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Utilization of Oligosaccharides from Banana Peel as Prebiotics on Feed Digestibility, Feed Consumption, Growth, and Survival Rate of the Milkfish (*Chanos chanos*)

Tuthy Tazkiah Mustari[1], Haryati[2]*, Siti Aslamyah[2]*

[1]Faculty of Marine Science and Fisheries, Hasanuddin University, Indonesia
[2]Department of Aquaculture, Faculty of Marine Science and Fisheries, Hasanuddin University, Indonesia
[*]Corresponding Author: haryati_fikpunhas@yahoo.com

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ABSTRACT

This study examined the potential utilization of banana peel waste as a source of prebiotic oligosaccharides to improve the biological performance of the milkfish (*Chanos chanos*). A comprehensive study was conducted to evaluate the effect of banana peel oligosaccharide supplementation on feed digestibility, feed consumption, growth, and survival rate. The milkfish specimens were fed diets supplemented with banana peel oligosaccharide extract at levels of 0 (control), 1, 2, 3, and 4% (initial weight 15± 5g/ ind) maintained at a density of 10 fish per aquaria, measuring 50 × 40 × 35cm^3, totaling 15 units. This study was designed in a completely randomized design (CRD) with five treatments and three replications. The experimental fish were fed with 5% of fish biomass per day for 50 days of rearing, with a frequency of feeding three times a day at 07:00, 12:00, and 17:00. The results showed that the oligosaccharide extract had a significant effect ($P < 0.05$) on digestibility (total, protein, fat, and energy) and growth (absolute length and relative length), while no effect was detected on the survival and feed consumption of the milkfish. The best dose varied depending on the parameters: 1% for fat digestibility (93.69 ± 0.76%), 2% for total digestibility (18.83 ± 0.19%), protein digestibility (92.58 ± 0.66%), energy digestibility (90.65 ± 1.23%), in addition to 3% for absolute length growth (11.04 ± 0.85cm) and relative length growth (8.29 ± 0.50%). In conclusion, the addition of banana peel oligosaccharide extract as a prebiotic improves the growth and nutritional performance of milkfish, with the best dose ranging from 2–3%, depending on the optimized parameters.

INTRODUCTION

The milkfish is one of the most important aquaculture commodities in Indonesia. The national milkfish production continues to increase from year to year, reaching 701,319 tons in 2020 **(Ministry of Marine Affairs and Fisheries, 2021)**. Feed is one of the components that determines the success of the business because it absorbs 60–70% of total production costs **(Mustari, 2022)**. To reduce the cost of feed procurement, it is necessary to increase its utilization, such as through the provision of feed additives. Prebiotics are one of the feed additives that are a source of nutrients for probiotics. The combination of probiotics and prebiotics is called synbiotics. Thus, when the mixture is given to feed, it can improve the digestibility, feed consumption, growth performance, and survival rate of the milkfish. Recently, prebiotics have been the focus of aquaculture research due to their potential to improve fish health and growth performance.

Oligosaccharides are one of the most promising types of prebiotics due to their ability to stimulate the growth of beneficial bacteria in the gastrointestinal tract **(Ringø *et al*., 2020)**. Banana peels are abundant agricultural wastes and potential source of oligosaccharides. Research by **Emaga *et al*. (2017)** showed that banana peels contain about 30–40% pectin, which can be hydrolyzed into oligosaccharides. The utilization of banana peels as prebiotics can be a solution to reduce waste while increasing the added value of banana industry by-products. Several studies have shown the positive effects of oligosaccharides on fish performance. **Hoseinifar *et al*. (2019)** reported that 0.2% mannanoligosaccharide supplementation improved growth, feed efficiency, and digestive enzyme activity in the goldfish. In *Oreochromis niloticus*, the addition of 0.5% fructooligosaccharide improved the survival rate and immune response **(Miao *et al*., 2018)**.

Feed digestibility is an important parameter in evaluating the effectiveness of prebiotics. **Song *et al*. (2020)** found that xylooligosaccharide supplementation increased protein and fat digestibility in *Paralichthys olivaceus*. This increased digestibility was correlated with the increased digestive enzyme activity and beneficial bacterial populations in the gut **(Ringø *et al*., 2020)**. **Ringø *et al*. (2010)** showed that prebiotics can increase the population of beneficial bacteria in the digestive tract of fish, which in turn can improve digestion and nutrient absorption. Feed consumption and efficiency can also be affected by prebiotics. Studies on *Clarias gariepinus* showed that mannanoligosaccharides improved appetite and feed conversion efficiency **(Aderolu *et al*., 2019)**. This has the potential to reduce feed costs, which is the largest component in fish farming. Feed efficiency and fish growth are strongly influenced by the composition of the gut microbiota. **Dawood *et al*. (2020)** reported that the addition of prebiotics to tilapia feed can modulate the gut microbial community, which in turn improves feed efficiency and growth rate. Fish growth and survival rates are key indicators of successful aquaculture. **Dawood *et al*. (2017)** reported that β-glucan supplementation increased the specific growth rate and survival rate of tilapia fish challenged with *Aeromonas hydrophila*. These effects were mediated by enhanced immune responses and stress resistance. Feed consumption and efficiency can also be affected by prebiotics. Studies on the African catfish (*Clarias gariepinus*) showed that mannanoligosaccharides improved appetite and feed conversion efficiency **(Aderolu *et al*., 2019)**. This has the potential to reduce feed costs, which is the largest component in fish farming.

