PAPER • OPEN ACCESS

The effect probiotics from the types of nuts in feeding to the for bacterial populations, enzyme activity and feed FCR for milkfish (Chanos chanos)

To cite this article: Wahyudi *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **763** 012024

The effect probiotics from the types of nuts in feeding to the for bacterial populations, enzyme activity and feed FCR for milkfish (*Chanos chanos*)

Wahyudi[1,2], Siti Aslamyah[3], Zainuddin[3] and Surianti[4]

[1]Postgraduate Program, Fisheries Science Study Program, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar 90245, Indonesia
[2]Department Fisheries Agribusiness, Politeknik Nusantara Makassar, Indonesia
[3]Study Program of Aquaculture, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar 90245, Indonesia
[4]Department of Fisheries Science, Faculty of Science and Technology, Universitas Muhammadiyah Sidenreng Rappang, Indonesia

Email: wahyudialimi@gmail.com

Abstract. Nuts contain oligosaccharides which can function as prebiotics. Prebiotics are fibers that the body cannot digest and become food for probiotics. This study aimed to analyze the bacterial population, enzyme activity and feed conversion ratio of milkfish after being given various prebiotics from legumes in the feed. Milkfish measuring 4.13 ± 2.75 g are reared in a glass aquarium measuring 50 x 45 x 45 cm filled with water with a salinity of 15-20 ppt. Fish are maintained at a density of 15 individuals /aquarium for 60 days and are fed 5% body weight/day. The study was designed in a completely randomized design with 5 prebiotic treatments of soybeans, peanuts, green beans, kidney beans, and control. The results showed that the treatment had a significant effect ($p < 0.05$) on the bacterial population, as well as the protease and amylase enzyme activity, but not on FCR. The highest bacterial population was red bean prebiotics (2,082 × 103 CFU / mL), followed by green beans (2,001 × 103 CFU/mL), soybeans (972 × 103 CFU/mL). The highest activity (0.243 u/mL) and amylase enzyme (0.745 u/mL) was the prebiotic kidney beans, followed by soybeans (0.236 u/mL) amylase enzyme (0.506 u/mL), peanuts, protease enzymes (0.129 u / mL) amylase enzyme (0.554 u/mL) and green bean protease enzyme (0.156 u/mL) amylase enzyme (0.543 u/mL). Meanwhile, the FCR ranges from 1.43 ± .0.85 to 2.87 ± .1.70. It can be concluded that the best type of prebiotic is red beans.

1. Introduction

Milkfish (Chanos chanos) is one of the superior brackish water commodities in Indonesia. Many pond fish farmers cultivate this species in traditional system which only relies on live food available in the pond. [1]. This fish is a favorite food in the people of South Sulawesi, but there are production efficiency problems in the milkfish production process, especially in intensive cultivation which is related to high production costs in line with the increase in feed prices. Feed as the biggest component in financing will determine the success of cultivation. The very expensive price of feed is due to the feed-producing component, namely protein derived from fish meal, which is an expensive source of feed energy but low digestibility[2].Various efforts have been made to improve feed digestibility in an effort to feed efficiency. Several types of research with the use of probiotics in feed have been carried out, in which probiotics are beneficial bacteria that can help digestion of feed in the digestive tract.

One solution that can be offered is to optimize the role of the digestive tract microflora as a producer of exogenous enzymes and as a source of protein. Optimizing the role of microflora can be done by administering prebiotics. Prebiotics act as feed supplements that are in the feed or are intentionally added to the feed, can play a role in maintaining the balance of the microflora population and can act as a growth promoter or activate several strains of beneficial bacteria found in the digestive tract of fish [3].

In the digestive tract of milkfish there are many microflora, where microflora is a group of microorganisms that are present in the digestive tract of the organism. In Aslamyah's research, (2012) states that there are microflora in the digestive tract of milkfish, namely 4 amylolytic microbial isolates (Moraxella sp., Aeromonas hydrophila, Citrobacter sp., And Carnobacterium sp.), 3 types of anaerobic amylolytic microbes (Staphylococcus sp. Flavobacterium sp. ., and Vibrio sp.), 5 types of aerobic proteolytic microbes (Streptococcus sp., Bacillus sp., Micrococcus sp., Pseudomonas sp., and Proteus sp.), 2 types of anaerobic proteolytic microbes (Vibrio alginoliticus and unidentified species), 2 types of aerobic lipolytic microbes (Planococcus sp., And Plesiomonas sp.) And 2 types of anaerobic lipolytic microbes (Kurthia sp., And Serratia sp.). Microflora is able to utilize fiber in feed that is not digested by the intestines of the organism, thus optimizing the digestibility of feed in the digestive tract. Microflora is in the form of probiotic bacteria that require nutrition in the development of its population [4].

