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### Synthesis of Tilapia Feed From a Mixture of Tarum Leaves (*Indigosfera sp*) and Fermented Shrimp Waste by EM-4 Probiotics

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#### ABSTRACT

Feed is the most important thing in tilapia fish farming. A feed required appropriate and balanced nutritional content so that fish growth becomes faster. Shrimp waste and tarum leaves are alternative raw materials for manufacturing tilapia feed because they have a fairly high protein content. The purpose of this study was to examine the comparative effect of adding the percentage of tarum leaf meal and EM-4 fermented shrimp waste on the water content, ash content, protein content, fat content, and crude fibre content of the feed. The research method used is using the EM-4 fermentation process on shrimp waste. The variations carried out were the concentration of the addition of EM-4 (14,16,18,20, and 22 ml) in 100 grams of feed mixture and the percentage of addition of Wet Shrimp Waste (WSW) and Tarum Leaf Powder (TLP) (90% WSW + 10% TLP, 80% WSW + 20% TLP, 70% WSW + 30% TLP, 60% WSW + 40% TLP, and 50% WSW + 50% TLP). The results showed that the addition of fermented WSW EM-4 and TLP had a significant effect on the protein, fat and crude fibre content, while the water and ash content did not have a significant effect. The best tilapia feed formulation results were obtained at a ratio of 60% WSW and 40% TLP using 18 ml of EM-4 with the results obtained for water, ash, protein, fat and crude fibre content each of 7.09% ; 12.59%; 29.98%; 7.45%; and 3.49%.

**Keywords:** fermented EM-4; fish feed; shrimp waste; tarum leaf; tilapia fish

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#### ABSTRAK

Pakan merupakan hal yang paling utama dalam melakukan suatu budidaya ikan nila. Dalam sebuah pakan dibutuhkan kandungan nutrisi yang sesuai dan seimbang agar pertumbuhan ikan menjadi lebih cepat. Limbah udang dan daun tarum merupakan sebuah alternatif bahan baku campuran pembuatan pakan ikan nila karena memiliki kandungan protein yang cukup tinggi. Tujuan penelitian ini yaitu untuk mengkaji pengaruh perbandingan penambahan persentase tepung daun tarum dan limbah udang terfermentasi EM-4 terhadap kadar air, kadar abu, kadar protein, kadar lemak, dan kadar serat kasar pakan. Metode penelitian yang digunakan yaitu menggunakan proses fermentasi EM-4 pada limbah udang. Variasi yang dilakukan yaitu konsentrasi penambahan EM-4 (14,16,18,20, dan 22 ml) dalam 100 gram campuran pakan serta persentase penambahan Limbah Udang Basah (LUB) dan Tepung Daun Tarum (TDT) (90%LUB + 10%TDT, 80%LUB + 20%TDT, 70%LUB + 30%TDT, 60%LUB + 40%TDT, dan 50%LUB + 50%TDT). Hasil penelitian menunjukkan bahwa penambahan LUB terfermentasi EM-4 dan TDT berpengaruh secara signifikan terhadap kadar protein, lemak, dan serat kasar, sedangkan terhadap kadar air dan abu tidak berpengaruh secara signifikan. Hasil formulasi pakan ikan nila terbaik diperoleh pada perbandingan 60% LUB dan 40% TDT menggunakan EM-4 sebesar 18 ml dengan hasil yang diperoleh untuk kadar air, abu, protein, lemak, dan serat kasar masing-masing sebesar 7,09%; 12,59%; 29,98%; 7,45%; dan 3,49%.

**Kata kunci:** fermentasi EM-4; pakan ikan; limbah udang; daun tarum; ikan nila

## INTRODUCTION

High market demand for tilapia causes tilapia cultivation to grow. Quality feed is needed in tilapia cultivation so that fish growth is getting better and faster. There are several types of tilapia feed, namely natural and commercial feed. The natural food for tilapia is moss, worms, and *Azolla microphylla*. The natural feed has deficiencies such as inappropriate nutrition and susceptibility to pests and diseases. Commercial feed is artificial feed to meet the nutritional needs of fish. One of the obstacles in tilapia aquaculture is the high price of commercial fish feed. The price of this feed is quite expensive because the production of fish meal (commercial feed raw material) in Indonesia is still lacking, so 200.000 tons/year of fish meal is still imported [1].

