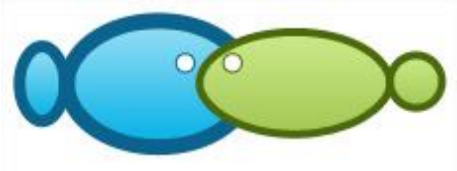

Increasing growth performances of Nile tilapia (*Oreochromis niloticus*) by supplementation of noni *Morinda citrifolia* fruit extract via diet

[1]Vini Kristiana, [2]Akhmad T. Mukti, [2]Agustono

[1] Study Program of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115, Indonesia; [2] Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115, Indonesia. Corresponding author: A. T. Mukti, akhmad-t-m@fpk.unair.ac.id

Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract to the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments of noni fruit extract dosages given to red tilapia. The treatments used were the dosage amount of noni extraction given to red tilapia, namely P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder); P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol; P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol; P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 0.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).
Key Words: fish meal substitution, noni fruit (*Morinda citrifolia*) extract, red tilapia (*Oreochromis niloticus*).

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand for consumption and cultivation. The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the community, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. Feed is an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu 2016). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most significant amount of

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2.	Under review oleh reviewer jurnal	15 Juli 2019
3.	Review dari reviewer/editor jurnal	14 September 2019
4.	Revisi manuskrip dan re-submit	17 September 2019
5.	Revisi manuskrip dari editor jurnal	22 Desember 2019
6.	Revisi manuskrip dan re-submit	25 Desember 2019
7.	Koreksi dari editor jurnal	25 Desember 2019
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the review for the paper "The potential of Morinda citrifolia in commercial feed on the growth and protein of Oreochromis niloticus"

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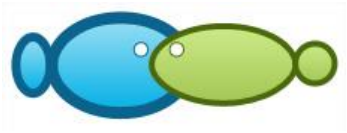

The potential of *Morinda citrifolia* in commercial feed on the growth and protein of *Oreochromis niloticus*

Vini Kristiana, Akhmad Taufiq Mukhti, Agustono

Department of Fisheries and Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Indonesia. Corresponding author: A. T. Mukhti, akhmadtaufiqmukhti@yahoo.com; akhmad-t-m@fpk.unair.ac.id

Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract to the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments. The treatments used were the dosage amount of noni extraction given to red tilapia. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 5.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).

Key Words: fish meal substitution, noni fruit (*Morinda citrifolia*) extract, red tilapia (*Oreochromis niloticus*).

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand by the community for consumption and cultivation. The provision of feed is considered necessary in fish farming, including the addition of feed additives to spur growth and production. The addition of natural ingredients to commercial feed is still limited in use so that efforts to provide natural additives in the form of noni fruit extract (*Morinda citrifolia*) are used to obtain the desired results, especially for the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the community, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. According to a study, feed as an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu 2015). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most

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significant amount of production costs component (Shepherd & Jackson 2013). One way to overcome this problem is to use feed additives. Feed additives in fish farming, especially red tilapia, are ingredients that are intentionally used to increase appetite, digestibility, endurance, reduce stress levels, and spur fish growth (Alemayehu et al 2018).

Phytobiotics is one type of natural feed additive derived from plants (Vidanarachchi et al 2005). The purpose of adding natural feed additives to the feed is to spur growth, as well as improve the health and productivity of fish (Citarasu 2001; Citarasu et al 1998, 1999, 2002; Sivaram et al 2004). Noni fruit can be used as phytobiotic (feed additive) because it has antibacterial effects and bioactive substances such as alkaloids, flavonoids, and tannins which are very effective in suppressing the growth of pathogenic bacteria and improving intestinal morphological characteristics (Murdiati & Adiwinatai 2000). Bioactive substances in noni include xeronine and precursor of xeronine (proxeronine). Proxeronine will be converted to xeronine in the intestine by proxeronase enzymes and other substances. Xeronine increases the permeability of cell membranes, facilitates the transfer of peptides and increases nutrient absorption, as well as effectuates amino acids, vitamins, and minerals. Xeronine can activate several enzymes and regulate protein synthesis (Solomon 2001). Therefore, noni fruit extract can be used as an additional ingredient in feed to help the digestion process of fish, so that the consumed feed can be optimally utilized for growth and feed efficiency, as well as the protein content of fish. This study aims to determine the potential of given noni fruit (*Morinda citrifolia*) extract to the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

Material and Method

Preparation of red tilapia (Oreochromis niloticus) and rearing conditions. This research was conducted from April to May 2017 at the Education Laboratory of the Airlangga University, Faculty of Fisheries and Marine, Surabaya, Indonesia. The proximate test of feed and protein retention test were conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The instruments used in this study were an aquarium measuring 45 cm x 30 cm x 30 cm, a set of aerators, digital scales, pH pens, thermometers, rulers, plastic bags, plastic hoses with a diameter of 1 cm, dissolved oxygen (DO) meters, and ammonia kits. The fish used were 140 red tilapia fry in healthy condition, measuring 5-6 cm, weighing 2-5 g/head. The red tilapia fry were obtained from the Gunung Sari fish market, Surabaya, East Java. Noni fruit extract was obtained from the result of squeezed noni fruit. The noni fruit used was ripe noni fruit, which is yellow and soft with a distinctive smell.

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Experimental design. This research method was experimental. The primary test parameters in this study were protein retention, specific growth rate, feed conversion ratio, and survival rate of red tilapia. The variables observed were independent variables, dependent variables, and control variables. The independent variable is the dose of noni extract. The dependent variables are specific growth rate, feed conversion ratio, survival rate, and protein retention. Control variables are water quality (temperature, pH, DO, and ammonia). This study used a Completely Randomized Design (CRD) with four treatments and four replications, which treatments explained as follows:
P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

The feed given in this study was PF 1000 commercial feed from PT Matahari Sakti, with a protein content of 39-41%, 5% lipid, 6% crude fiber, 16% ash, and 10% moisture content.

Preparation of the experimental diets. First, ripe noni fruits were cleaned with water, then washed with warm water. The noni fruits were crushed and then squeezed by using strain cloth. The squeezed noni fruit extract was put in a bottle and stored.

The commercial feed was mixed with noni fruit extract and 2 grams of Progol with a different amount in each treatment, as previously explained. The commercial feed and noni fruit extract were smoothly mixed. The mixed feed was dried in an open room and was aerated until dry. After that, the dried food was stored in a plastic jar. The mixed fish feed with different doses were then analyzed through the proximate test in the laboratory. The nutrient content of the feed used in this study can be seen in Table 1 as follows.

Table 1

Nutrient content of feed with various doses of noni fruit extract

Analysis	*P0 (Control)*	*P1 (100 mL kg^{-1})*	*P2 (300 mL kg^{-1})*	*P3 (500 mL kg^{-1})*
Dry ingredients (%)	90.1441	87.3484	86.7339	84.5036
Ash (%)	8.9591	8.7352	8.6875	8.5261
Crude protein (%)	29.1112	30.9015	30.2753	29.2539
Crude lipid (%)	8.3165	7.6176	6.9983	6.872
Crude fiber (%)	3.5026	2.488	2.6143	2.2593
Ca	2.5824	2.4109	2.4069	2.1481
Nitrogen-free extract (%)	40.2547	37.6061	38.1585	37.5923
Metabolism energy (Kcal kg^{-1})	3057.9622	2967.4828	2922.6623	2857.8501

The analytical procedure of the diets. The aquarium was then filled with 30 liters of water, aerated, and then left for 24 hours. The fish were selected based on a similar length and weight and its healthy condition. The fish were acclimatized for one week and were fasted for 24 hours before being treated. The fish were placed in fourteen aquariums according to the treatment given with a stocking density of 10 fish per aquarium. The fish were kept for thirty days and fed according to the treatment doses. Feeding was given three times a day: morning, afternoon, and evening, with a dose of 3% every day.

Below is the protein retention (PR) calculation based on a formula from Tung & Shiau (1991):

$$PR = \frac{Ln\ final\ body\ weight\ - Ln\ initial\ body\ weight\ (g)}{Total\ protein\ intake\ (g)} x100\%$$

The specific growth rate was calculated based on a formula from Effendie (1997):

$$SGR = \frac{LnWt - LnWo}{t} x\ 100\%$$

where: SGR = specific growth rate (% day^{-1});
W0 = initial body weight (g);
Wt = final body weight (g);
T = treatment time (in days).

The daily length growth rate (LPP) was calculated based on a formula from Aggraeni & Abdulgani 2013):

$$LPP = \frac{Ln\ Lt - Ln\ Lo}{t} x\ 100\%$$

where: LPP = daily length growth rate (% day^{-1});
Lt = average total length on day t (cm);
L0 = average total length on day 0 (cm);
t = total of observation time (in days).

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The survival rate was calculated based on a formula from Wirabakti (2006):

$$SR = \frac{Nt}{No} x\ 100\%$$

where: SR = survival rate (%);
Nt = the number of survived fish at the end of the experiment (in heads);
No = the number of survived fish at the start of the experiment (in heads).

The feed conversion ratio was calculated based on a formula from Council (1997):

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
Wo = the biomass weight of fish at the start of the experiment (g);
Wt = the biomass weight of fish at the end of the experiment (g);
D = total weight of dead fish (g);
F = feed intake (g).

Statistical analysis. Data analysis using Variant Analysis (ANOVA) with the study design was a Completely Randomized Design (CRD) to determine the differences in treatment. If there is a difference in treatment, Duncan's Multiple Range Test is applied.

Results. The calculation results of the average value of the specific growth rate, feed conversion ratio, and survival rate of red tilapia using statistical analysis after being treated for 30 days can be seen in Table 2.

Table 2

Average value of the specific growth rate, survival rate, feed conversion ratio, daily length growth, and protein retention of red tilapia

Treatment	*Specific growth rate (% day^{-1})*	*Survival rate (%)*	*Feed conversion ratio (%)*	*Daily length growth rate (% day^{-1})*	*Protein retention (%)*
P0	0.0105±0.000 7^{c}	100	7.712±0.588^{b}	1.070±0.189^{b}	7.3345±0.3434^{c}
P1	0.0135±0.0006^{b}	100	6.157±0.226^{a}	1.145±0.050^{b}	12.6246±0.3074^{b}
P2	0.0147±0.0005^{a}	100	5.846±0.234^{a}	1.435±0.186^{a}	11.5692±0.4054^{a}
P3	0.0150±0.0009^{a}	100	5.918±0.436^{a}	1.599±1.312^{a}	11.4289±0.7356^{a}

P0 (0% noni fruit extract), P1 (100 mL kg^{-1} noni fruit extract), P2 (300 mL kg^{-1} commercial feed with noni fruit extract), P3 (500 mL kg^{-1} commercial feed with noni fruit extract). Different superscripts show significant differences.

According to Table 2, the value of the specific growth rate (SGR) of red tilapia was 0.0105-0.0150% day^{-1}. The results of the statistical analysis of added noni fruit extract on commercial feed to the SGR of red tilapia showed significantly different results ($p < 0.05$). Duncan's Multiple Range Test (DMTR) results showed that the highest growth rate in this study was P3 (0.0150% day^{-1}) which was not significantly different from P2 (0.0147% day^{-1}), while it was significantly different with P0 (0.0105% day^{-1}) and P1 (0.0135% day^{-1}). The lowest specific growth rate was P0 (0.0105% day^{-1}) which was significantly different from P1 (0.0135% day^{-1}), P2 (0.147% day^{-1}), and P3 (0.0150% day^{-1}).

The feed conversion ratio (FCR) of red tilapia was 5.846-7.712%. The results of statistical analysis of the added noni fruit extract to commercial feed to FCR of red tilapia showed significantly different results ($p < 0.05$). Based on the results of DMTR, the highest FCR in this study was P0 (7.712%) which was significantly different from P1 (6.157%), P2 (5.846%), and P3 (5.9188%). While the lowest feed conversion ratio was P2 (5.846%) and was not significantly different from P1 (6.157%), P3 (5.918%), and significantly different from P0 (7.712%). These results indicate that the addition of noni fruit extract to commercial feed can reduce feed conversion ratio of red tilapia.

The survival rate of red tilapia from all treatments was 100%. The average survival rate of red tilapia showed no significant difference ($p > 0.05$). Based on the results of DMTR, the survival rates of P0, P1, P2, and P3 were not significantly different.

The value of the daily length growth rate of red tilapia for thirty days of preservation was 1.070-1.599% day^{-1}. The results showed significant differences ($p < 0.05$). The highest daily length growth value was at P3 (1.599% day^{-1}) which was not significantly different from P2 (1.435% day^{-1}) but was significantly different from P0 (1.070% day^{-1}) and P1 (1.145% day^{-1}). The lowest daily length growth rate was P0 (1.070% day^{-1}) which was not significantly different from P1 (1.145% day^{-1}) but significantly different from P2 (1.435% day^{-1}) and P3 (1.599% day^{-1}).

The results of the statistical analysis of the addition of noni fruit extract to commercial feed showed significantly different results ($p < 0.05$) to the protein retention of red tilapia. The results of DMTR showed that the highest protein retention value was P1 (12.6246%) which was significantly different from P0 (7.3345%), P2 (11.5692%), and P3 (11.4289%). Protein retention value in P2 (11.5692%) was not significantly different from P3 (11.4289%) but was significantly different from P0 (7.3345%) and P1 (12.6246%). The lowest protein retention value was P0 (7.3345%), which was significantly different from P1 (12.6246%), P2 (11.5692%), and P3 (11.4289%).

Data on the range of water quality parameters for red tilapia preservation for thirty days can be seen in Table 3.

Table 3

Water quality range value of red tilapia in 30 days of preservation

Parameter	*Range*
Temperature (°C)	27.4-29.2
pH	7.2-7.9
DO (mg L^{-1})	3.3-5
Ammonia (mg L^{-1})	0-0.5

The water quality was observed based on the measurements of temperature, pH, DO, and ammonia intensity in the tilapia fish for 30 days. The observations used temperatures ranging from 27.4-29.2°C, pH (acidity degree) ranging from 7.2-7.9, DO (dissolved oxygen level) ranging from 3.3 to 5 mg L^{-1}, and ammonia ranging from 0 to 0.5 mg L^{-1}.

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Discussion. The noni fruit extract mixed in the commercial feed had a significantly different effect on specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia for thirty days of preservation. These effects are due to the optimum absorption process or nutrient absorption of the feed in the digestive tract. Noni extract contains anthraquinone or acidic ingredients that can affect the pH of the digestive tract to be more acidic (Bayu et al 2015). The statement is right considering that in an acidic environment, protein-breaking enzymes (pepsin) can work optimally, so that protein intakes are absorbed more by the body which supports the optimal fish growth rate (ME et al 1990).

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The extract of noni fruit contains proxeronine and xeronine. Proxeronine is converted to xeronine with the help of proxeronase enzymes. Xeronine is an active compound that has a role in facilitating metabolism inside the body of the animals and increasing nutrient absorption (Solomon 2001). Xeronine in noni fruit can function to expand the absorption in the intestine surface and facilitate the absorption process of food so the nutrients in the feed can be absorbed optimally (Wang et al 2008). The optimal absorption process will affect increased protein retention (Schlegel 1987; William & Westhoff 1988; Cui et al 1992; Solomon 2001; Sjofjan 2003; Sujono 2006; Bayu et al 2015). Increased protein retention in the treatment results was caused by adding noni fruit extract in commercial feed, which can suppress the growth of microbial pathogens and increase non-pathogenic microbes in the digestive tract, resulting in the ability to decompose feed ingredients, especially crude fiber. The use of noni fruit extract can inhibit the growth of pathogenic bacteria in the intestine so that the number of non-pathogenic bacteria in the intestine was increased (Solomon 2001). Noni extract can increase the absorption process in the digestive tract and help decompose feed

ingredients, especially crude fiber (Bayu et al 2015). Non-pathogenic bacteria can produce enzymes that digest crude fiber, protein, and lipid (Sjofjan 2003). *Bacillus* sp. bacteria in the digestive tract can break down proteins into amino acids (Sujono 2006). These amino acids are used by bacteria to multiply themselves. Bacteria are a source of single-cell proteins so that the bacteria multiplication can increase protein feed and reduce crude fiber (Schlegel 1987).

Moreover, *Bacillus* sp. is a pectinolytic bacteria, which is an organism that can produce pectin or complex carbohydrates (William & Westhoff 1988). The characteristics of *Bacillus* sp. bacteria are proteolytic, saccharolytic, and pectinolytic, which increases protein and carbohydrate feed. Protein retention indicates the amount of additional body protein from the consumed feed protein. The feed consumed was a source of protein used for preservation, metabolic activity, and growth (Cui et al 1992). The high protein retention in P1 treatment was caused by the increase in protein and carbohydrate content by the activity of non-pathogenic microbes that increase due to the addition of noni fruit extract in the feed. The only issue was the more noni fruit extract dosage in the feed cannot help at breaking down the crude fiber and result in higher crude fiber amount in the feed.

The feed conversion ratio is one of the standards to determine the quality of feed given to livestock in fulfilling the required nutrition. The higher the value of feed conversion, the worse the quality of the nutritional value of the feed. Noni fruit contains saponins which are bioactive in the growth of animals and digestive microbes. Giving saponins can increase the permeability of cell walls in the intestine, increase absorption of food substances hence result in higher conversion value (Önning et al 1996).

Survival rates of red tilapia were not significantly different between treatments. Thirty days preservation of red tilapia with the treatments given did not cause death in fish. This proves that adding noni fruit extract in commercial feed did not contain toxic ingredients that cause death. One study using noni fruit extract with a dose of 15,000 mg per kg body weight in experimental animals was not found any sign of toxic effects (Peter & Abu Saleha 2006). The study had performed surgery on experimental animals but still did not find any signs of toxicity in the animals' organs. Noni fruit is categorized as non-toxic substances (Wang et al 2008; Younos et al 1990).

