IOP Conference Series: Earth and Environmental Science

PAPER • OPEN ACCESS

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To cite this article: E Hendalia *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **391** 012011

IOP Conf. Series: Earth and Environmental Science **391** (2019) 012011 doi:10.1088/1755-1315/391/1/012011

The Use of Prebiotics and Probiotics in Fish Meal Processing

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Abstract, The use of prebiotics and probiotics in fish meal processing is an alternative method to produce fish meal without boiling, steaming and pressing process. Processing fish meal using prebiotics and probiotics does not produce waste and bad odor that potentially pollutes the environment. This study aims to evaluate physical, chemical and microbiological quality of fish meal processed using two types of prebiotic sources i.e. coconut meal (CM) and palm kernel meal (PKM) and three levels (0; 1 and 2%) of Probio_FM probiotics. The experiment was carried out using a factorial 2 x 3 in complete randomized design with four replications. The variables observed were physical characteristics (aroma, color and texture), microbiological characteristics (number of *Lactobacillus* spp. and *Salmonella* colonies), and chemical composition of fish meal (moisture content, crude protein, ether extact and gross energy). The results showed that fish meal processed using prebiotics with or without probiotics had a specific odor of fish meal, light brown color to dark brown, not greasy, not clumping and not indicating physical changes and rancidity after four months of storage. The use of prebiotics combined with Probio_FM produces fish meal free of *Salmonella*, containing 10^{10} cfu/g of *Lactobacillus* spp.; 43,77 to 45,81% crude protein; 8,16 to 11,35% ether extract; and 4354 to 4529 kcal/kg Gross Energy. It was concluded that the use of coconut meal or palm kernel meal with or without Probio_FM could be applied to produce fish meal as a source of protein as well as source of energy and probiotics, however the combination of prebiotics and probiotics would be better for inhibiting Salmonella growth.

1. Introduction

Since some new policies applied to combating Illegal, Unreported, and Unregulated Fishing (IUU-F) in 2014, national fish stock has been continuing increase, including trash fish (garbage fish) that potentially to be used as protein source feed. Due to the lack of domestic fish meal processing industry and the absence of simple method that can be applied by the community, most of trash fish are still dumped back into sea rather than processed into feed, meanwhile Indonesia still imports fish meal to meet national needs.

Environmental issues in fish processing projects primarily include: solid waste and by-products; waste water; water consumption and management; and air emission and energy consumption (IFC, 2017). Related to these issues and to optimize the use of trash fish as feed, it is necessary to find a fish meal processing technique that is practically applied, producing less waste, using less water, using less energy and producing minimum odor that potentially pollutes the environment.

Figure 1. TFM in the drying process

Fish meal is generally processed follows six steps: cooking, pressing, separating, evaporation, drying and grinding (Likitrattanaporn, 2016). This conventional processing technique, besides producing waste and air emission, it also causes a damage of protein and loss of several dissolved nutrients such as amino acids, fatty acids, vitamins and minerals. An alternative to overcome this problems is processing trash fish using prebiotics feeds source i.e. coconut meal (CM) and palm kernel meal (PKM) combined with Probio_FM probiotics to produce trash fishmeal containing probiotics (TFM-PRO) (Hendalia et al. 2014; Hendalia et al. 2016). Probio_FM is a probiotics containing five species of lactic acid bacteria (*Lactobacillus* spp) produced by Manin et al. (2014).

Some advantages of producing TFM-PRO are: trash fish, garbage fish, and rotten fish can be easily processed into fishmeal without cooking (steaming or boiling) and pressing (squeezing) process. There are no nutrient damage or loss caused of heating process. The process is very simple and does not produce waste and bad odor that potentially pollutes the environment. The fishmeal by itself becomes a source of probiotics that is useful for maintaining animal health and reducing ammonia emissions from poultry cages and fish ponds (Hendalia et al., 2012; Hendalia et al., 2014a; Hendalia et al., 2014b). The price of the fishmeal is cheaper than commercial fishmeal. And it can increase fishermen income from selling trash fish.