The abundant shrimp waste has the potential to be used as an alternative raw material for making tilapia feed. Shrimp can be produced around 80.000 tons from a shrimp pond area of 380.000 hectares each year. Shrimp waste generated from the shrimp processing is equal to 30-40 percent of the weight of the shrimp [2]. Shrimp waste has a relatively high crude protein content of 45-55% [3]. This high protein content can be used to mix raw materials in the manufacture of tilapia feed.

## LITERATURE REVIEW

In shrimp waste, there is a compound, chitin, which can limit the protein content. Chitin is a cellulose-like polysaccharide compound which contains nitrogen in the form of N-acetylated-glucosamine-polysaccharida. Protein and nitrogen content in shrimp waste are tightly bound to chitin and calcium carbonate in the form of protein-chitin-calcium carbonate compounds so that the chitin content is difficult for fish to digest. One way to reduce the chitin content in shrimp waste can be done by EM-4 fermentation. EM-4 is a fermenting bacterium that has a mixed culture of several microorganisms such as *Lactobacillus* sp, photosynthetic bacteria, *Actinomycetes* sp, *Sreptomyces* sp, cellulose-decomposing fungi and yeast which can decompose cellulose or chitin content in shrimp waste [4].

The raw materials for making tilapia feed do not only come from animal ingredients, but vegetable ingredients also play an important role in fish feed nutrition. One of the vegetable ingredients that has the potential to be used as a raw material for making tilapia feed is tarum leaves (*Indigofera* sp). Tarum leaf (*Indigofera* sp) is a plant from the Leguminosae family. Tarum leaves have a protein content of 27,68 – 28,98% and a nutritional content 2 – 3 times greater than the content of cereal grains. The addition of tarum leaves has the potential to improve nutrition and feed quality for tilapia due to the high protein content in these leaves [5].

Previous research has done a lot of exploration regarding the manufacture of fish feed. Research conducted by Musdalifah (2019) found a protein content of 27,44%, a fat content of 5,11%; crude fiber content 16,55%; water content 8,36%; and ash content of 21,99% with raw material substitution of 30% shrimp head flour and 20% tarum leaves (*indigofera* sp). The difference between this research and Musdalifah's research is by fermenting EM-4 first on the shrimp waste, while Musdalifah's research did not fermentation the shrimp waste. Research from Mirzah & Filawati (2013) found crude protein levels of 42,23%, crude fiber 19,87%; fat 2,89%; water content 9,39%; and 9,56% chitin content by immersing shrimp waste in 20% Husk Ash Filtrate (FAAS) solution for 48 hours and heated in an autoclave for 45 minutes. The difference between this research and Mirzah & Filawati's research is that the manufacture of fish feed uses the EM-4 fermentation method, while Mirzah & Filawati's research uses the Husk Ash Water Filtrate (FAAS) method. Research from Yuriandala (2020) found a moisture content of 16,48%, an ash content of 31,66%, a protein content of 14,68%, a fat content of 3,63% and a crude fiber content of 8,61% with a ratio of fish bone meal; bran ; Mixed Flour; Starch ; Water = 17,81 gr; 17,81 grams; 64,38 gr; 5 grams; and 30%. The difference between this research and Yuriandala's research is the presence of EM-4 fermentation treatment on shrimp waste in the formulation of fish feed, while Yuriandala's research did not carry out fermentation in the manufacture of feed. This research updates some of the research that has been done, namely by carrying out the EM-4 fermentation process on shrimp waste, it can reduce chitin levels and increase protein levels so that good feed quality is obtained and in accordance with SNI 01-7242-2006 tilapia fish feed.

## METHOD

### Materials

The main material used in this study was fresh shrimp waste from PT. Surya Alam Tunggal and tarum leaves obtained from Bocek Village. Other supporting materials are EM-4 as an ingredient for shrimp waste fermentation and bran, starch, vitamins, and squid oil as complementary ingredients for tilapia feed formulations.