Microflora as an enzyme producer will increase the availability of digested feed that is ready for absorption by the body so that feed efficiency increases. Apart from acting as a producer of enzymes, the microflora of the digestive tract also functions as a source of protein. This occurs when the microflora undergoes a lethal phase and undergoes lysis and is then absorbed by the fish body. The microflora of the digestive tract can inhibit the development of pathogenic bacteria, thereby helping to balance the total population of the microflora. Nuts contain undigested oligosaccharides but are beneficial for probiotic bacteria[5].

Types of beans that contain oligosaccharides are soybeans, peanuts, green beans, and red beans, so they are expected to be prebiotics so that they can increase the bacterial population. Therefore, the synergy between probiotics and prebiotics will be even more beneficial, where the balanced combination of probiotics and prebiotics will support the continuity and growth of beneficial bacteria in the digestive tract. The addition of prebiotics in feed that is used by digestive bacteria causes an increase in enzyme activity in the digestive tract which can increase digestibility[6]. Good digestibility will optimize the use of feed consumed, so it is expected to increase feed efficiency and reduce feed organic waste in the form of feces and other metabolic waste.

2. Material and Method

This research was conducted in January 2019 at the Mini Hatchery Unit, Department of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University Makassar.

2.1. Milkfish juvenile

The test animal used in this study was juvenile milkfish measuring 4.13 ± 2.75g from the nursery site Marana Kab. Maros with a density of 15 fish / aquarium.

2.2. Feed

The feed used is formulated with a nutritional composition according to the needs of milkfish and added with soybean flour, green beans, peanuts and kidney beans as a prebiotic. The composition of feed raw materials is presented in Table 1.

Table 1.Feed formulations used during the study

Raw material	**Persentase**				
	A	B	C	D	E
Fish flour	35	32	43	42	45
Soybean flour	30	0	0	0	0
Peanut flour	0	30	0	0	0
Mung bean flour	0	0	30	0	0
Red bean flour	0	0	0	30	0
Coconut Cake Flour	20	23	12	13	40
Wheat flour	10	10	10	10	10
Fish oil	3	3	3	3	3
Vitamins & Minerals *	2	2	2	2	2
Total	100	100	100	100	100

Keterangan: *) *Komposisi vitamin & mineral mix. Setiap 10 kg mengandung Vitamin A 12.000.000 IU; Vitamin D 2.000.000 IU; Vitamin E 8.000 IU; Vitamin K 2.000 mg; Vitamin B1 2.000 mg; Vitamin B2 5.000; Vitamin B6 500 mg; Vitamin B12 12.000 µg; Asam askorbat 25.000 mg; Calsium-D-Phantothenate 6.000 mg; Niacin 40.000 mg; Cholin Chloride 10.000 mg; Metheonine 30.000 mg; Lisin 30.000 mg; Manganese 120.000 mg; Iron 20.000 mg; Iodine 200 mg; Zinc 100.000 mg; Cobalt 200.000 mg; Copper 4.000 mg; Santoquin (antioksidan) 10.000 mg; Zinc bacitracin 21.000 mg.*

Table 2. Results of the proximate analysis of the feed used in the study.

	Parameter (% BK)					
Feed	Protein	Fat	Crude Fiber	BETN	Ash	Energy(Kkal/kg)
A	35,49	7,34	9,65	30,99	17,53	4033,1
B	32,30	7,35	7,09	39,07	16,19	2816,0
C	29,43	7,00	8,67	35,82	19,08	3100,3
D	34,39	6,98	9,91	30,96	18,76	3287,8
E	34,13	7,36	8,27	34,50	15,75	3548,4

Descrition : Analysis in the nutrition laboratory of the Takalar Brackish Water Cultivation Research and Development Center (BPPBAP).

2.3. Bacterial Population

The population of bacteria found in the digestive tract of milkfish was counted at the beginning and end of the experiment using the plate count method. The sample of the digestive tract of milkfish is crushed and every 1 g of the digestive tract that has been crushed is diluted with 9 mL of sterile physiological solution. Then the dilution results are used to grow lactic acid bacteria on MRS medium to make it solid.