Water quality in this study is an essential supporting parameter for the survival rate of red tilapia. Water as a living medium for fish that is preserved must meet the requirements. Water quality needs to be controlled in order to maintain its optimal conditions, thus creating an environment that is suitable for the habitat of red tilapia. In order to keep the optimum quality, dirt in the water needs to be cleaned once every day and changing the water as much as 50% of the total volume. The supporting parameters of this study are water quality parameters such as temperature, DO, pH, and ammonia. The temperature in this study averaged around 27.4-29.2^{o}C. This temperature is good enough for the survival of red tilapia. The optimal water temperature for red tilapia is 25-30^{o}C (Kungvakij & Pudadera 1984). Temperature is an environmental factor that influences the body metabolism speed. The optimum speed metabolism for fish will be achieved at optimal temperatures. The pH value has an essential influence on the survival of fish in the water. The pH in this study ranged from 7.2 to 7.9, which is quite adequate for the survival rate of red tilapia. The pH range required by fish is 6-9 (DeLong et al 2009). The average DO content in this study ranged from 3.3 to 5 mg L^{-1}. Based on these results, the DO during the study indicated a suitable level for the survival of red tilapia (Yildiz et al 2017). Thus DO in the preservation of red tilapia met the requirements. Ammonia in the water is derived from fish feces and from the rest of the feed that is not consumed by fish. In general, the concentration of ammonia contained in the water must not exceed 1 mg L^{-1} (BFAR-NFFTC 2000; Indian Council of Agricultural Research 2006). Ammonia during this study was 0-0.5 mg L^{-1}, which is suitable for the preservation of red tilapia.

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Conclusions. Based on this research, it can be concluded that the addition of noni fruit extract in commercial feed can increase specific growth rate, daily length growth rate, lower the feed conversion ratio, and increase protein retention of red tilapia (*Oreochromis*

niloticus). Moreover, the addition of noni fruit extract in commercial feed did not show any toxic effect on the survival rate of red tilapia during the thirty days of preservation.

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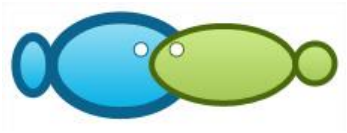

The potential of *Morinda citrifolia* in commercial feed on the growth and protein of *Oreochromis niloticus*

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Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract on the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments of noni fruit extract dosages given to red tilapia. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 5.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).
Key Words: fish meal substitution, noni fruit extract, red tilapia.

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand for consumption and cultivation. The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the commodity, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. Feed as an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu 2015). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most significant amount of production costs component (Shepherd & Jackson 2013). One way to overcome this problem is to use feed additives. Feed additives in fish farming, especially red tilapia, are ingredients that are intentionally used to increase appetite, digestibility, endurance, reduce stress levels, and spur fish growth (Alemayehu et al 2018).

The provision of feed is considered necessary in fish farming, including the addition of feed additives to spur growth and production. Phytobiotics is one type of natural feed additive derived from plants (Vidanarachchi et al 2005). The purpose of adding natural feed additives to the feed is to spur growth, as well as improve the health and productivity of fish (Citarasu 2001; Citarasu et al 1998, 1999, 2002; Sivaram et al 2004). Noni fruit can be used as phytobiotic (feed additive) because it has antibacterial effects and bioactive substances such as alkaloids, flavonoids, and tannins which are very effective in suppressing the growth of pathogenic bacteria and improving intestinal morphological characteristics (Murdiati et al 2000). Bioactive substances in noni include xeronine and precursor of xeronine (proxeronine). Proxeronine will be converted to xeronine in the intestine by proxeronase enzymes and other substances. Xeronine increases the permeability of cell membranes, facilitates the transfer of peptides and increases nutrient absorption, as well as effectuates amino acids, vitamins, and minerals. Xeronine can activate several enzymes and regulate protein synthesis (Solomon 2001). Therefore, noni fruit extract can be used as an additional ingredient in feed to help the digestion process of fish, so that the consumed feed can be optimally utilized for growth and feed efficiency, as well as the protein content of fish. This study aims to determine the potential of given noni fruit (*Morinda citrifolia*) extract to the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

Material and Method

Preparation of red tilapia (Oreochromis niloticus) and rearing conditions. This research was conducted from April to May 2017 at the Education Laboratory of the Airlangga University, Faculty of Fisheries and Marine, Surabaya, Indonesia. The proximate test of feed and protein retention test were conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The instruments used in this study were an aquarium measuring 45 cm × 30 cm × 30 cm, a set of aerators, digital scales, pH pens, thermometers, rulers, plastic bags, plastic hoses with a diameter of 1 cm, dissolved oxygen (DO) meters, and ammonia kits. The fish used were 140 red tilapia fry in healthy condition, measuring total length of 5-6 cm, weighing 2-5 g/head. The red tilapia fry were obtained from the Gunung Sari fish market, Surabaya, East Java. Noni fruit extract was obtained from the result of squeezed noni fruit. The noni fruit used was ripe noni fruit, which is yellow and soft with a distinctive smell. In order to keep the optimum quality, dirt in the water needs to be cleaned once every day and changing the water as much as 50% of the total volume.

Experimental design. This research method was experimental. The primary test parameters in this study were protein retention, specific growth rate, feed conversion ratio, and survival rate of red tilapia. The variables observed were independent variables, dependent variables, and control variables. The independent variable is the dose of noni extract. The dependent variables are specific growth rate, feed conversion ratio, survival rate, and protein retention. Control variables are water quality (temperature, pH, DO, and ammonia). This study used a Completely Randomized Design (CRD) with four treatments and four replications, which treatments explained as follows:
P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

The feed given in this study was PF 1000 commercial feed from PT Matahari Sakti, with a protein content of 39-41%, 5% lipid, 6% crude fiber, 16% ash, and 10% moisture content.

Preparation of the experimental diets. First, ripe noni fruits were cleaned with water, then washed with warm water. The noni fruits were crushed and then squeezed by using strain cloth. The squeezed noni fruit extract was put in a bottle and stored at room temperature.

The commercial feed was mixed with noni fruit extract and 2 grams of Progol with a different amount in each treatment, as previously explained. The commercial feed and noni fruit extract were smoothly mixed. The mixed feed was dried in an open room and was aerated until dry. After that, the dried food was stored in a plastic jar. The mixed fish feed with different doses were then analyzed through the proximate test in the laboratory. The nutrient content of the feed used in this study can be seen in Table 1 as follows.

Table 1

Nutrient content of feed with various doses of noni fruit extract

Analysis	*P0* *(Control)*	*P1* *(100 mL kg^{-1})*	*P2* *(300 mL kg^{-1})*	*P3* *(500 mL kg^{-1})*
Dry ingredients (%)	90.1441	87.3484	86.7339	84.5036
Ash (%)	8.9591	8.7352	8.6875	8.5261
Crude protein (%)	29.1112	30.9015	30.2753	29.2539
Crude lipid (%)	8.3165	7.6176	6.9983	6.872
Crude fiber (%)	3.5026	2.488	2.6143	2.2593
Ca	2.5824	2.4109	2.4069	2.1481
Nitrogen-free extract (%)	40.2547	37.6061	38.1585	37.5923
Metabolism energy (Kcal kg^{-1})	3057.9622	2967.4828	2922.6623	2857.8501

The analytical procedure of the diets. The aquarium was then filled with 30 liters of water, aerated, and then left for 24 hours. The fish were selected based on a similar length and weight and its healthy condition. The fish were acclimatized for one week and were fasted for 24 hours before being treated. The fish were placed in fourteen aquariums according to the treatment given with a stocking density of 10 fish per aquarium. The fish were kept for thirty days and fed according to the treatment doses. Feeding was given three times a day: morning, afternoon, and evening, with a dose of 3% of body weight, every day.

Below is the protein retention (PR) calculation based on a formula from Tung & Shiau (1991):

$$PR = \frac{Ln\ final\ body\ weight - Ln\ initial\ body\ weight\ (g)}{Total\ protein\ intake\ (g)} \times 100$$

The specific growth rate was calculated based on a formula from Effendie (1997):

$$SGR = \frac{LnWt - LnWo}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W0 = initial body weight (g);
Wt = final body weight (g);
T = treatment time (in days).

The daily length growth rate (LGR) was calculated based on a formula from Aggraeni & Abdulgani 2013):

$$LGR = \frac{LnLt - LnLo}{t} \times 100$$

where: LGR = daily length growth rate (% day^{-1});
Lt = average total length on day t (cm);
L0 = average total length on day 0 (cm);
t = total of observation time (in days).

The survival rate was calculated based on a formula from Wirabakti (2006):

$$SR = \frac{Nt}{No} \times 100$$

where: SR = survival rate (%);
Nt = the number of survived fish at the end of the experiment (in fish);
No = the number of survived fish at the start of the experiment (in fish).

The feed conversion ratio was calculated based on a formula from Council (1997):

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
Wo = the biomass weight of fish at the start of the experiment (g);
Wt = the biomass weight of fish at the end of the experiment (g);
D = total weight of dead fish (g);
F = feed intake (g).

Statistical analysis. Data analysis using Variant Analysis (ANOVA) with the study design was a Completely Randomized Design (CRD) to determine the differences in treatment. If there is a difference in treatment, Duncan's Multiple Range Test is applied.

Results. The calculation results of the average value of the specific growth rate, feed conversion ratio, and survival rate of red tilapia using statistical analysis after being treated for 30 days can be seen in Table 2.

Table 2

Average value of the specific growth rate, survival rate, feed conversion ratio, daily length growth, and protein retention of red tilapia

Treatment	*Specific growth rate (% day^{-1})*	*Survival rate (%)*	*Feed conversion ratio (%)*	*Daily length growth rate (% day^{-1})*	*Protein retention (%)*
P0	0.0105±0.000 7^{c}	100	7.712±0.588^{b}	1.070±0.189^{b}	7.3345±0.3434^{c}
P1	0.0135±0.0006^{b}	100	6.157±0.226^{a}	1.145±0.050^{b}	12.6246±0.3074^{b}
P2	0.0147±0.0005^{a}	100	5.846±0.234^{a}	1.435±0.186^{a}	11.5692±0.4054^{a}
P3	0.0150±0.0009^{a}	100	5.918±0.436^{a}	1.599±1.312^{a}	11.4289±0.7356^{a}

P0 (0% noni fruit extract), P1 (100 mL kg^{-1} noni fruit extract), P2 (300 mL kg^{-1} commercial feed with noni fruit extract), P3 (500 mL kg^{-1} commercial feed with noni fruit extract). Different superscripts show significant differences.

According to Table 2, the value of the specific growth rate (SGR) of red tilapia was 0.0105-0.0150% day^{-1}. The results of the statistical analysis of added noni fruit extract on commercial feed to the SGR of red tilapia showed significantly different results ($p < 0.05$). Duncan's Multiple Range Test (DMTR) results showed that the highest growth rate in this study was P3 (0.0150% day^{-1}) which was not significantly different from P2 (0.0147% day^{-1}), while it was significantly different with P0 (0.0105% day^{-1}) and P1 (0.0135% day^{-1}). The lowest specific growth rate was P0 (0.0105% day^{-1}) which was significantly different from P1 (0.0135% day^{-1}), P2 (0.147% day^{-1}), and P3 (0.0150% day^{-1}).

The feed conversion ratio (FCR) of red tilapia was 5.846-7.712%. The results of statistical analysis of the added noni fruit extract to commercial feed to FCR of red tilapia showed significantly different results ($p < 0.05$). Based on the results of DMTR, the highest FCR in this study was P0 (7.712%) which was significantly different from P1 (6.157%), P2 (5.846%), and P3 (5.9188%). While the lowest feed conversion ratio was P2 (5.846%) and was not significantly different from P1 (6.157%), P3 (5.918%), and significantly different from P0 (7.712%). These results indicate that the addition of noni fruit extract to commercial feed can reduce feed conversion ratio of red tilapia.

The survival rate of red tilapia from all treatments was 100%. The average survival rate of red tilapia showed no significant difference ($p > 0.05$). Based on the results of DMTR, the survival rates of P0, P1, P2, and P3 were not significantly different.

The value of the daily length growth rate of red tilapia for thirty days of preservation was 1.070-1.599% day^{-1}. The results showed significant differences ($p < 0.05$). The highest daily length growth value was at P3 (1.599% day^{-1}) which was not significantly different from P2 (1.435% day^{-1}) but was significantly different from P0 (1.070% day^{-1}) and P1 (1.145% day^{-1}). The lowest daily length growth rate was P0 (1.070% day^{-1}) which was not significantly different from P1 (1.145% day^{-1}) but significantly different from P2 (1.435% day^{-1}) and P3 (1.599% day^{-1}).

The results of the statistical analysis of the addition of noni fruit extract to commercial feed showed significantly different results ($p < 0.05$) to the protein retention of red tilapia. The results of DMTR showed that the highest protein retention value was P1 (12.6246%) which was significantly different from P0 (7.3345%), P2 (11.5692%), and P3 (11.4289%). Protein retention value in P2 (11.5692%) was not significantly different from P3 (11.4289%) but was significantly different from P0 (7.3345%) and P1 (12.6246%). The lowest protein retention value was P0 (7.3345%), which was significantly different from P1 (12.6246%), P2 (11.5692%), and P3 (11.4289%).

Data on the range of water quality parameters for red tilapia preservation for thirty days can be seen in Table 3.

Table 3

Water quality range value of red tilapia in 30 days of preservation

Parameter	*Range*
Temperature (°C)	27.4-29.2
pH	7.2-7.9
DO (mg L^{-1})	3.3-5
Ammonia (mg L^{-1})	0-0.5

Discussion. The noni fruit extract mixed in the commercial feed had a significantly different effect on specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia for thirty days of preservation. These effects are due to the optimum absorption process or nutrient absorption of the feed in the digestive tract. Noni extract contains anthraquinone or acidic ingredients that can affect the pH of the digestive tract to be more acidic (Bayu et al 2015). The statement is right considering that in an acidic environment, protein-breaking enzymes (pepsin) can work optimally, so that protein intakes are absorbed more by the body which supports the optimal fish growth rate (Me et al 1990).

The extract of noni fruit contains proxeronine and xeronine. Proxeronine is converted to xeronine with the help of proxeronase enzymes. Xeronine is an active compound that has a role in facilitating metabolism inside the body of the animals and increasing nutrient absorption (Solomon 2001). Xeronine in noni fruit can function to expand the absorption in the intestine surface and facilitate the absorption process of food so the nutrients in the feed can be absorbed optimally (Wang et al 2008). The optimal absorption process will affect increased protein retention (Schlegel 1987; William & Westhoff 1988; Cui et al 1992; Solomon 2001; Sjofjan 2003; Sujono 2006; Bayu et al 2015). Increased protein retention in the treatment results was caused by adding noni fruit extract in commercial feed, which can suppress the growth of microbial pathogens and increase non-pathogenic microbes in the digestive tract, resulting in the ability to decompose feed ingredients, especially crude fiber. The use of noni fruit extract can inhibit the growth of pathogenic bacteria in the intestine so that the number of non-pathogenic bacteria in the intestine was increased (Solomon 2001). Noni extract can increase the absorption process in the digestive tract and help decompose feed ingredients, especially crude fiber (Bayu et al 2015). Non-pathogenic bacteria can produce enzymes that digest crude fiber, protein, and lipid (Sjofjan 2003). *Bacillus* sp.

bacteria in the digestive tract can break down proteins into amino acids (Sujono 2006). These amino acids are used by bacteria to multiply themselves. Bacteria are a source of single-cell proteins so that the bacteria multiplication can increase protein feed and reduce crude fiber (Schlegel 1987).

Moreover, *Bacillus* sp. is a pectinolytic bacteria, which is an organism that can produce pectin or complex carbohydrates (William & Westhoff 1988). The characteristics of *Bacillus* sp. bacteria are proteolytic, saccharolytic, and pectinolytic, which increases protein and carbohydrate feed. Protein retention indicates the amount of additional body protein from the consumed feed protein. The feed consumed was a source of protein used for preservation, metabolic activity, and growth (Cui et al 1992). The high protein retention in P1 treatment was caused by the increase in protein and carbohydrate content by the activity of non-pathogenic microbes that increase due to the addition of noni fruit extract in the feed. The only issue was the more noni fruit extract dosage in the feed cannot help at breaking down the crude fiber and result in higher crude fiber amount in the feed.

The feed conversion ratio is one of the standards to determine the quality of feed given to livestock in fulfilling the required nutrition. The higher the value of feed conversion, the worse the quality of the nutritional value of the feed. Noni fruit contains saponins which are bioactive in the growth of animals and digestive microbes. Giving saponins can increase the permeability of cell walls in the intestine, increase absorption of food substances hence result in higher conversion value (Önning et al 1996).

Survival rates of red tilapia were not significantly different between treatments. Thirty days preservation of red tilapia with the treatments given did not cause death in fish. This proves that adding noni fruit extract in commercial feed did not contain toxic ingredients that cause death. One study using noni fruit extract with a dose of 15,000 mg per kg body weight in experimental animals was not found any sign of toxic effects (Krishnakumar et al 2015). The study had performed surgery on experimental animals but still did not find any signs of toxicity in the animals' organs. Noni fruit is categorized as non-toxic substances (Wang et al 2008; Younos et al 1990).

Water quality in this study is an essential supporting parameter for the survival rate of red tilapia. Water as a living medium for fish that is preserved must meet the requirements. Water quality needs to be controlled in order to maintain its optimal conditions, thus creating an environment that is suitable for the habitat of red tilapia. The supporting parameters of this study are water quality parameters such as temperature, DO, pH, and ammonia. The temperature in this study averaged around 27.4-29.2^{o}C. This temperature is good enough for the survival of red tilapia. The optimal water temperature for red tilapia is 25-30^{o}C (Kungvakij & Pudadera 1984). Temperature is an environmental factor that influences the body metabolism speed. The optimum speed metabolism for fish will be achieved at optimal temperatures. The pH value has an essential influence on the survival of fish in the water. The pH in this study ranged from 7.2 to 7.9, which is quite adequate for the survival rate of red tilapia. The pH range required by fish is 6-9 (DeLong et al 2009). The average DO content in this study ranged from 3.3 to 5 mg L^{-1}. Based on these results, the DO during the study indicated a suitable level for the survival of red tilapia (Yildiz et al 2017). Thus DO in the preservation of red tilapia met the requirements. Ammonia in the water is derived from fish feces and from the rest of the feed that is not consumed by fish. In general, the concentration of ammonia contained in the water must not exceed 1 mg L^{-1} (BFAR-NFFTC 2000; Indian Council of Agricultural Research 2006). Ammonia during this study was 0-0.5 mg L^{-1}, which is suitable for the preservation of red tilapia.