The principle of using prebiotics and probiotics (i.e. Probio_FM) in processing fishmeal is utilizing Lactic Acid Bacteria (LAB) to inhibit the growth of decomposing bacteria in fish, because the LAB are able to produce lactic acid and bacteriocin (Lopez, 2000). The LAB is also able to utilize ammonia come from decaying of fish so that it can reduce bad odors of fish meal (Hendalia et al. 2016). The growth of the LAB is very dependent on prebiotics. Therefore, the use of CM or PKM as source of nutrients for bacteria are needed (Manin et al. 2012; 2013; Hendalia et al. 2012; 2015). Based on the ability of LAB bacteria to produce antimicrobial compounds, there is an opportunity to utilize Probio_FM combined with CM or PKM to process trash fish to be fish meal containing probiotics (TFM-PRO).

The aim of this study was to evaluate the physical, microbiological, and chemical characteristics of TFM-PRO processed from trash fish using coconut meal or palm kernel meal combined with Probio_FM containing *Lactobacillus* spp.

2. Methods

2.1 Material

The materials used in this study were derived from Jambi Province consisting of trash fish, Probio_FM, coconut meal and palm kernel meal. The trash fish is obtained from fishermen in Tanjung Jabung Timur Regency. The coconut meal is obtained from coconut processing factories in Tanjung Jabung Barat Regency; while the palm kernel meal from palm oil processing plants in Sarolangun Regency. The probiotics used are Probio_FM produced by Faculty of Animal Husbandry Universitas Jambi, containing 10^{10} - 10^{11} cfu /ml of Lactobacillus spp. (Manin et al., 2014).

2.2 Fishmeal Processing

Fresh trash fish were milled, then the prebiotic source (coconut meal and palm kernel meal) added to milled fish in 10% of fish weight. Likewise, Probio_FM is added 0%, 1% and 2% according to the treatment applied. The mixture of ingredients were stirred until homogeneous and put in the tray for dried in the drying room naturally, until the water content of fishmeal is approximately 10%. The dried TFM-PRO was analyzed at the laboratory to evaluate the physical characteristics (aroma, color and texture), chemical composition (moisture content, crude protein, ether extract and gross energy) and microbiological characteristics (the number of Lactobacillus spp. and Salmonella colonies). To evaluate durability in storage, the TFM-PRO was put into plastic bags and stored at room temperature for 1, 2 and 4 months for observation with organoleptic test. The data was analyzed with Anova and Duncan post-test.

3. Findings and Discussion

3.1 Physical Characteristics of Trash Fishmeal containing Probiotics (TFM-PRO)

The fishmeal processed using prebiotic, with or without probiotic, had a specific odor of fishmeal and no bad or rancid odor. The fish meal had a bright brown color, however the use of palm kernel meal produced a darker color compared to coconut meal (Fig.1). The overall of fishmeal had soft texture, not greasy and not clumping (Fig.1). The fishmeal also did not show spoilages and rancidity after four months storage at room temperature.

The results of this study prove that fishmeal can be produced without process of cooking and pressing. Handoyo and Assadad (2016), reported that the step of fishmeal processing by industries or community were through washing, cooking (steaming or boiling), pressing, drying and grinding. The cooking process was the first step to stop enzymes and microorganisms activity in the waste and to prevent deterioration and spoilage (Likitrattanaporn, 2016). Besides to stop microorganisms activity, the purpose of cooking also to reduce fat content in the fish. High fat content causes fishmeal to look oily and to accelerate rancidity (Handoyo and Assadad, 2016). The entire TFM-PRO in this study had good physical characteristics, although there was no reduction in fish fat content in the process.

This study shows that prebiotic and the probiotics bacteria play an important role in maintaining the quality of fish meal. The presence of coconut flour or palm kernel cake as a prebiotic can stimulate the growth of LAB derived from Probio_FM or which grows naturally in prebiotic sources, so that lactic acid, bacteriocin and antimicrobial compounds will be produced (Lopez, 2000). From previous research, Hendalia et al. (2017) report that Lactobacilus spp. can use ammonia from the decay process and can also use fat as an energy source, so it can eliminate unpleasant odors and prevent rancidity.

