The number of lactic acid bacteria, growth, and feed utilization in catfish (*Pangasianodon hypophthalmus*) with the addition of banana peel flour

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Abstract. Banana peel is a waste that is not widely used in aquaculture. The dietary fiber content in banana peels has the potential as a prebiotic in fish. This research aims to analyze the effect of adding Kepok banana (*Musa acuminata* x *Musa balbisiana*) peel flour (KBPF) on the number of lactic acid bacteria, growth performance, feed utilization, and survival rate of catfish (*Pangasianodon hypophthalmus*) seeds. This research consists of four treatments with three replications, namely the addition of KBPF at 0%, 1%, 2%, and 3% in the catfish diet. Catfish fry of 30 sizes 1.43±0.08 g is reared in plastic containers with a volume of 40 L each with 15 fish per container. Fish were fed *ad libitum* in the morning and evening according to the treatments. The results show that the number of lactic acid bacteria in the intestine increased significantly with the addition of KBPF compared to the control. The best growth performance and feed utilization are shown in the addition of 1-2% KBPF to diet. The highest survival rate was shown in the addition of 1% KBPF in the diet at 97.78%, and the lowest was in the control treatment at 86.67%. These results indicate that KBPF can be used as a prebiotic at the beginning of catfish cultivation.
Key Words: feed additive, fish cultivation, Kepok banana peel, prebiotic.

Introduction. Kepok banana (*Musa acuminata* x *Musa balbisiana*) is widely grown in East Kalimantan Province, Indonesia. Kepok banana production is quite high in this area, reaching 1,340 tons and 1,650 tons per month in 2021 and 2022, respectively. This banana has a distinctive sweet taste, is very popular in the domestic market, and has been exported to various countries, such as Malaysia and Singapore (Rohmah 2022). Banana peel, which is considered waste, accounts for around 1/3 of a whole banana. The peel weight is around 25-40% of the Kepok banana (Koni et al 2013; Wadhwa et al 2015). This, of course, makes Kepok banana peels have the potential to become a large amount of organic waste. On the other hand, Kepok banana peel contains nutrients that are good enough to be used as animal feed, namely moisture of 8.91%, ash of 8.85%, protein of 7.08%, fat of 12.51%, and dietary fiber of 51.93% (Anwar et al 2021). Moreover, Kepok banana peel has quite a high antioxidant activity, namely 95.14% (Supriyanti et al 2015). Banana peel comprises a high content of dietary fiber, principally insoluble dietary fiber (11.04%) and saturated fatty acids, which constitutes 40-50% of the fatty acids. In addition, banana peel is rich in phytochemicals with potential antioxidant properties, essential amino acids, and vitamins (Venkateshwaran & Elayaperumal 2010; Pelissari et al 2013).

Prebiotics as feed additives are commonly utilized in aquaculture and have demonstrated promising outcomes in enhancing fish growth and well-being. Prebiotics are any substance, fiber, long-chain sugar, vitamin, or substrate that the host's digestive system uses as food for good microbes (Mountzouris 2022). Another name for prebiotics is a source of nourishment for beneficial bacteria in the digestive system (Tran & Li 2022). Fruit, nuts, and vegetables are the primary plant sources of prebiotics found in

nature. β-glucan, inulin, arabinoxylan oligosaccharide (AXOS), mannan oligosaccharide (MOS), galactooligosaccharide (GOS), fructooligosaccharides (FOS), and oligosaccharides are among the nutritional components that are frequently employed as prebiotics in aquaculture. According to Wee et al (2024), the fish's immune system can be strengthened by this prebiotic, which will accelerate growth and increase feed consumption efficiency.

Prebiotic oligosaccharides may be produced in part by the banana peel's total oligosaccharide content (142 mg/100 g dry weight) (Pereira et al 2017). The use of banana peel as a prebiotic source in fish rearing has produced fruitful outcomes. By raising the quantity of lactic acid bacteria in the intestines of Nile tilapia (*Oreochromis niloticus*), Ambon banana (*Musa acuminata*) peel flour at a dose of 2% kg^{-1} diet might promote growth and feed utilization (Susanto & Agustina 2023). According to Mir and Singh (2019), snakehead fish (*Channa striata*) grew and survived better when given 6% of banana flour as a prebiotic. At a dosage of 5% in the diet, adding banana peel flour (*M. acuminata*) as a prebiotic increased the weight and specific growth rate of rohu fish (*Labeo rohita*) at the end of rearing (Giri et al 2016).