Conclusions. Based on this research, it can be concluded that the addition of noni fruit extract in commercial feed can increase specific growth rate, daily length growth rate, lower the feed conversion ratio, and increase protein retention of red tilapia (*Oreochromis niloticus*). Moreover, the addition of noni fruit extract in commercial feed did not show any toxic effect on the survival rate of red tilapia during the thirty days of preservation.

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For the journals write the full names, then provide the volume's number, issue (if possible) and pages

For all the other references (paper, theses, report...) provide the pages too

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the paper "The potential of M. citrifolia in commercial feed on the growth and protein of O. niloticus" - still not the final form

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I am sending you back the paper. There are many comments you simply ignored. And there are few more new comments too.
We also did lots of corrections within the references section.

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Dear
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Herewith, we re-submitted our revised article based on the editor's comments. Thank you very much.

Best regards

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gavriloaie ionel claudiu <ionelclaudiu@yahoo.com> Wed, Dec 25, 2019 at 1:01 AM
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Dear Dr. Mukti,

Read again the comments. You still did not addrees few of them. The title is the same, same problems in the Abstract, same lack of info in the last comment.
Thank you!

Yours,
Claudiu Gavriloaie

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Dear
Editor-in-Chief AACL Bioflux

Herewith, we re-submitted the revised manuscript based on editor's comments and corrections. Thank you very much

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Herewith, we re-submitted the revised manuscript based on the editor's comments. In addition, we have revised several points including feed conversion ratio (FCR) value because we found that there was a miscalculation on the previous manuscript.
Thank you very much

Best regards,

Akhmad Taufiq Mukti
[Quoted text hidden]
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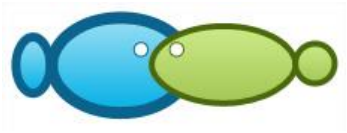

The potential of *Morinda citrifolia* in commercial feed on the growth and protein of *Oreochromis niloticus*

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Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract to the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments of noni fruit extract dosages given to red tilapia. The treatments used were the dosage amount of noni extraction given to red tilapia. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 5.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).
Key Words: fish meal substitution, noni fruit (*Morinda citrifolia*) extract, red tilapia (*Oreochromis niloticus*).

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand for consumption and cultivation. The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the commodity, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. Feed is an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu 2016). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most significant amount of production costs component (Shepherd & Jackson 2013). One way to overcome this problem is to use feed additives. Feed additives in fish farming, especially red tilapia, are ingredients that are intentionally used to increase appetite, digestibility, endurrance, reduce stress levels, and spur fish growth (Alemayehu et al 2018).

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Commented [indra2]: clearly provide the treatments
you did not address the comment. Clearly name the treatments: P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

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The provision of feed is considered necessary in fish farming, including the addition of feed additives to spur growth and production. Phytobiotics is one type of natural feed additive derived from plants (Vidanarachchi et al 2005). The purpose of adding natural feed additives to the feed is to spur growth, as well as improve the health and productivity of fish (Citarasu 2001; Citarasu et al 1998, 1999, 2002; Sivaram et al 2004). Noni fruit can be used as phytobiotic (feed additive) because it has antibacterial effects and bioactive substances such as alkaloids, flavonoids, and tannins which are very effective in suppressing the growth of pathogenic bacteria and improving intestinal morphological characteristics (Murdiati & Adiwinatai 2000). Bioactive substances in noni include xeronine and precursor of xeronine (proxeronine). Proxeronine will be converted to xeronine in the intestine by proxeronase enzymes and other substances. Xeronine increases the permeability of cell membranes, facilitates the transfer of peptides and increases nutrient absorption, as well as effectuates amino acids, vitamins, and minerals. Xeronine can activate several enzymes and regulate protein synthesis (Solomon 2001). Therefore, noni fruit extract can be used as an additional ingredient in feed to help the digestion process of fish, so that the consumed feed can be optimally utilized for growth and feed efficiency, as well as the protein content of fish. This study aims to determine the potential of given noni fruit (*Morinda citrifolia*) extract to the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

Material and Method

Preparation of red tilapia (Oreochromis niloticus) and rearing conditions. This research was conducted from April to May 2017 at the Education Laboratory of the Airlangga University, Faculty of Fisheries and Marine, Surabaya, Indonesia. The proximate test of feed and protein retention test were conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The instruments used in this study were an aquarium measuring 45 cm x 30 cm x 30 cm, a set of aerators, digital scales, pH pens, thermometers, rulers, plastic bags, plastic hoses with a diameter of 1 cm, dissolved oxygen (DO) meters, and ammonia kits. The fish used were 140 red tilapia fry in healthy condition, measuring 5-6 cm total length, weighing 2-5 g head^{-1}. The red tilapia fry were obtained from the Gunung Sari fish market, Surabaya, East Java. Noni fruit extract was obtained from the result of squeezed noni fruit. The noni fruit used was ripe noni fruit, which is yellow and soft with a distinctive smell.

Experimental design. This research method was experimental. The primary test parameters in this study were protein retention, specific growth rate, feed conversion ratio, and survival rate of red tilapia. The variables observed were independent variables, dependent variables, and control variables. The independent variable is the dose of noni extract. The dependent variables are specific growth rate, feed conversion ratio, survival rate, and protein retention. Control variables are water quality (temperature, pH, DO, and ammonia). This study used a Completely Randomized Design (CRD) with four treatments and four replications, which treatments explained as follows:
P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

The feed given in this study was PF 1000 commercial feed from PT Matahari Sakti, with a protein content of 39-41%, 5% lipid, 6% crude fiber, 16% ash, and 10% moisture content.

Preparation of the experimental diets. First, ripe noni fruits were cleaned with water, then washed with warm water. The noni fruits were crushed and then squeezed by using strain cloth. The squeezed noni fruit extract was put in a bottle and stored at room temperature.

The commercial feed was mixed with noni fruit extract and 2 grams of Progol with a different amount in each treatment, as previously explained. The commercial feed and noni fruit extract were smoothly mixed. The mixed feed was dried in an open room and was aerated until dry. After that, the dried food was stored in a plastic jar. The mixed fish feed with different doses were then analyzed through the proximate test in the laboratory. The nutrient content of the feed used in this study can be seen in Table 1 as follows.

Table 1

Nutrient content of feed with various doses of noni fruit extract

Analysis	*P0*	*P1*	*P2*	*P3*
	(Control)	*(100 mL kg^{-1})*	*(300 mL kg^{-1})*	*(500 mL kg^{-1})*
Dry ingredients (%)	90.1441	87.3484	86.7339	84.5036
Ash (%)	8.9591	8.7352	8.6875	8.5261
Crude protein (%)	29.1112	30.9015	30.2753	29.2539
Crude lipid (%)	8.3165	7.6176	6.9983	6.872
Crude fiber (%)	3.5026	2.488	2.6143	2.2593
Ca	2.5824	2.4109	2.4069	2.1481
Nitrogen-free extract (%)	40.2547	37.6061	38.1585	37.5923
Metabolism energy (Kcal kg^{-1})	3057.9622	2967.4828	2922.6623	2857.8501

The analytical procedure of the diets. The aquarium was then filled with 30 liters of water, aerated, and then left for 24 hours. The fish were selected based on a similar length and weight and its healthy condition. The fish were acclimatized for one week and were fasted for 24 hours before being treated. The fish were placed in fourteen aquariums according to the treatment given with a stocking density of 10 fish per aquarium. The fish were kept for thirty days and fed according to the treatment doses. Feeding was given three times a day: morning, afternoon, and evening, with a dose of 3% of body weight.

Below is the protein retention (PR) calculation based on a formula from Tung & Shiau (1991):

$$PR = \frac{Ln\ final\ body\ weight - Ln\ initial\ body\ weight\ (g)}{Total\ protein\ intake\ (g)} \times 100$$

The specific growth rate was calculated based on a formula from Effendie (1997):

$$SGR = \frac{LnWt - LnWo}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W0 = initial body weight (g);
Wt = final body weight (g);
T = treatment time (in days).

The daily length growth rate (LGR) was calculated based on a formula from Aggraeni & Abdulgani 2013):

$$LGR = \frac{LnLt - LnLo}{t} \times 100$$

where: LGR = daily length growth rate (% day^{-1});
Lt = average total length on day t (cm);
L0 = average total length on day 0 (cm);
t = total of observation time (in days).

The survival rate was calculated based on a formula from Wirabakti (2006):

$$SR = \frac{Nt}{No} \times 100$$

where: SR = survival rate (%);
Nt = the number of survived fish at the end of the experiment (in fish);
No = the number of survived fish at the start of the experiment (in fish).

The feed conversion ratio was calculated based on a formula from Council (1997):

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
Wo = the biomass weight of fish at the start of the experiment (g);
Wt = the biomass weight of fish at the end of the experiment (g);
D = total weight of dead fish (g);
F = feed intake (g).

Statistical analysis. Data analysis using Variant Analysis (ANOVA) with the study design was a Completely Randomized Design (CRD) to determine the differences in treatment. If there is a difference in treatment, Duncan's Multiple Range Test is applied.

Results. The calculation results of the average value of the specific growth rate, feed conversion ratio, and survival rate of red tilapia using statistical analysis after being treated for 30 days can be seen in Table 2.

Table 2

Average value of the specific growth rate, survival rate, feed conversion ratio, daily length growth, and protein retention of red tilapia

Treatment	*Specific growth rate (% day^{-1})*	*Survival rate (%)*	*Feed conversion ratio (%)*	*Daily length growth rate (% day^{-1})*	*Protein retention (%)*
P0	0.0105±0.000 7^{c}	100	7.712±0.588^{b}	1.070±0.189^{b}	7.3345±0.3434^{c}
P1	0.0135±0.0006^{b}	100	6.157±0.226^{a}	1.145±0.050^{b}	12.6246±0.3074^{b}
P2	0.0147±0.0005^{a}	100	5.846±0.234^{a}	1.435±0.186^{a}	11.5692±0.4054^{a}
P3	0.0150±0.0009^{a}	100	5.918±0.436^{a}	1.599±1.312^{a}	11.4289±0.7356^{a}

P0 (0% noni fruit extract), P1 (100 mL kg^{-1} noni fruit extract), P2 (300 mL kg^{-1} commercial feed with noni fruit extract), P3 (500 mL kg^{-1} commercial feed with noni fruit extract). Different superscripts show significant differences.

According to Table 2, the value of the specific growth rate (SGR) of red tilapia was 0.0105-0.0150% day^{-1}. The results of the statistical analysis of added noni fruit extract on commercial feed to the SGR of red tilapia showed significantly different results ($p < 0.05$). Duncan's Multiple Range Test (DMTR) results showed that the highest growth rate in this study was P3 (0.0150% day^{-1}) which was not significantly different from P2 (0.0147% day^{-1}), while it was significantly different with P0 (0.0105% day^{-1}) and P1 (0.0135% day^{-1}). The lowest specific growth rate was P0 (0.0105% day^{-1}) which was significantly different from P1 (0.0135% day^{-1}), P2 (0.147% day^{-1}), and P3 (0.0150% day^{-1}).

The feed conversion ratio (FCR) of red tilapia was 5.846-7.712%. The results of statistical analysis of the added noni fruit extract to commercial feed to FCR of red tilapia showed significantly different results ($p < 0.05$). Based on the results of DMTR, the highest FCR in this study was P0 (7.712%) which was significantly different from P1 (6.157%), P2 (5.846%), and P3 (5.9188%). While the lowest feed conversion ratio was P2 (5.846%) and was not significantly different from P1 (6.157%), P3 (5.918%), and significantly different from P0 (7.712%). These results indicate that the addition of noni fruit extract to commercial feed can reduce feed conversion ratio of red tilapia.

The survival rate of red tilapia from all treatments was 100%. The average survival rate of red tilapia showed no significant difference ($p > 0.05$). Based on the results of DMTR, the survival rates of P0, P1, P2, and P3 were not significantly different.

The value of the daily length growth rate of red tilapia for thirty days of preservation was 1.070-1.599% day^{-1}. The results showed significant differences ($p < 0.05$). The highest daily length growth value was at P3 (1.599% day^{-1}) which was not

significantly different from P2 (1.435% day^{-1}) but was significantly different from P0 (1.070% day^{-1}) and P1 (1.145% day^{-1}). The lowest daily length growth rate was P0 (1.070% day^{-1}) which was not significantly different from P1 (1.145% day^{-1}) but significantly different from P2 (1.435% day^{-1}) and P3 (1.599% day^{-1}).

The results of the statistical analysis of the addition of noni fruit extract to commercial feed showed significantly different results ($p < 0.05$) to the protein retention of red tilapia. The results of DMTR showed that the highest protein retention value was P1 (12.6246%) which was significantly different from P0 (7.3345%), P2 (11.5692%), and P3 (11.4289%). Protein retention value in P2 (11.5692%) was not significantly different from P3 (11.4289%) but was significantly different from P0 (7.3345%) and P1 (12.6246%). The lowest protein retention value was P0 (7.3345%), which was significantly different from P1 (12.6246%), P2 (11.5692%), and P3 (11.4289%).

Data on the range of water quality parameters for red tilapia preservation for thirty days can be seen in Table 3.

Table 3

Water quality range value of red tilapia in 30 days of preservation

Parameter	*Range*
Temperature (°C)	27.4-29.2
pH	7.2-7.9
DO (mg L^{-1})	3.3-5
Ammonia (mg L^{-1})	0-0.5

Discussion. The noni fruit extract mixed in the commercial feed had a significantly different effect on specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia for thirty days of preservation. These effects are due to the optimum absorption process or nutrient absorption of the feed in the digestive tract. Noni extract contains anthraquinone or acidic ingredients that can affect the pH of the digestive tract to be more acidic (Widianto et al 2015). The statement is right considering that in an acidic environment, protein-breaking enzymes (pepsin) can work optimally, so that protein intakes are absorbed more by the body which supports the optimal fish growth rate (Esminger et al 1990).

The extract of noni fruit contains proxeronine and xeronine. Proxeronine is converted to xeronine with the help of proxeronase enzymes. Xeronine is an active compound that has a role in facilitating metabolism inside the body of the animals and increasing nutrient absorption (Solomon 2001). Xeronine in noni fruit can function to expand the absorption in the intestine surface and facilitate the absorption process of food so the nutrients in the feed can be absorbed optimally (Wang et al 2008). The optimal absorption process will affect increased protein retention (Schlegel 1987; Frazier & Westhoff 1988; Cui et al 1992; Solomon 2001; Sjofjan 2003; Sujono 2006; Widianto et al 2015). Increased protein retention in the treatment results was caused by adding noni fruit extract in commercial feed, which can suppress the growth of microbial pathogens and increase non-pathogenic microbes in the digestive tract, resulting in the ability to decompose feed ingredients, especially crude fiber. The use of noni fruit extract can inhibit the growth of pathogenic bacteria in the intestine so that the number of non-pathogenic bacteria in the intestine was increased (Solomon 2001). Noni extract can increase the absorption process in the digestive tract and help decompose feed ingredients, especially crude fiber (Widianto et al 2015). Non-pathogenic bacteria can produce enzymes that digest crude fiber, protein, and lipid (Sjofjan 2003). *Bacillus* sp. bacteria in the digestive tract can break down proteins into amino acids (Sujono 2006). These amino acids are used by bacteria to multiply themselves. Bacteria are a source of single-cell proteins so that the bacteria multiplication can increase protein feed and reduce crude fiber (Schlegel 1987).

Moreover, *Bacillus* sp. is a pectinolytic bacteria, which is an organism that can produce pectin or complex carbohydrates (Frazier & Westhoff 1988). The characteristics of *Bacillus* sp. bacteria are proteolytic, saccharolytic, and pectinolytic, which increases

protein and carbohydrate feed. Protein retention indicates the amount of additional body protein from the consumed feed protein. The feed consumed was a source of protein used for preservation, metabolic activity, and growth (Cui et al 1992). The high protein retention in P1 treatment was caused by the increase in protein and carbohydrate content by the activity of non-pathogenic microbes that increase due to the addition of noni fruit extract in the feed. The only issue was the more noni fruit extract dosage in the feed cannot help at breaking down the crude fiber and result in higher crude fiber amount in the feed.

The feed conversion ratio is one of the standards to determine the quality of feed given to livestock in fulfilling the required nutrition. The higher the value of feed conversion, the worse the quality of the nutritional value of the feed. Noni fruit contains saponins which are bioactive in the growth of animals and digestive microbes. Giving saponins can increase the permeability of cell walls in the intestine, increase absorption of food substances hence result in higher conversion value (Önning et al 1996).

Survival rates of red tilapia were not significantly different between treatments. Thirty days preservation of red tilapia with the treatments given did not cause death in fish. This proves that adding noni fruit extract in commercial feed did not contain toxic ingredients that cause death. One study using noni fruit extract with a dose of 15,000 mg per kg body weight in experimental animals was not found any sign of toxic effects (Krishnakumar et al 2015). The study had performed surgery on experimental animals but still did not find any signs of toxicity in the animals' organs. Noni fruit is categorized as non-toxic substances (Wang et al 2008; Younos et al 1990).

Water quality in this study is an essential supporting parameter for the survival rate of red tilapia. Water as a living medium for fish that is preserved must meet the requirements. Water quality needs to be controlled in order to maintain its optimal conditions, thus creating an environment that is suitable for the habitat of red tilapia. The supporting parameters of this study are water quality parameters such as temperature, DO, pH, and ammonia. The temperature in this study averaged around 27.4-29.2^{o}C. This temperature is good enough for the survival of red tilapia. The optimal water temperature for red tilapia is 25-30^{o}C (Kungvakij & Pudadera 1984). Temperature is an environmental factor that influences the body metabolism speed. The optimum speed metabolism for fish will be achieved at optimal temperatures. The pH value has an essential influence on the survival of fish in the water. The pH in this study ranged from 7.2 to 7.9, which is quite adequate for the survival rate of red tilapia. The pH range required by fish is 6-9 (DeLong et al 2009). The average DO content in this study ranged from 3.3 to 5 mg L^{-1}. Based on these results, the DO during the study indicated a suitable level for the survival of red tilapia (Yildiz et al 2017). Thus DO in the preservation of red tilapia met the requirements. Ammonia in the water is derived from fish feces and from the rest of the feed that is not consumed by fish. In general, the concentration of ammonia contained in the water must not exceed 1 mg L^{-1} (BFAR-NFFTC 2000; Indian Council of Agricultural Research 2006). Ammonia during this study was 0-0.5 mg L^{-1}, which is suitable for the preservation of red tilapia.