Based on this, research on the utilization of oligosaccharides from banana peels as prebiotics for the milkfish can have a significant potential. This study aimed to provide new insights into strategies to improve the growth performance and nutrition of the milkfish through the sustainable utilization of agricultural waste. A comprehensive study was conducted to determine the best dose of banana peel oligosaccharides as prebiotics for feed digestibility, feed consumption, growth, and survival rate.

MATERIALS AND METHODS

Test animals

The experimental fish used were the juvenile milkfish weighing 15± 5g/ ind gatheredfrom the Takalar Lama breeding pond, Mappakasunggu sub-district, Takalar district. The stocking density used was 10 fish per 55 liters.

Rearing containers

The rearing animal test used 15 glass aquaria measuring 50, 40, and 35cm^3. Each aquarium was filled with 55L of ± 25ppt sterilized salinity water. The layout of the rearing containers was randomly arranged based on a completely randomized design (CRD) pattern.

Probiotics and prebiotics

The probiotics used were a mixed microorganism collection of **Aslamyah *et al*. (2022)** consisting of a mixture of bacteria, yeast, and fungi, namely *Bacillus* sp., *Streptococcus* sp., *Lactobacillus* sp., *Saccharomyces* sp., *Aspergillus* sp., and *Rhizophus* sp. Before use, the probiotic starter was refreshed by taking 2mL of starter and inoculating it in a substrate solution, which was a mixture of 2 liters of old coconut water and 500g of sugar. Then, it was incubated for 24 hours, and the culture was ready for use. Oligosaccharides used as prebiotics were extracted from banana peels following a modified method from **Syafura *et al*. (2016)**. The key steps in this process include: first, banana peels were prepared by cleaning, drying, and grinding them into powder. Next, extraction was carried out using the hot water extraction method at 90°C for 2 hours. Afterward, filtration and concentration were performed to separate the solid residue and concentrate the extract. The next step was precipitation, where ethanol was added to precipitate the oligosaccharides. Finally, oligosaccharide powder was obtained through a freeze-drying process.

Experimental diet

The feed used in this study is an artificial feed formulated by **Aslamyah *et al*. (2020)**, which can be seen in Table (1) and proximate analysis in Table (2).

The test feed was prepared following the method of **Aslamyah *et al.* (2022)**. The mixture of feed ingredients was formed into pellets and dried. The pellets were then added with a mixture of microorganisms (10mL/ kg feed) and prebiotic oligosaccharides according to the treatment. The feed was coated with 2% egg white and aerated for 15 minutes before being given to the experimental fish.

Table 1. Composition of feed raw materials without and with Cr_2O_3

Ingredient	Composition (%)	
	Feed without Cr_2O_3	Feed with Cr_2O_3
Fishmeal	26	26
Soy flour	22	22
Coconut meal flour	16	15.4
Corn flour	18	18
Pollard flour	10	10
Fat	4	4
Vitamin and mineral mix	4	4
Cr_2O_3	0	0.6
Total	100	100

Table 2. Results of feed proximate analysis

Ingredient	Composition (%)
Protein	26.43
Fat	8.37
Ash	24.22
Crude fiber	7.45
NFE	33.53
DE (kcal/kg)	2627.52
C/P (DE/g protein)	9.94

Research treatment

This study used a completely randomized design (CRD) with five treatments and three replicates for each treatment, resulting in 15 experimental units. The treatments tested were various doses of banana peel oligosaccharide extract in feed, namely 0 (control), 1, 2, 3, and 4%. Each treatment was coded with letters A, B, C, D, and E.

Research procedure

The experimental fish were stocked in the rearing container and acclimatized for 7 days to the environment and artificial feed. During acclimatization, a control feed at a dose of 5% of body weight per day was given three times a day, at 07:00, 12:00, and 17:00. After the acclimatization period was completed, the experimental fish were fasted for 24 hours to remove the remaining feed from their bodies. Next, weighing was carried out to determine the initial weight of the experimental fish. Fish were maintained for 50 days and given test feed at the same dose and frequency at the time of acclimatization. Sampling was done every 10 days to determine the increase in weight of the experimental fish and to adjust the weight of the feed to be given. Water quality was maintained by flushing out the remaining feed and feces at the bottom of the container and daily changing the water by 25%.

Research parameters

1. Feed digestibility

Feed digestibility was measured using the indirect method of **Takeuchi (1988)** by mixing 0.6% chromium oxide (Cr_2O_3) evenly into the feed. The experimental fish were fasted for 24 hours and then fed the test feed at the same dose and feeding frequency as previously mentioned. Feces were daily collected using a fecal collection device for 2 weeks, starting two days after the first feeding with Cr_2O_3 mixed feed. The collected feces were oven-dried at 60°C to avoid changes in the compounds. Dried feces were stored at -10 °C for further analysis.

1.1. Total digestibility

Total digestibility was calculated using the equation of **Takeuchi (1988)** as follows:

$$\text{Total digestibility (\%)} = 100 - (100 \times a/a')$$

Explanation: a = % Cr_2O_3 in feed (%)

a' = % Cr_2O_3 in feces (%)

1.2. Nutrient digestibility (Protein, Fat, and Energy)

Nutrient digestibility was calculated using the **Takeuchi (1988)** equation as follows:

$$\text{Nutrient digestibility (\%)} = (1 - a'/a \times b'/b) \times 100$$

Explanation: a' = nutrients in feces (%)

a = nutrients in feed (%)

b' = indicator in feces (%)

b = indicator in feed (%)

2. Feed consumption

Feed consumption was calculated by subtracting the feed given from the remaining uneaten feed, which was removed after 1 hour from the end of feeding. It was then filtered using a fine cloth, taken, and safely put into a sealed plastic bag and stored in the refrigerator. The uneaten feed was stored for up to ten days and then dried in direct sunlight. Feed consumption was calculated using the following formula **(Malah, 2022)**:

$$\text{Feed Consumption (g)} = \text{Amount of feed fed (g)} - \text{Amount of uneaten feed (g)}$$

3. Growth

Growth was observed every 10 days by weighing the experimental fish.

