Faculty of Resource Science and Technology

ASSESSMENT OF NATURAL ORGANIC WASTE FOR AQUACULTURE FEED FORMULATION (FISH WASTE)

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Bachelor of Science with Honours
(Resource Biotechonology)
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ASSESSMENT OF NATURAL ORGANIC WASTE FOR AQUACULTURE FEED FORMULATION (FISH WASTE)

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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours
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Programme of Resource Biotechnology
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ASSESSMENT OF NATURAL ORGANIC WASTE FOR AQUACULTURE FEED FORMULATION (FISH WASTE)

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ABSTRACT

In recent years, the cost of feeding ingredients has increased drastically, causing the price of animals to become higher. To solve this problem, fish wastes were used as an alternative ingredient in aquaculture feed formulation. The objectives of this project were to formulate fish silage from fish wastes by using lactic acid fermentation (Lactobacillus plantarum) for aquaculture feed formulation, to evaluate the nutritional composition of the fish wastes as an alternative ingredient for the aquaculture feed production, and to investigate the effect of experimental diets formulated on the animal model. The fish silage was carried out using a fermentation procedure by lactic acid fermentation (L. plantarum). In this study, 112 tilapia's fingerlings were used and cultured into 4 compartments with different concentration of fish waste, the experimental diet of Fish waste 30% showed the highest increment in CP (36.527) compared to the other treatment. Fish waste 30% shows the lowest FCR (1.39) and high survival rate (92.86%). In the ADC% analysis, Fish waste 30% shows a higher increment in CL (22.82%) and lowest CF (6.27%). Fish waste experimental diet with 30% inclusion exhibited the best performance on body weight gained (0.36 g) with significant difference ($P<0.05$) recorded compared to control treatment (0.34 g). This showed that the fish waste has better potential to be used as main protein source in the aquaculture feed formulation.

Key words: fish wastes, fish silage, aquaculture feed formulation, fermentation, Lactobacillus plantarum,

ABSTRAK

Sejak beberapa tahun ni, kos bahan makanan ternakan meningkat secara drastik menyebabkan harga ternakan meningkat. Untuk menyelesaikan masalah ini, sisa ikan digunakan dalam formulasi makanan akuakultur. Sehingga kini, terdapat kajian yang terhad mengenai penilaian sisa ikan untuk formulasi makanan ternakan. Objektif projek ini adalah untuk merumus silaj ikan daripada sisa ikan dengan menggunakan penapaian asid laktik (Lactobacillus plantarum) untuk formulasi makanan ternakan, untuk menilai komposisi pemakanan sisa ikan sebagai bahan alternatif untuk pengeluaran makanan akuakultur, dan untuk menyiasat kesannya. diet eksperimen yang dirumuskan pada model haiwan ternakan. Silaj ikan dijalankan menggunakan prosedur penapaian secara penapaian asid laktik (L. plantarum). Dalam kajian ini, 112 anak ikan tilapia telah digunakan dan dipecahkan kepada 4 bahagian ynag mempunyai kepekatan sisa ikan ynag berbeza, diet eksperimen sisa Ikan 30% menunjukkan peningkatan tertinggi dalam CP (36.527) berbanding rawatan lain. Sisa ikan 30% menunjukkan nilai FCR (1.39) yang terendah dan kadar kemandirian yang tingg (92.86). Didalam ADC% analisis, sisa ikan 30% menunjukkan peningkatan tertinggi dalam CL dan penurunan dalam CF (6.27%). Pemakanan eksperimen sisa ikan dengan kemasukan 30% menunjukkan prestasi terbaik ke atas berat ikan yang diperolehi (0.36 g) dengan perbezaan yang ketara ($P<0.05$) direkodkan berbanding rawatan kawalan (0.34 g). Ini menunjukkan bahawa sisa ikan mempunyai potensi yang lebih baik untuk digunakan sebagai sumber protein utama dalam formulasi makanan akuakultur.