### Experiment

#### Pretreatment of Raw Materials

Shrimp waste is washed and cleaned first to remove dirt. Shrimp waste is drained for 10 minutes, then blended with a blender to become Wet Shrimp Waste (WSW). Furthermore tarum leaves are washed first, then dried in the sun for about 3 days. Tarum leaves that have been dried in the sun are then blended until smooth and then sieved with a sieve size of  $\pm 60$  mesh.

#### Shrimp Waste Fermentation

Fermentation is carried out to reduce the chitin content contained in shrimp waste. The fermentation process is carried out by adding EM-4 to a container containing shrimp waste and tightly closed so that the fermentation process runs anaerobically. Wet Shrimp Waste (WSW) was fermented using EM-4 with a variable concentration of EM-4 addition of 14, 16, 18, 20, and 22 ml which was carried out for 9 days.

#### Feed Formulation

The main raw materials include fermented WSW and tarum leaves mixed with additional raw materials in the form of 13% bran, 5% starch, 2% vitamins, and 10% squid oil from 100 grams of feed to be made. The feed formulation that has been made is steamed for approximately 5-10 minutes, after steaming it is left to cool and then molded into pellets. Bake the pellets for approximately 2-3 hours at 100-110°C until dry. The product in the form of tilapia feed was then analyzed for its water content, ash, protein, fat and crude fiber.

## RESULTS AND DISCUSSION

### Results of Analysis of Raw Materials

Raw material analysis in this study was carried out to determine the protein content of the main raw materials including wet shrimp waste and tarum leaves as formulations in the manufacture of tilapia feed. Analysis of raw materials also aims to produce tilapia feed in accordance with SNI 01-7242-2006 tilapia fish feed. Raw material analysis was carried out by testing the protein content of shrimp waste and tarum leaves. The results of the protein content test of shrimp waste and tarum leaves (*Indigofera sp.*) are presented in Table 1.

Table 1. Results of protein content test of Shrimp Waste and Tarum Leaves (*Indigofera Sp*)

Material	Protein Content (%)
Shrimp Waste	13,84
Tarum Leaf	29,98

Source : Fish and Environmental Health Laboratory (2022)

Based on Table 1. it can be seen that the results of the initial protein content for raw materials for shrimp waste and tarum leaves are 13.84% and 29.98%. Fresh unfermented shrimp waste contains quite high crude protein, which is equal to 36.75% [6]. The results of the protein content of shrimp waste before being fermented were not too high in the results of the raw material analysis because the shrimp waste was not analyzed directly, but was stored first in the refrigerator. This causes the water content in the shrimp waste to become more so that the protein in the shrimp waste will dissolve and result in a decrease in protein levels when analyzed [7]. The low protein content in shrimp waste

is due to the presence of a limiting factor in the form of chitin content. The chitin content can limit the digestibility of protein in shrimp waste which results in protein not being used optimally.

Table 1 shows that the results of the protein content test on tarum leaf raw material was 29.98%. The protein content of tarum leaves that can be used as a raw material for the synthesis of tilapia feed is 27.08% with most of the essential amino acid composition [5].

#### The Effect of EM-4 Dosage on Water Content of Tilapia Feed at Various Ratio of WSW:TLP (w/w% (90:10; 80:20; 70:30; 60:40; 50:50))

The effect of the percentage addition of tarum leaf flour and fermented shrimp waste with EM-4 probiotics on water content is presented in Figure 1.