Aktivitas Enzim

The activity of the protease and amylase enzymes follows the method of [7]as follows:

A. Protease enzyme activity

The protease activity was calculated according to the equation:

$$U = \left\{ \frac{Act - Abl}{Ast - Abl} \right\} x \ \frac{P}{T}$$

Description:

U = unit of protease enzyme activity

Act = Sample absorbance value

Abl = Blank absorbance value
Ast = Standard absorbance value
P = dilution factor
T = Incubation time in minutes.

2.4. A-amylase enzyme activity
The activity of the α-amylase enzyme was measured using the following formula:

$$\text{A-amylase activity} = \left\{ \frac{\text{Ass - Abl}}{\text{Ast - Abl}} \right\} \times \frac{P}{T}$$

Description:
Ass = Absorbance value of sample
Abl = blank absorbance value
Ast = standard absorbance value
P = dilution factor (mL)
T = Incubation time (minutes).

2.5. Rasio konversi pakan (FCR)
Feed conversion ratio (FCR)The feed conversion was calculated using [8]:

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

Description :
FCR = Feed conversion ratio
Wo = weight of test animals at the beginning of the study
Wt = weight of the test animal at the end of the study
D = Number of fish that died

2.6. Data analysis
The data to be obtained was tested using Analysis of Variance (ANOVA) at a 95% level ofconfidencethrough SPSS statistical software. If the results of statistical analysis show a significantly differenteffect, a further test will be carried out to determine the best treatment.

3. Results anddiscussion

3.1. Bacterial Population
The results of measurements of bacterial populations in the digestive tract of test fish that received various prebiotic treatments from nuts in the feed at the beginning and end of the study are presented in Appendix 4 and the mean is in Table 3.

Table 3. Average population of bacteria in the digestive tract of milkfish at the beginning and end of the study

Prebiotics in Feed	Parameter ±Std (CFU/mL)	
	Early	End
Soybeans	324×10^{3}	$1{,}296 \times 10^{6} \pm .60{,}277^{b}$
Peanuts	204×10^{2}	$816 \times 10^{5} \pm .64{,}291^{a}$
Green beans	642×10^{3}	$2{,}643 \times 10^{6} \pm .113{,}724^{c}$
Red beans	694×10^{3}	$2{,}776 \times 10^{6} \pm .152{,}752^{c}$

Control	609 x10^3	2,436 x10^6±.236,924^c

Description: Different superscript letters in the same column indicate a significant difference between treatments at the 95% confidence level (P <0.05).

The results of the analysis of variance showed that the various prebiotic treatments of legumes in the feed had a significant effect (p <0.05) on the bacterial population in the milkfish digestive tract. The W-Tuckey further test showed that the milkfish bacterial population was the same in the prebiotic treatment of mung bean, red bean, and control, but it was different from the prebiotic treatment of soybeans and peanuts.In Table 3, it can be seen that there was an increase in the bacterial population in the digestive tract of milkfish, the highest was in the prebiotic treatment of red beans (2.082 × 103 CFU / mL), followed by green beans (2,001 × 103 CFU / mL), soybeans (972 × 103 CFU / mL).

The lowest digestive tract bacterial population in the prebiotic treatment of peanuts (612x103CFU / mL) was due to the lowest fiber content in the feed among treatments (Table 3). The increase in bacterial population in the digestive tract of milkfish is closely related to the fiber content in the feed. Crude fiber is a nutrient in feed that the host body cannot digest and absorb, but is a source of nutrients for the microflora of the digestive tract.[9]prebiotics are food ingredients that cannot be digested and absorbed by the body, but have a positive effect by stimulating the growth of beneficial bacteria in the digestive tract of its host. [10]explained that microorganisms in the digestive tract need nutrients to grow and reproduce. Nutrients are obtained from feed that enters the host's body. Feed with enough nutrients, causing microorganisms to grow and develop properly. [11]stated that crude fiber contains fructose oligosaccharide (FOS) Galacto-Oligo-Sakarida (GOS) or inulin which is a prebiotic that functions to increase the microflora population in the digestive tract.