Kata kunci: sisa ikan, silaj ikan, formulasi makanan akuakultur, penapaian, Lactobacillus plantarum.

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ºC	Degree Celsius
t	Ton
g	Gram
kg	Kilogram
%	Percentage
mL	Millilitre
mm	Millimeter
mg/l	Milligram per liter
pH	potential Hydrogen
CP	Crude Protein
CF	Crude Fibre
CL	Crude Lipid
MC	Moisture Content
DM	Dry Matter
AM	Ash Matter
FLFAM	Federation of Livestock Farmers Associations of Malaysia
NFE	Nitrogen Free Extract

CHAPTER 1

INTRODUCTION

In 2019, fish production fell by 4.5%, from 1.87 million T in 2019 to 1.79 million T in 2020. The value of the contribution made by the fishing sector dropped by 9.3%, decreasing from RM15.26 billion (in 2019) to RM13.84 billion (in 2020). (Fisheries Industry Scenario - Department of Fisheries Malaysia Official Portal, 2021). In 2019, Malaysia's aquaculture industry is expected to generate 391,000 metric tonnes of cultured organisms yearly, with a market value of about USD 700 million. The COVID-19 pandemic produced a major human pandemic over the world, it has had a severe impact on aquaculture in Malaysia, which is essential for the availability of seafood and the guarantee of its security. The new coronavirus known as SARS-CoV-2 was responsible for causing severe social and economic damage. Aquaculture was also impacted in some way, either directly or indirectly, by the repercussions that COVID-19 caused in the agricultural food industries (Stephens et al., 2020).

Furthermore, in recent years, the high cost of commercial feed has increased the price of animal products. According to the Federation of Livestock Farmers Associations Of Malaysia (FLFAM), the price of raw materials for feed has increased by at least 40% in the last year, resulting in a higher price for animals, especially chickens and fish. From January 2020 to March 2020, the cost of maize per metric tonne grew by 41.3 percent, while the price of soya bean meal per metric tonne increased by 57.6 percent over the same period. The cost of palm oil increased from RM3,000 per tonne to RM4,300 per tonne, representing a 43.3 percent rise (The Star Online, 2021). The higher price of the feed components has increased the price of the livestock. Feed costs are a crucial issue in the livestock sector since they make up the most significant amount (about 60–70 %) of total animal production expenses. To provide a foundation for future research, this study was done to determine fish wastes as an alternative component for the feed formulation that would reduce the cost of feeding.

The objectives of this project were to formulate fish meal from fish wastes for feed formulation, to evaluate the nutritional composition of the fish wastes as an alternative ingredient for the aquaculture feed production, and to investigate the effect of experimental diets formulated on the animal model. In addition, the price of the commercial feed in the market can be marked down if these fish wastes prove to have the potential as alternative ingredients to replace the conventional components. This indirectly contributes to thesocio-economy of our community here in Malaysia. On the other hand, this research also targeted reducing the environmental pollution caused by these fish wastes as a long-term objective.

The findings obtained from the study on chemical analysis of fish wastes will provide additional information for fish waste as an alternative ingredient for developing feed formulation products. Furthermore, the collected data contributed to the importance of the production of feed formulation in Malaysia.

CHAPTER 2

LITERATURE REVIEW

The rapid expansion of the global aquaculture sector is driving an increase in demand for processed fish feed and its component ingredients. The expenses of running aquaculture operations across the globe are significantly impacted in a negative way by even a little rise in the market price of these components. Farmers face a significant amount of stress as a result of price fluctuations in fish meal and other commodities that are worldwide traded. This is especially true for agricultural products such as wheat and maize. Furthermore, fluctuations in the pricing of petroleum fuels and other expenditures associated with transportation have an additional impact on the cost of feed. Rising feed and other expenses are too expensive for small-scale fish growers in developing countries to handle. When it comes to the overall expenses of production, feed may account for as much as 80% in smaller-scale aquaculture enterprises. If the price of fish food continues to rise, many subsistence farmers may be left with no alternative except to quit feeding their fish immediately.