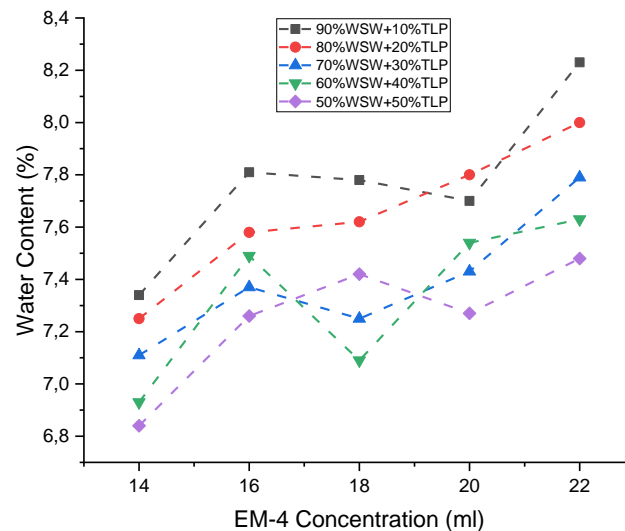


Figure 1. The effect of EM-4 dosage on water content of Tilapia feed at various ratio of WSW:TLP (w/w% (90:10; 80:20; 70:30; 60:40; 50:50))

Based on Figure 1. it can be seen that the increasing percentage of Wet Shrimp Waste (WSW) and decreasing percentage of Tarum Leaf Powder (TLP) with the addition of the same EM-4 probiotic, the resulting water content is relatively stable. The lowest water content was the addition of 50% WSW and 50% TLP with 14 ml of EM-4 probiotics resulting in 6.84% water content, while the highest water content was 90% WSW and 10% TLP with EM-4 probiotics of 22 ml yields a moisture content of 8.23%. The difference in water content is affected by the water content in the raw materials for shrimp waste and tarum leaves, the greater the percentage of addition of shrimp waste, the higher the water content produced [8]. Factors that affect the water content in a material are storage method, storage climate, and drying time [9]. Appropriate water content will prevent fish feed from growing fungi so that the shelf life and life of the feed can be longer [10]. The water content in each parameter is in accordance with SNI for tilapia feed 01-7242-2006, which is a maximum of 12%.

#### The Effect of EM-4 Dosage on Ash Content of Tilapia Feed at Various Ratio of WSW:TLP (w/w% (90:10; 80:20; 70:30; 60:40; 50:50))

The comparative effect of adding the percentage of tarum leaf powder and fermented shrimp waste with EM-4 probiotics on ash content is presented in Figure 2.

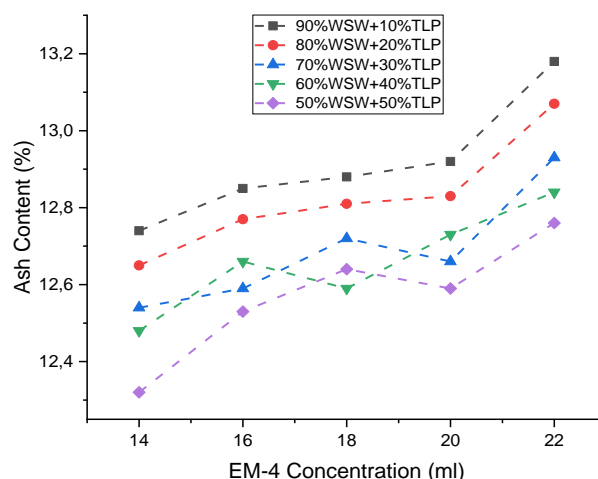


Figure 2. The effect of EM-4 dosage on ash content of Tilapia feed at various ratio of WSW:TLP (w/w% (90:10; 80:20; 70:30; 60:40; 50:50))

Based on Figure 2. it can be seen that the increasing percentage of Wet Shrimp Waste (WSW) and decreasing percentage of Tarum Leaf Powder (TLP) with the addition of the same EM-4 probiotic, the resulting ash content tends to increase. The lowest ash content was found in the percentage of 50% WSW and 50% TLP with 14 ml of EM-4 probiotic, namely 12.32%, while the highest ash content was found in the percentage of 90% WSW and 10% TLP with 22 ml of EM-4 probiotic, namely 13.18%. The high ash content is due to differences in the ash content of the high raw materials for shrimp waste and tarum leaves (*Indigofera* sp) [5]. Ash in feed includes inorganic components that cannot be consumed. The higher the ash content, the worse the quality of the feed (*Suparjo, 2010*). Appropriate ash content indicates no over cooking (*Romadhon, et al, 2013*). Fish feed that has experienced over cooking causes a high ash content in the feed (*Romadhon, et al, 2013*). The results of the ash content in each parameter did not experience a significant difference and were in accordance with SNI for tilapia feed 01-7242-2006, the ideal ash content for tilapia feed in the rearing phase is less than 15% [11].