Obstacles to the growing catfish (*Pangasianodon hypophthalmus*) cultivation include low feed utilization and a high requirement for commercial feed. The use of Kepok banana peel flour as a source of prebiotics in catfish cultivation could increase production. Therefore, this research analyzes the effect of Kepok banana peel flour on the number of lactic acid bacteria in the intestine, growth performance, feed utilization, and survival rate of catfish seeds.

Material and Method. This research was conducted for 3 months, namely in September-November 2023, which included material preparation, fish maintenance and data processing. Preparation of test feed and fish maintenance were carried out in the Fish Nutrition Laboratory, and the number of lactic acid bacteria measurements were carried out in the Microbiology and Aquaculture Biotechnology Laboratory, Faculty of Fisheries and Marine Science, Mulawarman University, Samarinda, East Kalimantan, Indonesia.

Preparation of fish and Kepok banana peel flour. Catfish seeds of 1.43 ± 0.08 g were obtained from a local hatchery. The fish were adapted for five days in plastic containers with a diameter of 1 m. Fish were given commercial feed *ad libitum* in the morning and evening. Fish were fasted one day before treatment. Kepok banana peel flour (KBPF) came from ripe Kepok bananas sold in the local market. After peeling, bananas were thoroughly cleaned to get rid of any debris that clung to the peel. After cleaning, the banana peel was cut into small pieces and dried for 12 hours at 60 ± 5^{O}C in an oven. A flouring machine was used to grind the dried banana peels until they became fine flour and kept in a tight container for use in further tests (Gupta et al 2020).

Diet preparation. The feed used in this research was commercial pellets with a protein content of 38%. As stated by Susanto and Agustina (2023), the pellets are crushed and then combined with a dose of 0% (control), 1%, 2%, and 3% of Kepok banana peel flour. Water is added as much as 20% to this mixture and then the pellet machine is used to form 2 mm sized pellets. After that, the formed pellets were dried for two hours at 50^{O}C in an oven, and the dehydrated pellets were stored in a tight container.

Treatment design and fish culture. This study used a completely randomized design, with four treatments and three replications consisting of adding KBPF at doses of 0%, 1%, 2%, and 3% in the diet. Fish were reared in plastic containers filled with 40L water for 30 days. Each container was filled with 15 catfish. Treatment feed was given twice a day in the morning and evening with *ad libitum*. To maintain the water quality in the fish-rearing container, siphoning is carried out every day, and adding as much water as is released during siphoning. During the experiment, the water temperature ranged between 28.3-28.8^{o}C, pH ranged between 6.9-7.3, dissolved oxygen ranged between 4.75-5.20 ppm, and total ammonia nitrogen ranged between 0.06-0.07 ppm.

The number of lactic acid bacteria in fish intestines. The number of lactic acid bacteria (LAB) in the intestines of catfish was measured at the beginning and end of rearing. Using Yunita et al (2015) technique, fish intestinal samples were cultured in MRS agar media.

Fish growth performance. Fish growth was measured based on weight at the beginning and end of rearing. Growth performance parameters include weight gain, specific growth rate, average daily growth, and relative growth rate of catfish during 30 days of rearing.

Feed utilization and survival rate. Parameters for catfish feed utilization include feed consumption, feed efficiency, feed conversion ratio, and protein efficiency ratio, referring to Morais et al (2001). Survival rate was measured based on the number of fish that live until the end of rearing divided by the number of fish at the beginning multiplied by 100%.

Statistical analysis. The Bartlett test was used to check for homogeneity in all of the data. One-way ANOVA was used to assess the differences between the treatments, and Duncan's post hoc test was then performed. A difference was considered significant if $p<0.05$.

Results. Aquaculture's need for feed is quite large, meaning that innovations related to using natural ingredients as feed additives continue to be carried out. Fruit peel is an alternative nutritional source that is widely studied in aquaculture (Giri et al 2016; Gupta et al 2020). Kepok banana peels thrown away have become interesting research material regarding their benefits as feed additives in catfish cultivation. The results of research on adding KBPF to catfish diets based on several test parameters show positive results, as described below.

The number of lactic acid bacteria in catfish intestines. Adding Kepok banana peel flour (KBPF) to catfish feed at different doses significantly affected the number of lactic acid bacteria (LAB) in their intestines. Figure 1 shows that the number of LAB in the 1, 2, and 3% KBPF treatments in the diet were higher than the control, respectively 4.40, 4.35, 4.46, and 3.64 log CFU mL^{-1} ($p<0.05$). In general, there was an increase in the number of LAB in the intestines of catfish during 30 days of rearing in both the control treatment and the KBPF addition treatment, each amounting to 0.83, 21.98, 20.59, and 23.64% (Figure 2). The increase in the number of LAB in the intestines of catfish was greater with the addition of KBPF. This shows that KBPF in feed can be utilized by LAB as a source of nutrition, so the amount has increased significantly compared to the control.

