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Research article

Effects of Diet Supplemented with *Morinda citrifolia* Fermented Extract on the Growth Performance and Feed Conversion Ratio of *Oreochromis niloticus*

Duangjai Pisuttharachai[1*], Saichon Lerdsuwan[2], Nataporn Sangkhonkhet[3] and Warrapong Nalinanon[4]

[1] Fishery Science and Aquatic Resources, Disciplines of Technology Agriculture, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand; e-mail: duangjai.pi@kmitl.ac.th

[2] Animal Science, Disciplines of Technology Agriculture, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand; e-mail: saichon.le@kmitl.ac.th

[3] Scientist, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand; e-mail: nataporn.sa@kmitl.ac.th

[4] Fishery Science and Aquatic Resources, Disciplines of Technology Agriculture, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon, 86160, Thailand; e-mail: warrapong.na@kmitl.ac.th

* Correspondence: duangjai.pi@kmitl.ac.th

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Abstract: The use of additives in feeds to promote the growth of farmed fish has been rapidly expanding; among these are supplements derived from indigenous plants. Here, we analyzed the effects of satiation feeding of diet supplemented with noni (*Morinda citrifolia*) fermented extract on the feeding rate, growth performance and feed conversion ratio of Tilapia, *Oreochromis niloticus*. Fish with an initial average weight of 2.83 ± 0.01 g were fed with diets supplemented with noni fermented extract at 0 (control), 100-, 200- and 300-ml kg^{-1} for 12 weeks. Subsequent results showed that growth rate was correlated with or dependent on the concentration of noni fermented extract in the diet. The best result for growth performance, such as weight gain, average daily growth rate and specific growth rate, was found in a fish-fed diet supplemented with noni fermented extract at 200 ml kg^{-1}. However, the feed conversion ratio (FCR) is still higher than the control. The high FCR compared to control may be due to the anthraquinone in noni fermented extract, which decreases nutrient absorption. Fish fed with a 300 ml kg^{-1} noni fermented extract supplemented diet was the lowest growth performance and highest in FCR, suggesting that concentrations over 200 ml kg^{-1} might be detrimental to fish. Taken together, noni fermented extract can be a natural source of feed supplement to improve fish growth performance and may act as an appetite stimulant. However, due to its laxative effect, noni fermented extract supplementation should be given at appropriate levels and effective feeding management.

Keywords: *Morinda citrifolia*; *Oreochromis niloticus*; growth performance; satiate feeding

1. Introduction

Over the years, the aquaculture of tilapia (*Oreochromis niloticus*) has rapidly expanded to more than 130 countries worldwide [1]. Global production of tilapia farms is up 3.3% in 2020 and peaked at 6 million tons for the first time despite the impact of COVID-19. The global expansion of tilapia is because tilapia is a fish that can be grown in a diverse aquatic environment and can potentially replace marine fish products [2]. Currently, there are various methods used to increase the productivity of tilapia farming. Such approaches include using additives in feed formulas such as antimicrobials, antioxidants and enzymes to improve feed quality [3] and or supplementing with probiotics, prebiotics, immunostimulants and phytobiotics to increase the growth rate and health of tilapia [3-5].

Noni (*Morinda citrifolia*), a fruit-bearing tree native to Southeast Asia, is reported to exhibit phytobiotic properties, meaning compounds derived from plants that, when added to feed, affect the growth of animals [6]. Heinicke [7] and Widjastuti et al. [8] revealed that noni contains the precursor xeronine, which is important for metabolic processes that influence growth. Furthermore, earlier studies on noni suggest that the plant has antimicrobial properties [9, 10], antioxidant [11] and an appetite stimulant [12]. Based on the properties mentioned above, experimental application of noni as a component of feed formula for raising animals was reported. Examples include using noni, in fresh fruit form, for raising cattle [13] and noni fruit extract for raising red tilapia [14]. Their results suggest that, by feeding according to the percentage of body weight, noni in both the fresh fruit form and fruit extracts increased the growth rate of experimental animals and did not affect the efficiency of food use. However, in practice, most farmed tilapia are fed several times a day depending on the size and age of the cultured fish and each time, the fish are fed until they are full or until satiation. Therefore, this study's objective was to determine the effects of a fish diet supplemented with noni fruit extract on tilapia's feeding rate, growth, and feed conversion ratio. The supplemented diets were fed until satiation to simulate farm rearing conditions.