#### The Effect of Ratio of WSW:TLP:EM-4 (w/w%/v) on Protein Content of Tilapia Feed

The comparative effect of adding the percentage of tarum leaf powder and fermented shrimp waste with EM-4 probiotics on protein content is presented in Figure 3.

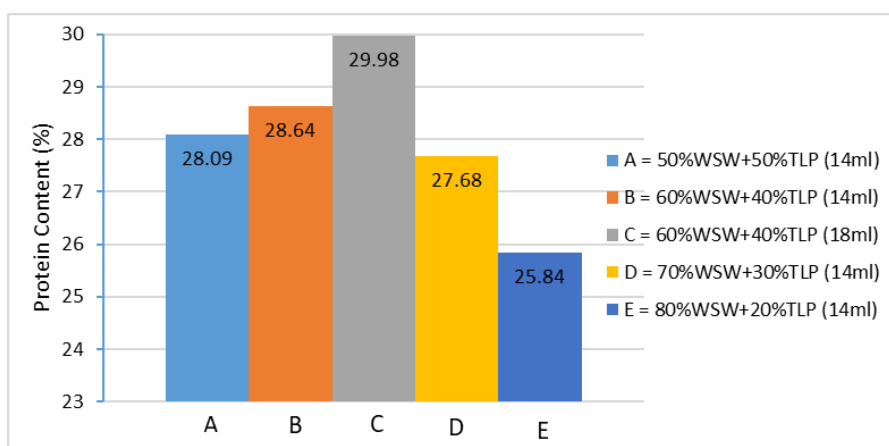


Figure 3. The effect of EM-4 dosage on protein content of Tilapia feed at various ratio of WSW:TLP:EM-4 (w/w%/v) (50:50:14ml; 60:40:14ml; 60:40:18ml; 70:30:14ml; 80:20:14ml))

Based on Figure 3. it can be seen that the increasing percentage of addition of Wet Shrimp Waste (WSW) and decreasing percentage of Tarum Leaf Powder (TLP) in the feed, the resulting protein content tends to increase. This can be seen from Figure 3. where the highest protein content was in treatment C with a percentage of 60% WSW and 40% TLP with 18 ml EM-4 of 29.98%, while in treatment D and E with the addition of an excess percentage of WSW and a low TLP percentage results in a decrease in protein content in the feed. The addition of EM-4 to shrimp waste can increase protein production in feed [12].

Shrimp waste and tarum leaves (*Indigofera* sp) are the main sources of protein resulting in a significant increase in protein [5]. A significant increase in protein levels was due to the high protein content in the raw materials for fermented shrimp waste and tarum leaves (*Indigofera* sp) [11]. Fermentation of shrimp waste can reduce the chitin content in shrimp waste, where chitin acts as a protein barrier. The increase in protein in fermented shrimp waste was caused by the addition and development of photosynthetic bacteria, lactic acid bacteria, yeast, and fungi contained in EM-4. Photosynthetic bacteria in fermentation will produce single cell proteins [13] Single cell protein will increase the amount of protein in the feed. In addition, the fermentation process will lead to the formation of simple compounds that can reduce the chitin content, so that the protein content produced is greater than that of unfermented shrimp waste [14]. The results of the analysis of protein content in the feed showed that all parameters met SNI for tilapia feed 01-7242-2006, namely at least 25%.

#### The Effect of Ratio of WSW:TLP:EM-4 (w/w%/v) on Fat Content of Tilapia Feed

The comparative effect of adding the percentage of tarum leaf powder and fermented shrimp waste with EM-4 probiotics on fat content is presented in Figure 4.

