Effect of Prebiotic and Probiotic Fish Feed on Physical, Chemical and Biological Quality of Feed

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Abstract

The limitations of fish in digesting food depend on the presence of enzymes protease, amylase, and lipase that react with substrates in the digestive channel of fish. Supplementation methods can be performed to increase fish growth by adding prebiotics and probiotics to the feed. This study aims to determine the influence of the difference in the length of time fermented prebiotic feed (sweet potato extract) and probiotics (*Bacillus megaterium*) on the quality of feed. The results of the physical analysis of fish feed showed the color looks brown, fishy smell, texture, and general conditions of feed seemed completely normal. Chemical analysis showed that the proteins were best improved in B3 treatment, with the period of fermentation approximately 72 hours. Biological analysis suggests that the longer the fermentation time taken, the higher the abundance of bacteria obtained.

Keywords: *B. megaterium*, chemistry and biology of fish feed, physical analysis, sweet potatoes *(Ipomoea batatas L)*

INTRODUCTION

Certainly, there are obstacles in doing cultivation activities, one of them is the decrease in feed quality caused by the limitations of fish in digesting food depending on the presence of enzymes that react with substrates in the fish digestive system. Therefore, additional ingredients are needed to increase the fish growth and the feed efficiency which are added into feed additives in order to minimize the production costs [1]. Normally, only 20-25% of protein is consumed by fish in intensive cultivation systems [2].

The limitations of fish in digesting food depend on the presence of enzymes protease, amylase, and lipase that react with substrates in the fish digestive system. Supplementation methods are able to be applied in order to increase fish growth by adding prebiotics and probiotics to the feed. The addition of prebiotics and probiotics into the feed with the process of fermentation may increase the quality of fish feed as the microorganisms assist in breakdown the difficult-to-digest substance into smaller pieces [3].

Prebiotics are generally carbohydrate compounds formed as oligosaccharides (oligofructose) and dietary fiber (inulin) [4]. Oligosaccharides are generally found in grains, nuts, and tubers, such as sweet potatoes. Oligosaccharides contained in sweet potatoes *(Ipomoea batatas L)* are maltotriose, raffinose, and oligofructose [5]. Meanwhile, probiotics in aquaculture have several advantages, such as improving the growth physically, contributing enzymes into nutrition, inhibiting the colonization of pathogenic bacteria in the gastrointestinal tract, modulating the intestinal microbiota, and improving the hematological and immune response [6]. Bacterium derived from the group of Bacillus spp. is *Bacillus megaterium* bacteria that are used as probiotics. Thus, the purpose of this study is to figure out the effect of the difference in the length of duration in making fermentation prebiotic feed (sweet potato extract) and probiotics (*Bacillus megaterium*) on the quality of feed.

MATERIAL AND METHOD

This research was conducted at the Laboratory of Technology of Fisheries Products Division of Food Safety, Faculty of Fisheries and Marine Sciences, Brawijaya University. This study used commercial feed of fish with a protein content of 32%. Prebiotics used are derived from sweet potato extract with total dissolved solids (TPT) of 5%., while probiotics with density 10^8 CFU.mL^{-1} [5].

This research uses a Complete Factorial Randomized Design (RALF), which consists of two factors with six treatments and three replays. Factor A with addition (prebiotics by 1% [7] + probiotic *Bacillus megaterium* of 20 mL.kg^{-1} of feed [8] at a concentration of 10^8 CFU.mL^{-1} with a fermentation time of 24, 48, and 72 hours), and factor B (prebiotics by 2% [9] + probiotic *Bacillus megaterium* of 20 mL.kg^{-1} of feed [10] at a concentration of 10^8 CFU.mL^{-1} with a fermentation time of 24, 48, and 72 hours).

The analysis data is obtained from research such as biological parameters (abundance of

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bacteria in fermented feed) where it is processed by using fingerprint analysis (ANOVA) in accordance with the Complete Factorial Random Design (RALF) using Microsoft Excel 2013. If there is a real effect, then the next step is the Smallest Real Difference (SRD) test with a confidence level of 95%. Physical parameters (color, aroma, texture, and general condition of feed) and chemical (fermented feed proximate) are analyzed descriptively.

RESULT AND DISCUSSION

Physical Analysis

Physical observation consists of the color, aroma, texture, and general condition of prebiotic and probiotic fish feed against physical qualities based on the different fermentation time lengths. The results of the study are shown in Table 1. It shows the physical condition of feed that has not changed in the physical state for the treatment of factors A and B compared to control. Factors A and B with fermentation time from 24 to 72 hours, on the color, parameters indicate the physical state of brown feed. Feed aroma has a distinctive smell such as the fishy smell of fish, normal texture, not soft and easily destroyed, and for the general condition, the feed shows the normal conditions with feed conditions that do not clump and unmold. Based on the results of the previous study [3], feed containing probiotics with time of fermentation of seven days did not experience physical changes. It was resulting in a normal texture, unsoft and easily destroyed with brown feed color, feed aroma is most likely a fishy smell of fish and general condition feed does not clump, and there are no fungi.