Conclusions. Based on this research, it can be concluded that the addition of noni fruit extract in commercial feed can increase specific growth rate, daily length growth rate, lower the feed conversion ratio, and increase protein retention of red tilapia (*Oreochromis niloticus*). Moreover, the addition of noni fruit extract in commercial feed did not show any toxic effect on the survival rate of red tilapia during the thirty days of preservation.

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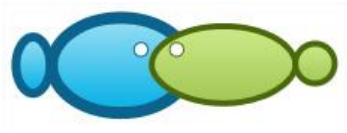

The potential of *Morinda citrifolia* in commercial feed on the growth and protein of *Oreochromis niloticus*

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Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract to the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments of noni fruit extract dosages given to red tilapia. The treatments used were the dosage amount of noni extraction given to red tilapia. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 5.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).
Key Words: fish meal substitution, noni fruit (*Morinda citrifolia*) extract, red tilapia (*Oreochromis niloticus*).

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand for consumption and cultivation. The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the community, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. Feed is an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu 2016). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most significant amount of production costs component (Shepherd & Jackson 2013). One way to overcome this problem is to use feed additives. Feed additives in fish farming, especially red tilapia, are ingredients that are intentionally used to increase appetite, digestibility, endurrance, reduce stress levels, and spur fish growth (Alemayehu et al 2018).

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P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

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The provision of feed is considered necessary in fish farming, including the addition of feed additives to spur growth and production. Phytobiotics is one type of natural feed additive derived from plants (Vidanarachchi et al 2005). The purpose of adding natural feed additives to the feed is to spur growth, as well as improve the health and productivity of fish (Citarasu 2001; Citarasu et al 1998, 1999, 2002; Sivaram et al 2004). Noni fruit can be used as phytobiotic (feed additive) because it has antibacterial effects and bioactive substances such as alkaloids, flavonoids, and tannins which are very effective in suppressing the growth of pathogenic bacteria and improving intestinal morphological characteristics (Murdiati & Adiwinatai 2000). Bioactive substances in noni include xeronine and precursor of xeronine (proxeronine). Proxeronine will be converted to xeronine in the intestine by proxeronase enzymes and other substances. Xeronine increases the permeability of cell membranes, facilitates the transfer of peptides and increases nutrient absorption, as well as effectuates amino acids, vitamins, and minerals. Xeronine can activate several enzymes and regulate protein synthesis (Solomon 2001). Therefore, noni fruit extract can be used as an additional ingredient in feed to help the digestion process of fish, so that the consumed feed can be optimally utilized for growth and feed efficiency, as well as the protein content of fish. This study aims to determine the potential of given noni fruit (*Morinda citrifolia*) extract to the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

Material and Method

Preparation of red tilapia (Oreochromis niloticus) and rearing conditions. This research was conducted from April to May 2017 at the Education Laboratory of the Airlangga University, Faculty of Fisheries and Marine, Surabaya, Indonesia. The proximate test of feed and protein retention test were conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The instruments used in this study were an aquarium measuring 45 cm x 30 cm x 30 cm, a set of aerators, digital scales, pH pens, thermometers, rulers, plastic bags, plastic hoses with a diameter of 1 cm, dissolved oxygen (DO) meters, and ammonia kits. The fish used were 140 red tilapia fry in healthy condition, measuring 5-6 cm total length, weighing 2-5 g head^{-1}. The red tilapia fry were obtained from the Gunung Sari fish market, Surabaya, East Java. Noni fruit extract was obtained from the result of squeezed noni fruit. The noni fruit used was ripe noni fruit, which is yellow and soft with a distinctive smell.

Experimental design. This research method was experimental. The primary test parameters in this study were protein retention, specific growth rate, feed conversion ratio, and survival rate of red tilapia. The variables observed were independent variables, dependent variables, and control variables. The independent variable is the dose of noni extract. The dependent variables are specific growth rate, feed conversion ratio, survival rate, and protein retention. Control variables are water quality (temperature, pH, DO, and ammonia). This study used a Completely Randomized Design (CRD) with four treatments and four replications, which treatments explained as follows:
P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

The feed given in this study was PF 1000 commercial feed from PT Matahari Sakti, with a protein content of 39-41%, 5% lipid, 6% crude fiber, 16% ash, and 10% moisture content.

Preparation of the experimental diets. First, ripe noni fruits were cleaned with water, then washed with warm water. The noni fruits were crushed and then squeezed by using strain cloth. The squeezed noni fruit extract was put in a bottle and stored at room temperature.

The commercial feed was mixed with noni fruit extract and 2 grams of Progol with a different amount in each treatment, as previously explained. The commercial feed and noni fruit extract were smoothly mixed. The mixed feed was dried in an open room and was aerated until dry. After that, the dried food was stored in a plastic jar. The mixed fish feed with different doses were then analyzed through the proximate test in the laboratory. The nutrient content of the feed used in this study can be seen in Table 1 as follows.

Table 1

Nutrient content of feed with various doses of noni fruit extract

Analysis	*P0*	*P1*	*P2*	*P3*
	(Control)	*(100 mL kg^{-1})*	*(300 mL kg^{-1})*	*(500 mL kg^{-1})*
Dry ingredients (%)	90.1441	87.3484	86.7339	84.5036
Ash (%)	8.9591	8.7352	8.6875	8.5261
Crude protein (%)	29.1112	30.9015	30.2753	29.2539
Crude lipid (%)	8.3165	7.6176	6.9983	6.872
Crude fiber (%)	3.5026	2.488	2.6143	2.2593
Ca	2.5824	2.4109	2.4069	2.1481
Nitrogen-free extract (%)	40.2547	37.6061	38.1585	37.5923
Metabolism energy (Kcal kg^{-1})	3057.9622	2967.4828	2922.6623	2857.8501

The analytical procedure of the diets. The aquarium was then filled with 30 liters of water, aerated, and then left for 24 hours. The fish were selected based on a similar length and weight and its healthy condition. The fish were acclimatized for one week and were fasted for 24 hours before being treated. The fish were placed in fourteen aquariums according to the treatment given with a stocking density of 10 fish per aquarium. The fish were kept for thirty days and fed according to the treatment doses. Feeding was given three times a day: morning, afternoon, and evening, with a dose of 3% of body weight.

Below is the protein retention (PR) calculation based on a formula from Tung & Shiau (1991):

$$PR = \frac{Ln\ final\ body\ weight - Ln\ initial\ body\ weight\ (g)}{Total\ protein\ intake\ (g)} \times 100$$

The specific growth rate was calculated based on a formula from Effendie (1997):

$$SGR = \frac{LnWt - LnWo}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W0 = initial body weight (g);
Wt = final body weight (g);
T = treatment time (in days).

The daily length growth rate (LGR) was calculated based on a formula from Aggraeni & Abdulgani 2013):

$$LGR = \frac{LnLt - LnLo}{t} \times 100$$

where: LGR = daily length growth rate (% day^{-1});
Lt = average total length on day t (cm);
L0 = average total length on day 0 (cm);
t = total of observation time (in days).

The survival rate was calculated based on a formula from Wirabakti (2006):

$$SR = \frac{Nt}{No} \times 100$$

where: SR = survival rate (%);
Nt = the number of survived fish at the end of the experiment (in fish);
No = the number of survived fish at the start of the experiment (in fish).

The feed conversion ratio was calculated based on a formula from Council (1997):

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
Wo = the biomass weight of fish at the start of the experiment (g);
Wt = the biomass weight of fish at the end of the experiment (g);
D = total weight of dead fish (g);
F = feed intake (g).

Statistical analysis. Data analysis using Variant Analysis (ANOVA) with the study design was a Completely Randomized Design (CRD) to determine the differences in treatment. If there is a difference in treatment, Duncan's Multiple Range Test is applied.

Results. The calculation results of the average value of the specific growth rate, feed conversion ratio, and survival rate of red tilapia using statistical analysis after being treated for 30 days can be seen in Table 2.

Table 2

Average value of the specific growth rate, survival rate, feed conversion ratio, daily length growth, and protein retention of red tilapia

Treatment	*Specific growth rate (% day^{-1})*	*Survival rate (%)*	*Feed conversion ratio (%)*	*Daily length growth rate (% day^{-1})*	*Protein retention (%)*
P0	0.0105±0.000 7^{c}	100	7.712±0.588^{b}	1.070±0.189^{b}	7.3345±0.3434^{c}
P1	0.0135±0.0006^{b}	100	6.157±0.226^{a}	1.145±0.050^{b}	12.6246±0.3074^{b}
P2	0.0147±0.0005^{a}	100	5.846±0.234^{a}	1.435±0.186^{a}	11.5692±0.4054^{a}
P3	0.0150±0.0009^{a}	100	5.918±0.436^{a}	1.599±1.312^{a}	11.4289±0.7356^{a}

P0 (0% noni fruit extract), P1 (100 mL kg^{-1} noni fruit extract), P2 (300 mL kg^{-1} commercial feed with noni fruit extract), P3 (500 mL kg^{-1} commercial feed with noni fruit extract). Different superscripts show significant differences.

According to Table 2, the value of the specific growth rate (SGR) of red tilapia was 0.0105-0.0150% day^{-1}. The results of the statistical analysis of added noni fruit extract on commercial feed to the SGR of red tilapia showed significantly different results ($p < 0.05$). Duncan's Multiple Range Test (DMTR) results showed that the highest growth rate in this study was P3 (0.0150% day^{-1}) which was not significantly different from P2 (0.0147% day^{-1}), while it was significantly different with P0 (0.0105% day^{-1}) and P1 (0.0135% day^{-1}). The lowest specific growth rate was P0 (0.0105% day^{-1}) which was significantly different from P1 (0.0135% day^{-1}), P2 (0.147% day^{-1}), and P3 (0.0150% day^{-1}).

The feed conversion ratio (FCR) of red tilapia was 5.846-7.712%. The results of statistical analysis of the added noni fruit extract to commercial feed to FCR of red tilapia showed significantly different results ($p < 0.05$). Based on the results of DMTR, the highest FCR in this study was P0 (7.712%) which was significantly different from P1 (6.157%), P2 (5.846%), and P3 (5.9188%). While the lowest feed conversion ratio was P2 (5.846%) and was not significantly different from P1 (6.157%), P3 (5.918%), and significantly different from P0 (7.712%). These results indicate that the addition of noni fruit extract to commercial feed can reduce feed conversion ratio of red tilapia.

The survival rate of red tilapia from all treatments was 100%. The average survival rate of red tilapia showed no significant difference ($p > 0.05$). Based on the results of DMTR, the survival rates of P0, P1, P2, and P3 were not significantly different.

The value of the daily length growth rate of red tilapia for thirty days of preservation was 1.070-1.599% day^{-1}. The results showed significant differences ($p < 0.05$). The highest daily length growth value was at P3 (1.599% day^{-1}) which was not

significantly different from P2 (1.435% day^{-1}) but was significantly different from P0 (1.070% day^{-1}) and P1 (1.145% day^{-1}). The lowest daily length growth rate was P0 (1.070% day^{-1}) which was not significantly different from P1 (1.145% day^{-1}) but significantly different from P2 (1.435% day^{-1}) and P3 (1.599% day^{-1}).

The results of the statistical analysis of the addition of noni fruit extract to commercial feed showed significantly different results ($p < 0.05$) to the protein retention of red tilapia. The results of DMTR showed that the highest protein retention value was P1 (12.6246%) which was significantly different from P0 (7.3345%), P2 (11.5692%), and P3 (11.4289%). Protein retention value in P2 (11.5692%) was not significantly different from P3 (11.4289%) but was significantly different from P0 (7.3345%) and P1 (12.6246%). The lowest protein retention value was P0 (7.3345%), which was significantly different from P1 (12.6246%), P2 (11.5692%), and P3 (11.4289%).

Data on the range of water quality parameters for red tilapia preservation for thirty days can be seen in Table 3.

Table 3

Water quality range value of red tilapia in 30 days of preservation

Parameter	*Range*
Temperature (°C)	27.4-29.2
pH	7.2-7.9
DO (mg L^{-1})	3.3-5
Ammonia (mg L^{-1})	0-0.5

Discussion. The noni fruit extract mixed in the commercial feed had a significantly different effect on specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia for thirty days of preservation. These effects are due to the optimum absorption process or nutrient absorption of the feed in the digestive tract. Noni extract contains anthraquinone or acidic ingredients that can affect the pH of the digestive tract to be more acidic (Widianto et al 2015). The statement is right considering that in an acidic environment, protein-breaking enzymes (pepsin) can work optimally, so that protein intakes are absorbed more by the body which supports the optimal fish growth rate (Esminger et al 1990).

The extract of noni fruit contains proxeronine and xeronine. Proxeronine is converted to xeronine with the help of proxeronase enzymes. Xeronine is an active compound that has a role in facilitating metabolism inside the body of the animals and increasing nutrient absorption (Solomon 2001). Xeronine in noni fruit can function to expand the absorption in the intestine surface and facilitate the absorption process of food so the nutrients in the feed can be absorbed optimally (Wang et al 2008). The optimal absorption process will affect increased protein retention (Schlegel 1987; Frazier & Westhoff 1988; Cui et al 1992; Solomon 2001; Sjofjan 2003; Sujono 2006; Widianto et al 2015). Increased protein retention in the treatment results was caused by adding noni fruit extract in commercial feed, which can suppress the growth of microbial pathogens and increase non-pathogenic microbes in the digestive tract, resulting in the ability to decompose feed ingredients, especially crude fiber. The use of noni fruit extract can inhibit the growth of pathogenic bacteria in the intestine so that the number of non-pathogenic bacteria in the intestine was increased (Solomon 2001). Noni extract can increase the absorption process in the digestive tract and help decompose feed ingredients, especially crude fiber (Widianto et al 2015). Non-pathogenic bacteria can produce enzymes that digest crude fiber, protein, and lipid (Sjofjan 2003). *Bacillus* sp. bacteria in the digestive tract can break down proteins into amino acids (Sujono 2006). These amino acids are used by bacteria to multiply themselves. Bacteria are a source of single-cell proteins so that the bacteria multiplication can increase protein feed and reduce crude fiber (Schlegel 1987).

Moreover, *Bacillus* sp. is a pectinolytic bacteria, which is an organism that can produce pectin or complex carbohydrates (Frazier & Westhoff 1988). The characteristics of *Bacillus* sp. bacteria are proteolytic, saccharolytic, and pectinolytic, which increases

protein and carbohydrate feed. Protein retention indicates the amount of additional body protein from the consumed feed protein. The feed consumed was a source of protein used for preservation, metabolic activity, and growth (Cui et al 1992). The high protein retention in P1 treatment was caused by the increase in protein and carbohydrate content by the activity of non-pathogenic microbes that increase due to the addition of noni fruit extract in the feed. The only issue was the more noni fruit extract dosage in the feed cannot help at breaking down the crude fiber and result in higher crude fiber amount in the feed.

The feed conversion ratio is one of the standards to determine the quality of feed given to livestock in fulfilling the required nutrition. The higher the value of feed conversion, the worse the quality of the nutritional value of the feed. Noni fruit contains saponins which are bioactive in the growth of animals and digestive microbes. Giving saponins can increase the permeability of cell walls in the intestine, increase absorption of food substances hence result in higher conversion value (Önning et al 1996).

Survival rates of red tilapia were not significantly different between treatments. Thirty days preservation of red tilapia with the treatments given did not cause death in fish. This proves that adding noni fruit extract in commercial feed did not contain toxic ingredients that cause death. One study using noni fruit extract with a dose of 15,000 mg per kg body weight in experimental animals was not found any sign of toxic effects (Krishnakumar et al 2015). The study had performed surgery on experimental animals but still did not find any signs of toxicity in the animals' organs. Noni fruit is categorized as non-toxic substances (Wang et al 2008; Younos et al 1990).

Water quality in this study is an essential supporting parameter for the survival rate of red tilapia. Water as a living medium for fish that is preserved must meet the requirements. Water quality needs to be controlled in order to maintain its optimal conditions, thus creating an environment that is suitable for the habitat of red tilapia. The supporting parameters of this study are water quality parameters such as temperature, DO, pH, and ammonia. The temperature in this study averaged around 27.4-29.2^{o}C. This temperature is good enough for the survival of red tilapia. The optimal water temperature for red tilapia is 25-30^{o}C (Kungvakij & Pudadera 1984). Temperature is an environmental factor that influences the body metabolism speed. The optimum speed metabolism for fish will be achieved at optimal temperatures. The pH value has an essential influence on the survival of fish in the water. The pH in this study ranged from 7.2 to 7.9, which is quite adequate for the survival rate of red tilapia. The pH range required by fish is 6-9 (DeLong et al 2009). The average DO content in this study ranged from 3.3 to 5 mg L^{-1}. Based on these results, the DO during the study indicated a suitable level for the survival of red tilapia (Yildiz et al 2017). Thus DO in the preservation of red tilapia met the requirements. Ammonia in the water is derived from fish feces and from the rest of the feed that is not consumed by fish. In general, the concentration of ammonia contained in the water must not exceed 1 mg L^{-1} (BFAR-NFFTC 2000; Indian Council of Agricultural Research 2006). Ammonia during this study was 0-0.5 mg L^{-1}, which is suitable for the preservation of red tilapia.

Conclusions. Based on this research, it can be concluded that the addition of noni fruit extract in commercial feed can increase specific growth rate, daily length growth rate, lower the feed conversion ratio, and increase protein retention of red tilapia (*Oreochromis niloticus*). Moreover, the addition of noni fruit extract in commercial feed did not show any toxic effect on the survival rate of red tilapia during the thirty days of preservation.

