The potency of synbiotics in improving the growth rate, feed conversion ratio, protein retention and lipid retention in Nile tilapia (*Oreochromis niloticus*)

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Abstract. Synbiotics are defined as the combination products of probiotics and prebiotics in supporting the growth of microorganisms in the host's digestive tract. The purpose of this research was to determine the effect of synbiotic addition in feed formulation towards the growth rate (length and weight), feed conversion ratio, protein retention and lipid retention of tilapia (*Oreochromis niloticus*). This research used a Completely Randomized Design with 5 treatments and 4 repetitions. The treatments were: T0 (Control), T1 (prebiotic 1% and probiotic 1%), T2 (prebiotic 1% and probiotic 2%), T3 (prebiotic 2% and probiotic 1%) and T4 (prebiotic 2% and probiotic 2%). The prebiotics used are commercial products and the probiotics used are *Lactobacillus casei* and *Lactobacillus rhamnosus*, in concentrations of 2×10^8 CFU g^{-1}. The data were analyzed with the Analysis of Variance (ANOVA). The results showed that the use of synbiotics significantly improved ($p<0.05$) the growth rate, length and weight of tilapia, but did not significantly improve ($p>0.05$) protein and lipid retention. Based on the results of the study, the use of synbiotics improve the growth rate of tilapia.

Key Words: FCR, growth rate, prebiotics, probiotics, synbiotic.

Introduction. One of the freshwater fish species with high potential in Indonesia is Nile tilapia (*Oreochromis niloticus*). Based on statistical data from the Ministry of Maritime and Fisheries, tilapia production in Indonesia has increased from 2011 to 2015 with an average increase ratio 22.41% (Ministry of Marine and Fisheries 2015). The efforts to increase tilapia production face some problems. One of them is the high price of feed, which can result in decreased fish production (Kurniati & Jumanto 2017). Feed costs in fish farming activities can reach 60-70% of production costs (Putra & Utomo 2015).

A synbiotic is defined as a synergistic combination consisting of probiotics and prebiotics in supporting the survival and growth of microorganisms in the host's digestive tract (Schrezenmeir & de Vrese 2001; Wang et al 2017). Synbiotic use is one of the safest and most effective solutions to reduce infection, especially by pathogenic bacteria. The promising effects of synbiotics are generally associated with the benefits of prebiotics, probiotic function, and combinational effectiveness of both. Synbiotics have a role in controlling the growth and pathogen infection of enteric bacteria (Peng et al 2019).

Probiotics are defined as food supplements of non-pathogenic living microorganisms. When consumed, they provide benefits to the health of the host. In increasing nutrient digestibility, probiotic microorganisms can be useful by producing exogenous enzymes (Wang et al 2017). Prebiotics are indigestible fibers that increase beneficial intestinal bacteria, improving the health of the host. The beneficial effects of prebiotics occur due to byproducts that come from commensal intestinal bacterial fermentation (Akhter et al 2015). Prebiotics are substrates that function as nutrients for the proliferation of probiotic microorganisms in the digestive tract (Schrezenmeir & de Vrese 2001).

Protein retention is an estimation of the ability of the body of fish to absorb and utilize proteins from feed, while lipid retention is an estimation of the ability of the body of the fish to absorb and utilize the fat consumed (Setiawati et al 2013). Synbiotics seem to increase protein retention by 30.47% and fat retention by 36.32% in Nile tilapia (Putra & Utomo 2015).

The study was conducted to determine the effect of synbiotic in formulated feeds on growth rates (length and weight gain), feed conversion ratio (FCR), protein retention and lipid retention in Nile tilapia.

Material and Method

Materials and treatments. The research was conducted in 2019, in the Faculty of Fisheries and Marine, University of Airlangga, Surabaya, Indonesia. The proximate analysis was carried out at the Laboratory of Animal Feed, Unit of Veterinary Services and Feed Analysis, Faculty of Veterinary Medicine, University of Airlangga, Surabaya, Indonesia. The main tools used in this study are aquariums with a capacity of 20 L, aerators, pH meter, hose aerators, filters, filter hoses, thermometer, ruler (accuracy 0.1 cm), digital scale (SF-400) with an accuracy of 1 g, bowls, measuring cups (Pyrex).