The good quality of the feed, physically, has a distinctive smell, brown color, and no fungi [10]. Feed that undergoes fermentation process for 22 to 34 hours has a brown color [11]. The standard aroma and color of fishmeal that is suitable for use are that it has a distinctive aroma such as fishy smell and the color looks mostly like yellowish-brown to dark brown [12].

Chemical analysis

Based on the fish feed proximate analysis method [13] in Table 2, the change in nutrient content in the feed that is added prebiotics and probiotics during the fermentation process, occur from the hour of 24th to 72nd. Ash content in factor A decreased after fermentation of the hour of 24th to 72nd from 9.67% to 9.45%, while factor B of the hour 24th, ash content by 9.69% increased in 48 hours to 9.72 and decreased in 72 hours by 9.53%. but the results of the analysis of ash levels in a whole, both factors A and B decreased compared to the treatment without the addition of prebiotics and probiotics and did not undergo the fermentation process. The decrease in ash content is caused by the activity of microbial growth due to the fermentation process that occurs in feed. Good ash content as feed is when it has low ash content because it will be easy to digest by the fish [14]. In protein, fats, and carbohydrates increased after fermentation in both factors A and B. Protein content increased from 32.14% (before fermentation), increased at the hour of 24th in factor A by 32.60% and factor B by 33.58%, by the hour of 48th, it is increased in factor A by 32.90% and factor B by 33.95% and increased again until the hour of 72nd in factor A by 33.13% and factor B by 34.14%.

Table 1. Physical analysis of fish feed

Parameters	No Fermentation	Fermentation Time (hours)					
		A1 (28 hours)	A2 (28 hours)	A3 (72 hours)	B1 (48 hours)	B2 (72 hours)	B3 (72 hours)
Color	Brown	Brown	Brown	Brown	Brown	Brown	Brown
Aroma	Feed Aroma	Feed Aroma	Feed Aroma	Feed Aroma	Feed Aroma	Feed Aroma	Feed Aroma
Texture	Normal	Normal	Normal	Normal	Normal	Normal	Normal
General Conditions	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Table 2. Chemical analysis (fermented feed proximate)

Feed (%)	No Fermentation	Fermentation Time of Feed (hours)					
		A1 (24 hours)	A2 (48 hours)	A3 (72 hours)	B1 (24 hours)	B2 (48 hours)	B3 (72 hours)
Ash	9.96	9.67	9.50	9.45	9.69	9.72	9.53
Coarse Protein	32.14	32.60	32.90	33.13	33.58	33.95	34.14
Fat	6.29	7.08	7.09	7.59	7.00	7.06	7.34
Carbohydrates	49.45	50.06	50.12	50.91	50.27	50.73	50.99

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Based on these results, it can be concluded that the highest protein content is found in factor B, with a fermentation time of 72 hours of 34.14%. The increase of crude protein in fermented feed as a result of the growth process of microbial cells that develop in feed is seen in Table 3. The possibility of crude proteins to increase is due to the increasing growth of cells of the microbes during the fermentation process [15]. The growth of probiotic bacteria cannot be separated by prebiotics because probiotics desperately need prebiotics to spur their growth [16]. It is suspected that in the fermentation process, there is exogenous enzyme activity by means of macromolecular hydrolysis reactions into simpler molecules such as proteins into amino acids. Microorganisms that can adjust to the surrounding environment are rich in complex molecules by secreting exogenous enzymes by catalyzing macromolecular hydrolysis reactions into simpler molecules such as proteins into amino acids [17].

Fat content increased from 6.29% (before fermentation), it increased at the hour of 24th in factor A by 7.08% and factor B by 7.00%, by the hour of 48th, it increased in factor A by 7.09% and factor B by 7.06%, then it increased for more at the hour of 72nd in factor A by 7.59% and factor B by 7.34%. The increasing levels of fats are suspected due to the microorganisms that are occurred from living cells are able to produce microbial oil or fat. During the fermentation process, the fat content increases due to microorganisms that are able to produce microbial oil, where microorganisms occurred from the living cells are able to produce lipids or fats [18].

Carbohydrate content increased from 49.45% (before fermentation), increased at the hour of 24th in factor A by 50.06% and factor B by 50.27%, at the hour of 48th, it increased in factor A by 50.12% and factor B by 50.73% and increased for more at the hour of 72nd in factor A by 50.91% and factor B by 50.99%. Increased carbohydrate levels are caused by carbohydrate content i.e. oligosaccharides derived from sweet potato extract can affect the growth of *Bacillus megaterium* bacterial activity during the fermentation process. It is suspected that with the addition of oligosaccharides, bacteria will grow to the maximum and the activity of exogenous enzymes that work in the fermentation process is catalyzed macromolecular hydrolysis reactions into simpler molecules, such as polysaccharides into sugars. Probiotic bacteria can produce exogenous enzymes such as cellulase, amylase, lipase, and protease [19].