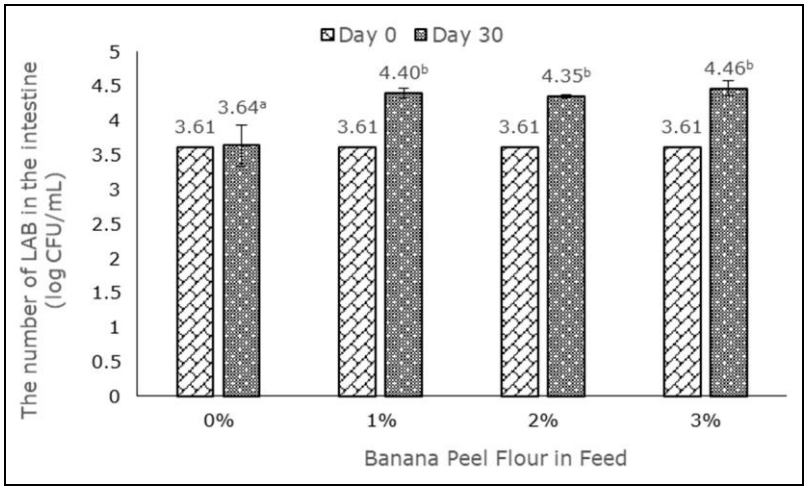

Figure 1. The number of lactic acid bacteria in the intestines of catfish during observation. Data is presented as mean ± SD. The different superscript letter within the same column shows a significantly different effect ($p<0.05$).

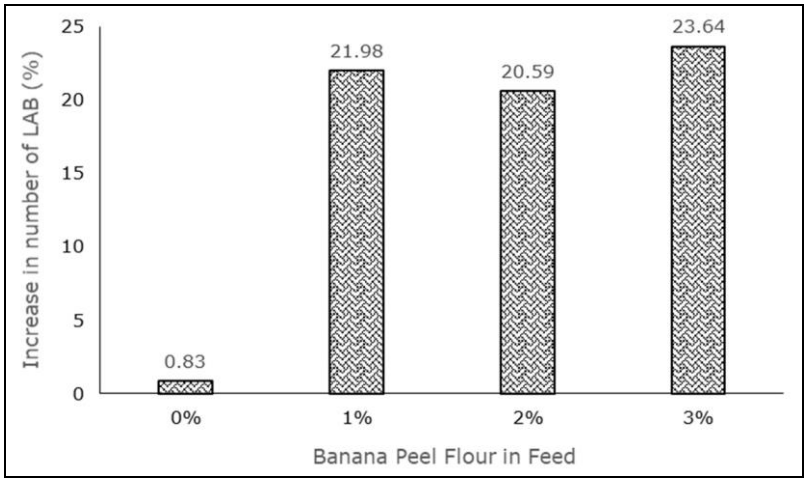

Figure 2. Percentage increase in the number of lactic acid bacteria in the intestines of catfish during rearing

Fish growth performance. In general, the addition of KBPF at different doses in the diet significantly affected the growth performance parameters of catfish during 30 days of rearing (Table 1). The weight gain and average daily growth of catfish in the treatment with the addition of 1 and 2% KBPF in feed was significantly different from the control but not significantly different from the 3% dose ($p>0.05$). Meanwhile, the specific growth rate and relative growth rate of catfish in the treatment with the addition of 1-3% KBPF in the diet were significantly different from the control ($p<0.05$).

Table 1

Growth performance of catfish with the addition of Kepok banana peel flour (KBPF) in the diet

Parameters	*0%*	*1%*	*2%*	*3%*
Initial weight (g)	1.48±0.01^{a}	1.44±0.13^{a}	1.40±0.07^{a}	1.39±0.03^{a}
Final weight (g)	3.11±0.18^{a}	3.57±0.34^{a}	3.47±0.25^{a}	3.26±0.09^{a}
Weight gain (g)	1.63±0.18^{a}	2.13±0.23^{b}	2.07±0.23^{b}	1.87±0.06^{ab}
Specific growth rate (%/day)	2.45±0.18^{a}	3.03±0.14^{b}	3.02±0.22^{b}	2.85±0.02^{b}
Average daily growth (g/day)	0.81±0.09^{a}	1.07±0.11^{b}	1.03±0.11^{b}	0.94±0.03^{ab}
Relative growth rate (%)	3.64±0.38^{a}	4.95±0.35^{b}	4.92±0.53^{b}	4.50±0.64^{b}

Note: Data is presented as mean ± SD. The different superscript letter within the same column shows a significantly different effect ($p<0.05$).