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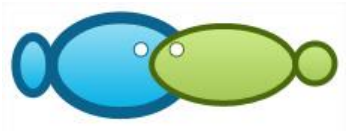

Increasing growth performances of Nile tilapia (*Oreochromis niloticus*) by supplementation of noni *Morinda citrifolia* fruit extract via diet ~~The potential of *Morinda citrifolia* in commercial feed on the growth and protein of~~

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Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract to the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments of noni fruit extract dosages given to red tilapia. The treatments used were the dosage amount of noni extraction given to red tilapia, namely P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder); P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol; P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol; P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 5.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).
Key Words: fish meal substitution, noni fruit (*Morinda citrifolia*) extract, red tilapia (*Oreochromis niloticus*).

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand for consumption and cultivation. The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the community, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. Feed is an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu

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P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

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2016). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most significant amount of production costs component (Shepherd & Jackson 2013). One way to overcome this problem is to use feed additives. Feed additives in fish farming, especially red tilapia, are ingredients that are intentionally used to increase appetite, digestibility, endurrance, reduce stress levels, and spur fish growth (Alemayehu et al 2018).

The provision of feed is considered necessary in fish farming, including the addition of feed additives to spur growth and production. Phytobiotics is one type of natural feed additive derived from plants (Vidanarachchi et al 2005). The purpose of adding natural feed additives to the feed is to spur growth, as well as improve the health and productivity of fish (Citarasu 2001; Citarasu et al 1998, 1999, 2002; Sivaram et al 2004). Noni fruit can be used as phytobiotic (feed additive) because it has antibacterial effects and bioactive substances such as alkaloids, flavonoids, and tannins which are very effective in suppressing the growth of pathogenic bacteria and improving intestinal morphological characteristics (Murdiati & Adiwinatai 2000). Bioactive substances in noni include xeronine and precursor of xeronine (proxeronine). Proxeronine will be converted to xeronine in the intestine by proxeronase enzymes and other substances. Xeronine increases the permeability of cell membranes, facilitates the transfer of peptides and increases nutrient absorption, as well as effectuates amino acids, vitamins, and minerals. Xeronine can activate several enzymes and regulate protein synthesis (Solomon 2001). Therefore, noni fruit extract can be used as an additional ingredient in feed to help the digestion process of fish, so that the consumed feed can be optimally utilized for growth and feed efficiency, as well as the protein content of fish. This study aims to determine the potential of given noni fruit (*Morinda citrifolia*) extract to the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

Material and Method

Preparation of red tilapia (Oreochromis niloticus) and rearing conditions. This research was conducted from April to May 2017 at the Education Laboratory of the Airlangga University, Faculty of Fisheries and Marine, Surabaya, Indonesia. The proximate test of feed and protein retention test were conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The instruments used in this study were an aquarium measuring 45 cm x 30 cm x 30 cm, a set of aerators, digital scales, pH pens, thermometers, rulers, plastic bags, plastic hoses with a diameter of 1 cm, dissolved oxygen (DO) meters, and ammonia kits. The fish used were 140 red tilapia fry in healthy condition, measuring 5-6 cm total length, weighing 2-5 g head^{-1}. The red tilapia fry were obtained from the Gunung Sari fish market, Surabaya, East Java. Noni fruit extract was obtained from the result of squeezed noni fruit. The noni fruit used was ripe noni fruit, which is yellow and soft with a distinctive smell.

Experimental design. This research method was experimental. The primary test parameters in this study were protein retention, specific growth rate, feed conversion ratio, and survival rate of red tilapia. The variables observed were independent variables, dependent variables, and control variables. The independent variable is the dose of noni extract. The dependent variables are specific growth rate, feed conversion ratio, survival rate, and protein retention. Control variables are water quality (temperature, pH, DO, and ammonia). This study used a Completely Randomized Design (CRD) with four treatments and four replications, which treatments explained as follows:
P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

The feed given in this study was PF 1000 commercial feed from PT Matahari Sakti, with a protein content of 39-41%, 5% lipid, 6% crude fiber, 16% ash, and 10% moisture content.

Preparation of the experimental diets. First, ripe noni fruits were cleaned with water, then washed with warm water. The noni fruits were crushed and then squeezed by using strain cloth. The squeezed noni fruit extract was put in a bottle and stored at room temperature.

The commercial feed was mixed with noni fruit extract and 2 grams of Progol with a different amount in each treatment, as previously explained. The commercial feed and noni fruit extract were smoothly mixed. The mixed feed was dried in an open room and was aerated until dry. After that, the dried food was stored in a plastic jar. The mixed fish feed with different doses were then analyzed through the proximate test in the laboratory. The nutrient content of the feed used in this study can be seen in Table 1 as follows.

Table 1

Nutrient content of feed with various doses of noni fruit extract

Analysis	*P0*	*P1*	*P2*	*P3*
	(Control)	*(100 mL kg^{-1})*	*(300 mL kg^{-1})*	*(500 mL kg^{-1})*
Dry ingredients (%)	90.1441	87.3484	86.7339	84.5036
Ash (%)	8.9591	8.7352	8.6875	8.5261
Crude protein (%)	29.1112	30.9015	30.2753	29.2539
Crude lipid (%)	8.3165	7.6176	6.9983	6.872
Crude fiber (%)	3.5026	2.488	2.6143	2.2593
Ca	2.5824	2.4109	2.4069	2.1481
Nitrogen-free extract (%)	40.2547	37.6061	38.1585	37.5923
Metabolism energy (Kcal kg^{-1})	3057.9622	2967.4828	2922.6623	2857.8501

The analytical procedure of the diets. The aquarium was then filled with 30 liters of water, aerated, and then left for 24 hours. The fish were selected based on a similar length and weight and its healthy condition. The fish were acclimatized for one week and were fasted for 24 hours before being treated. The fish were placed in fourteen aquariums according to the treatment given with a stocking density of 10 fish per aquarium. The fish were kept for thirty days and fed according to the treatment doses. Feeding was given three times a day: morning, afternoon, and evening, with a dose of 3% of body weight.

Below is the protein retention (PR) calculation based on a formula from Tung & Shiau (1991):

$$PR = \frac{Ln\ final\ body\ weight - Ln\ initial\ body\ weight\ (g)}{Total\ protein\ intake\ (g)} \times 100$$

The specific growth rate was calculated based on a formula from Effendie (1997):

$$SGR = \frac{LnWt - LnWo}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W0 = initial body weight (g);
Wt = final body weight (g);
T = treatment time (in days).

The daily length growth rate (LGR) was calculated based on a formula from Aggraeni & Abdulgani 2013):

$$LGR = \frac{LnLt - LnLo}{t} \times 100$$

where: LGR = daily length growth rate (% day^{-1});
Lt = average total length on day t (cm);
L0 = average total length on day 0 (cm);
t = total of observation time (in days).

The survival rate was calculated based on a formula from Wirabakti (2006):

$$SR = \frac{Nt}{No} \times 100$$

where: SR = survival rate (%);
Nt = the number of survived fish at the end of the experiment (in fish);
No = the number of survived fish at the start of the experiment (in fish).

The feed conversion ratio was calculated based on a formula from Council (1997):

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
Wo = the biomass weight of fish at the start of the experiment (g);
Wt = the biomass weight of fish at the end of the experiment (g);
D = total weight of dead fish (g);
F = feed intake (g).

Statistical analysis. Data analysis using Variant Analysis (ANOVA) with the study design was a Completely Randomized Design (CRD) to determine the differences in treatment. If there is a difference in treatment, Duncan's Multiple Range Test is applied.

Results. The calculation results of the average value of the specific growth rate, feed conversion ratio, and survival rate of red tilapia using statistical analysis after being treated for 30 days can be seen in Table 2.

Table 2

Average value of the specific growth rate, survival rate, feed conversion ratio, daily length growth, and protein retention of red tilapia

Treatment	*Specific growth rate (% day^{-1})*	*Survival rate (%)*	*Feed conversion ratio (%)*	*Daily length growth rate (% day^{-1})*	*Protein retention (%)*
P0	0.0105±0.000 7^{c}	100	7.712±0.588^{b}	1.070±0.189^{b}	7.3345±0.3434^{c}
P1	0.0135±0.0006^{b}	100	6.157±0.226^{a}	1.145±0.050^{b}	12.6246±0.3074^{b}
P2	0.0147±0.0005^{a}	100	5.846±0.234^{a}	1.435±0.186^{a}	11.5692±0.4054^{a}
P3	0.0150±0.0009^{a}	100	5.918±0.436^{a}	1.599±1.312^{a}	11.4289±0.7356^{a}

P0 (0% noni fruit extract), P1 (100 mL kg^{-1} noni fruit extract), P2 (300 mL kg^{-1} commercial feed with noni fruit extract), P3 (500 mL kg^{-1} commercial feed with noni fruit extract). Different superscripts show significant differences.

According to Table 2, the value of the specific growth rate (SGR) of red tilapia was 0.0105-0.0150% day^{-1}. The results of the statistical analysis of added noni fruit extract on commercial feed to the SGR of red tilapia showed significantly different results ($p < 0.05$). Duncan's Multiple Range Test (DMTR) results showed that the highest growth rate in this study was P3 (0.0150% day^{-1}) which was not significantly different from P2 (0.0147% day^{-1}), while it was significantly different with P0 (0.0105% day^{-1}) and P1 (0.0135% day^{-1}). The lowest specific growth rate was P0 (0.0105% day^{-1}) which was significantly different from P1 (0.0135% day^{-1}), P2 (0.147% day^{-1}), and P3 (0.0150% day^{-1}).

The feed conversion ratio (FCR) of red tilapia was 5.846-7.712%. The results of statistical analysis of the added noni fruit extract to commercial feed to FCR of red tilapia showed significantly different results ($p < 0.05$). Based on the results of DMTR, the highest FCR in this study was P0 (7.712%) which was significantly different from P1 (6.157%), P2 (5.846%), and P3 (5.9188%). While the lowest feed conversion ratio was

P2 (5.846%) and was not significantly different from P1 (6.157%), P3 (5.918%), and significantly different from P0 (7.712%). These results indicate that the addition of noni fruit extract to commercial feed can reduce feed conversion ratio of red tilapia.

The survival rate of red tilapia from all treatments was 100%. The average survival rate of red tilapia showed no significant difference ($p > 0.05$). Based on the results of DMTR, the survival rates of P0, P1, P2, and P3 were not significantly different.

The value of the daily length growth rate of red tilapia for thirty days of preservation was 1.070-1.599% day^{-1}. The results showed significant differences ($p < 0.05$). The highest daily length growth value was at P3 (1.599% day^{-1}) which was not significantly different from P2 (1.435% day^{-1}) but was significantly different from P0 (1.070% day^{-1}) and P1 (1.145% day^{-1}). The lowest daily length growth rate was P0 (1.070% day^{-1}) which was not significantly different from P1 (1.145% day^{-1}) but significantly different from P2 (1.435% day^{-1}) and P3 (1.599% day^{-1}).

The results of the statistical analysis of the addition of noni fruit extract to commercial feed showed significantly different results ($p < 0.05$) to the protein retention of red tilapia. The results of DMTR showed that the highest protein retention value was P1 (12.6246%) which was significantly different from P0 (7.3345%), P2 (11.5692%), and P3 (11.4289%). Protein retention value in P2 (11.5692%) was not significantly different from P3 (11.4289%) but was significantly different from P0 (7.3345%) and P1 (12.6246%). The lowest protein retention value was P0 (7.3345%), which was significantly different from P1 (12.6246%), P2 (11.5692%), and P3 (11.4289%).

Data on the range of water quality parameters for red tilapia preservation for thirty days can be seen in Table 3.

Table 3

Water quality range value of red tilapia in 30 days of preservation

Parameter	*Range*
Temperature (°C)	27.4-29.2
pH	7.2-7.9
DO (mg L^{-1})	3.3-5
Ammonia (mg L^{-1})	0-0.5

Discussion. The noni fruit extract mixed in the commercial feed had a significantly different effect on specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia for thirty days of preservation. These effects are due to the optimum absorption process or nutrient absorption of the feed in the digestive tract. Noni extract contains anthraquinone or acidic ingredients that can affect the pH of the digestive tract to be more acidic (Widianto et al 2015). The statement is right considering that in an acidic environment, protein-breaking enzymes (pepsin) can work optimally, so that protein intakes are absorbed more by the body which supports the optimal fish growth rate (Esminger et al 1990).

The extract of noni fruit contains proxeronine and xeronine. Proxeronine is converted to xeronine with the help of proxeronase enzymes. Xeronine is an active compound that has a role in facilitating metabolism inside the body of the animals and increasing nutrient absorption (Solomon 2001). Xeronine in noni fruit can function to expand the absorption in the intestine surface and facilitate the absorption process of food so the nutrients in the feed can be absorbed optimally (Wang et al 2008). The optimal absorption process will affect increased protein retention (Schlegel 1987; Frazier & Westhoff 1988; Cui et al 1992; Solomon 2001; Sjofjan 2003; Sujono 2006; Widianto et al 2015). Increased protein retention in the treatment results was caused by adding noni fruit extract in commercial feed, which can suppress the growth of microbial pathogens and increase non-pathogenic microbes in the digestive tract, resulting in the ability to decompose feed ingredients, especially crude fiber. The use of noni fruit extract can inhibit the growth of pathogenic bacteria in the intestine so that the number of non-pathogenic bacteria in the intestine was increased (Solomon 2001). Noni extract can increase the absorption process in the digestive tract and help decompose feed

ingredients, especially crude fiber (Widianto et al 2015). Non-pathogenic bacteria can produce enzymes that digest crude fiber, protein, and lipid (Sjofjan 2003). *Bacillus* sp. bacteria in the digestive tract can break down proteins into amino acids (Sujono 2006). These amino acids are used by bacteria to multiply themselves. Bacteria are a source of single-cell proteins so that the bacteria multiplication can increase protein feed and reduce crude fiber (Schlegel 1987).

Moreover, *Bacillus* sp. is a pectinolytic bacteria, which is an organism that can produce pectin or complex carbohydrates (Frazier & Westhoff 1988). The characteristics of *Bacillus* sp. bacteria are proteolytic, saccharolytic, and pectinolytic, which increases protein and carbohydrate feed. Protein retention indicates the amount of additional body protein from the consumed feed protein. The feed consumed was a source of protein used for preservation, metabolic activity, and growth (Cui et al 1992). The high protein retention in P1 treatment was caused by the increase in protein and carbohydrate content by the activity of non-pathogenic microbes that increase due to the addition of noni fruit extract in the feed. The only issue was the more noni fruit extract dosage in the feed cannot help at breaking down the crude fiber and result in higher crude fiber amount in the feed.

The feed conversion ratio is one of the standards to determine the quality of feed given to livestock in fulfilling the required nutrition. The higher the value of feed conversion, the worse the quality of the nutritional value of the feed. Noni fruit contains saponins which are bioactive in the growth of animals and digestive microbes. Giving saponins can increase the permeability of cell walls in the intestine, increase absorption of food substances hence result in higher conversion value (Önning et al 1996).

Survival rates of red tilapia were not significantly different between treatments. Thirty days preservation of red tilapia with the treatments given did not cause death in fish. This proves that adding noni fruit extract in commercial feed did not contain toxic ingredients that cause death. One study using noni fruit extract with a dose of 15,000 mg per kg body weight in experimental animals was not found any sign of toxic effects (Krishnakumar et al 2015). The study had performed surgery on experimental animals but still did not find any signs of toxicity in the animals' organs. Noni fruit is categorized as non-toxic substances (Wang et al 2008; Younos et al 1990).

Water quality in this study is an essential supporting parameter for the survival rate of red tilapia. Water as a living medium for fish that is preserved must meet the requirements. Water quality needs to be controlled in order to maintain its optimal conditions, thus creating an environment that is suitable for the habitat of red tilapia. The supporting parameters of this study are water quality parameters such as temperature, DO, pH, and ammonia. The temperature in this study averaged around 27.4-29.2^{o}C. This temperature is good enough for the survival of red tilapia. The optimal water temperature for red tilapia is 25-30^{o}C (Kungvakij & Pudadera 1984). Temperature is an environmental factor that influences the body metabolism speed. The optimum speed metabolism for fish will be achieved at optimal temperatures. The pH value has an essential influence on the survival of fish in the water. The pH in this study ranged from 7.2 to 7.9, which is quite adequate for the survival rate of red tilapia. The pH range required by fish is 6-9 (DeLong et al 2009). The average DO content in this study ranged from 3.3 to 5 mg L^{-1}. Based on these results, the DO during the study indicated a suitable level for the survival of red tilapia (Yildiz et al 2017). Thus DO in the preservation of red tilapia met the requirements. Ammonia in the water is derived from fish feces and from the rest of the feed that is not consumed by fish. In general, the concentration of ammonia contained in the water must not exceed 1 mg L^{-1} (BFAR-NFFTC 2000; Indian Council of Agricultural Research 2006). Ammonia during this study was 0-0.5 mg L^{-1}, which is suitable for the preservation of red tilapia.

Conclusions. Based on this research, it can be concluded that the addition of noni fruit extract in commercial feed can increase specific growth rate, daily length growth rate, lower the feed conversion ratio, and increase protein retention of red tilapia (*Oreochromis niloticus*). Moreover, the addition of noni fruit extract in commercial feed did not show any toxic effect on the survival rate of red tilapia during the thirty days of preservation.

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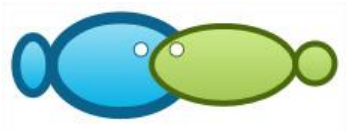

Increasing growth performances of Nile tilapia (*Oreochromis niloticus*) by supplementation of noni *Morinda citrifolia* fruit extract via diet ~~The potential of *Morinda citrifolia* in commercial feed on the growth and protein of~~

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Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract to the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments of noni fruit extract dosages given to red tilapia. The treatments used were the dosage amount of noni extraction given to red tilapia, namely P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder); P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol; P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol; P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 0.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).
Key Words: fish meal substitution, noni fruit (*Morinda citrifolia*) extract, red tilapia (*Oreochromis niloticus*).

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand for consumption and cultivation. The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the community, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. Feed is an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu

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Authors response: We have revised the title of the manuscript to make it clearly clearer.