3.1. Absolute length growth of milkfish

Absolute length growth was calculated by using the following formula **(Surbakti *et al*., 2024)**:

$$ALG = SLt - SL0$$

Explanation:

ALG = absolute length growth rate (cm)

SL0 = initial fish length (cm)

SLt = final fish length (cm)

T = period (days)

3.2. Relative length growth of milkfish

Relative length growth was calculated using the formula **(Science, 2020)**:

$$GR = \frac{W_t - W_0}{t} \times 100$$

Explanation:

GR = relative length growth (%)

W_t = final fish length (cm)

W_0 = initial fish length (cm)

t = time (day)

4. Survival rate

The percentage survival rate was calculated based on the formula from **Effendie (1979)**:

$$SR = N_t/N_0 \times 100\%$$

Explanation:

SR = survival rate (%)

N_t = total of final experimental fish (ind)

N_0 = total of initial experimental fish (ind)

5. Water quality

During the research, several physico-chemical parameters of the research medium, water, were measured, including temperature, salinity, pH, DO, and ammonia.

Temperature was measured using a thermometer; salinity was measured using a refractometer; pH was measured using a pH meter; dissolved oxygen was measured using a DO meter, and ammonia was measured using a spectrophotometer. Temperature, salinity, pH, and dissolved oxygen were measured twice a day at 07:00 and 17:00. The ammonia was measured twice during the study, at the beginning and end of the study.

Data analysis

Data on feed digestibility, feed consumption, growth, and survival rate were analyzed by the analysis of variance (ANOVA) to determine the effect of treatment. If there was a significant effect, then the W-Tuckey further test was conducted to assign the differences between treatments. SPSS version 24.0 was used as a data analysis tool. Media water quality was analyzed descriptively by comparing literature based on the milkfish survival rates.

RESULTS

1. Feed digestibility

1.1. Total digestibility

Data on the total digestibility of the milkfish at various doses of oligosaccharide extract from banana peels in feed are presented in Table (3).

Table 3. Total digestibility of *Chanos chanos*

Treatment of banana peel oligosaccharide extract in feed	Total digestibility (%)
A (0%)	18.37 ± 0.12[a]
B (1%)	18.83 ± 0.19[b]
C (2%)	18.79 ± 0.11[b]
D (3%)	18.63 ± 0.11[ab]
E (4%)	18.82 ± 0.10[b]

Note: Different letters in the same column indicate significant effects (*P*< 0.05).

The results of the analysis of variance (ANOVA) showed that feeding with different doses of banana peel oligosaccharide extract had a significant effect (*P*< 0.05) on the total digestibility of the milkfish. The results of the W-Tukey test indicated that the highest total digestibility was achieved with the 1% dose, measuring 18.83 ± 0.19%. This value was not significantly different from the 4% dose, which had a digestibility of 18.82 ± 0.10%, or the 2% dose at 18.79 ± 0.11%, and the 3% dose at 18.63 ± 0.11%. However, these doses were significantly different from the 0% dose, which had the lowest total digestibility value of 18.37 ± 0.12%.

1.2. Nutrient digestibility

The average nutrient digestibility of the milkfish with the treatment dose of oligosaccharides from banana peel in feed is presented in Table (4).

Table 4. Nutrient digestibility of *Chanos chanos*

Treatment of banana peel oligosaccharide extract in feed	Protein digestibility (%)	Fat digestibility (%)	Energy digestibility (%)
A (0%)	91.14 ± 0.44[a]	91.25 ± 0.22[a]	89.28 ± 1.26[ab]
B (1%)	92.58 ± 0.66[b]	93.53 ± 0.54[b]	89.48 ± 0.64[ab]
C (2%)	92.45 ± 0.38[b]	93.69 ± 0.76[b]	90.65 ± 1.23[b]
D (3%)	91.92 ± 0.38[ab]	93.19 ± 0.61[b]	87.13 ± 0.65[a]
E (4%)	92.57 ± 0.36[b]	93.30 ± 0.19[b]	87.94 ± 0.58[a]

Note: Different letters in the same column indicate significant effects (*P*< 0.05).

The results of the ANOVA showed that feeding with different doses of banana peel oligosaccharide extract had a significant effect (*P*< 0.05) on the protein digestibility of the milkfish. The results of the W-Tuckey further test showed that the highest protein digestibility was obtained from the 1% dose of 92.58 ± 0.66%, which was not significantly different from the 4% dose of 92.57 ± 0.36%, the 2% dose of 92.45 ± 0.38%, and the 3% dose of 91.92 ± 0.38%, while significantly different from the 0% dose of 91.14 ± 0.44%. Protein digestibility at a dose of 3% was not significantly different from the other treatments.

The results of the ANOVA showed that feeding with different doses of oligosaccharide extract from banana peel had a significant effect (*P*< 0.05) on the fat digestibility of the milkfish. The results of the W-Tuckey further test showed that the highest fat digestibility was obtained from a 2% dose of 93.69 ± 0.76%, not significantly different from a 1% dose of 93.52 ± 0.54%, a 4% dose of 93.30 ± 0.19%, and a 3% dose of 93.19 ± 0.61%, which was significantly different from a 0% dose with a value of 91.25 ± 0.22%.