2. Materials and Methods

2.1 Experimental design

The experiment was designed completely randomized with four treatments and four replications. Noni fermented extract was supplemented into the diets at 0, 100, 200 and 300 ml kg^{-1} feed. Nile tilapia was purchased from the Aquaculture Genetics Research and Development Center, Department of Fisheries, Chumphon Province, Thailand. A total of 800 Nile tilapia with an average weight of 2.83 ± 0.01 g and an average length of 5.27 ± 0.03 cm were used in this study. Fish were reared in hapa nets or hapas 1.5 m x 1.5 m x 1.5 m in size; each hapa holds 50 fish, and 16 hapas were used in total. The hapas were placed in an earthen pond 25 x 40 x 2.5 m in size. The tilapia was trained to feed on sinker pellet feed using a control diet for 7 days before the start of the experiment.

2.2 Preparation of Noni fruit extract and experimental diet

Noni fruit was cleaned with tap water and dried. After that, the fruit was cut into pieces about 1 cm thick. Three kilograms of noni fruit were mixed with 1 kg of sugar and kept in containers for 3 months. The extract was collected by squeezing the fermented fruit and filtering to separate the extract from the waste. The filtered extract was mixed with other ingredients to make the experimental diets following the methods described earlier with some modifications [15]. The contents of each diet are shown in Table 1. The ingredients of each diet were equivalently mixed using a Hobart mincer (model HL200) for 15 min and pelleted to make a pelleted diet of 1.5 mm in size. All diets were dried in a hot air oven at 60 ºC for 12 hr. The experimental diets were stored in plastic bags and refrigerated at 4 °C until use.

2.3 pH measure of experimental diet

Experimental diets were ground with a medicinal mortar. One gram of the experimental diet was mixed thoroughly with 20 ml of distilled water. The pH of experimental diets was measured using a pH meter (model C835)

Table 1. Ingredients of experimental diets

Ingredient	Noni fruit extract in diets (ml)			
	0	100	200	300
Fish meal	220.00	220.00	220.00	220.00
Soybean meal	90.00	90.00	90.00	90.00
Broken rice	330.00	330.00	330.00	330.00
Rice bran	350.00	350.00	350.00	350.00
Vitamins and minerals mix[1]	10.00	10.00	10.00	10.00
Total (g)	1000.00	1000.00	1000.00	1000.00
Noni (ml)	0.00	100.00	200.00	300.00
Water (ml)	300.00	200.00	100.00	0.00
Proximate composition				
Moisture (%)	9.98	9.98	9.98	9.98
Crude protein (%)	25.03	25.03	25.03	25.03
Crude lipid (%)	6.19	6.19	6.19	6.19
Crude fiber (%)	4.66	4.66	4.66	4.66
Ash (%)	9.16	9.16	9.16	9.16
Gross energy (Kcal/100g)	387.30	387.30	387.30	387.30
pH of Experimental feed	5.66 ± 0.02	5.60 ± 0.01	5.16 ± 0.01	5.06 ± 0.03
pH of Noni fermented extract	3.08 ± 0.00			

[1] Vitamins and minerals mix/kg: vitamin A 1.20 MIU; vitamin D3 0.24 MIU; vitamin E 8,000 IU; vitamin B1 2.00 g; vitamin B2 2.00 g; vitamin B6 2.00 g; vitamin B12 0.001 g; vitamin C 22.00 g; pantothenic acid 5.00 g; nicotinic acid 5.00 g; folic acid 0.20 g; biotin 0.002 g; choline 2.00 g; iron (Fe) 2.00 g; copper (Cu) 2.00 g; zinc (Zn) 5.00 g; calcium 5.00 g; manganese (Mn) 0.8 g; selenium (Se) 0.01 g; lysine 8.50 g and methionine 3.00 g