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P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

Authors response: We have added the treatments used in the present study

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2016). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most significant amount of production costs component (Shepherd & Jackson 2013). One way to overcome this problem is to use feed additives. Feed additives in fish farming, especially red tilapia, are ingredients that are intentionally used to increase appetite, digestibility, endurrance, reduce stress levels, and spur fish growth (Alemayehu et al 2018).

The provision of feed is considered necessary in fish farming, including the addition of feed additives to spur growth and production. Phytobiotics is one type of natural feed additive derived from plants (Vidanarachchi et al 2005). The purpose of adding natural feed additives to the feed is to spur growth, as well as improve the health and productivity of fish (Citarasu 2001; Citarasu et al 1998, 1999, 2002; Sivaram et al 2004). Noni fruit can be used as phytobiotic (feed additive) because it has antibacterial effects and bioactive substances such as alkaloids, flavonoids, and tannins which are very effective in suppressing the growth of pathogenic bacteria and improving intestinal morphological characteristics (Murdiati & Adiwinatai 2000). Bioactive substances in noni include xeronine and precursor of xeronine (proxeronine). Proxeronine will be converted to xeronine in the intestine by proxeronase enzymes and other substances. Xeronine increases the permeability of cell membranes, facilitates the transfer of peptides and increases nutrient absorption, as well as effectuates amino acids, vitamins, and minerals. Xeronine can activate several enzymes and regulate protein synthesis (Solomon 2001). Therefore, noni fruit extract can be used as an additional ingredient in feed to help the digestion process of fish, so that the consumed feed can be optimally utilized for growth and feed efficiency, as well as the protein content of fish. This study aims to determine the potential of given noni fruit (*Morinda citrifolia*) extract to the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

Material and Method

Preparation of red tilapia (Oreochromis niloticus) and rearing conditions. This research was conducted from April to May 2017 at the Education Laboratory of the Airlangga University, Faculty of Fisheries and Marine, Surabaya, Indonesia. The proximate test of feed and protein retention test were conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The instruments used in this study were an aquarium measuring 45 cm x 30 cm x 30 cm, a set of aerators, digital scales, pH pens, thermometers, rulers, plastic bags, plastic hoses with a diameter of 1 cm, dissolved oxygen (DO) meters, and ammonia kits. The fish used were 140 red tilapia fry in healthy condition, measuring 5-6 cm total length, weighing 2-5 g head^{-1}. The red tilapia fry were obtained from the Gunung Sari fish market, Surabaya, East Java. Noni fruit extract was obtained from the result of squeezed noni fruit. The noni fruit used was ripe noni fruit, which is yellow and soft with a distinctive smell.

Experimental design. This research method was experimental. The primary test parameters in this study were protein retention, specific growth rate, feed conversion ratio, and survival rate of red tilapia. The variables observed were independent variables, dependent variables, and control variables. The independent variable is the dose of noni extract. The dependent variables are specific growth rate, feed conversion ratio, survival rate, and protein retention. Control variables are water quality (temperature, pH, DO, and ammonia). This study used a Completely Randomized Design (CRD) with four treatments and four replications, which treatments explained as follows:
P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

The feed given in this study was PF 1000 commercial feed from PT Matahari Sakti, with a protein content of 39-41%, 5% lipid, 6% crude fiber, 16% ash, and 10% moisture content.

Preparation of the experimental diets. First, ripe noni fruits were cleaned with water, then washed with warm water. The noni fruits were crushed and then squeezed by using strain cloth. The squeezed noni fruit extract was put in a bottle and stored at room temperature.

The commercial feed was mixed with noni fruit extract and 2 grams of Progol with a different amount in each treatment, as previously explained. The commercial feed and noni fruit extract were smoothly mixed. The mixed feed was dried in an open room and was aerated until dry. After that, the dried food was stored in a plastic jar. The mixed fish feed with different doses were then analyzed through the proximate test in the laboratory. The nutrient content of the feed used in this study can be seen in Table 1 as follows.

Table 1

Nutrient content of feed with various doses of noni fruit extract

Analysis	*P0*	*P1*	*P2*	*P3*
	(Control)	*(100 mL kg^{-1})*	*(300 mL kg^{-1})*	*(500 mL kg^{-1})*
Dry ingredients (%)	90.1441	87.3484	86.7339	84.5036
Ash (%)	8.9591	8.7352	8.6875	8.5261
Crude protein (%)	29.1112	30.9015	30.2753	29.2539
Crude lipid (%)	8.3165	7.6176	6.9983	6.872
Crude fiber (%)	3.5026	2.488	2.6143	2.2593
Ca	2.5824	2.4109	2.4069	2.1481
Nitrogen-free extract (%)	40.2547	37.6061	38.1585	37.5923
Metabolism energy (Kcal kg^{-1})	3057.9622	2967.4828	2922.6623	2857.8501

The analytical procedure of the diets. The aquarium was then filled with 30 liters of water, aerated, and then left for 24 hours. The fish were selected based on a similar length and weight and its healthy condition. The fish were acclimatized for one week and were fasted for 24 hours before being treated. The fish were placed in fourteen aquariums according to the treatment given with a stocking density of 10 fish per aquarium. The fish were kept for thirty days and fed according to the treatment doses. Feeding was given three times a day: morning, afternoon, and evening, with a dose of 3% of body weight.

Below is the protein retention (PR) calculation based on a formula from Tung & Shiau (1991):

$$PR = \frac{Ln\ final\ body\ weight - Ln\ initial\ body\ weight\ (g)}{Total\ protein\ intake\ (g)} \times 100$$

The specific growth rate was calculated based on a formula from Effendie (1997):

$$SGR = \frac{LnWt - LnWo}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W0 = initial body weight (g);
Wt = final body weight (g);
T = treatment time (in days).

The daily length growth rate (LGR) was calculated based on a formula from Aggraeni & Abdulgani 2013):

$$LGR = \frac{LnLt - LnLo}{t} \times 100$$

where: LGR = daily length growth rate (% day^{-1});
Lt = average total length on day t (cm);
L0 = average total length on day 0 (cm);
t = total of observation time (in days).

The survival rate was calculated based on a formula from Wirabakti (2006):

$$SR = \frac{Nt}{No} \times 100$$

where: SR = survival rate (%);
Nt = the number of survived fish at the end of the experiment (in fish);
No = the number of survived fish at the start of the experiment (in fish).

The feed conversion ratio was calculated based on a formula from Council (1997):

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
Wo = the biomass weight of fish at the start of the experiment (g);
Wt = the biomass weight of fish at the end of the experiment (g);
D = total weight of dead fish (g);
F = feed intake (g).

Statistical analysis. Data analysis using Variant Analysis (ANOVA) with the study design was a Completely Randomized Design (CRD) to determine the differences in treatment. If there is a difference in treatment, Duncan's Multiple Range Test is applied.

Results. The calculation results of the average value of the specific growth rate, feed conversion ratio, and survival rate of red tilapia using statistical analysis after being treated for 30 days can be seen in Table 2.

Table 2

Average value of the specific growth rate, survival rate, feed conversion ratio, daily length growth, and protein retention of red tilapia

Treatment	*Specific growth rate (% day^{-1})*	*Survival rate (%)*	*Feed conversion ratio (%)*	*Daily length growth rate (% day^{-1})*	*Protein retention (%)*
P0	0.0105±0.000 7^{c}	100	2.712±0.588^{b}	1.070±0.189^{b}	7.3345±0.3434^{c}
P1	0.0135±0.0006^{b}	100	1.157±0.226^{a}	1.145±0.050^{b}	12.6246±0.3074^{b}
P2	0.0147±0.0005^{a}	100	0.846±0.234^{a}	1.435±0.186^{a}	11.5692±0.4054^{a}
P3	0.0150±0.0009^{a}	100	0.918±0.436^{a}	1.599±1.312^{a}	11.4289±0.7356^{a}

P0 (0% noni fruit extract), P1 (100 mL kg^{-1} noni fruit extract), P2 (300 mL kg^{-1} commercial feed with noni fruit extract), P3 (500 mL kg^{-1} commercial feed with noni fruit extract). Different superscripts show significant differences.

According to Table 2, the value of the specific growth rate (SGR) of red tilapia was 0.0105-0.0150% day^{-1}. The results of the statistical analysis of added noni fruit extract on commercial feed to the SGR of red tilapia showed significantly different results ($p < 0.05$). Duncan's Multiple Range Test (DMTR) results showed that the highest growth rate in this study was P3 (0.0150% day^{-1}) which was not significantly different from P2 (0.0147% day^{-1}), while it was significantly different with P0 (0.0105% day^{-1}) and P1 (0.0135% day^{-1}). The lowest specific growth rate was P0 (0.0105% day^{-1}) which was significantly different from P1 (0.0135% day^{-1}), P2 (0.147% day^{-1}), and P3 (0.0150% day^{-1}).

The feed conversion ratio (FCR) of red tilapia was 0.846-2.712%. The results of statistical analysis of the added noni fruit extract to commercial feed to FCR of red tilapia showed significantly different results ($p < 0.05$). Based on the results of DMTR, the highest FCR in this study was P0 (2.712%) which was significantly different from P1 (1.157%), P2 (0.846%), and P3 (0.9188%). While the lowest feed conversion ratio was

P2 (0.846%) and was not significantly different from P1 (1.157%), P3 (0.918%), and significantly different from P0 (2.712%). These results indicate that the addition of noni fruit extract to commercial feed can reduce feed conversion ratio of red tilapia.

The survival rate of red tilapia from all treatments was 100%. The average survival rate of red tilapia showed no significant difference ($p > 0.05$). Based on the results of DMTR, the survival rates of P0, P1, P2, and P3 were not significantly different.

The value of the daily length growth rate of red tilapia for thirty days of preservation was 1.070-1.599% day^{-1}. The results showed significant differences ($p < 0.05$). The highest daily length growth value was at P3 (1.599% day^{-1}) which was not significantly different from P2 (1.435% day^{-1}) but was significantly different from P0 (1.070% day^{-1}) and P1 (1.145% day^{-1}). The lowest daily length growth rate was P0 (1.070% day^{-1}) which was not significantly different from P1 (1.145% day^{-1}) but significantly different from P2 (1.435% day^{-1}) and P3 (1.599% day^{-1}).

The results of the statistical analysis of the addition of noni fruit extract to commercial feed showed significantly different results ($p < 0.05$) to the protein retention of red tilapia. The results of DMTR showed that the highest protein retention value was P1 (12.6246%) which was significantly different from P0 (7.3345%), P2 (11.5692%), and P3 (11.4289%). Protein retention value in P2 (11.5692%) was not significantly different from P3 (11.4289%) but was significantly different from P0 (7.3345%) and P1 (12.6246%). The lowest protein retention value was P0 (7.3345%), which was significantly different from P1 (12.6246%), P2 (11.5692%), and P3 (11.4289%).

Data on the range of water quality parameters for red tilapia preservation for thirty days can be seen in Table 3.

Table 3

Water quality range value of red tilapia in 30 days of preservation

Parameter	*Range*
Temperature (°C)	27.4-29.2
pH	7.2-7.9
DO (mg L^{-1})	3.3-5
Ammonia (mg L^{-1})	0-0.5

Discussion. The noni fruit extract mixed in the commercial feed had a significantly different effect on specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia for thirty days of preservation. These effects are due to the optimum absorption process or nutrient absorption of the feed in the digestive tract. Noni extract contains anthraquinone or acidic ingredients that can affect the pH of the digestive tract to be more acidic (Widianto et al 2015). The statement is right considering that in an acidic environment, protein-breaking enzymes (pepsin) can work optimally, so that protein intakes are absorbed more by the body which supports the optimal fish growth rate (Esminger et al 1990).

The extract of noni fruit contains proxeronine and xeronine. Proxeronine is converted to xeronine with the help of proxeronase enzymes. Xeronine is an active compound that has a role in facilitating metabolism inside the body of the animals and increasing nutrient absorption (Solomon 2001). Xeronine in noni fruit can function to expand the absorption in the intestine surface and facilitate the absorption process of food so the nutrients in the feed can be absorbed optimally (Wang et al 2008). The optimal absorption process will affect increased protein retention (Schlegel 1987; Frazier & Westhoff 1988; Cui et al 1992; Solomon 2001; Sjofjan 2003; Sujono 2006; Widianto et al 2015). Increased protein retention in the treatment results was caused by adding noni fruit extract in commercial feed, which can suppress the growth of microbial pathogens and increase non-pathogenic microbes in the digestive tract, resulting in the ability to decompose feed ingredients, especially crude fiber. The use of noni fruit extract can inhibit the growth of pathogenic bacteria in the intestine so that the number of non-pathogenic bacteria in the intestine was increased (Solomon 2001). Noni extract can increase the absorption process in the digestive tract and help decompose feed

ingredients, especially crude fiber (Widianto et al 2015). Non-pathogenic bacteria can produce enzymes that digest crude fiber, protein, and lipid (Sjofjan 2003). *Bacillus* sp. bacteria in the digestive tract can break down proteins into amino acids (Sujono 2006). These amino acids are used by bacteria to multiply themselves. Bacteria are a source of single-cell proteins so that the bacteria multiplication can increase protein feed and reduce crude fiber (Schlegel 1987).

Moreover, *Bacillus* sp. is a pectinolytic bacteria, which is an organism that can produce pectin or complex carbohydrates (Frazier & Westhoff 1988). The characteristics of *Bacillus* sp. bacteria are proteolytic, saccharolytic, and pectinolytic, which increases protein and carbohydrate feed. Protein retention indicates the amount of additional body protein from the consumed feed protein. The feed consumed was a source of protein used for preservation, metabolic activity, and growth (Cui et al 1992). The high protein retention in P1 treatment was caused by the increase in protein and carbohydrate content by the activity of non-pathogenic microbes that increase due to the addition of noni fruit extract in the feed. The only issue was the more noni fruit extract dosage in the feed cannot help at breaking down the crude fiber and result in higher crude fiber amount in the feed.

The feed conversion ratio is one of the standards to determine the quality of feed given to livestock in fulfilling the required nutrition. The higher the value of feed conversion, the worse the quality of the nutritional value of the feed. Noni fruit contains saponins which are bioactive in the growth of animals and digestive microbes. Giving saponins can increase the permeability of cell walls in the intestine, increase absorption of food substances hence result in higher conversion value (Önning et al 1996).

Survival rates of red tilapia were not significantly different between treatments. Thirty days preservation of red tilapia with the treatments given did not cause death in fish. This proves that adding noni fruit extract in commercial feed did not contain toxic ingredients that cause death. One study using noni fruit extract with a dose of 15,000 mg per kg body weight in experimental animals was not found any sign of toxic effects (Krishnakumar et al 2015). The study had performed surgery on experimental animals but still did not find any signs of toxicity in the animals' organs. Noni fruit is categorized as non-toxic substances (Wang et al 2008; Younos et al 1990).

Water quality in this study is an essential supporting parameter for the survival rate of red tilapia. Water as a living medium for fish that is preserved must meet the requirements. Water quality needs to be controlled in order to maintain its optimal conditions, thus creating an environment that is suitable for the habitat of red tilapia. The supporting parameters of this study are water quality parameters such as temperature, DO, pH, and ammonia. The temperature in this study averaged around 27.4-29.2^{o}C. This temperature is good enough for the survival of red tilapia. The optimal water temperature for red tilapia is 25-30^{o}C (Kungvakij & Pudadera 1984). Temperature is an environmental factor that influences the body metabolism speed. The optimum speed metabolism for fish will be achieved at optimal temperatures. The pH value has an essential influence on the survival of fish in the water. The pH in this study ranged from 7.2 to 7.9, which is quite adequate for the survival rate of red tilapia. The pH range required by fish is 6-9 (DeLong et al 2009). The average DO content in this study ranged from 3.3 to 5 mg L^{-1}. Based on these results, the DO during the study indicated a suitable level for the survival of red tilapia (Yildiz et al 2017). Thus DO in the preservation of red tilapia met the requirements. Ammonia in the water is derived from fish feces and from the rest of the feed that is not consumed by fish. In general, the concentration of ammonia contained in the water must not exceed 1 mg L^{-1} (BFAR-NFFTC 2000; Indian Council of Agricultural Research 2006). Ammonia during this study was 0-0.5 mg L^{-1}, which is suitable for the preservation of red tilapia.

Conclusions. Based on this research, it can be concluded that the addition of noni fruit extract in commercial feed can increase specific growth rate, daily length growth rate, lower the feed conversion ratio, and increase protein retention of red tilapia (*Oreochromis niloticus*). Moreover, the addition of noni fruit extract in commercial feed did not show any toxic effect on the survival rate of red tilapia during the thirty days of preservation.

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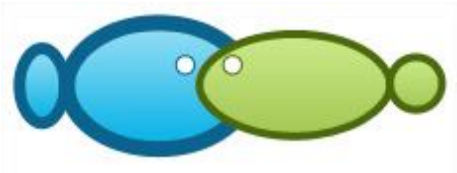

Increasing growth performances of Nile tilapia (*Oreochromis niloticus*) by supplementation of noni *Morinda citrifolia* fruit extract via diet

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Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract to the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments of noni fruit extract dosages given to red tilapia. The treatments used were the dosage amount of noni extraction given to red tilapia, namely P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder); P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol; P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol; P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 0.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).
Key Words: fish meal substitution, noni fruit (*Morinda citrifolia*) extract, red tilapia (*Oreochromis niloticus*).

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand for consumption and cultivation. The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the community, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. Feed is an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu 2016). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most significant amount of

production costs component (Shepherd & Jackson 2013). One way to overcome this problem is to use feed additives. Feed additives in fish farming, especially red tilapia, are ingredients that are intentionally used to increase appetite, digestibility, endurrance, reduce stress levels, and spur fish growth (Alemayehu et al 2018).