The results of the W-Tuckey further test showed that the highest energy digestibility was obtained from a 2% dose of 90.65 ± 1.23%, which was not significantly different from a 1% dose of 89.48 ± 0.64% and a 0% dose of 89.28 ± 1.26%, but significantly different from a 4% dose with a value of 87.94 ± 0.58% and a 3% dose of 87.13 ± 0.65%.

2. Feed consumption

Feed consumption data of milkfish at various doses of oligosaccharide extract from banana peel in the average feed is presented in Table (5).

Table 5. Feed consumption of *Chanos chanos*

Treatment of banana peel oligosaccharide extract in feed	Feed vonsumption (g)
A (0%)	368.39 ± 28.54[a]
B (1%)	319.00 ± 32.74[a]
C (2%)	379.34 ± 25.18[a]
D (3%)	369.81 ± 75.83[a]
E (4%)	422.85 ± 50.41[a]

Note: Different letters in the same column indicate significant effects ($P< 0.05$).

The results of the analysis of variance showed that various doses of banana peel oligosaccharide extract in feed had no significant effect ($P> 0.05$) on the milkfish feed consumption.

3. Growth

3.1. Absolute length growth of the milkfish

Data on the absolute length growth of the milkfish at various doses of oligosaccharide extract from banana peel in the average feed are presented in Table (6).

Table 6. Absolute length growth of *Chanos chanos*

Treatment of banana peel oligosaccharide extract in feed	Absolute length growth (cm)
A (0%)	5.76 ± 0.15[a]
B (1%)	6.57 ± 0.42[a]
C (2%)	8.72 ± 1.01[b]
D (3%)	11.04 ± 0.85[c]
E (4%)	10.21 ± 0.23[bc]

Note: Different letters in the same column indicate significant effects ($P< 0.05$).

The 3% extract content produced the highest absolute length growth (11.04 ± 0.85 cm) and was significantly different from the other treatments, except for the 4% extract content. 0% extract content and 1% extract content produced the lowest absolute length growth and were not significantly different from each other.

3.2. Relative length growth of the milkfish

Data on the absolute length growth of milkfish at various doses of oligosaccharide extract from banana peel in feed and the average are presented in Table (7).

Table 7. Relative length growth of *Chanos chanos*

Treatment of banana peel oligosaccharide extract in feed	Relative length growth (%)
A (0%)	4.21 ± 0.17^{a}
B (1%)	4.91 ± 0.20^{a}
C (2%)	6.62 ± 0.69^{b}
D (3%)	8.29 ± 0.50^{c}
E (4%)	$7.47 \pm 0.10b^{c}$

Note: Different letters in the same column indicate significant effects ($P< 0.05$).

The results of the analysis of variance (ANOVA) showed that the treatment had a significant effect ($P< 0.05$) on the growth of relative length. The 3% extract level again showed the highest results (82.93 ± 5.14%) and was significantly different from other treatments, except for the 4% extract level. Whereas, the 0 and 1% extract level had the lowest relative length growth and were not significantly different from one another.

4. Survival rate

The survival rate data of milkfish at various doses of oligosaccharide extract from banana peel in the average feed are presented in Table (8).

Table 8. Survival rate of *Chanos chanos*

Treatment of banana peel oligosaccharide extract in feed	Survival rate (%)
A (0%)	60.00 ± 10.00^{a}
B (1%)	43.33 ± 11.54^{a}
C (2%)	43.33 ± 25.16^{a}
D (3%)	53.33 ± 25.16^{a}
E (4%)	80.00 ± 10.00^{a}

Note: Different letters in the same column indicate significant effects ($P < 0.05$).

The results of the analysis of variance showed that various doses of banana peel oligosaccharide extract in feed had no significant effect ($P> 0.05$) on the milkfish survival rate.

5. Water quality

Parameters measured during 50 days of the milkfish rearing include physical, biological, and chemical parameters, namely: temperature, salinity, pH, dissolved oxygen, and ammonia. The results of the water quality measurements during the study are within the optimal range for rearing the milkfish (In-text citation), as illustrated in Table (9).

Table 9. Water quality of *Chanos chanos* during the research

Parameter	Value range obtained	Tolerance range	Reference
Temperature (°C)	27-32	27-30	**Syahid *et al.* (2006)**
Salinity (ppt)	30-35	28-32	**Mandal *et al.* (2018)**
pH	6,9-8	6,5-9	**SNI: 6148.3 -2013**
DO (ppm)	2,1-5	3-8	**Hendrajat *et al*. (2018)**
Ammonia (ppm)	0,0002	<0,01	**Winarsih *et al*. (2011)**

DISCUSSION

1. Feed digestibility

1.1. Total digestibility

The results showed that the addition of oligosaccharide extract from banana peels to the milkfish feed had a positive effect on total digestibility. Treatments B (1% extract content), C (2%), and E (4%) showed a significant increase in total digestibility compared to the control (treatment A), while treatment D (3%) showed results that were not significantly different from the control or other treatments.

The increase in total digestibility in the treatment with the addition of banana peel oligosaccharides is in line with the research of **Grisdale-Helland *et al*. (2008)** on *Salmo salar*, where mannanoligosaccharide (MOS) supplementation increased nutrient digestibility. Treatment D (3%) showed lower results than treatments B, C, and E, although it was still higher than the control. This phenomenon is similar to that reported by **Hoseinifar *et al*. (2013)** for carp, where the optimal dose of MOS was in the range of 2%, and the higher doses did not always produce better effects. This may be due to the presence of a negative feedback mechanism or saturation at a certain level **(Torrecillas *et al*., 2014)**.