Table 1. Physical Characteristics of Trash Fishmeal Containing Probiotics during the storage periode

Treatments		Physical Characteristics		
Prebiotic Source	Probio_FM leves	Aroma	Color	Texture
Coconut Meal (CM)	0%	specific fish odor, no rancidity	light brown	soft, not greasy and not clumping
	1%	specific fish odor, no rancidity	light brown	soft, not greasy and not clumping
	2%	specific fish odor, no rancidity	light brown	soft, not greasy and not clumping
Palm Kernel Meal (PKM)	0%	specific fish odor, no rancidity	dark brown	soft, not greasy and not clumping

1%	specific fish odor, no rancidity	dark brown	soft, not greasy and not clumping
2%	specific fish odor, no rancidity	dark brown	soft, not greasy and not clumping

Remark: There were no differences in physical characteristics during the 4-month storage period

3.2 The Number of Lactobacillus and Salmonella Colonies

The microbiological characteristics of fishmeal can be observed from the number of *Lactobacillus* spp. and *Salmonella* on the TFM-PRO. Total *Lactobacillus* spp. and *Salmonella* on the fishmeal was not significantly (P>0,05) influenced by the kind of prebiotics source, but was significantly influenced by the addition of Probio_FM and the interaction of both. Number of colonies of *Lactobacillus* spp. and *Salmonella* in fishmeal are showed in Table 2.

The data in Table 2. show that *Lactobacillus* spp. can grow evenly on all fish meal produced, including fish meal without probiotics. The presence of *Lactobacillus* spp. in fish meal without probiotics it is predicted to originate from bacteria that grow naturally in CM and PKM. These findings suggest that Lactobacillus grown in prebiotic feed sources can inhibit the growth of decomposing bacteria in the fish, but cannot completely inhibit Salmonella growth. It means that probiotics must be added to produce a TFM-PRO that is free of Salmonella. Previous experiments showed that *Bacillus* spp. and *Lactobacillus* spp. can grow on chicken feather substrate added with coconut meal without the addition of probiotics (Hendalia et al., 2017). Manin et al. (2012; 2013) reported that some feed ingredients such as soybean meal, palm kernel meal and coconut meal can be used as prebiotic sources, but the best is palm kernel meal.

Table 2. The Number of *Lactobacillus* spp. and *Salmonella* Colonies on the Trash Fish Meal Containing Probiotics (TFM-PRO)

TREATMENTS		*Lactobacillus* spp. (cfu/gr)	Salmonella (cfu/gr)
PREBIOTICS	CM	$3{,}24 \times 10^{10}$	$2{,}1 \times 10^{1}$
	PKM	$1{,}66 \times 10^{10}$	$2{,}2 \times 10^{1}$
Significance level		***P>0,05***	***P>0,05***
PROBIOTICS	0%	$1{,}23 \times 10^{10}$	$9{,}6 \text{ X } 10^{1}$
	1%	$3{,}98 \times 10^{10}$	0
	2%	$2{,}57 \times 10^{10}$	0
Significance level		***P>0,05***	***P<0,05***
PREBIOTICS	CM - PRO 0%	$1{,}55 \times 10^{10}$	$8{,}8 \times 10^{1}$
vs	CM - PRO 1%	$3{,}31 \times 10^{10}$	0
PROBIOTICS	CM - PRO 2%	$6{,}61 \times 10^{10}$	0
	PKM - PRO 0%	$9{,}77 \times 10^{9}$	$10{,}4 \times 10^{1}$
	PKM - PRO 1%	$4{,}68 \times 10^{10}$	0
	PKM - PRO 2%	$1{,}0 \times 10^{10}$	0
Significance level		***P>0,05***	***P>0,05***

Remarks: CM = Coconut Meal; PKM = Palm Kernel Meal, PRO = Probio_FM Probiotics; TFM-PRO = Trash Fish Meal containing Probiotics

Salmonella only grows in fishmeal without Probio_FM (0% PRO). the addition of 1% and 2% Probio_FM produces negative salmonella TFM-PRO. Lopez (2000), reports that the mechanism of probiotic activity consists of two ways. First, it produces acid to make pH lower. This condition is not beneficial for pathogenic microorganisms. Second, some probiotic microbes can produce bacteriocin and antimicrobial substances which can inhibit the growth of other unfavorable microbes.