In the recent year, the cost of feed materials used in aquaculture feed has increased over the last year. Aquaculture feed prices have risen dramatically, putting a strain on small-scale aquaculture operators' capacity to make ends meet. This is because the rising cost of feed components combined with rising manufacturing and shipping costs has a compounding impact on both worldwide output and prices. Even smallholders and rural farmers may be especially vulnerable in the face of this global phenomena, which might further compound their poverty and susceptibility as a result of this global transformation. Thus, fish wastes have been proposed as a great potential to be further as natural sources for aquaculture feed. However, the documentation on the fish wastes is still limited. Therefore, this study is aimed to determine the chemical and biological profile of fish wastes in order to identify the chemical analysis of the fish waste as an alternative component for aquaculture feed formulation.

Moreover, there was no denying the fact that the first half of 2020 has presented feed millers located across Asia with a significant number of obstacles. This is undeniably the fact beyond a shadow of a doubt. COVID 19 virus epidemic and the subsequent lockdowns have continued to have an influence on the sector, including the supply chain, the cost of feed components, and other factors. There is now a great deal of uncertainty over the pricing of fish meal and soybean meal that is sourced from South America because of recent reports of labour unrest and port blockades in the region. Other nations that manufacture fish meal, such as those in Africa, North America, and India, are at a greater risk of suffering supply interruptions because of the fast spread of the pandemic. Instead, fish waste was introduced as a feed component with an acceptable nutritional component. This resulted in lowered expenses associated with the manufacturing of feed while simultaneously maintaining or even enhancing the growth performance of the livestock.

2.1 Malaysia Fish Production

The Malaysian fishing industry has long been recognised as a vital source of fish protein for many countries. In 2017, the country's total fishing output exceeded 1.5 million tonnes, which includes close to 1.5 million tonnes of catch as well as 0.2 million tonnes of aquaculture production. Coastal fisheries and offshore sub-sector of marine capture fisheries are classified. Malaysia is a net importer of fishery products in terms of quantity since the country offers fishery items at high prices. The value of fish and fisheries products imported into Malaysia in 2017 was USD 976.6 million. The high-end fishery products that Malaysia exports, on the other hand, include sashimi and shrimp. Total export revenue for 2017 was USD 714.1 million (FAO Fisheries & Aquaculture, 2021).