The positive effect of oligosaccharides on digestibility may be attributed to the increased digestive enzyme activity, as reported in the study of **Xu *et al*. (2009)** on the tilapia. In addition, oligosaccharides may increase the population of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* in the digestive tract, which contributes to enhancing the fermentation and the production of short-chain fatty acids **(Burr *et al*., 2008)**. The increase in total digestibility observed in this study has a positive impact on the growth and feed utilization efficiency of the milkfish. This is consistent with the findings of **Akter *et al*. (2016)** for the tilapia, where increased nutrient digestibility was positively correlated with growth rate and feed efficiency.

1.2. Nutrient digestibility

Protein digestibility

The results showed that the addition of oligosaccharide extract from banana peels to the milkfish feed had a positive effect on protein digestibility. Treatments B (1% extract content), C (2%), and E (4%) showed a significant increase in protein digestibility compared to the control (treatment A), while treatment D (3%) showed results that were not significantly different from the control or other treatments. The observed increase in protein digestibility is in line with the study of **Akter *et al*. (2016)** on the catfish (*Pangasianodon hypophthalmus*), where mannanoligosaccharide (MOS) supplementation improved the species' protein digestibility. This positive effect can be explained through several mechanisms. **Ringø *et al.* (2010)** postulated that prebiotics can modulate the gut microbiota, which in turn can increase the activity of digestive enzymes including proteolytic enzymes. Treatment D (3%) showed lower results than treatments B, C, and E, although still higher than the control. A similar phenomenon was reported by **Torrecillas *et al*. (2014)** on the European sea bass (*Dicentrarchus labrax*), where the effect of MOS was not always linear with increasing the dose. This may be due to the presence of a feedback mechanism or saturation at certain levels. The phenomenon of increased protein, fat and energy digestibility at the 4% level after a decrease at the 3% level can be interpreted as a non-linear effect of xylooligosaccharides (XOS) on the fish digestive system. This suggests a physiological threshold and complex interactions between XOS and factors in the digestive system. Possibly, a 3% dose was not enough to achieve an optimal effect, while a 4% dose was able to surpass this threshold. Adaptation of the gut microbiota could also play a role, where a higher dose might promote a more effective adaptation. Given the observation of **Torrecillas *et al*. (2014)**, saturation and feedback mechanisms may occur at some levels, with the 4% dose again increasing effectiveness after a decline at 3%. Biological variability between individual fish and possible synergistic effects of XOS with other feed components at higher doses could also contribute to this phenomenon. However, this interpretation remains speculative and requires further research to understand the exact mechanisms behind the observed nutrient digestibility patterns. The positive effect of oligosaccharides on protein digestibility can also be explained by an increase in the population of beneficial bacteria in the digestive tract. **Hoseinifar *et al*. (2013)** reported that MOS supplementation in the beluga fish (*Huso huso*) increased the population of *Lactobacillus* spp., which is known to increase digestive enzyme activity and contribute to improving protein digestibility. The increase in protein digestibility observed in this study could potentially have a positive impact on the growth and feed utilization efficiency of the milkfish. This concurs with the findings of **Genc *et al*. (2007)** on the tilapia (*Oreochromis niloticus*), where increased protein digestibility was positively correlated with growth rate and feed efficiency.

Fat digestibility

The results showed that the addition of oligosaccharide extract from banana peels to the feed of the milkfish had a significant positive effect on fat digestibility. All treatments with the addition of oligosaccharides (B, C, D, and E) exhibited a significant increase in fat digestibility compared to the control (treatment A), with the highest value in treatment C (2% extract content).

The observed increase in fat digestibility corroborates with that of of **Torrecillas *et al*. (2015)** regarding the European sea bass (*Dicentrarchus labrax*), where mannanoligosaccharide (MOS) supplementation improved fat digestibility. This positive effect can be explained through several mechanisms. **Ringø *et al*. (2010)** explained that prebiotics can modulate the gut microbiota, which in turn can increase the activity of digestive enzymes, including lipolytic enzymes. Although all treatments with oligosaccharides showed a significant increase, there was no significant difference among treatments B, C, D, and E. This suggests that the positive effect of oligosaccharides on fat digestibility may reach a saturation point at certain concentrations. A similar phenomenon was recorded by **Grisdale-Helland *et al*. (2008)** for the Atlantic salmon (*Salmo salar*), where increasing the dose of fructooligosaccharides above a certain level did not result in a higher increase in digestibility. The increased fat digestibility can be attributed to the improved gut structure and increased digestive enzyme activity. In addition, **Xu *et al*. (2009)** found that xylooligosaccharides increased lipase enzyme activity in the crucian carp (*Carassius auratus gibelio*), which may contribute to improving the fat digestibility.

The positive effect of oligosaccharides on fat digestibility can also be explained by an increase in the population of beneficial bacteria in the gastrointestinal tract. **Hoseinifar *et al*. (2015)** reported that fructooligosaccharide supplementation in carp (*Cyprinus carpio*) increased the population of *Lactobacillus* spp. and *Bifidobacterium* spp., which are known to increase the digestive enzyme activity and contribute to improving the fat digestibility. The increased fat digestibility observed in this study could potentially have a positive impact on the growth and feed utilization efficiency of the milkfish. This finding collaborates with those of **Yilmaz *et al*. (2007)** investigating the Nile tilapia (*Oreochromis niloticus*), where increased fat digestibility was positively correlated with growth rate and feed efficiency.

Energy digestibility

The results of research on the utilization of oligosaccharides from banana peel as prebiotics in the milkfish feed showed varying effects on energy digestibility. Treatment C with 2% banana peel oligosaccharide extract showed a significant increase in energy digestibility compared to the control (treatment A) and other treatments. However, treatments D (3%) and E (4%) showed a decrease in energy digestibility compared to the control, although it was not significant for treatment E.