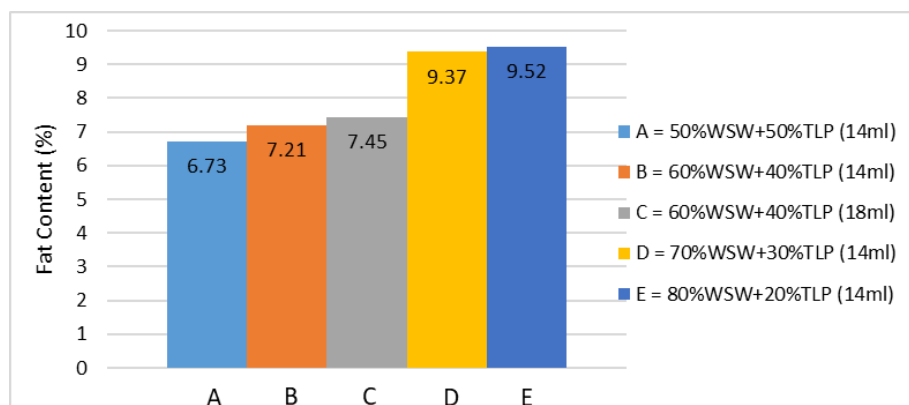


Figure 4. The effect of EM-4 dosage on the fat content of Tilapia feed at various ratios of WSW:TLP: EM-4 (w/w%/v) (50:50:14ml; 60:40:14ml; 60:40:18ml; 70:30:14ml; 80:20:14ml))

Based on Figure 4. it can be seen that the increasing the percentage of Wet Shrimp Waste (WSW) and the decreasing the percentage of Tarum Leaf Powder (TLP), the greater the fat content produced. The lowest fat content was obtained in treatment A with a percentage ratio of 50% WSW and 50% TLP with 18 ml EM-4 of 6.73%, while the highest fat content was obtained in treatment E with a percentage ratio of 80% WSW and 20% TLP with 14 ml EM-4 of 9.52%. The addition of a high percentage of WSW causes the fat content to increase. This is because the fat content contained in shrimp waste is quite large [8]. Good feed generally contains 2-10% fat. The fat content of the feed tested ranged from 6.73% - 9.52%, so it can be categorized as good [9]. The appropriate fat content is due to the fact that in the feed ingredients, there is an additional source of fat from squid oil [15]. Squid oil contains EPA (Eicosa Pentanoid Acid) fatty acids of 13.4% -17.4% and DHA (Docosa Hexanoid Acid) of 12.8% -15.6% which can increase the fat content in fish feed and can be a source of fish energy. The fat contained in the feed will affect the taste and texture so it will affect the fish's interest in the feed [16]. The results of the analysis of fat content in the feed showed that all parameters met SNI for tilapia feed 01-7242-2006, namely 5-10%.

### The Effect of Ratio of WSW:TLP: EM-4 (w/w%/v) on Crude Fiber Content of Tilapia Feed

The comparative effect of adding the percentage of tarum leaf powder and fermented shrimp waste with EM-4 probiotics on crude fibre content content is presented in Figure 5.

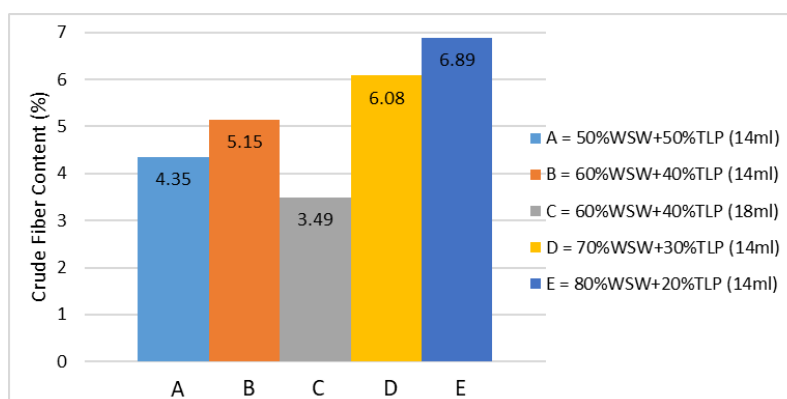


Figure 5. The effect of EM-4 dosage on the crude fiber content of Tilapia feed at various ratios of WSW:TLP: EM-4 (w/w%/v) (50:50:14ml; 60:40:14ml; 60:40:18ml; 70:30:14ml; 80:20:14ml))