Feed utilization and survival rate. During 30 days of catfish rearing in this study, feed utilization performance was significantly influenced by the addition of KBPF at different doses in the diet ($p<0.05$). The same thing was shown by the survival rate of catfish, where there was a significant difference between the treatment with the addition of KBPF in the diet and the control ($p<0.05$). Table 2 presents the average values of feed utilization parameters consisting of feed consumption, feed efficiency, feed conversion ratio, protein efficiency ratio, and the survival rate of catfish.

Table 2

Feed utilization and survival rate of catfish with the addition of Kepok banana peel flour (KBPF) in the diet

Parameters	*0%*	*1%*	*2%*	*3%*
Feed consumption (g)	40.97±2.39^{a}	43.74±0.97^{a}	40.11±4.23^{a}	39.92±1.32^{a}
Feed efficiency (%)	59.72±9.83^{a}	73.00±6.25^{b}	77.24±2.39^{b}	70.49±2.60^{ab}
Feed conversion ratio	1.70±0.26^{b}	1.37±0.12^{a}	1.29±0.04^{a}	1.42±0.05^{a}
Protein efficiency ratio (%)	1.57±0.26^{a}	1.92±0.16^{b}	2.03±0.06^{b}	1.86±0.07^{ab}
Survival rate (%)	86.87±6.67^{a}	97.78±3.85^{b}	95.55±3.85^{ab}	93.33±6.67^{ab}

Note: Data is presented as mean ± SD. The different superscript letter within the same column shows a significantly different effect (p<0.05).

Discussion. Among prebiotics characteristics, they selectively stimulate beneficial bacteria in specific amounts (Teng & Kim 2018). The increased number of beneficial bacteria in the fish intestine indicates the ability of feed additives to act as prebiotics. Fiber is one of the "prebiotic compounds" that is mainly supplied from the cell walls of fruits, vegetables, and grains and includes polysaccharides, oligosaccharides, and lignin (Zhang et al 2005). Kepok banana peels have a fiber content of 51.93% (Anwar et al 2021), while Gupta et al (2020) found that the fiber content of banana peels is around 29.52%. The fiber content in Kepok banana peels plays a role in increasing the number of lactic acid bacteria in the intestines of catfish.

According to Pereira et al (2017), banana peels had a total oligosaccharide concentration of 142 mg/100 g dry weight. Interestingly, the oligosaccharides from banana peels greatly boosted the growth of *Lactobacillus plantarum* TISTR2075, but did not stimulate the development of pathogenic *Escherichia coli* (Pereira et al 2021). Three commercial probiotic strains: *L. rhamnosus*, *L. casei*, and *Bifidobacterium lactis*, showed notable growth in culture media supplemented with 2% powdered banana peels, according to Zahid et al (2021). The most prevalent and extensively researched probiotic lactic acid bacteria (LAB) are *Lactobacillus* spp. and *Bifidobacterium lactis*. According to studies on catfish, adding Ambon banana peel flour at a dose of 1-3% kg^{-1} diet resulted in a considerable rise in the quantity of lactic acid bacteria in the intestines of Nile tilapia (*Oreochromis niloticus*) (Susanto & Agustina 2023).

Fish growth is the increase in weight and length of the fish body in a given period. Banana peel is expected to provide an energy source for catfish because it contains high carbohydrates, namely around 62.65% (Anwar et al 2021). Leucine, valine, phenylalanine, threonine, and tryptophan are among the nutrients found in banana peels (Emaga et al 2007). In this study, this macronutrient contributed to the growth of the catfish. Esteban (2012) states that feed including non-protein energy sources, like lipids and carbs, may contribute to lower protein energy use. This includes a mechanism to reduce protein catabolism to be used for energy, thereby increasing protein retention and ultimately increasing animal growth.

In the local community, Kepok banana peels are mostly thrown away when the bananas are ripe, and then the ripe bananas are used as processed food products. Facts show that there is a higher increase in most banana capacities, such as soluble sugars, proteins, lipids, and antioxidants, after the fruit ripens (Emaga et al 2007). In this study, the Kepok banana peel used was also ripe, so it is suspected that the sugar, protein, and lipid content as an energy source for catfish was better than the control. Moreover, Palintorn et al (2019) found that tilapia fish fed with an additional 1%, 5%, and 10% kg^{-1} diet of ripe Kluai Nam Wa banana showed higher weight gain, average daily growth, and specific growth rate compared to the control.