[12] reported that the addition of one type of green bean prebiotic with various concentrations (0, 5, 10, and 15%) into the feed on the test animals of vanemai shrimp had a significant effect (p <0.05) on the probiotic population with a value the best (19,0x105 ± 468,501CFU / mL) was the addition of 10% green bean prebiotic concentration. This is because probiotics have the ability to remodel nutrients that are not digested by the body, so that prebiotics that cannot be digested in the digestive tract are used to stimulate probiotic growth. According to [13], prebiotics are components that cannot be separated from probiotics because the target of prebiotics is to stimulate probiotic growth. Previous studies have also reported that several types of oligosaccharides that have potential as prebiotics in fish feed can increase the growth and composition of probiotics in the gut[14].

3.2. Enzyme Activity

The results of measurements of protease and amylase enzyme activity from the digestive tract of milkfish treated with various prebiotics from nuts are presented in Appendix 5 and the average is in Table 4.

Table 4. Results of the enzyme activity analysis

Prebiotics in Feed	Enzyme (u/mL)	
	Protease	Amylase
Early	0,095	0,283
Soybeans	0,236	0,506
Peanuts	0,129	0,554
Green beans	0,156	0,543
Red beans	0,243	0,745
Control	0,112	0,520

Description: Different superscript letters in the same column indicate a significant difference between treatments at the 95% confidence level (P <0.05).

Table 4 shows that there was an increase in enzyme activity at the end of the study. The highest enzyme activity in the prebiotic type of kidney beans, namely the protease enzyme (0.243 u / mL) and the amylase enzyme (0.745 u / mL). This is thought to be high in crude fiber content in undigested feed, so that it is utilized by digestive tract microorganisms and provides positive benefits to the host. This concurs [15]that increasing the substrate concentration can increase probiotics and increase enzyme activity until the maximum limit is reached. [16]reported that enzyme activity was influenced by substrate concentration. At low substrate concentrations, the enzyme does not reach the maximum conversion due to the difficulty of the enzyme finding the substrate to be reacted. As the substrate concentration increases, the reaction speed will also increase due to the faster the substrate is bound to the enzyme. Increasing the substrate concentration at the saturation point can no longer increase the rate of reaction.

The increase in enzyme activity in the digestive tract was the result of an increase in the bacterial population in the digestive tract of test fish and the highest increase was produced in the prebiotic treatment of kidney beans. The addition of prebiotics in feed aims to increase the microflora population in the digestive tract of the host, so that the mechanism of action is to produce exogenous enzymes for digestion. According to[14] exogenous enzymes will help endogenous enzymes in the host to hydrolyze feed nutrients such as breaking or breaking down long chains of carbohydrates, proteins and fats that make up feed.

The addition of prebiotics in feed aims to increase the population of beneficial bacteria (probiotics) in the milkfish digestive tract so that the mechanism of action of probiotics in producing exogenous enzymes for digestion increases, so that the breakdown of complex molecules into simple molecules will facilitate digestion and absorption in the digestive tract. fish. [14]reported in a study that the addition of prebiotic oligosaccharides from sweet potato extracts (0, 1, 2, and 3%) in feed showed the highest increase in bacterial population was found in the 2% prebiotic treatment of 7.44 ± 0.02log CFU / g . The same result was also obtained by [17], the addition of rafinose in feed has increased the composition of probiotic bacteria in the digestive tract of turbot fish.

3.3. Feed Conversion Ratio(FCR)

The ratio of feed conversion for milkfish treated with various types of nuts in the feed is presented in attachment 5.

Table 5. Average ratio of milkfish feed conversion during the study

Prebiotikc in feed	Parameter ±Std
	FCR
Soybeans	1,43±.0,85 [a]
Peanuts	2,87±.1,70 [a]
Green beans	2,23±.0,81 [a]
Red beans	1,90±.0,79 [a]
Control	2,07±.1,50 [a]

The results of the analysis of variance showed that the treatment of various prebiotics from legumes in the feed had no significant effect ($p > 0.05$) on the feed conversion ratio.

The feed conversion ratio is the ratio between the amount of feed consumed and the resulting weight gain. The feed conversion value shows how much fish can use the feed given to form 1 kg of meat. The value of the feed conversion ratio that is getting smaller shows the better quality of the feed which is the higher the digestibility of the feed [18].

The value of the same feed conversion ratio in each treatment is thought to be related to good feed quality and according to the needs of milkfish and Sutikno (2011) [19]reported the nutritional needs of milkfish feed, namely protein (30-40%), carbohydrates (12-50%). and fat (7-10%), as well as the test feed can be consumed properly in sufficient quantity. According to [20], the size of the feed conversion ratio is influenced by several factors, namely the quality and quantity of feed, species, size and water quality. The size of the feed conversion ratio determines the effectiveness of the feed. [21]reported that the use of probiotics (bacteria Lactobacillus casei and Saccharomyces cereviceae) in feed with doses (0, 3, 4 and 5%) did not significantly influence the FCR value of tilapia during the study.