The increase in energy digestibility in treatment C (2%) is in line with the research of **Grisdale-Helland *et al*. (2008)** on the Atlantic salmon (*Salmo salar*), where fructooligosaccharide supplementation at optimal levels increased the energy digestibility. This positive effect can be explained through various mechanisms. **Ringø *et al*. (2010)** argued that prebiotics can modulate the gut microbiota, which in turn can increase the activity of digestive enzymes and the fermentation of complex carbohydrates, producing short-chain fatty acids that can be used as an energy source by fish. The decrease in energy digestibility at higher concentrations (3 and 4%) suggests a non-linear dose-response effect. Similarly, the study of**Torrecillas *et al*. (2015)** conducted on the European sea bass (*Dicentrarchus labrax*), elucidated that an extremely high dose of mannanoligosaccharides could reduce their effectiveness. This may be due to changes in the digesta viscosity or unfavorable interactions with other nutrients at higher concentrations **(Sinha *et al*., 2011)**.

Xu *et al*. (2009) found that xylooligosaccharides increased amylase enzyme activity in the crucian carp (*Carassius auratus gibelio*), which may contribute to improving carbohydrate and energy digestibility. The difference in energy digestibility response to various concentrations of oligosaccharides indicates the importance of determining the optimal dose in prebiotic application. Furthermore, **Hoseinifar *et al*. (2015)** emphasized that the effectiveness of prebiotics may vary depending on the type of prebiotic, fish species, and environmental conditions.

2. Feed consumption

Based on the results of the study, the utilization of oligosaccharides from banana peel as prebiotics showed varying effects on the feed consumption of the milkfish. Treatment B (1% oligosaccharide) showed the lowest feed consumption, which may be related to the concept of "optimal dose" in the use of prebiotics, where too low a dose may not have a significant effect, whereas higher doses may provide more optimal benefits. This is supported by the study of **Hoseinifar *et al*. (2015)**, who showed that the effectiveness of prebiotics is highly dependent on the dose administered. The increase in feed consumption at higher doses (especially treatment E) can be explained by the mechanism of the increased digestive enzyme activity and improved gut structure induced by prebiotics, as reported by **Cerezuela *et al*. (2013)**. This improvement in the gut condition may increase the efficiency of digestion and nutrient absorption, which in turn stimulates fish appetite.

3. Growth

The results of the study on the utilization of oligosaccharides from banana peels as prebiotics showed significant differences between treatments on the absolute and relative length growth of the milkfish. Treatment D with 3% banana peel oligosaccharide extract in feed showed the highest absolute length growth (11.04 ± 0.85cm) and the highest relative length growth (8.29 ± 0.50cm), which was statistically significantly different from the other treatments. The increase in growth coincides with the findings of **Ringø *et al*. (2010)** and **Hoseinifar *et al*. (2014)**, postulating that prebiotics can increase the population of beneficial bacteria in the digestive tract of fish, thereby improving the digestion and nutrient absorption. The growth pattern that increased significantly up to 3% concentration and slightly decreased at 4% indicates the optimal level of prebiotic use in accordance with the study of **Grisdale-Helland *et al*. (2008)**. The significant increase in growth in the prebiotic treatment can also be explained by the immunomodulatory effect of oligosaccharides, as reported by **Song *et al*. (2014)**. These results are also in line with the research of **Mahious *et al*. (2006)** and **Torrecillas *et al*. (2007)**, who found that oligosaccharide supplementation can improve growth and feed efficiency in various fish species. Treatment E (4%) showed results that were not significantly different from treatment C (2%) for absolute growth, while for relative growth it was not significantly different from treatments C (2%) and D (3%), but all three were still higher than the control. This suggests the presence of a fairly wide concentration range where oligosaccharides from banana peels can provide growth benefits for the milkfish. The growth enhancement observed in this study could potentially have a positive impact on the digestibility and feed utilization efficiency of the milkfish, in line with the findings of **Akter *et al*. (2016)** concerning tilapia.

4. Survival rate

Various doses of oligosaccharides in the feed did not cause differences in survival, this occurred because prebiotics are growth promoters of microorganisms in the digestive tract; nonetheless, the experimental fish were still able to survive, even without the provision of prebiotics with a microorganism mix. This occurred since the survival of the experimental fish received the same quality of feed nutrition, which is in accordance with the nutritional needs of the milkfish. In addition, the quality of the housing facilities is also in the range that is suitable for the survival of the milkfish. This aligns with the statement of **Wahyuningsih *et al*. (2015)** that survival can be influenced by biotic and abiotic factors. Biotic factors consist of age and the ability to adapt to the environment, while abiotic factors include food availability and the quality of living media.

The high variability in the results, especially in treatments C and D, suggests the presence of other factors that may affect fish survival. As described by **Grisdale-Helland *et al*. (2008)**, the effectiveness of prebiotics may vary depending on the fish species and rearing conditions. In addition, **Song *et al*. (2014)** emphasized that the immunomodulatory effects of prebiotics may vary depending on the dosage and type of oligosaccharides used. The control treatment (A) showed a relatively high survival rate compared to several treatments with prebiotics. This may indicate that the milkfish specimens displayed a good level of natural resistance under the conditions of this experiment. However, the increased survival at the highest prebiotic concentration (4%)

indicates the potential benefits of banana peel oligosaccharides at the correct dosage. This result copes with that of **Mahious *et al*. (2006)**, who reported that the addition of oligosaccharides in feed can increase the survival of the turbot fish (*Psetta maxima*) larvae. In addition, **Torrecillas *et al*. (2007)** also found that mannanoligosaccharide supplementation can increase resistance to infection in the sea bass (*Dicentrarchus labrax*).