2.4 Feeding management

Tilapia were fed with experimental diets twice daily, at 7:00 a.m. and 4:00 p.m., by satiation feeding for 12 weeks. The weight of the experimental diet of each hapa was recorded before feeding. The experimental diets were half sinking baskets, half floating in the water. The experimental diets were given to fish little by little. When the fish start to feel full, the fish will not come up to eat at the basket. The weight of the experimental diet for each hapa was re-recorded after feeding. Water quality, including temperature, dissolved oxygen, pH, ammonia and nitrites, was checked every 2 weeks. Water exchange (30 %) was done every 15 days.

2.5 Data collection and analysis

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, increase in length, specific growth rate, feed conversion ratio, feed intake and survival rate. The statistical results were analyzed using the obtained values to analyze variance (ANOVA) and comparing the mean difference in each experiment by Duncan's new multiple range test with a statistical program at 95% confidence level.

3. Results and Discussion

Feeding Nile tilapia with diet supplemented with noni fermented extract at 0 (control), 100, 200 and 300 ml kg^{-1} by satiation feeding for 12 weeks showed that fish fed diet with supplemented noni fermented extract at 200 ml kg^{-1} had the best growth performance in terms of final weight (34.31 ± 0.52 g), weight gain (31.49 ± 0.52 g), average daily gain (0.37 ± 0.01 g/fish/day), final length (12.38 ± 0.10 cm), length gain (7.13 ± 0.05 cm) and specific growth rate (2.97 ± 0.02 %/day). These parameters were significantly different ($P < 0.05$) from a fish-fed supplemented with noni fermented extract at 0 (control), 100 and 300 ml kg^{-1}. For the survival rate, fish-fed supplemented with noni fermented extract at all concentrations, including the control, had a survival rate of more than 90% (Table 2). The results indicated that noni fermented extract supplementation could enhance the growth of Nile tilapia. This is consistent with the results conducted earlier in cattle [13], in which they were fed 0, 0.09% and 0.18% noni supplementation in the diets. The cattle were fed diets at 2.5 % of their initial body weight for 28 days. Noni supplementation improved the average daily gain weight of the cattle as the percentage of noni increased in the diet. Moh et al. [16] reported that noni fruit extract supplementation could improve the growth of whiteleg shrimp in terms of body weight, total length, average daily growth and feed conversion ratio. They mentioned that their study's improvement of whiteleg shrimp's growth performance might be associated with the improved digestive enzymes induced by noni fruit extract. Their result showed increasing activities of amylase in whiteleg shrimp with increasing concentration of noni fruit extract. This affects the efficiency of using carbohydrates for growth. The fermentation of noni fruit for 3 months resulted in a noni fermented extract with a pH of 3.08 caused by the microbial product. The pH of experimental diets were also mesured, as shown in Table 1. The pH of experimental diets decreased following increased noni fermented extract. The noni fermented extract, therefore, will make the environment in the digestive tract of fish slightly more acidic, which is suitable for the functioning of the enzyme pepsin and resulting in the efficiency of protein digestion and better absorption [17]. Our result showed that a fish-fed diet supplemented with noni fermented extract at 300 ml $kg^{-1,}$ which has the lowest pH, fared the worst in terms of growth performance. This can be attributed to alcohol compounds in the fermented fruit, which might be toxic to the fish. In addition, the fermentation of noni has been shown to promote the survival of probiotic strains such as *Gluconobacter*, *Acetobacter*, *Lactobacillus*, and *Bifidobacterium* spp. [18, 19]. It is well known that probiotics could improve digestion by reducing the growth of harmful bacteria in the intestines so that good bacteria in the intestine increases. Moreover, the increased growth may be due to the proxeronine in noni that is converted to xeronine by the proxeroninase enzyme in the body. Xeronine is important for biochemical processes in the body, including protein synthesis, glucose absorption and other metabolic processes that affect growth [8, 20, 21].