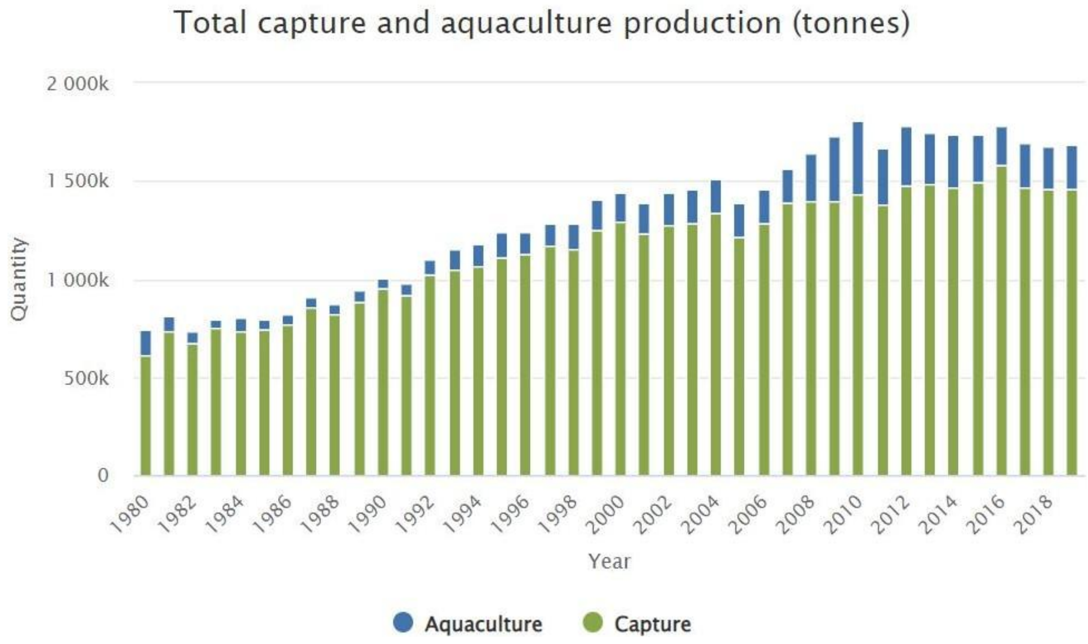

Figure 1 : Total capture and aquaculture production in tonnes in 1980 - 2018 (FAO Fisheries & Aquaculture, 2021)

Aquaculture is playing a key role in increasing global fish output and satisfying expanding demand for fisheries products, and it will continue to do so in the future. The world's aquaculture output rose from 1.7 million tonnes in 1957 to 110,2 million tonnes in 2016. From 2011 to 2018, the annual output of Nile tilapia fish in Kenyir Lake averaged 145.13 tonnes, which represents a modest increase when compared to the previous seven years. The annual growth in fish output is reflected in the rise in the number of operators, fish farm cages, and new production methods. In Malaysia, the production of Nile tilapia accounts for almost 90% of overall tilapia output (Dullah et al., 2020). Tilapia is the world's second most farmed fish, and its output has doubled in the last decade due to its adaptability for aquaculture, marketability, and steady market pricing.

Nowadays, tilapias are currently one of the most extensively imported fish worldwide, and a highly promising aquaculture species (Prabu Elangovan et al., 2019). Malaysia has one of the world's largest fish demands, with 59 kg per capita consumption in 2016. As a result, the Malaysian government places a significant amount of reliance on the country's seafood producing industry to provide the country's primary sources of protein. Aquaculture is responsible for about 20% of all seafood produced in Malaysia. Since 1980, the overall production of aquaculture in Malaysia has increased rapidly, reaching 1.69 million tonnes in 2017. The primary species with the largest production value generated by the aquaculture industry in Malaysia in 2018 were the Whiteleg shrimp (value: 203.82 million USD; quantity: 36,007 t), the giant tiger prawn (value: 70.19 million USD; quantity: 9906.0 t), and the tilapia (value: 73.79 million USD; quantity: 31,766 t) (FAO, 2022).

2.2 Fish Wastes

The fish waste consists of intestines, heads, fins, and fish skin. Fish skins are a significant source of protein. These waste products were a potential cause of contamination and effects of pollution since they deteriorate quickly in mild temperatures. The improper management of these fish wastes may result in unsightly aesthetic issues as well as unpleasant odours created by bacterial biodegradation, which can be detrimental to human health. While on the other hand, those wastes contain a higher concentration of nutrients like protein, fat, and minerals. According to Picot et al. (2010), North Atlantic dry fish and grass carp show that the skin of these fish species is a rich source of protein that can convert to protein hydrolysates. Protein hydrolysates are good antioxidants and, therefore, beneficial for feed formation.

2.3 Use of Fish Waste in Animal Feed

Making animal feed from fish wastes helps reduce pollution and ensure affordable livestock production. Fish wastes may be utilised in animal feed because they are easily accessible in animal feed because they are not employed in human diet and because they are a low-cost source of nutrients. In addition, these fish wastes are used as a feed element because of their high protein content, they are employed as a feed ingredient in the production of aquaculture, pig, poultry, and other animal feed. The completed fish or lungfish pieces are dried and processed to make fish meal, which is a protein source. Fish meal is used to make animal feed, which is then combined with other components. The basic chemical components of fishmeal include 70% protein, 10% minerals, 9% fats, 8% water, pantothenic acid, vitamins, ash, and a variety of other minerals.