4. Conclusion

Prebiotic application of legumes has a significant effect on milkfish. The best type of prebiotic is used as feed raw material and to maximize probitic performance in milkfish, namely red beans.

References

[1] S A and Karim 2012 *Pengembangan pakan buatan berbasis bahan baku lokal dan limbah dengan subtitusi kacang merah untuk mendukung produksi ikan bandeng ukuran ekspor* (Universitas Hasanuddin)
[2] Sudradjat A 2011 *Panen bandeng 50 hari* (Penebar Swadaya Grup)
[3] Mazurkiewicz J, Przybył A and Golski J 2008 Usability of Fermacto prebiotic in feeds for common carp (Cyprinus carpio L.) fry *Nauk. Przyr. Technol.***2** 15
[4] Pelczar M J, Chan E C S and Hadioetomo R S 1988 *Dasar-dasar mikrobiologi* (Universitas Indonesia)
[5] Misgiyarta & Widowati S 2002 Seleksi dan Karakterisasi Bakteri Asam Laktat (BAL) Indigenous *Prosiding Seminar Hasil Penelitian Rintisan dan Bioteknologi Tanaman*
[6] Murni 2004 *Pengaruh Penambahan Bakteri Probiotik Bacillus sp. dalam Pakan Buatan Terhadap aktivitas Enzim Pencernaan, Efesiensi Pakan dan Pertumbuhan Ikan Gurame* (Institut Pertanian Bogor)
[7] Bergmeyer H U 1974 *Methods of enzymatic analysis* (Verlag Chemie)
[8] Djajasewaka H 1985 Pakan ikan *Yasaguna. Jakarta*
[9] 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 *Aquac. Nutr.***16** 117–36
[10] Iriyanti N, ER A and S R 2002 *Suplementasi kultur Lactobacillus sp. dan Bacillus sp. sebagai probiotik dan antibiotik dalam ransum terhadap peningkatan kecernaan nutrien ayam broiler* (Universitas Jenderal Soedirman)
[11] Kusharto C M 2006 Serat makanan dan perannya bagi kesehatan *J. gizi dan pangan***1** 45–54
[12] Basir B 2013 Kinerja prebiotik Lactococcus lactis dalam saluran pencernaan udang Vanamei (Litopenaeus vannamei) dengan pemberian pakan disuplemen prebiotik kacang hijau
[13] J. S and M V 2001 Probiotics, Prebiotiks dan Synbiotic Approaching a Definitions *J. Clin. Nutr.***73** 361–4
[14] A.N P 2010 *Aplikasi Probiotik, Prebiotik dan Sinbiotik untuk Meningkatkan Kinerja Pertumbuhan Ikan Nila (Oreochromis niloticus).* (Institut Pertanian Bogor)
[15] Adugna S, Alemu L A, Kelemu T, Tekola H, Kibret B and Genet S 2004 Medical Biochemistry (Lecture Notes For Health Science Students) Gondar University *Jimma Univ. Debub Univ. Collab. with Ethiop. Public Heal. Train. Initiat. Cart. Center, Ethiop. Minist. Heal. Ethiop. Minist. Educ.* 13–9
[16] R P 2009 *Isolasi Bakteri dan Uji Aktivitas Protease Termofilik dari Sumber Air Panas Sipoholon Tapanuli Utara Sumatera Utara* (Universitas Sumatera Utara Medan)
[17] 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) *Aquac. Int.***14** 219
[18] Nur 2011 *Manajemen Pemeliharaan Udang Vaname*

[19] Sutikno and Erik 2011 *Pembuatan pakan buatan ikan bandeng." Direktorat Jenderal Perikanan Budidaya Balai Besar Pengembangan Budidaya Air Payau* (Jepara)
[20] Handayani H 2008 *Pengujian Tepung Azolla Terfermentasi Sebagai Penyusun Pakan Ikan Terhadap Pertumbuhan dan Daya Cerna Ikan Nila Gift* (Universitas Muhammadiyah Malang.)
[21] Ardita N 2013 Pertumbuhan dan rasio konversi pakan ikan nila (Oreochromis niloticus) dengan penambahan probiotik