The efficacy of noni fermented extract, in addition to enhancing growth, is that it can also stimulate appetite [12, 22]. Here, we employed feeding until satiation to simulate the current and most popular feeding practice for farmed tilapia. Our subsequent results showed that noni fermented extract could stimulate fish's appetite. The feeding rate of fish increased with the concentration of noni fermented extract supplemented in the diet. The feed conversion ratio analysis revealed that fish fed with a diet supplemented with noni fermented extract at 100 ml kg^{-1} did not differ significantly from the control group ($P > 0.05$). Still, those fed with noni fermented extract at 200 and 300 ml kg^{-1} had feed conversion ratios significantly higher ($P < 0.05$) than the control (Table 2). The results indicated that the amount of noni fermented extract in the diet affected the feed utilization of fish. It is to be noted that If the fish receives an excessive amount of noni fermented extract, the feed utilization of the fish will decrease. The decrease in feed utilization may be due to the anthraquinone found in noni [23]. Van Gorkom et al. [24] and Mueller et al. [25] reported that anthraquinone has laxative properties by causing damage to the intestinal lining cells, which may result in a reduction in the efficiency of nutrient absorption, enzyme secretion and intestine movements. In addition, excessive exposure to noni fruit extract carries a risk of hepatotoxicity. The liver is a vital organ involved in the digestive process. Shalan et al. [26] evaluated chronic toxicity of noni fruit in mice. The mice drank water mixed with noni fruit extract at 1 and 2 mg ml^{-1} for 6 months. The result showed that the mice who drank water mixed with noni fruit extract at 2 mg ml^{-1} had symptoms of hepatotoxicity with the destruction of liver cells and a 40% mortality rate after just 3 months of drinking water mixed with noni fruit extract. Moreover, Millonig et al. [27] and Stadlbauer et al. [28] also reported that noni fruit extract could induce hepatitis symptoms in humans.

The results of this experiment showed that noni fermented extract could stimulate appetite and increase growth as well as can be a laxative. Therefore, the use of noni fermented extract supplemented diets to enhance the development of fish must be applied at an appropriate rate as too high concentrations of noni fermented extract in the diet may negatively affect fish growth. This was evident in fish fed with a diet supplemented with noni fermented extract at 300 ml kg^{-1}. This experimental group has the worst feed conversion ratio, which is significantly different ($P < 0.05$) compared to other experimental diets. Our results indicate that satiation feeding with noni fermented extract supplemented diets should not be done as this will increase the level of noni fermented extract and should be given based on the percentage of fish body weight. The experiment of Kristiana et al. [14] investigated the effect of noni fruit extract supplementation on growth rates in red tilapia. The fish were fed a diet supplemented with noni fruit extract at 0, 100, 300 and 500 ml kg^{-1} of feed and provided at a rate of 3% of the body weight for 30 days. Noni fruit extract at 500 ml kg^{-1} of the meal was most effective in terms of specific growth rate and fish feed conversion ratio, which were the best and significantly different compared to the control ($P < 0.05$). The growth of tilapia in this study was lower than the normal growth rate of tilapia in commercial farms and lower than data reports in other publications, such as in Figueiredo-Silva *et al*. [29], wherein red tilapia cultured in the laboratory increased their body weight from 1.7 g to 67-83 g within 10 weeks. In this study, fish grew from 2 g to 34 g in 12 weeks. The growth performance of aquatic animals depends on several factors such as proximate diet composition, especially the percentage of protein, number of feeding per day, rate of feeding, etc. In our experiment, fish were fed diets containing 25% crude protein and 6% crude lipid with providing twice a day. Still, in the investigation of Figueiredo-Silva *et al*. [29], fish were fed with diets containing 35% crude protein and 12% crude lipid and fed three times a day, which suggests that the percentage of crude protein and crude lipid, as well as feeding frequency, were higher than in our study. In typical commercial farms, on the other hand, fish are fed with commercial diets containing at least 18% protein, but the feeding frequency is at least three times a day. These factors contributed to why growth performance was different in those settings.