The provision of feed is considered necessary in fish farming, including the addition of feed additives to spur growth and production. Phytobiotics is one type of natural feed additive derived from plants (Vidanarachchi et al 2005). The purpose of adding natural feed additives to the feed is to spur growth, as well as improve the health and productivity of fish (Citarasu 2001; Citarasu et al 1998, 1999, 2002; Sivaram et al 2004). Noni fruit can be used as phytobiotic (feed additive) because it has antibacterial effects and bioactive substances such as alkaloids, flavonoids, and tannins which are very effective in suppressing the growth of pathogenic bacteria and improving intestinal morphological characteristics (Murdiati & Adiwinatai 2000). Bioactive substances in noni include xeronine and precursor of xeronine (proxeronine). Proxeronine will be converted to xeronine in the intestine by proxeronase enzymes and other substances. Xeronine increases the permeability of cell membranes, facilitates the transfer of peptides and increases nutrient absorption, as well as effectuates amino acids, vitamins, and minerals. Xeronine can activate several enzymes and regulate protein synthesis (Solomon 2001). Therefore, noni fruit extract can be used as an additional ingredient in feed to help the digestion process of fish, so that the consumed feed can be optimally utilized for growth and feed efficiency, as well as the protein content of fish. This study aims to determine the potential of given noni fruit (*Morinda citrifolia*) extract to the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

Material and Method

Preparation of red tilapia (Oreochromis niloticus) and rearing conditions. This research was conducted from April to May 2017 at the Education Laboratory of the Airlangga University, Faculty of Fisheries and Marine, Surabaya, Indonesia. The proximate test of feed and protein retention test were conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The instruments used in this study were an aquarium measuring 45 cm x 30 cm x 30 cm, a set of aerators, digital scales, pH pens, thermometers, rulers, plastic bags, plastic hoses with a diameter of 1 cm, dissolved oxygen (DO) meters, and ammonia kits. The fish used were 140 red tilapia fry in healthy condition, measuring 5-6 cm total length, weighing 2-5 g head^{-1}. The red tilapia fry were obtained from the Gunung Sari fish market, Surabaya, East Java. Noni fruit extract was obtained from the result of squeezed noni fruit. The noni fruit used was ripe noni fruit, which is yellow and soft with a distinctive smell.

Experimental design. This research method was experimental. The primary test parameters in this study were protein retention, specific growth rate, feed conversion ratio, and survival rate of red tilapia. The variables observed were independent variables, dependent variables, and control variables. The independent variable is the dose of noni extract. The dependent variables are specific growth rate, feed conversion ratio, survival rate, and protein retention. Control variables are water quality (temperature, pH, DO, and ammonia). This study used a Completely Randomized Design (CRD) with four treatments and four replications, which treatments explained as follows:

P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

The feed given in this study was PF 1000 commercial feed from PT Matahari Sakti, with a protein content of 39-41%, 5% lipid, 6% crude fiber, 16% ash, and 10% moisture content.

Preparation of the experimental diets. First, ripe noni fruits were cleaned with water, then washed with warm water. The noni fruits were crushed and then squeezed by using strain cloth. The squeezed noni fruit extract was put in a bottle and stored at room temperature.

The commercial feed was mixed with noni fruit extract and 2 grams of Progol with a different amount in each treatment, as previously explained. The commercial feed and noni fruit extract were smoothly mixed. The mixed feed was dried in an open room and was aerated until dry. After that, the dried food was stored in a plastic jar. The mixed fish feed with different doses were then analyzed through the proximate test in the laboratory. The nutrient content of the feed used in this study can be seen in Table 1 as follows.

Table 1

Nutrient content of feed with various doses of noni fruit extract

Analysis	*P0*	*P1*	*P2*	*P3*
	(Control)	*(100 mL kg^{-1})*	*(300 mL kg^{-1})*	*(500 mL kg^{-1})*
Dry ingredients (%)	90.1441	87.3484	86.7339	84.5036
Ash (%)	8.9591	8.7352	8.6875	8.5261
Crude protein (%)	29.1112	30.9015	30.2753	29.2539
Crude lipid (%)	8.3165	7.6176	6.9983	6.872
Crude fiber (%)	3.5026	2.488	2.6143	2.2593
Ca	2.5824	2.4109	2.4069	2.1481
Nitrogen-free extract (%)	40.2547	37.6061	38.1585	37.5923
Metabolism energy (Kcal kg^{-1})	3057.9622	2967.4828	2922.6623	2857.8501

The analytical procedure of the diets. The aquarium was then filled with 30 liters of water, aerated, and then left for 24 hours. The fish were selected based on a similar length and weight and its healthy condition. The fish were acclimatized for one week and were fasted for 24 hours before being treated. The fish were placed in fourteen aquariums according to the treatment given with a stocking density of 10 fish per aquarium. The fish were kept for thirty days and fed according to the treatment doses. Feeding was given three times a day: morning, afternoon, and evening, with a dose of 3% of body weight.

Below is the protein retention (PR) calculation based on a formula from Tung & Shiau (1991):

$$PR = \frac{Ln\ final\ body\ weight - Ln\ initial\ body\ weight\ (g)}{Total\ protein\ intake\ (g)} \times 100$$

The specific growth rate was calculated based on a formula from Effendie (1997):

$$SGR = \frac{LnWt - LnWo}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W0 = initial body weight (g);
Wt = final body weight (g);
T = treatment time (in days).

The daily length growth rate (LGR) was calculated based on a formula from Aggraeni & Abdulgani 2013):

$$LGR = \frac{LnLt - LnLo}{t} \times 100$$

where: LGR = daily length growth rate (% day^{-1});
Lt = average total length on day t (cm);
L0 = average total length on day 0 (cm);
t = total of observation time (in days).

The survival rate was calculated based on a formula from Wirabakti (2006):

$$SR = \frac{Nt}{No} \times 100$$

where: SR = survival rate (%);
Nt = the number of survived fish at the end of the experiment (in fish);
No = the number of survived fish at the start of the experiment (in fish).

The feed conversion ratio was calculated based on a formula from Council (1997):

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
Wo = the biomass weight of fish at the start of the experiment (g);
Wt = the biomass weight of fish at the end of the experiment (g);
D = total weight of dead fish (g);
F = feed intake (g).

Statistical analysis. Data analysis using Variant Analysis (ANOVA) with the study design was a Completely Randomized Design (CRD) to determine the differences in treatment. If there is a difference in treatment, Duncan's Multiple Range Test is applied.

Results. The calculation results of the average value of the specific growth rate, feed conversion ratio, and survival rate of red tilapia using statistical analysis after being treated for 30 days can be seen in Table 2.

Table 2

Average value of the specific growth rate, survival rate, feed conversion ratio, daily length growth, and protein retention of red tilapia

Treatment	*Specific growth rate (% day^{-1})*	*Survival rate (%)*	*Feed conversion ratio (%)*	*Daily length growth rate (% day^{-1})*	*Protein retention (%)*
P0	0.0105±0.000 7^{c}	100	2.712±0.588^{b}	1.070±0.189^{b}	7.3345±0.3434^{c}
P1	0.0135±0.0006^{b}	100	1.157±0.226^{a}	1.145±0.050^{b}	12.6246±0.3074^{b}
P2	0.0147±0.0005^{a}	100	0.846±0.234^{a}	1.435±0.186^{a}	11.5692±0.4054^{a}
P3	0.0150±0.0009^{a}	100	0.918±0.436^{a}	1.599±1.312^{a}	11.4289±0.7356^{a}

P0 (0% noni fruit extract), P1 (100 mL kg^{-1} noni fruit extract), P2 (300 mL kg^{-1} commercial feed with noni fruit extract), P3 (500 mL kg^{-1} commercial feed with noni fruit extract). Different superscripts show significant differences.

According to Table 2, the value of the specific growth rate (SGR) of red tilapia was 0.0105-0.0150% day^{-1}. The results of the statistical analysis of added noni fruit extract on commercial feed to the SGR of red tilapia showed significantly different results ($p < 0.05$). Duncan's Multiple Range Test (DMTR) results showed that the highest growth rate in this study was P3 (0.0150% day^{-1}) which was not significantly different from P2 (0.0147% day^{-1}), while it was significantly different with P0 (0.0105% day^{-1}) and P1 (0.0135% day^{-1}). The lowest specific growth rate was P0 (0.0105% day^{-1}) which was significantly different from P1 (0.0135% day^{-1}), P2 (0.147% day^{-1}), and P3 (0.0150% day^{-1}).

The feed conversion ratio (FCR) of red tilapia was 0.846-2.712%. The results of statistical analysis of the added noni fruit extract to commercial feed to FCR of red tilapia showed significantly different results ($p < 0.05$). Based on the results of DMTR, the highest FCR in this study was P0 (2.712%) which was significantly different from P1 (1.157%), P2 (0.846%), and P3 (0.9188%). While the lowest feed conversion ratio was P2 (0.846%) and was not significantly different from P1 (1.157%), P3 (0.918%), and significantly different from P0 (2.712%). These results indicate that the addition of noni fruit extract to commercial feed can reduce feed conversion ratio of red tilapia.

The survival rate of red tilapia from all treatments was 100%. The average survival rate of red tilapia showed no significant difference ($p > 0.05$). Based on the results of DMTR, the survival rates of P0, P1, P2, and P3 were not significantly different.

The value of the daily length growth rate of red tilapia for thirty days of preservation was 1.070-1.599% day^{-1}. The results showed significant differences ($p < 0.05$). The highest daily length growth value was at P3 (1.599% day^{-1}) which was not significantly different from P2 (1.435% day^{-1}) but was significantly different from P0 (1.070% day^{-1}) and P1 (1.145% day^{-1}). The lowest daily length growth rate was P0 (1.070% day^{-1}) which was not significantly different from P1 (1.145% day^{-1}) but significantly different from P2 (1.435% day^{-1}) and P3 (1.599% day^{-1}).

The results of the statistical analysis of the addition of noni fruit extract to commercial feed showed significantly different results ($p < 0.05$) to the protein retention of red tilapia. The results of DMTR showed that the highest protein retention value was P1 (12.6246%) which was significantly different from P0 (7.3345%), P2 (11.5692%), and P3 (11.4289%). Protein retention value in P2 (11.5692%) was not significantly different from P3 (11.4289%) but was significantly different from P0 (7.3345%) and P1 (12.6246%). The lowest protein retention value was P0 (7.3345%), which was significantly different from P1 (12.6246%), P2 (11.5692%), and P3 (11.4289%).

Data on the range of water quality parameters for red tilapia preservation for thirty days can be seen in Table 3.

Table 3

Water quality range value of red tilapia in 30 days of preservation

Parameter	*Range*
Temperature (^oC)	27.4-29.2
pH	7.2-7.9
DO (mg L^{-1})	3.3-5
Ammonia (mg L^{-1})	0-0.5

Discussion. The noni fruit extract mixed in the commercial feed had a significantly different effect on specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia for thirty days of preservation. These effects are due to the optimum absorption process or nutrient absorption of the feed in the digestive tract. Noni extract contains anthraquinone or acidic ingredients that can affect the pH of the digestive tract to be more acidic (Widianto et al 2015). The statement is right considering that in an acidic environment, protein-breaking enzymes (pepsin) can work optimally, so that protein intakes are absorbed more by the body which supports the optimal fish growth rate (Esminger et al 1990).

The extract of noni fruit contains proxeronine and xeronine. Proxeronine is converted to xeronine with the help of proxeronase enzymes. Xeronine is an active compound that has a role in facilitating metabolism inside the body of the animals and increasing nutrient absorption (Solomon 2001). Xeronine in noni fruit can function to expand the absorption in the intestine surface and facilitate the absorption process of food so the nutrients in the feed can be absorbed optimally (Wang et al 2008). The optimal absorption process will affect increased protein retention (Schlegel 1987; Frazier & Westhoff 1988; Cui et al 1992; Solomon 2001; Sjofjan 2003; Sujono 2006; Widianto et al 2015). Increased protein retention in the treatment results was caused by adding noni fruit extract in commercial feed, which can suppress the growth of microbial pathogens and increase non-pathogenic microbes in the digestive tract, resulting in the ability to decompose feed ingredients, especially crude fiber. The use of noni fruit extract can inhibit the growth of pathogenic bacteria in the intestine so that the number of non-pathogenic bacteria in the intestine was increased (Solomon 2001). Noni extract can increase the absorption process in the digestive tract and help decompose feed ingredients, especially crude fiber (Widianto et al 2015). Non-pathogenic bacteria can produce enzymes that digest crude fiber, protein, and lipid (Sjofjan 2003). *Bacillus* sp. bacteria in the digestive tract can break down proteins into amino acids (Sujono 2006).

These amino acids are used by bacteria to multiply themselves. Bacteria are a source of single-cell proteins so that the bacteria multiplication can increase protein feed and reduce crude fiber (Schlegel 1987).

Moreover, *Bacillus* sp. is a pectinolytic bacteria, which is an organism that can produce pectin or complex carbohydrates (Frazier & Westhoff 1988). The characteristics of *Bacillus* sp. bacteria are proteolytic, saccharolytic, and pectinolytic, which increases protein and carbohydrate feed. Protein retention indicates the amount of additional body protein from the consumed feed protein. The feed consumed was a source of protein used for preservation, metabolic activity, and growth (Cui et al 1992). The high protein retention in P1 treatment was caused by the increase in protein and carbohydrate content by the activity of non-pathogenic microbes that increase due to the addition of noni fruit extract in the feed. The only issue was the more noni fruit extract dosage in the feed cannot help at breaking down the crude fiber and result in higher crude fiber amount in the feed.

The feed conversion ratio is one of the standards to determine the quality of feed given to livestock in fulfilling the required nutrition. The higher the value of feed conversion, the worse the quality of the nutritional value of the feed. Noni fruit contains saponins which are bioactive in the growth of animals and digestive microbes. Giving saponins can increase the permeability of cell walls in the intestine, increase absorption of food substances hence result in higher conversion value (Önning et al 1996).

Survival rates of red tilapia were not significantly different between treatments. Thirty days preservation of red tilapia with the treatments given did not cause death in fish. This proves that adding noni fruit extract in commercial feed did not contain toxic ingredients that cause death. One study using noni fruit extract with a dose of 15,000 mg per kg body weight in experimental animals was not found any sign of toxic effects (Krishnakumar et al 2015). The study had performed surgery on experimental animals but still did not find any signs of toxicity in the animals' organs. Noni fruit is categorized as non-toxic substances (Wang et al 2008; Younos et al 1990).

Water quality in this study is an essential supporting parameter for the survival rate of red tilapia. Water as a living medium for fish that is preserved must meet the requirements. Water quality needs to be controlled in order to maintain its optimal conditions, thus creating an environment that is suitable for the habitat of red tilapia. The supporting parameters of this study are water quality parameters such as temperature, DO, pH, and ammonia. The temperature in this study averaged around 27.4-29.2^{o}C. This temperature is good enough for the survival of red tilapia. The optimal water temperature for red tilapia is 25-30^{o}C (Kungvakij & Pudadera 1984). Temperature is an environmental factor that influences the body metabolism speed. The optimum speed metabolism for fish will be achieved at optimal temperatures. The pH value has an essential influence on the survival of fish in the water. The pH in this study ranged from 7.2 to 7.9, which is quite adequate for the survival rate of red tilapia. The pH range required by fish is 6-9 (DeLong et al 2009). The average DO content in this study ranged from 3.3 to 5 mg L^{-1}. Based on these results, the DO during the study indicated a suitable level for the survival of red tilapia (Yildiz et al 2017). Thus DO in the preservation of red tilapia met the requirements. Ammonia in the water is derived from fish feces and from the rest of the feed that is not consumed by fish. In general, the concentration of ammonia contained in the water must not exceed 1 mg L^{-1} (BFAR-NFFTC 2000; Indian Council of Agricultural Research 2006). Ammonia during this study was 0-0.5 mg L^{-1}, which is suitable for the preservation of red tilapia.

Conclusions. Based on this research, it can be concluded that the addition of noni fruit extract in commercial feed can increase specific growth rate, daily length growth rate, lower the feed conversion ratio, and increase protein retention of red tilapia (*Oreochromis niloticus*). Moreover, the addition of noni fruit extract in commercial feed did not show any toxic effect on the survival rate of red tilapia during the thirty days of preservation.

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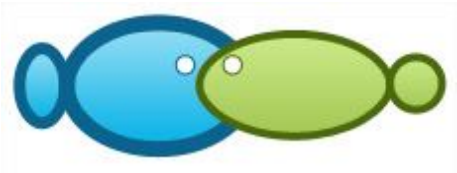

Increasing growth performances of Nile tilapia (*Oreochromis niloticus*) by supplementation of noni *Morinda citrifolia* fruit extract via diet

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Abstract. High feed prices and its low nutritional quality are the inhibitions in the process of red tilapia fish cultivation. Therefore, the use of feed additives in feed becomes significant in order to optimize fish growth. Especially the ones made of natural ingredients, such as noni (*Morinda citrifolia*) fruit. The purpose of this study was to determine the effect of added noni fruit extract to the growth rate and protein retention of red tilapia fish. The method used was an experimental method with a completely randomized design, consisting of four replications and four treatments of noni fruit extract dosages given to red tilapia. The treatments used were the dosage amount of noni extraction given to red tilapia, namely P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder); P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol; P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol; P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol. The parameters observed were specific growth rates, feed conversion ratio, survival rate, and protein retention of red tilapia. The result showed that the addition of noni fruit extract in commercial feed with different doses significantly affected the specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia. The survival rate from the preservation of red tilapia is not significantly different between treatments. The best result for the specific growth rate is P3 (500 mL kg^{-1}) of 0.0150% day^{-1}, daily length growth rate is P3 (500 mL kg^{-1}) of 1.599% day^{-1}, feed conversion ratio is P2 (300 mL kg^{-1}) of 0.846%, survival rate of 100% in all treatments, and protein retention with the best results is P1 (10 mL kg^{-1}) of 12.6246%. The addition of noni fruit extract in commercial feed can increase the specific growth rate and daily length, lowering the value of feed conversion ratio in red tilapia, and increasing the protein retention of red tilapia (*Oreochromis niloticus*).
Key Words: fish meal substitution, noni fruit (*Morinda citrifolia*) extract, red tilapia (*Oreochromis niloticus*).