The main materials of this study were Nile tilapia, numbering 300 individuals with an average weight of 22.5±0.41 g. Other materials included fish feed with the following ingredients: fishmeal, soybean meal, green beans, tapioca flour, premix, corn flour, probiotics (*Lactobacillus rhamnosus* and *Lactobacillus casei*, with a concentration of $2x10^8$ CFU g^{-1} from the collection of Widya Paramita Lokapirnasari, Animal Husbandry Department, Faculty of Veterinary Medicine, University of Airlangga) and commercial prebiotics (oligosaccharides). The tilapia were purchased from Pasuruan, East Java, Indonesia. The tilapia were randomized with a Completely Randomized Design into 5 treatments (T0, T1, T2, T3, and T4), each treatment consisting of 4 replications, with 15 fish in each: T0 - control (standard feed); T1 - 1% prebiotic + 1% probiotic addition to standard feed; T2 - 1% prebiotic + 2% probiotic addition to standard feed; T3 - 2% prebiotic + 1% probiotic addition to standard feed; T4 - 2% prebiotic + 2% probiotic addition to standard feed.

Preparation of tilapia feed. Feed formulations were prepared by mixing all ingredients, namely fishmeal (30%), soybean meal (32%), green beans (15%), tapioca flour (3%), premix (1%) and corn flour (19%). Then the proximate analysis was conducted to determine the nutrient content. Afterwards, pellets with a diameter of 3 mm were produced (Standard National Indonesia 2006).

Synbiotic preparation. Synbiotics were prepared by culturing each synbiotic treatment into 1 L of water (chlorine free and antiseptic) in anaerobic conditions. The treatment was incubated for 24 hours, and was ready to be mixed into the feed (Lokapirnasari et al 2017). The synbiotic solution was sprayed into the feed and left to dry at room temperature for 24 hours, so that the synbiotic could be absorbed by the feed. Tilapia was fed twice daily at 7 am and 4 pm. The feed was administered in an amount of 3% of biomass (Shiau 2002).

Aquarium preparation. 20 aquariums with a capacity of 20 L were used. First, the aquariums were cleaned, after which filters and aerators were installed. The water in the tanks was aerated for 24 hours, then the tilapia were placed in the water.

Maintenance of tilapia. The tilapia was stocked in each aquarium with a density of 15 fish per aquarium (1.33 ind L^{-1}). For one week, the fish were normally fed and acclimatized to the new environment. Then, the tilapia were administered the synbiotic treatments for 28 days. Water quality parameters were also monitored. Temperature, pH and dissolved oxygen (DO) were measured daily from the beginning to the end of the experiment, using thermometer, pH meter and DO meter.

Data collection. Data collection was conducted once a week, in the morning. The data collected was: the average growth rate (length and weight gain), feed conversion ratio (FCR), protein retention and fat retention of tilapia. The growth rate was measured by using a ruler (0.1 cm accuracy) to determine the total length, and a digital balance (accuracy 0.1 g) to measure the total weight.

Length gain (cm) = final length (cm) – initial length (cm)

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

The protein and lipid retention were obtained from analyzing the meat of tilapia. 5 muscle samples from each treatment was tested with the proximate analysis to determine the protein and lipid content. Calculating the protein and lipid retention was carried out at the beginning and at the end of the study. The calculation of protein and lipid retention was conducted using the following formulas (Pirarat et al 2015; Ma et al 2015):

Protein retention (%) = 100 x [(% dry matter of sample at the end of experiment x % crude protein of sample at the end of experiment x final body weight) - (% dry matter of sample at the start of experiment x % crude protein of sample at the start of experiment x initial body weight)] / (% dry matter of feed x % crude protein of feed x feed intake)

Lipid retention (%) = 100 x [(% dry matter of sample at the end of experiment x % lipid of sample at the end of experiment x final body weight) - (% dry matter of sample at the start of experiment x % lipid of sample at the start of experiment x initial body weight)] / (% dry matter of feed x % lipid of feed x feed intake)

The FCR was determined using the next formula:

FCR = feed intake (g) / weight gain (g)

The experimental feed and the meat samples of fish were analyzed according to the standard methods of AOAC (1995) for dry matter, crude protein and crude fat determination.