In *Labeo rohita*, the addition of banana peel flour at a dose of 5% in the diet showed the best growth performance, namely weight gain and specific growth rate of 40.97 g and 3.14%/day, respectively (Giri et al 2016). Susanto and Agustina (2023) found that the addition of 1-2% kg^{-1} diet of Ambon banana peel flour in feed showed the best growth performance in Nile tilapia (*Oreochromis niloticus*). An increase in the number of lactic acid bacteria in the activity of digestive enzymes in the intestines of Nile

tilapia with the addition of the Ambon banana peel flour in the diet supports these results. In this study, catfish showed a greater increase in the number of lactic acid bacteria with the addition of Kepok banana peel flour (KBPF) in the diet when compared to the control (Figure 2). This is thought to be the influence of fiber from KBPF, which acts as a prebiotic that is a source of nutrition for microbes in the digestive tract of catfish. The presence of lactic acid bacteria in the digestive tract has a beneficial effect on fish with its ability to ferment dietary fiber in feed, thereby increasing the activity of digestive enzymes and ultimately increasing the absorption of nutrients from feed. In accordance with the opinion of Fuentes-Zaragoza et al (2010), prebiotics promote full body absorption of food, resulting in rapid growth and health.

The increase in feed efficiency and the decrease in FCR value in the treatment where KBPF was added, especially in the treatment with 2% KBPF in the diet, shows that the catfish could utilize the feed better than in the control treatment. The oligosaccharide content in banana peel flour is thought to help increase digestive activity and absorption of nutrients in the feed so that the efficiency value and feed conversion ratio are better than in the control treatment. Oligosaccharides are a component that functions as a prebiotic in fish (Wee et al 2024). The addition of prebiotics to the diet is intended to increase the microbiota population in the host's digestive tract so the working mechanism of the microbiota in producing enzymes for digestion (Merrifield et al 2010) and will increase the number of nutrients to be absorbed by the fish. Prebiotics can also increase feed utilization efficiency. Therefore, it can reduce the feed conversion rate (FCR) and increase the growth rate of aquatic animals (Ganguly et al 2013). The results of this study are in line with the addition of ABPF at a dose of 2% kg^{-1} of the diet, which can produce better feed utilization in Nile tilapia (Susanto & Agustina 2023). In tilapia, the combination of two oligosaccharide components, namely xylooligosaccharide (XOS) and galactooligosaccharide (GOS), was able to increase feed utilization by reducing the feed conversion ratio, going 20.2% lower than the control (Xu et al 2022).

Adding KBPF in the feed resulted in a better survival rate for catfish compared to the control, namely a dose of 1% in the diet. The survival rate is closely related to the health condition of the fish. Banana peels contain antioxidants and antibacterials that can support health during cultivation. Banana peel and seed fractions include more phenolic compounds and antioxidants than the pulp (González-Montelongo et al 2010). Ascorbic acid, phenolic groups, tocopherol, dopamine, beta carotene, and gallocatechol are the most frequently occurring antioxidant components in banana peels (Bankar et al 2010).

Banana peels contain bioactive substances such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, anthocyanins, and terpenoids. These substances have been shown to have a variety of biological and pharmacological effects, including antibacterial and anti-inflammatory properties (Pereira & Maraschin 2015). Ibrahim (2015) discovered that *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* are all susceptible to the antibacterial effects of banana peels. This suggests that including banana peels into fish diet may help lower the risk of diseases brought on by these microorganisms. Furthermore, research has demonstrated that giving fish banana peels improves their immune system. Giri et al (2016) discovered that diets with banana peel flour contents of 5% showed the greatest benefits in immunological parameters such lysozyme, the alternative complement pathway, phagocytic leukocyte, superoxide dismutase, and catalase activity. Additional studies demonstrate that the use of banana peel flour can lower the degree of cannibalism in giant prawns (*Macrobrachium rosenbergii*) (Rakhmawati et al 2020) and catfish (Haetami et al 2019), resulting in a greater survival rate compared to the control group.

Conclusions. This study has succeeded in analyzing the ability of Kepok banana peel flour (KBPF) in catfish feed to improve the number of lactic acid bacteria, growth performance and feed utilization. The results show that KBPF has the potential as a prebiotic in catfish feed at a dose of 1-2% in the diet. In addition, it is recommended to be given at the beginning of the catfish cultivation period because it has the potential to increase their survival rate.

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

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