Number of colonies of *Lactobacillus* spp. on all TFM-PRO around 10^{10} cfu/g. This number is close to the amount of *Lactobacillus* spp. contained in Probio_FM probiotics (Manin et al. 2014). The presence of *Lactobacillus* spp. in fish meal can be used as a source of probiotics to maintain animal health and improve animal performance. Previous experiments proved that Probio_FM use in livestock was able to reduce the number of pathogenic bacteria in the digestive tract of poultry, improve animal health, improve feed efficiency, reduce ammonia pollution from broiler cages, and reduce odor in fish pond (Manin et al. 2010; Hendalia et al., 2012; Hendalia et al., 2013). Probiotic microbes can multiply in the digestive tract and compete with pathogenic microbes and other microbes to bind to the same receptors (Lopez, 2000).

3.3 Nutrient Composition of the TFM-PRO

Water. Moisture content of the fishmeal was significantly (P <0.05) influenced by prebiotic sources, but it was not significantly influenced by the probiotic level and the interaction of both. Fishmeal processed using CM contained a higher moisture content compared to the fishmeal processed using palm kernel meal (fig.2). It was difficult to know the cause of this difference because the drying process was carried out manually. Likitrattanaporn (2016) reported that tray drying takes 72 hours to dry catfish waste meal to 10% moisture contents compared to sun drying which takes 5 days to around 15% moisture content.

Table 3. Nutrients Composition of TFM-PRO (DM)

TREATMENT		WATER	CRUDE PROTEIN	ETHER EXTRACT	GROSS ENERGY
		(%)	(%)	(%)	(kkal/kg)
PREBIOTICS	CM	14,33^{a}±1,19	45,96^{a}± 1,75	9,69 ± 1,87	4458,36^{a}
	PKM	10,84^{b}±1,69	43,99^{b}± 1,76	9,84 ± 1,56	4273,71^{b}
Significance		P<0,05	P<0.05	P>0,05	P<0,05
PROBIOTICS	0%	13,06±1,61	44,99 ± 2,96	10,55a± 1,76	4184,45^{a}
	1%	12,03±2,70	44,35 ± 1,17	8,99^{b} ± 1,26	4441,46^{b}
	2%	12,66±2,58	45,60 ± 1,41	9,75b± 1,81	4472,18^{b}
Significance		P>0,05	P>0,05	P<0,05	P<0,05
PREBIOTICS vs	CM*PRO 0%	13,81±0,65	47,57^{a} ± 0,86	12,07^{a} ± 0,62	4367,94
PROBIOTICS	CM*PRO 1%	14,29±1,61	44,93^{c} ± 0,89	8,84^{b} ± 0,30	4529,35
	CM*PRO 2%	14,90±1,17	45,38^{b}± 2,01	8,16^{b} ± 0,81	4477,77
	PKM*PRO 0%	12,31±2,03	42,40^{c} ± 1,33	9,03^{b} ± 0,84	4000,95
	PKM*PRO 1%	9,76±0,83	43,77^{c}± 1,23	9,14^{b}± 1,88	4353,58
	PKM*PRO 2%	10,43±0,96	45,81^{ab}±0,41	11,35^{a}±0,41	4466,59
Significance		P>0,05	P<0,05	P<0,05	P>0,05

Remarks: The same letter shows no significant differences (P<0,05)

Although, the moisture content in fish meal processed using CM was higher than fish meal processed using PKM, there was no significant difference in aroma, color and texture. This fact showed that probiotic bacteria contained in the fishmeal could work well to inhibit the growth of decomposing bacteria.