Statistical analysis. All data were analyzed by using SPSS. The data were tested for normality and homogeneity using the Kolmogorov-Smirnov and Levene's test. If the data generated is normal and homogeneous, it is analyzed using one-way Analysis of Variance (ANOVA) (One Way ANOVA) with a significance level of 5%. If the data shows significant differences, then the data are analyzed with Duncan's Multiple Range Test (DMRT) to determine the differences among the treatments.

Results and Discussion

Effect of synbiotics use towards growth rate (length, weight, FCR) of tilapia. The length of tilapia from all experimental treatments was significantly different ($p<0.05$) than the length from control. The best length was obtained in T3 (13.17±1.34 cm), not significantly different ($p>0.05$) from T1, T2 and T4. The average values of length are presented in Table 1.

The result showed that the weight of tilapia was significantly higher ($p<0.05$) in treatments T1, T2, T3 and T4 compared to T0. The highest weight was obtained in T3 (35.36±6.48 g), not significantly different ($p>0.05$) than the weights from T1, T2 and T4. The average value of the weight is presented in Table 1.

The result of FCR showed that was no significant difference ($p>0.05$) among T0, T1, T2 and T4, but the better values of FCR of tilapia were present in T3 (1.35±0.22) and T4 (1.36±0.2). The average value of weight is presented in Table 1.

Table 1

Average value of length, weight and feed conversion ratio of Nile tilapia (*Oreochromis niloticus*)

Treatments	*Length (cm)*	*Weight (g)*	*FCR*
T0	10.65^{a}±0.65	25.19^{a}±4.06	1.48±0.25
T1	12.37^{b}±0.82	30.25^{ab}±2.98	1.56±0.19
T2	12.67^{b}±1.14	32.90^{b}±2.42	1.44±0.11
T3	13.17^{b}±1.34	35.36^{b}±6.48	1.35±0.22
T4	12.32^{b}±1.21	33.38^{b}±5.12	1.36±0.20

Note: T0 - control; T1 - 1% prebiotic + 1% probiotic addition to standard feed; T2 - 1% prebiotic + 2% probiotic addition to standard feed; T3 - 2% prebiotic + 1% probiotic addition to standard feed; T4 - 2% prebiotic + 2% probiotic addition to standard feed; different superscripts show significant differences ($p<0.05$).

Effect of synbiotics on protein and lipid retention of tilapia meat. The results of protein and lipid retention showed that there were no significant differences ($p>0.05$) among T0, T1, T2 and T4, but the highest value of protein retention of tilapia was present in T1 (3.066±0.939%), and the highest value of lipid retention was in T0 (0.809±0.126). Data on average protein and lipid retention is described in Table 2.

Table 2

Average value of protein and fat retention of Nile tilapia (*Oreochromis niloticus*)

Treatments	*Protein retention (%)*	*Lipid retention (%)*
T0	2.329^{a} ± 0.449	0.809^{a} ± 0.126
T1	3.066^{a} ± 0.939	0.545^{a} ± 0.250
T2	2.310^{a} ± 1.027	0.61^{a} ± 0.275
T3	2.490^{a} ± 0.233	0.368^{a} ± 0.566
T4	2.451^{a} ± 0.090	0.531^{a} ± 0.135

Note: T0 - control; T1 - 1% prebiotic + 1% probiotic addition to standard feed; T2 - 1% prebiotic + 2% probiotic addition to standard feed; T3 - 2% prebiotic + 1% probiotic addition to standard feed; T4 - 2% prebiotic + 2% probiotic addition to standard feed; same superscripts show no significant differences ($p>0.05$) between treatments.

The effect of synbiotics on the length and weight of tilapia. Based on the research, it can be said that the use of synbiotics has an effect on increasing the growth of tilapia in terms of length. The use of synbiotics was most influential in T3 (2% prebiotics and 1% probiotics), with 13.17 cm length and 35.36 g weight. In treatment T3, there were more prebiotics than probiotics, different from the other treatments. This condition could have possibly helped in increasing the growth traits.