The average water quality during the experiment was within the range suitable for aquaculture as specified by Boyd and Lichkoppler [30]. Specifically, the dissolved oxygen was at 5.60 - 5.90 mg l^{-1}; pH 7.20 - 7.70; water temperature 26.60 - 28.70 °C; ammonia 0.13 - 0.28 mg l^{-1}; and nitrites 0.01 - 0.03 mg l^{-1}.

Table 2. Growth performance of Nile tilapia fed with a diet supplemented with noni fermented extract at different concentrations for 12 weeks. Feed was given until satiation twice a day.

Growth performance	Noni fermented extract in diets (ml kg^{-1})				
	0	100	200	300	p-values
Initial weight (g/fish)	2.82 ± 0.02	2.84 ± 0.04	2.83 ± 0.02	2.83 ± 0.03	0.9041
Final weight (g/fish)	30.51 ± 0.94^{b}	30.64 ± 2.04^{b}	34.31 ± 0.52^{a}	25.79 ± 1.72^{c}	0.0001
Weight gain (g/fish)	27.69 ± 0.95^{b}	27.80 ± 2.05^{b}	31.49 ± 0.52^{a}	22.95 ± 1.72^{c}	0.0001
ADG (g/fish/day)[2]	0.33 ± 0.01^{b}	0.33 ± 0.02^{b}	0.37 ± 0.01^{a}	0.27 ± 0.02^{c}	0.0001
Initial length (cm)	5.30 ± 0.08	5.27 ± 0.06	5.27 ± 0.05	5.26 ± 0.02	0.9383
Final length (cm)	11.83 ± 0.26^{b}	11.85 ± 0.37^{b}	12.38 ± 0.10^{a}	11.38 ± 0.22^{c}	0.0013
Length gain (cm)	6.55 ± 0.31^{b}	6.58 ± 0.41^{b}	7.13 ± 0.05^{a}	6.13 ± 0.19^{c}	0.0023
SGR (%/day)[3]	2.83 ± 0.04^{b}	2.83 ± 0.09^{b}	2.97 ± 0.02^{a}	2.63 ± 0.08^{c}	0.0001
Feed conversion ratio	1.76 ± 0.13^{c}	1.90 ± 0.19^{c}	2.50 ± 0.04^{b}	3.78 ± 0.17^{a}	0.0001
Feed intake (g/fish)	48.75 ± 2.97^{c}	52.63 ± 4.75^{c}	78.61 ± 1.55^{b}	86.66 ± 6.02^{a}	0.0001
Survival rate (%)	91.00 ± 2.00^{b}	95.50 ± 3.00^{a}	93.50 ± 2.52^{ab}	97.50 ± 3.00^{a}	0.0269

[1] Different letter in the same row indicates a statistically significant difference at $P < 0.05$.
[2] ADG = Average daily gain
[3] SGR = Specific growth rate

4. Conclusions

The effect of diet supplementation with noni fermented extract in tilapia is concentration-dependent. Specifically, our results revealed that fish fed with a diet supplemented with noni fermented extract at 200 ml kg^{-1} by satiation feeding for 12 weeks showed the best growth rates. However, the feed conversion ratio was still higher than the control. The high feed conversion ratio may be attributed to the anthraquinone in noni fermented extract, which has a laxative effect and results in feed being pushed along faster in the digestive tract. This would then cause the efficiency of nutrient absorption to decrease. Our results revealed that feeding until satiation with diets supplemented with noni extract is not suitable. Therefore, to increase the efficiency of noni extract supplementation on tilapia growth rates, the amount of noni extract that the fish should receive must be controlled appropriately. Taken together, we have shown that noni fermented extract can be a potential natural growth enhancer for fish when given at appropriate levels coupled with sound feeding management. Further studies on the most suitable feeding program using noni fermented extract are currently being undertaken.

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