Introduction. Red tilapia is one of the freshwater fish commodities that are currently in high demand for consumption and cultivation. The meat of red tilapia is delicious, solid, easy to serve, does not have many thorns, is a source of animal protein, and is relatively inexpensive (Siddiqui & Al-Harbi 1995; Zonneveld & Fadholi 1991). Red tilapia is popular within the community, making it an opportunity for fish farmers to increase the productivity of their ponds. Therefore, the availability of red tilapia for consumption needs to be increased. The demand for red tilapia continues to increase in the international market, mainly the American and European Union markets (Widanarni et al 2012). Red tilapia is one of the export fish which provided as a whole or in the form of a filet. The effort to increase the export of red tilapia is by increasing its production through cultivation.

The feed is one of the essential factors in the agriculture business. Feed is an energy source for fish to grow and develop (Younos et al 1990). The availability of adequate and balanced feed will affect the growth and survival of cultivated fish (Tiamiyu 2016). Feed must contain all necessary nutrients such as carbohydrates, fats, proteins, minerals, vitamins, and feed additives with sufficient and balanced amounts (Halver & Hardy 2003). High feed prices and low nutritional quality are inhibitions in the fish farming process. Feed as an energy source for growth is the most significant amount of

production costs component (Shepherd & Jackson 2013). One way to overcome this problem is to use feed additives. Feed additives in fish farming, especially red tilapia, are ingredients that are intentionally used to increase appetite, digestibility, endurrance, reduce stress levels, and spur fish growth (Alemayehu et al 2018).

The provision of feed is considered necessary in fish farming, including the addition of feed additives to spur growth and production. Phytobiotics is one type of natural feed additive derived from plants (Vidanarachchi et al 2005). The purpose of adding natural feed additives to the feed is to spur growth, as well as improve the health and productivity of fish (Citarasu 2001; Citarasu et al 1998, 1999, 2002; Sivaram et al 2004). Noni fruit can be used as phytobiotic (feed additive) because it has antibacterial effects and bioactive substances such as alkaloids, flavonoids, and tannins which are very effective in suppressing the growth of pathogenic bacteria and improving intestinal morphological characteristics (Murdiati & Adiwinatai 2000). Bioactive substances in noni include xeronine and precursor of xeronine (proxeronine). Proxeronine will be converted to xeronine in the intestine by proxeronase enzymes and other substances. Xeronine increases the permeability of cell membranes, facilitates the transfer of peptides and increases nutrient absorption, as well as effectuates amino acids, vitamins, and minerals. Xeronine can activate several enzymes and regulate protein synthesis (Solomon 2001). Therefore, noni fruit extract can be used as an additional ingredient in feed to help the digestion process of fish, so that the consumed feed can be optimally utilized for growth and feed efficiency, as well as the protein content of fish. This study aims to determine the potential of given noni fruit (*Morinda citrifolia*) extract to the growth rate and protein retention of red tilapia (*Oreochromis niloticus*).

Material and Method

Preparation of red tilapia (Oreochromis niloticus) and rearing conditions. This research was conducted from April to May 2017 at the Education Laboratory of the Airlangga University, Faculty of Fisheries and Marine, Surabaya, Indonesia. The proximate test of feed and protein retention test were conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The instruments used in this study were an aquarium measuring 45 cm x 30 cm x 30 cm, a set of aerators, digital scales, pH pens, thermometers, rulers, plastic bags, plastic hoses with a diameter of 1 cm, dissolved oxygen (DO) meters, and ammonia kits. The fish used were 140 red tilapia fry in healthy condition, measuring 5-6 cm total length, weighing 2-5 g head^{-1}. The red tilapia fry were obtained from the Gunung Sari fish market, Surabaya, East Java. Noni fruit extract was obtained from the result of squeezed noni fruit. The noni fruit used was ripe noni fruit, which is yellow and soft with a distinctive smell.

Experimental design. This research method was experimental. The primary test parameters in this study were protein retention, specific growth rate, feed conversion ratio, and survival rate of red tilapia. The variables observed were independent variables, dependent variables, and control variables. The independent variable is the dose of noni extract. The dependent variables are specific growth rate, feed conversion ratio, survival rate, and protein retention. Control variables are water quality (temperature, pH, DO, and ammonia). This study used a Completely Randomized Design (CRD) with four treatments and four replications, which treatments explained as follows:

P0: commercial feed as the control group without noni fruit extract + 2 g Progol (fish feed binder);
P1: commercial feed 1 kg + noni fruit 100 mL kg^{-1} feed + 2 g Progol;
P2: commercial feed 1 kg + noni fruit 300 mL kg^{-1} feed + 2 g Progol;
P3: commercial feed 1 kg + noni fruit 500 mL kg^{-1} feed + 2 g Progol.

The feed given in this study was PF 1000 commercial feed from PT Matahari Sakti, with a protein content of 39-41%, 5% lipid, 6% crude fiber, 16% ash, and 10% moisture content.

Preparation of the experimental diets. First, ripe noni fruits were cleaned with water, then washed with warm water. The noni fruits were crushed and then squeezed by using strain cloth. The squeezed noni fruit extract was put in a bottle and stored at room temperature.

The commercial feed was mixed with noni fruit extract and 2 grams of Progol with a different amount in each treatment, as previously explained. The commercial feed and noni fruit extract were smoothly mixed. The mixed feed was dried in an open room and was aerated until dry. After that, the dried food was stored in a plastic jar. The mixed fish feed with different doses were then analyzed through the proximate test in the laboratory. The nutrient content of the feed used in this study can be seen in Table 1 as follows.

Table 1

Nutrient content of feed with various doses of noni fruit extract

Analysis	*P0*	*P1*	*P2*	*P3*
	(Control)	*(100 mL kg^{-1})*	*(300 mL kg^{-1})*	*(500 mL kg^{-1})*
Dry ingredients (%)	90.1441	87.3484	86.7339	84.5036
Ash (%)	8.9591	8.7352	8.6875	8.5261
Crude protein (%)	29.1112	30.9015	30.2753	29.2539
Crude lipid (%)	8.3165	7.6176	6.9983	6.872
Crude fiber (%)	3.5026	2.488	2.6143	2.2593
Ca	2.5824	2.4109	2.4069	2.1481
Nitrogen-free extract (%)	40.2547	37.6061	38.1585	37.5923
Metabolism energy (Kcal kg^{-1})	3057.9622	2967.4828	2922.6623	2857.8501

The analytical procedure of the diets. The aquarium was then filled with 30 liters of water, aerated, and then left for 24 hours. The fish were selected based on a similar length and weight and its healthy condition. The fish were acclimatized for one week and were fasted for 24 hours before being treated. The fish were placed in fourteen aquariums according to the treatment given with a stocking density of 10 fish per aquarium. The fish were kept for thirty days and fed according to the treatment doses. Feeding was given three times a day: morning, afternoon, and evening, with a dose of 3% of body weight.

Below is the protein retention (PR) calculation based on a formula from Tung & Shiau (1991):

$$PR = \frac{Ln\ final\ body\ weight - Ln\ initial\ body\ weight\ (g)}{Total\ protein\ intake\ (g)} \times 100$$

The specific growth rate was calculated based on a formula from Effendie (1997):

$$SGR = \frac{LnWt - LnWo}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W0 = initial body weight (g);
Wt = final body weight (g);
T = treatment time (in days).

The daily length growth rate (LGR) was calculated based on a formula from Aggraeni & Abdulgani 2013):

$$LGR = \frac{LnLt - LnLo}{t} \times 100$$

where: LGR = daily length growth rate (% day^{-1});
Lt = average total length on day t (cm);
L0 = average total length on day 0 (cm);
t = total of observation time (in days).

The survival rate was calculated based on a formula from Wirabakti (2006):

$$SR = \frac{Nt}{No} \times 100$$

where: SR = survival rate (%);
Nt = the number of survived fish at the end of the experiment (in fish);
No = the number of survived fish at the start of the experiment (in fish).

The feed conversion ratio was calculated based on a formula from Council (1997):

$$FCR = \frac{F}{(Wt + D) - Wo}$$

where: FCR = feed conversion ratio;
Wo = the biomass weight of fish at the start of the experiment (g);
Wt = the biomass weight of fish at the end of the experiment (g);
D = total weight of dead fish (g);
F = feed intake (g).

Statistical analysis. Data analysis using Variant Analysis (ANOVA) with the study design was a Completely Randomized Design (CRD) to determine the differences in treatment. If there is a difference in treatment, Duncan's Multiple Range Test is applied.

Results. The calculation results of the average value of the specific growth rate, feed conversion ratio, and survival rate of red tilapia using statistical analysis after being treated for 30 days can be seen in Table 2.

Table 2

Average value of the specific growth rate, survival rate, feed conversion ratio, daily length growth, and protein retention of red tilapia

Treatment	*Specific growth rate (% day^{-1})*	*Survival rate (%)*	*Feed conversion ratio (%)*	*Daily length growth rate (% day^{-1})*	*Protein retention (%)*
P0	0.0105±0.000 7^{c}	100	2.712±0.588^{b}	1.070±0.189^{b}	7.3345±0.3434^{c}
P1	0.0135±0.0006^{b}	100	1.157±0.226^{a}	1.145±0.050^{b}	12.6246±0.3074^{b}
P2	0.0147±0.0005^{a}	100	0.846±0.234^{a}	1.435±0.186^{a}	11.5692±0.4054^{a}
P3	0.0150±0.0009^{a}	100	0.918±0.436^{a}	1.599±1.312^{a}	11.4289±0.7356^{a}

P0 (0% noni fruit extract), P1 (100 mL kg^{-1} noni fruit extract), P2 (300 mL kg^{-1} commercial feed with noni fruit extract), P3 (500 mL kg^{-1} commercial feed with noni fruit extract). Different superscripts show significant differences.

According to Table 2, the value of the specific growth rate (SGR) of red tilapia was 0.0105-0.0150% day^{-1}. The results of the statistical analysis of added noni fruit extract on commercial feed to the SGR of red tilapia showed significantly different results ($p < 0.05$). Duncan's Multiple Range Test (DMTR) results showed that the highest growth rate in this study was P3 (0.0150% day^{-1}) which was not significantly different from P2 (0.0147% day^{-1}), while it was significantly different with P0 (0.0105% day^{-1}) and P1 (0.0135% day^{-1}). The lowest specific growth rate was P0 (0.0105% day^{-1}) which was significantly different from P1 (0.0135% day^{-1}), P2 (0.147% day^{-1}), and P3 (0.0150% day^{-1}).

The feed conversion ratio (FCR) of red tilapia was 0.846-2.712%. The results of statistical analysis of the added noni fruit extract to commercial feed to FCR of red tilapia showed significantly different results ($p < 0.05$). Based on the results of DMTR, the highest FCR in this study was P0 (2.712%) which was significantly different from P1 (1.157%), P2 (0.846%), and P3 (0.9188%). While the lowest feed conversion ratio was P2 (0.846%) and was not significantly different from P1 (1.157%), P3 (0.918%), and significantly different from P0 (2.712%). These results indicate that the addition of noni fruit extract to commercial feed can reduce feed conversion ratio of red tilapia.

The survival rate of red tilapia from all treatments was 100%. The average survival rate of red tilapia showed no significant difference ($p > 0.05$). Based on the results of DMTR, the survival rates of P0, P1, P2, and P3 were not significantly different.

The value of the daily length growth rate of red tilapia for thirty days of preservation was 1.070-1.599% day^{-1}. The results showed significant differences ($p < 0.05$). The highest daily length growth value was at P3 (1.599% day^{-1}) which was not significantly different from P2 (1.435% day^{-1}) but was significantly different from P0 (1.070% day^{-1}) and P1 (1.145% day^{-1}). The lowest daily length growth rate was P0 (1.070% day^{-1}) which was not significantly different from P1 (1.145% day^{-1}) but significantly different from P2 (1.435% day^{-1}) and P3 (1.599% day^{-1}).

The results of the statistical analysis of the addition of noni fruit extract to commercial feed showed significantly different results ($p < 0.05$) to the protein retention of red tilapia. The results of DMTR showed that the highest protein retention value was P1 (12.6246%) which was significantly different from P0 (7.3345%), P2 (11.5692%), and P3 (11.4289%). Protein retention value in P2 (11.5692%) was not significantly different from P3 (11.4289%) but was significantly different from P0 (7.3345%) and P1 (12.6246%). The lowest protein retention value was P0 (7.3345%), which was significantly different from P1 (12.6246%), P2 (11.5692%), and P3 (11.4289%).

Data on the range of water quality parameters for red tilapia preservation for thirty days can be seen in Table 3.

Table 3

Water quality range value of red tilapia in 30 days of preservation

Parameter	*Range*
Temperature (^oC)	27.4-29.2
pH	7.2-7.9
DO (mg L^{-1})	3.3-5
Ammonia (mg L^{-1})	0-0.5

Discussion. The noni fruit extract mixed in the commercial feed had a significantly different effect on specific growth rate, daily length growth rate, feed conversion ratio, and protein retention of red tilapia for thirty days of preservation. These effects are due to the optimum absorption process or nutrient absorption of the feed in the digestive tract. Noni extract contains anthraquinone or acidic ingredients that can affect the pH of the digestive tract to be more acidic (Widianto et al 2015). The statement is right considering that in an acidic environment, protein-breaking enzymes (pepsin) can work optimally, so that protein intakes are absorbed more by the body which supports the optimal fish growth rate (Esminger et al 1990).

The extract of noni fruit contains proxeronine and xeronine. Proxeronine is converted to xeronine with the help of proxeronase enzymes. Xeronine is an active compound that has a role in facilitating metabolism inside the body of the animals and increasing nutrient absorption (Solomon 2001). Xeronine in noni fruit can function to expand the absorption in the intestine surface and facilitate the absorption process of food so the nutrients in the feed can be absorbed optimally (Wang et al 2008). The optimal absorption process will affect increased protein retention (Schlegel 1987; Frazier & Westhoff 1988; Cui et al 1992; Solomon 2001; Sjofjan 2003; Sujono 2006; Widianto et al 2015). Increased protein retention in the treatment results was caused by adding noni fruit extract in commercial feed, which can suppress the growth of microbial pathogens and increase non-pathogenic microbes in the digestive tract, resulting in the ability to decompose feed ingredients, especially crude fiber. The use of noni fruit extract can inhibit the growth of pathogenic bacteria in the intestine so that the number of non-pathogenic bacteria in the intestine was increased (Solomon 2001). Noni extract can increase the absorption process in the digestive tract and help decompose feed ingredients, especially crude fiber (Widianto et al 2015). Non-pathogenic bacteria can produce enzymes that digest crude fiber, protein, and lipid (Sjofjan 2003). *Bacillus* sp. bacteria in the digestive tract can break down proteins into amino acids (Sujono 2006).

These amino acids are used by bacteria to multiply themselves. Bacteria are a source of single-cell proteins so that the bacteria multiplication can increase protein feed and reduce crude fiber (Schlegel 1987).

Moreover, *Bacillus* sp. is a pectinolytic bacteria, which is an organism that can produce pectin or complex carbohydrates (Frazier & Westhoff 1988). The characteristics of *Bacillus* sp. bacteria are proteolytic, saccharolytic, and pectinolytic, which increases protein and carbohydrate feed. Protein retention indicates the amount of additional body protein from the consumed feed protein. The feed consumed was a source of protein used for preservation, metabolic activity, and growth (Cui et al 1992). The high protein retention in P1 treatment was caused by the increase in protein and carbohydrate content by the activity of non-pathogenic microbes that increase due to the addition of noni fruit extract in the feed. The only issue was the more noni fruit extract dosage in the feed cannot help at breaking down the crude fiber and result in higher crude fiber amount in the feed.

The feed conversion ratio is one of the standards to determine the quality of feed given to livestock in fulfilling the required nutrition. The higher the value of feed conversion, the worse the quality of the nutritional value of the feed. Noni fruit contains saponins which are bioactive in the growth of animals and digestive microbes. Giving saponins can increase the permeability of cell walls in the intestine, increase absorption of food substances hence result in higher conversion value (Önning et al 1996).

Survival rates of red tilapia were not significantly different between treatments. Thirty days preservation of red tilapia with the treatments given did not cause death in fish. This proves that adding noni fruit extract in commercial feed did not contain toxic ingredients that cause death. One study using noni fruit extract with a dose of 15,000 mg per kg body weight in experimental animals was not found any sign of toxic effects (Krishnakumar et al 2015). The study had performed surgery on experimental animals but still did not find any signs of toxicity in the animals' organs. Noni fruit is categorized as non-toxic substances (Wang et al 2008; Younos et al 1990).

Water quality in this study is an essential supporting parameter for the survival rate of red tilapia. Water as a living medium for fish that is preserved must meet the requirements. Water quality needs to be controlled in order to maintain its optimal conditions, thus creating an environment that is suitable for the habitat of red tilapia. The supporting parameters of this study are water quality parameters such as temperature, DO, pH, and ammonia. The temperature in this study averaged around 27.4-29.2^{o}C. This temperature is good enough for the survival of red tilapia. The optimal water temperature for red tilapia is 25-30^{o}C (Kungvakij & Pudadera 1984). Temperature is an environmental factor that influences the body metabolism speed. The optimum speed metabolism for fish will be achieved at optimal temperatures. The pH value has an essential influence on the survival of fish in the water. The pH in this study ranged from 7.2 to 7.9, which is quite adequate for the survival rate of red tilapia. The pH range required by fish is 6-9 (DeLong et al 2009). The average DO content in this study ranged from 3.3 to 5 mg L^{-1}. Based on these results, the DO during the study indicated a suitable level for the survival of red tilapia (Yildiz et al 2017). Thus DO in the preservation of red tilapia met the requirements. Ammonia in the water is derived from fish feces and from the rest of the feed that is not consumed by fish. In general, the concentration of ammonia contained in the water must not exceed 1 mg L^{-1} (BFAR-NFFTC 2000; Indian Council of Agricultural Research 2006). Ammonia during this study was 0-0.5 mg L^{-1}, which is suitable for the preservation of red tilapia.

Conclusions. Based on this research, it can be concluded that the addition of noni fruit extract in commercial feed can increase specific growth rate, daily length growth rate, lower the feed conversion ratio, and increase protein retention of red tilapia (*Oreochromis niloticus*). Moreover, the addition of noni fruit extract in commercial feed did not show any toxic effect on the survival rate of red tilapia during the thirty days of preservation.

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