CONCLUSION

The addition of oligosaccharide extract from banana peel to the milkfish feed increased total digestibility, protein digestibility, and fat digestibility significantly at doses of 1–4% with an optimal dose of 2%. Moreover, the energy digestibility increased at a 2% dose, while a decrease was witnessed at 3–4% doses. It is worthy to note that, feed with banana peel oligosaccharide at a dose of 3% produced the highest absolute and relative length growth in the milkfish. Although there was no significant difference, there was a trend: the higher the dose of oligosaccharide, the higher the feed consumption and survival of the milkfish. Overall, this study showed that the use of banana peel oligosaccharides as prebiotics can improve the nutrient digestibility, growth, and feed efficiency of milkfish at an optimal dose of 2–4%.

REFERENCES

Aderolu, A.Z.; Seriki, B.M.; Apatira, A.L. and Ajaegbo, C.U. (2019). Effects of dietary mannan oligosaccharide on growth, body composition, haematology and biochemical parameters of African catfish (*Clarias gariepinus*). Aquaculture Research, 50(9): 2489‒2498.

Akter, M.N.; Sutriana, A.; Talpur, A.D. and Hashim, R. (2016). Dietary supplementation with mannan oligosaccharide influences growth, digestive enzymes, gut morphology, and microbiota in juvenile striped catfish, *Pangasianodon hypophthalmus*. Aquaculture International, 24(1): 127‒144.

Aslamyah, S.; Zainuddin; Badraeni; Umam, M.K.; Lestari, A.D.; and Haslinda. (2020). Organoleptic, physical, and chemical tests of artificial feed for milk fish substituted by earthworm meal (*Lumbricus* sp.). Jurnal Akuakultur Indonesia, 11(2): 124‒131.

Aslamyah, S.; Zainuddin, Z. and Badraeni, B. (2022). The effect of microorganisms combination as probiotics in feed for growth performance, gastric evacuation rates, and blood glucose levels of milkfish, *Chanos chanos* (Forsskal, 1775). Jurnal Iktiologi Indonesia, 22(1): 77‒91.

Burr, G.; Hume, M.; Neill, W.H. and Gatlin III, D.M. (2008). Effects of prebiotics on nutrient digestibility of a soybean-meal-based diet by red drum *Sciaenops ocellatus* (Linnaeus). Aquaculture Research, 39(15): 1680‒1686.

Cerezuela, R.; Meseguer, J. and Esteban, M.Á. (2013). Effects of dietary inulin, *Bacillus subtilis* and microalgae on intestinal gene expression in gilthead seabream (*Sparus aurata* L.). Fish & Shellfish Immunology, 34(3): 843–848.

Dawood, M.A.; Koshio, S.; Ishikawa, M. and Yokoyama, S. (2017). β-Glucan improved growth performance, innate immunity, and disease resistance in Nile tilapia. Fish & Shellfish Immunology, 67: 104–114.

Dawood, M.A.; Koshio, S. and Esteban, M.Á. (2020). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Reviews in Aquaculture, 12(4): 2225–2242.

Effendie, M.I. (1979). Fisheries Biology. Nusantara Library Foundation Bogor, 112p.

Emaga, T.H.; Andrianaivo, R.H.; Wathelet, B.; Tchango, J.T. and Paquot, M. (2017). Pectic substances from banana peel: Fractionation and structural characterization. Food Chemistry, 225: 197–205.

Genc, M.A.; Aktas, M.; Genc, E. and Yilmaz, E. (2007). Effects of dietary mannan oligosaccharide on growth, body composition and hepatopancreas histology of *Penaeus semisulcatus* (de Haan 1844). Aquaculture Nutrition, 13(2): 156–161.

Grisdale-Helland, B.; Helland, S.J. and Gatlin III, D.M. (2008). The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). Aquaculture, 283(1–4): 163–167.

Hendrajat, E.A.; Ratnawati, E. and Mustafa, A. (2018). Determining the Effect of Soil and Water Quality on Total Production of Vaname Shrimp and Milkfish Polyculture Ponds in Lamongan Regency, East Java Province through Path Analysis Application. Journal of Tropical Marine Science and Technology, 10(1): 179–195.

Hoseinifar, S.H.; Mirvaghefi, A. and Merrifield, D.L. (2013). The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. ellipsoideus on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). Aquaculture, 397: 1–7.

Hoseinifar, S.H.; Soleimani, N. and Ringø, E. (2014). Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (*Cyprinus carpio*) fry. British Journal of Nutrition, 112(8): 1296–1302.

Hoseinifar, S.H.; Esteban, M.Á.; Cuesta, A. and Sun, Y.Z. (2015). Prebiotics and fish immune response: a review of current knowledge and future perspectives. Reviews in Fisheries Science & Aquaculture, 23(4): 315–328.

Hoseinifar, S.H.; Safari, R.; Hosseini, M.; Saei, M.M. and Darvish Bastami, K. (2019). Dietary supplementation of mannanoligosaccharide (MOS) modulates gut microbiota, growth, and hemato-immunological responses of common carp (*Cyprinus carpio*). Aquaculture, 508: 336–342.

Mahious, A.S.; Gatesoupe, F.J.; Hervi, M.; Metailler, R. and Ollevier, F. (2006). Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). Aquaculture International, 14: 219–229.

Malah, Z. R. (2022). Effect of Various Source of Prebiotics in Functional Feed on Feed Intake and Feed Efficiency of Milkfish (*Chanos chanos*). Universitas Hasanuddin.