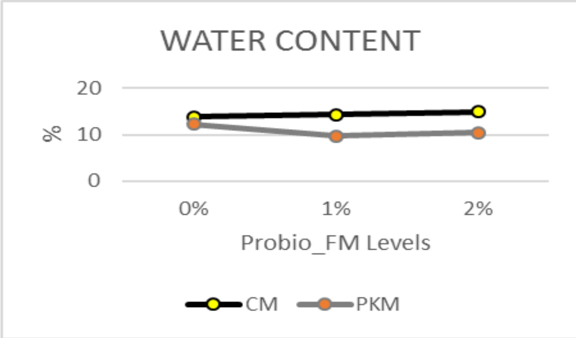

Figure. 2. Water content of TFM-PRO

Based on the Indonesian National Standard (SNI 01-2715-1996), the maximum water content of fishmeal for 1st quality is 10%, while for 2nd and 3rd is 12%. Only the fishmeal processed using PKM that meets the 2nd and 3rd quality standards. According to Fatmawati and Mardiana (2014), if the water content of fishmeal is more than 10% it will reduce the quality of fishmeal because it can increase the activity of microorganisms, especially *Salmonella* bacteria. Handoyo and Assadad (2016) reported that water content of local fishmeal from small industries were 8,19 – 24,8 %. Water content greatly is influenced by the processing and drying techniques carried out.

The study showed that even though the water content of TFM-PRO was higher than specified by SNI, the fishmeal in all treatments showed no change physically and no damage or rancidity during four months storage at room temperature. This study proves that the presence of probiotic bacteria in fish meal can maintain the quality of fish meal during storage.

Crude Protein. Crude protein content (CP) of fish meal was significantly ($P < 0.05$) influenced by prebiotic sources and interactions between prebiotics and probiotics, but not significantly affected by probiotic levels. Overall, the protein content in the TFM-PRO ranged from 42.40 - 47.51%, but the protein content in the TFM-PRO free of Salmonella was 43.77 to 45.81% in dry matter. (Fig. 3).

The highest CP content was obtained on the fishmeal with CM-PRO 0%, while the lowest CP is on the fishmeal PKM-PRO 0%. Higher levels of CP on the fishmeal processed using CM influenced by higher levels of protein of CM (21.2%) than PKM (16.6%) (Amrullah, 2003). There is an interaction between prebiotic type and the level of probiotics, where the fishmeal with CM-PRO 2% and with PKM-PRO 2% had a higher protein content.

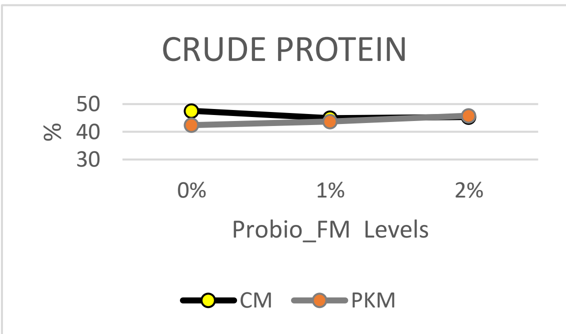

Figure 3. Crude protein content of TFM-PRO

Based on the Indonesian National Standard (SNI 01-2715-1996), the minimum protein content of fishmeal for 1st, 2nd and 3rd quality respectively are 65%; 55% and 45%. Protein content of the fishmeal in TFM-PRO classified in the range of 3rd quality standard. Assadad et al. (2015), reported that protein content of trash fish treated with cooking was 51.47% (steamed); 55.93% (pressure cooker) and 58.02% (boiled). Rahim et al. (2017) reported that proximate composition of fishmeal samples collected from different units showed that protein contents were 50.51% – 61.26% and energy was 4042.0 cal/g –

4558.0 cal/g. Variation in fishmeal protein content was influenced by the species of raw material used. Trash fish used in the study were mostly thin and scaly small fish.

Ether Extract. The ether extract content of the fishmeal was significantly (P <0.05) influenced by the probiotic level and the interaction between prebiotics and probiotics. Prebiotics and probiotics had an inconsistent effect on the ether extract content on the fishmeal (Figure 4). The ether extract content of the fishmeal in this study was ranged from 8.16% to 12.07%, lower than the results obtained by Assadad et al. (2015) which is 13.39%; 13.45% and 12.59% in trash fish meal processed through boiling, steaming and presto respectively. According to SNI 01-2715-1996, the maximum fat content in fishmeal is 8% for 1st quality, 10% for 2nd quality and 12% for 3rd quality.