Based on Table 1, it can be seen that the use of synbiotics in tilapia feed can increase the digestibility, so that it can increase the body length and weight. This can also occur because the microorganisms contained in probiotics play a role in improving metabolic products beneficial to the body of tilapia. Probiotics have varied effects depending on differences in fish species. Improved performance due to decreased stress, maintaining balance of microflora in the digestive tract, availability of nutrients and increased digestibility are some positive effects (Gatesoupe 2007; Kesarcodi-Watson et al 2008; Wang et al 2017; Pirarat et al 2015).

Probiotics act as an antimicrobial agent by secreting products called bacteriocins and substances such as organic acids (lactic, acetic and butyric acid) and H_2O_2 (De Keersmaecker et al 2006). Its antimicrobial properties can suppress the growth of *Escherichia coli* bacteria in the digestive tract. In addition, it can affect the density and length of villi, so that absorption of nutrients in the intestine is greater because of the wider intestinal surface. *L. casei* and *L. rhamnosus* are able to survive in the extreme acidic environment of the digestive tract and colonizes the intestinal lumen. These bacteria can balance microflora along the intestinal wall when working at various levels. After that, *L. rhamnosus* is able to produce acidic conditions, which are an unsuitable environment for pathogenic bacteria. *L. rhamnosus* works on the intestinal tract to inhibit pathogens from the surface wall of the small intestine. Decreasing pathogenic bacteria in the intestine causes the digestive system of tilapia to function better, so the absorption of feed will increase (Pirarat et al 2011; Prachom et al 2020; Negash & Tsehai 2020).

In addition to probiotics and prebiotics, the increasing growth rate of tilapia is also supported by good environmental conditions and appropriate water quality. This helps the proper function of synbiotics. Probiotic strains can inhibit pathogenic organisms by their competition for the limited substrates needed for fermentation. They prevent the adherence of pathogenic bacteria to host cells by strengthening the barrier effect of the intestinal mucosa (Eizaguirre et al 2002; Mangell et al 2002) and release gut-protective metabolites (arginine, glutamine, short-chain fatty acids and conjugated linoleic acids). Probiotic bacteria directly uptake or decompose organic matter or toxic material and improve the quality of water (Hemaiswarya et al 2013).

Several studies have shown that supplementation with probiotics greatly influences host growth, nutrient value and physiological functions in shrimp and fish (El-Rhman et al 2009; Hoseinifar et al 2010; Zokaeifar et al 2012; Nimrat et al 2012). This is because probiotics produce extracellular enzymes such as protease, amylase, lipase, cellulase and growth factors (Balcazar et al 2006; Hemaiswarya et al 2013). Enzymes can increase the activity of the digestive tract and feed digestibility. The existence of these probiotic mechanisms that have a role in the digestion of commercial feed in the intestine facilitate metabolism and growth processes of the host (Sahu et al 2008; Tzuc et al 2014). The presence of exogenous enzymes can help endogenous enzymes in hydrolyzing long chains of macromolecules such as carbohydrates, proteins and fats into simpler molecules, so that they will be more easily digested and absorbed by the intestine (Widanarni & Jusadi 2015).

The addition of prebiotics and probiotics in the composition of tilapia feed can affect growth factors of specific microflora by competing glycoconjugates, which are equivalent to epithelial cell walls, manipulating the pH of the digestive tract, inducing short chain fatty acid production and increasing mucus production. So that there is colonization of the microflora and probiotic bacteria to prevent adhesion and penetration of pathogenic microorganisms in intestinal epithelial cells (Akhter et al 2015).

