene (provitamin A), ascorbic acid (vitamin C), vitamin B6 and B12 etc., 2) minerals; potassium and magnesium etc., (Palintorn et al., 2019; Lau et al., 2020). They are the main vitamins and minerals composition as similar as dietary vitamins and minerals premix supplementation in the positive control group.

Lysozyme, a mucolytic enzyme occurring bacteriolysis, is a fish defense element of the innate immune system, which is important in activating protection against microbial invasion (Magnadóttir, 2006; Saurabh & Sahoo, 2008). The total lysozyme levels can be used as an indicator of humoral component of the non-specific defense function (Wang et al., 2016) and could be elevated in response to the immunostimulants (Zahran et al., 2014). In the present study, the lysozyme activity of fish fed with 30RCB and 50RCB groups was observed on 8 weeks, which were consistent with the positive control group. In addition, the serum lysozyme activity of fish was most influenced by synthetic vitamins and minerals, followed by 50RCB and 30RCB, with 10RCB having the least effect, respectively. Although, a significant increase of the elevated lysozyme level was not observed among the positive control group and RCB supplementary diet groups but a significant difference in the percent survival of fish after challenge with *S. agalactiae* was found. The percent survival of the negative control group and 10RCB group were significantly lower than other groups. This could be attributed to the resistant starch effect, bioactive compounds and polysaccharides in RCB as prebiotics which used by gut microbial community in the small intestine and its direct bactericidal effects on pathogenic gut microflora (Bird et al., 2010; Singh et al., 2016).

In light of health concern, chemical usage in agricultural products are likely to be declining. Clean food is not only pathogen-free but should also be proved to be organic. Thus, products with less chemical would be the choice. Food from farm, which tends to accumulate antibiotics and chemicals used during growing-out period, is subjected to inspection. An option of using prebiotic supplementation could be used not only for growth but also health improvement. Prebiotics are non-digestible carbohydrates providing nutrition for gut microbiome to compete with pathogenic bacteria. There are many of prebiotics reported in fish, for example, glucan from yeast, microalgae, etc. Banana (*Musa* spp.) is one of fruit containing oligo-fructose which works as prebiotic (Ganguly et al., 2013; Hoseinifar et al., 2015). Not only its edible part, but also peel which has been focused in many researches for application as animal feed as shown in giant freshwater prawn (Rattanavichai et al., 2015), *Labeo rohita* (Giri et al., 2016). Recently, Pimpimon et al. (2018) reported a result using onion, garlic and banana as feed supplementation fed to juvenile climbing perch (*Anabas testudineus*). The result indicated that using these natural prebiotics did not affect growth performance and feed utilization for the climbing perch. However, banana fed fish resulted in increasing percentage of protein in edible flesh and survival rate. Thus, it could be used as a prebiotic for aqua lives.

Because of various natural prebiotics influence on type, location and chemical contents, supplementation in practical diets were concerned with certain nutritional values and anti-nutrients for cultured fish. Further research is needed to define clearly the quantity of various vitamin and mineral contents in RCB to negate improper supplementation levels that could be encountered with varying fish age and size.

Conclusion

Base on this study, the strength of ripe cultivated banana CV. Kluai Namwa (RCB) appears to improve growth, survival and non-specific immune function against fish pathogens as well as vitamin and mineral supplementation. RCB supplement at a dose of 30-50 g/kg (3-5%) is recommended in the diet to potentially decrease the cost of commercially vitamin and mineral premix. Easy access and the cheap price for RBC are also encouraging factors for their use on a large scale in hybrid tilapia aquaculture to provide better growth and protection at the same time.

Ethical Statement

All animal care and handling procedures in the present study were approved by the Animal Care Committee of Naresuan University and Kasetsart University (certificate number ACKU 61-FIS-001).

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Author Contribution

Thuchapol Karaket designed the study, analyzed the data and prepared the draft. Chanuwat Somtua ran the feeding trial and collected the samples. Pattareeya Ponza collected the blood parameters and tested the resistance of the pathogen. Nontawith Areechon supplied the pathogen. All authors have read and approved the manuscript.

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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