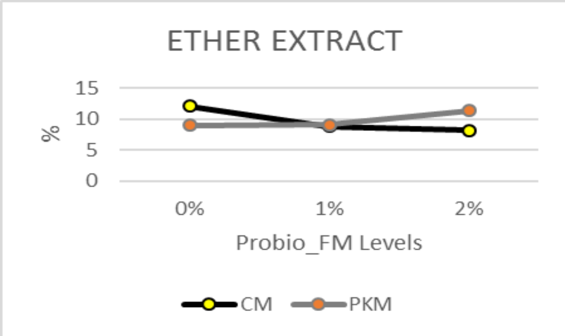

Figure 4. Ether extract content of TFM-PRO

Based on the fat content of the fishmeal, the TFM-PRO fulfilled the 2nd and 3rd quality. Handoyo and Assadad (2016) reported that high fat content (> 12%) caused the appearance of fishmeal to look wet and oily and accelerated the occurrence of rancidity. Hendalia et al. (2017a) reported that Lactobacilus spp. could utilize fat as an energy source, so it would influence on the extract ether content of fishmeal.

Gross Energy. Gross Energy (GE) content of TFM-PRO was significantly (P <0.05) influenced by the type of prebiotic source and probiotic level, but it was not significantly influenced by the interaction of both. The GE content of the fishmeal processed using coconut meal was higher (P <0.05) compared with the fishmeal processed using palm kernel meal. The Addition of probiotics significantly (P <0.05) increased GE of the fishmeal. However, there was no difference between the use of probiotics 1% and 2% (figure 5).

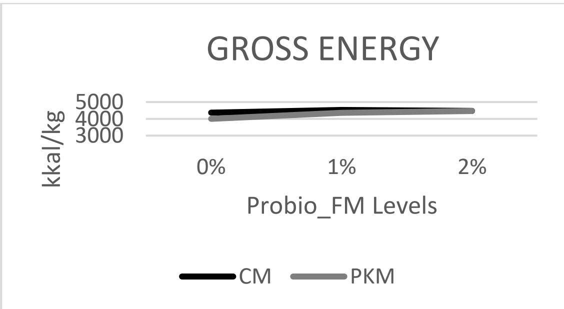

Figure 5. Gross Energi content of TFM-PRO

The GE content in all TFM-PRO was range from 4001 - 4529 Kcal/kg. If it was assumed that ME (metabolizable energy) was 72.5% from GE, approximate ME value of the fishmeal was about 2900 - 3280 kcal / kg. The ME value of fishmeal varied according to the type of fish and processing method. According to Hartadi et al. (2017), ME value of fishmeal was ranging from 2219 to 2580 kcal/kg, meanwhile according to NRC (1994) was ranging from 2580 to 3190 kcal/kg. The causes of these differences are the variety of raw materials and methods of fishmeal processing (Handoyo and Assadad, 2016). Fatmawati (2014) defined that fishmeal is a dry solid product produced by removing most of the liquid and fat contained in the body of the fish. Likitrattanaporn (2016) explained that Fishmeal production consisted of five processes which began with cooking. Water and oil were then squeezed from the cooked waste.

A higher ME content on the TFM-PRO has a positive influence on nutritional value of feed because the produced fishmeal can be used as a source of protein as well as an energy and probiotic source.

4. Conclusion

It can be concluded that the use of coconut meal, palm kernel meal with or without Probio_FM can be applied to process fishmeal as a source of protein as well as source of energy and probiotics. However, to inhibit the growth of *Salmonella*, it would be better if prebiotics was combined with probiotics.

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Acknowledgment

The author would like to thank to the Director of Research and Community Services, Directorate General of Strengthening Research and Development, Ministry of Research, Technology and Higher Education who has funded this research and the Head of the Institute for Research and Community Services, Universitas Jambi which has facilitated the implementation of this research.