Based on the analysis of feed chemically, it can be concluded that fermentation technology conducted with the addition of prebiotics (sweet potato extract) by 2% and probiotics (*Bacillus megaterium*) as much as 10^8 CFU.mL^{-1} in the feed may increase the content of nutritional level ssuch as proteins, fats, and carbohydrates that can be used as animal feed or fish. Fermentation is a process of breaking down organic compounds into simpler compounds by involving microorganisms. This organic compound consists of carbohydrates, fats, proteins, and other organic matters undergoing a process of chemical changes in an aerobic and anaerobic state through the work of enzymes produced by microbes [20]. Protein levels increase because microbes have the ability to convert complex proteins into simple compounds such as amino acids with the help of protease enzymes. High nutritional levels among others will undergo the fermentation process first by the process of breaking down the food ingredients that contain fats, carbohydrates, and proteins, which are difficult to digest to be easier to digest, and there is a distinctive smell and aroma [21].

Biological Analysis

$SRD_{0.05}$ test results of the abundance of bacteria at the hour of 24th, 48th and 72nd, after the fermentation process of feed showed a noticeable effect on the length of time fermentation of feed containing prebiotics and probiotics. The abundance of bacteria on the main effect of feed fermentation time showed the treatment 3 (72 hours) differed markedly higher than the other treatments. In interactions between B3 treatment factors differed markedly higher if compared to the other treatments.

The duration of fermentation of prebiotic administration at a dose of 1% with probiotics of 20 mL.kg^{-1} affects 97% (coefficient of determination = 0.97) against the abundance of bacteria. Meanwhile, the administration of prebiotic doses 2% with probiotics of 20 mL.kg^{-1} affects the abundance of bacteria by 85% (coefficient of determination = 0.85) seen on the chart, where it can be concluded that the longer the the duration of fermentation time taken, the higher the abundance of bacteria obtained.

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Table 3. $SRD_{0.05}$ test result of bacterial abundance 10^{-4} (CFU.mL^{-1}) in fermented feed

Effect of Single Dose	The Influence of Single Time ($SRD_{0.05}$ = 20.96)			Main Effects of Fermentation Doses ($SRD_{0.05}$ = 14.82)
	1 (24 hours)	2 (48 hours)	3 (72 hours)	
A (1%)	65[a]	118[b]	179[c]	121[a]
B (2%)	271[d]	441[e]	477[f]	396[b]
The Main Influence of Fermentation Time ($SRD_{0.05}$ = 12.10)	168[a]	279[b]	328[c]	

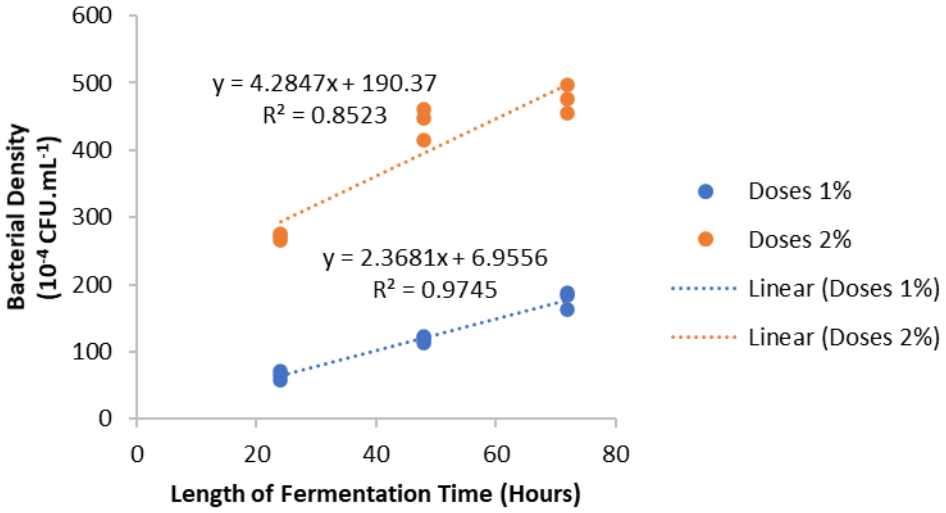

Figure 1. Abundance of fermented feed bacteria

The length of fermentation time is directly related to the growth of microbes that will undergo a phase that changes every time and optimizes the temperature. Extending the fermentation duration time in the fermentation process may be able to provide opportunities for microbes to remodel the components inside the substrate into simpler components to digest. The microbes will undergo a change in growth that binds directly with the increase in the number of microbes as they increase in the number of cells by utilizing the nutrients that have been broken down into simpler sugars forms which can be used as a source of energy [22].

Based on the results of this study, B3 treatment has the highest content of feed ingredients such as protein (43%), fat (7.34%), and carbohydrates (50.99%). According to Indonesian National Standards (SNI) [23], SNI 01-7242-2006 protein content of 25-30% and fat content of at least 5% in tilapia enlargement. Feed ingredients necessary for optimal growth and health of fish such as protein (38-42%), carbohydrates (30-40%), and fat (7-15%) [24].

CONCLUSION

The length of time of fermented fish feed, which is added prebiotics and probiotics, has a noticeable effect on the abundance of bacteria. The length of fermentation time is good for the physical, chemical, and biological state of feed i.e. B3, with a fermentation time of 72 hours that can increase protein and bacteria number to be more abundant.

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