Based on Figure 5. it can be seen that with the increasing percentage of Wet Shrimp Waste (WSW) and decreasing percentage of Tarum Leaf Powder (TLP), the crude fibre content produced tends to increase. The lowest crude fibre content was obtained in treatment C with a ratio of 60% WSW and 40% TLP with 18 ml EM-4 of 3.49%, while the highest crude fibre content was obtained in treatment E with a percentage ratio of 80% WSW and 20% TLP with 14 ml EM-4 of 6.89%. The addition of a high percentage of WSW causes the crude fibre content to increase. This is because there is a barrier in the form of chitin content [17]. Chitin is a coarse fibre that is difficult to digest, chitin is included in the fibre group from shrimp waste which has a structure similar to cellulose so it is grouped into foods with high fibre content [17]. Appropriate levels of crude fibre because EM-4 probiotics contain *Streptomyces* Sp bacteria which are capable of producing chitinase enzymes by fermenting using EM-4 probiotics can reduce chitin content which results in decreased levels of crude fibre [18]. The more use of EM-4 probiotics, the more chitinase enzymes are produced and the crude fiber content will decrease [18]. The results of the analysis of crude fibre content showed that all parameters met SNI for tilapia feed 01-7242-2006, namely a maximum of 8%.

### Comparison of Tilapia Feed Formulation Results with Tilapia Feed SNI 01-7242-2006

Table 2. Test Results of Moisture, Ash, Protein, Fat, and Crude Fiber Content of 5 Best Tilapia Feed Treatments

Parameters Tested	Feed					
	SNI	Treatment A (%)	Treatment B (%)	Treatment C (%)	Treatment D (%)	Treatment E (%)
Water Content	<12%	6,84	6,93	7,09	7,11	7,25
Ash Content	<15%	12,32	12,48	12,59	12,54	12,65
Protein Content	>25%	28,09	28,64	29,98	27,68	25,84
Fat Content	5-10%	6,73	7,21	7,45	9,37	9,52
Crude Fiber Content	<8%	4,35	5,15	3,49	6,08	6,89

Based on Table 2, it can be seen that all treatments have met the SNI of tilapia feed 01-7242-2006. The best tilapia feed results were found in treatment C where the addition of wet shrimp waste



by 60% and tarum leaf flour by 40% with 18 ml EM-4 had a moisture content of 7.09%, ash by 12.59%, protein by 29.98%, fat by 7.45%, and crude fiber by 3.49%. In addition, tilapia feed in treatment C can float on the water. This is because the feed specific gravity value is smaller than the water specific gravity value ( $\rho = 1$ ) [19]. Research conducted by Musdalifah (2019) on the manufacture of fish feed made from shrimp head flour and tarum leaves (*Indigofera sp*) without the fermentation process obtained the best results, namely protein content of 27.44%, fat by 5.11%, crude fiber by 16.55%, water by 8.36%, and ash by 21.99%. Fermentation of shrimp waste with EM-4 probiotics affects the results of water content, ash, protein, fat, and crude fiber obtained, where the fermentation process can reduce the chitin content in shrimp waste so that the crude fiber content becomes less and the protein content will be greater [6]. Tilapia feed with fermentation process produces better feed quality than previous studies conducted without fermentation process and has been in accordance with SNI tilapia feed 01-7242-2006.

## CONCLUSION

Based on the results of the research that has been done, it can be concluded that the addition of fermented wet shrimp waste (WSW) with EM-4 probiotics and tarum leaf powder (TLP) has a significant effect on protein content, fat content, and crude fiber content. Meanwhile, the water content and ash content did not significantly affect. The best results were obtained in treatment C with the addition of 60% WSW and 40% TLP with 18 ml of EM-4 probiotics and in accordance with SNI for tilapia feed 01-7242-2006. The results of the water content obtained were 7.09%; ash content of 12.59%; protein content of 29.98%; fat content of 7.45%; and crude fiber content of 3.49%.

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