Mandal, B.; Bera, A.; Kailasam, M.; Padiyar, A.; Ambasankar, K.; Alavandi, S.V. and Vijayan, K.K. (2018). A guide to milkfish (*Chanos chanos*) aquaculture. Central Institute of Brackishwater Aquaculture. Chennai, India.

Miao, S.; Zhao, C.; Zhu, J.; Hu, J.; Dong, X. and Sun, L. (2018). Dietary supplementation of fructooligosaccharide (FOS) improves the immunological and antioxidant activity of juvenile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology, 83: 396–404.

Ministry of Marine Affairs and Fisheries (2021). KKP Performance Report 2020. Jakarta: KKP RI.

Mustari, T.T. (2022). Effect of Various Prebiotic Sources in Functional Feed on Body Chemical Composition and Liver and Muscle Glycogen Levels of Milkfish (*Chanos chanos*). Universitas Hasanuddin.

Ringø, E.; Olsen, R.E.; Gifstad, T.Ø.; Dalmo, R.A.; Amlund, H.; Hemre, G. and Bakke, A.M. (2010). Prebiotics in aquaculture: a review. Aquaculture Nutrition, 16(2): 117–136.

Ringø, E.; Van Doan, H.; Lee, S.H.; Soltani, M.; Hoseinifar, S.H.; Harikrishnan, R. and Song, S.K. (2020). Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. Journal of Applied Microbiology, 129(1): 116–136.

Science, E. (2020). Survival rate and growth rate of milkfish (*Chanos chanos*, Forsskal 1775) seeds in the acclimatization process at Ir. H. Djuanda Reservoir. IOP Conference Series: Earth and Environmental Science, 535(1): 012046.

Sinha, A.K.; Kumar, V.; Makkar, H.P.S.; De Boeck, G. and Becker, K. (2011). Non-starch polysaccharides and their role in fish nutrition–A review. Food Chemistry, 127(4): 1409–1426.

SNI: 6148.3. (2013). Milkfish (*Chanos chanos,* Forskal) - Part 3: Seed Production.

Song, S.K.; Beck, B.R.; Kim, D.; Park, J.; Kim, J.; Kim, H.D. and Ringø, E. (2014). Prebiotics as immunostimulants in aquaculture: a review. Fish & Shellfish Immunology, 40(1): 40–48.

Song, S.K.; Yoon, J.S.; Kim, K.W.; Han, H.S.; Yun, H.H.; Choi, Y.J.; Kang, D.J. and Kim, K.D. (2020). Effects of dietary supplementation of xylo-oligosaccharide on growth performance, innate immunity and disease resistance of olive flounder (*Paralichthys olivaceus*) against *Edwardsiella tarda*. Fish & Shellfish Immunology, 102: 21–30.

Surbakti, J.A.; Ayu, I.; Dewi, L.; Alamsjah, M.A. and Lamid, M. (2024). Growth of milkfish (*Chanos chanos* forsskal) maintained in multitrophic seafarming with different. March. https://doi.org/10.53550/EEC.2022.v28i05s.009.

Susanto, A. and Agustina, A. (2023). Utilization of Ambon Banana (Musa acuminata) Peel Flour as a Prebiotic in the Nile tilapia (Oreochromis niloticus). Egyptian Journal of Aquatic Biology & Fisheries, 27(5): 973 – 986.

Syafura, S.K.; Rani, H. and Zulfahmi. (2016). Characterization of Fructooligosaccharide (FOS) Isolated from Banana Peel as Prebiotic in Livestock. Lampung State Polytechnic.

Syahid M.; Subhan A. and Armando R. (2006). Organic Milkfish Cultivation in Polyculture. Jakarta: Penebar Swadaya.

Takeuchi, T. (1988). Laboratory work-chemical evaluation of dietry nutrients. Fish Nutrition and Mariculture, 179‒226.

Torrecillas, S.; Makol, A.; Caballero, M.J.; Montero, D.; Robaina, L.; Real, F.; Sweetman, J.; Tort, L. and Izquierdo, M.S. (2007). Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish & Shellfish Immunology, 23(5): 969‒981.

Torrecillas, S.; Montero, D. and Izquierdo, M. (2014). Improved health and growth of fish fed mannan oligosaccharides: potential mode of action. Fish & Shellfish Immunology, 36(2): 525‒544.

Torrecillas, S.; Montero, D.; Caballero, M.J.; Robaina, L.; Zamorano, M.J.; Sweetman, J. and Izquierdo, M. (2015). Effects of dietary concentrated mannan oligosaccharides supplementation on growth, gut mucosal immune system and liver lipid metabolism of European sea bass (*Dicentrarchus labrax*) juveniles. Fish & Shellfish Immunology, 42(2): 508‒516.

Wahyuningsih, Y. and Widowati, L.L. (2015). Effect of different types of fresh feed on growth rate and survival of soft-shell mangrove crabs (*Scylla serrata*) using the Popeye method. Journal of Aquaculture Management and Technology, 4(2): 109‒116.

Winarsih, W.H.; Priyambodo, T.; Rahardjo, A. and Husein. (2011). Milkfish Cultivation and Management. Airlangga University. ISBN 978-602-8967-37-2.

Xu, B.; Wang, Y.; Li, J. and Lin, Q. (2009). Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). Fish Physiology and Biochemistry, 35: 351‒357.

Yilmaz, E.; Genc, M.A. and Genc, E. (2007). Effects of dietary mannan oligosaccharides on growth, body composition, and intestine and liver histology of rainbow trout, *Oncorhynchus mykiss.*