The effect of synbiotic usage on the FCR value of tilapia. The FCR value is closely related to the growth of tilapia. A low FCR value indicates feed efficiency. This was presumably due to the addition of probiotics, which could improve the digestibility of feed. The high and low feed conversion ratio is influenced by several factors, especially the quality, and amount of feed, fish species, fish size and water quality. Feed efficiency describes the ability of fish to utilize feed optimally (Winfree & Stickney 1981; Omasaki et al 2017). The feed quality in this study was considered normal. The results of the analysis of the feed samples were: 90.32% dry matter, 7.81% ash, 30.76% crude protein, 9.05% extract ether, 10.27% crude fiber, 2893.92 Kcal kg^{-1} metabolizable energy. The addition of prebiotics in feed can increase the use of carbohydrates from feed more effectively, so that the use of feed protein is more efficient and provides a better response to the value of feed efficiency. This is because a feed deficient in carbohydrates, renders fish less efficient in using protein in metabolic needs. The use of carbohydrates can save protein. It is estimated that 0.23 g of carbohydrates per 100 g of feed saves 0.05 g of protein (Nanariain et al 2017). More digested protein means more body protein, which is reflected in protein retention, growth and feed conversion in tilapia. This is due to probiotics that can enhance exogenous enzymes activities, subsequently increasing the digestibility of feed, so that the digestion of feed nutrients will increase and growth will also increase. This was observed in the case of *Penaeus monodon* shrimp (Chandran et al 2014). *L. casei* and *L. rhamnosus* have high tolerance to the acidity of the stomach and bile salts, which is one of the important conditions that must be possessed by probiotic candidates (Kesarcodi-Watson et al 2008; Lokapirnasari et al 2017). When probiotics enter the body of aquatic organisms, they will pass through the stomach, which has acidic conditions and the presence of bile salts. Probiotics must also have antagonistic activities that can inhibit the growth of pathogenic bacteria in the digestive tract (Verschuere et al 2000).

Based on the results, the FCR value did not show significant differences between treatments, but it decreased in T3 and T4 compared to T0 and T1. The effects of prebiotics may vary depending on the concentration of the prebiotic in the diet, its solubility, the fish species, the water temperature and length of the feeding period (Dawood & Koshio 2016).

Effect of synbiotics use towards protein and lipid retention of tilapia. Based on the ANOVA test, the highest protein retention value is in T1 (3.066%), while T2 had the lowest protein retention value (2.31%). This is because the proteolytic enzymes produced by probiotics help the process of protein hydrolysis into amino acids, so that amino acids can be absorbed in the digestive tract and used to regenerate damaged cells and increase the growth of tilapia. This is in accordance with a previous study, which showed that the use of synbiotics was able to improve the performance of protein absorption towards fish growth (Putra & Utomo 2015).

Based on the ANOVA test, the highest lipid retention value was in T0 (0.809%), while treatment T3 had the lowest lipid retention value (0.368%). The low lipid retention value in each synbiotic treatment when compared with T0 is suspected to occur because *Lactobacillus* sp. contains the BSH gene. The mechanism of probiotics reduce cholesterol levels in the body through bile deconjugation due to bile hydrolase enzyme activity, binding of cholesterol to the cellular surface of probiotics and combining of cholesterol molecules into cellular probiotic membranes, production of short chain fatty acids from oligosaccharides, cholesterol co-precipitation with deconjugated bile and conversion of cholesterol to coprostanol (Lew et al 2018; Kriaa et al 2019). The *Lactobacillus* sp. contains the BSH gene and produces bile salt hydrolases, which break down amide bonds. Probiotics bile salts can hydrolyze conjugated bile acids to release free primary bile acids, which are not efficiently reabsorbed from the intestinal lumen and excreted in feces (Debbarma 2013; Suman 2015). However, if excess fat intake in the body will cause health problems, namely atherosclerosis (blockage of arteries) and liver lipoid disease (Roberts 2012; Rodwell et al 2015).

Water quality is a major factor in supporting fish growth. The water temperature during the study ranged from 26.3 to 30°C. This temperature is in accordance with the optimum growth temperature of tilapia, which is 20-35°C. The degree of acidity during the study ranged from 6-8, this pH value being in accordance with the optimal pH for growth. DO during the study ranged from 5.81 to 6.95 mg L^{-1}. The minimum DO level for the growth of tilapia is more than 0.5 mg L^{-1} (El-Sayed 2019).

Conclusions. The use of synbiotics containing *L. casei* and *L. rhamnosus* as probiotics and prebiotics in the feed of tilapia influenced the growth (length and weight) and the FCR value (especially in T3, with 2% prebiotics and 1% probiotics), but did not influence protein and lipid retention of tilapia.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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