For the aquaculture feed, fish wastes can be added to make it more nutritious. They are a good source of protein, minerals, and vitamins. They were also used to supplement protein deficiencies in animal feed ingredients (Afreen&Ucak, 2019). Normally these wastes will be converted into fish meals. But the manufacturing operation of converting fish wastes to fish meals can be pricey. Thus, fish silage has been proposed as a feasible approach for increasing the utilisation of fish waste by offering a high-quality protein source for animal feed while simultaneously minimizing waste disposal expenses. According to Ramirez et al. (2013), fish silage can be generated by adding acids to fish or fish waste by fermenting with lactobacillus species. But in this study, the fermentation method was used to produce fish silage for aquaculture feed formulation.

2.4 Fish silage

Fish silage production was more environmentally beneficial, safer, and low-cost than the manufacture of fish meal since it uses a simple process and uses less resources (Palkar et al., 2017). The high protein content of fish silage indicates that it may be a useful source of protein for animals that live in arid conditions. Fish silage is a liquid product formed by mixing whole fish or undesirable components with enzymes, acids, or bacteria to produce a nutrient-dense product that is high in protein. During this formation phase, the activation of fish enzymes aids the process. Fish silage can be used in place of fish meal and is healthier than fishmeal. Moreover, it is inexpensive, convenient for large-scale production and fish silage reduces odours. Fish silage has been proposed as a potential alternative approach for increasing the utilisation of fish waste or fisheries by-products by providing a high-quality protein source for livestock. Fish silage might be produced by chemical silage or biological silage (Ferraz De Arruda et al., 2007).

Table 2 - Proximate composition (%)*, acidity and protein digestibility *in vitro* of fish waste and fish silage.

Component	Fish waste	Fish silage
Moisture	70.3 ± 0.4	65.5 ± 0.3
Crude protein (Nx6.25)	52.4 ± 0.9	39.9 ± 0.7
Ether extract	24.5 ± 0.6	14.5 ± 0.4
Ash	19.0 ± 0.5	18.0 ± 0.6
Nitrogen free extract	3.6 ± 0.2	26.9 ± 0.6
pH	6.49 ± 0.03	4.4 ± 0.02
Lactic acid (%)	0.34 ± 0.003	4.45 ± 0.01
Protein digestibility (%)	69.00 ± 1.51	81.61 ± 0.9

* = Mean ± SD values, (n=3), expressed in dry basis.

Figure 2: Proximate composition (%) of fish waste and fish silage (Ramírez et al., 2013)

Lactobacilli are gram-positive, microaerophilic, cocci- or rod-shaped bacteria that are fermentative, facultative anaerobes. Lactic Acid Bacteria (LAB) has been utilised as a bio-additive in ensiling forage to promote fermentation and retain nutritional value. LAB play an important role in the maintenance of silage nutritional quality by producing organic acids and inhibiting deterioration from the remaining microbial population. Secondary fermentation in silage is an unfavourable acidification process carried out mostly by Enterobacteria, Clostridia (butyric acid producer), and yeasts (ethanol producer) (Kim et al., 2021).

The utilisation of fish silage using lactic acid bacteria was formulated using Lactobacillus plantarum, cassava and molasses as the carbohydrate source. In fish silage by biological fermentation, sulphuric acid and molasses were used in the first method whole in the second method involved the utilisation of molasses and LAB. LAB produce lactic acid during fermentation, LAB transform sugars into lactic acid with the concomitant decrease in the pH of the mixture (around 4.5). An acidic environment and bacteria that generate antimicrobial compounds may prevent the growth of pathogens such as Clostridium Botulinum, Enterococci and Coliforms. It is possible to transform an underutilised protein into a useful hydrolyzed by-product via the production of biological fish silage.

In order for fermentation to take place properly, the raw material must include lactic acid bacteria, as well as an appropriate nutritional substrate for the bacteria and a temperature that allows for rapid growth of the bacteria. Fish waste enzymes breakdown proteins into polypeptide chains and free amino acids in a general autolytic process, resulting in more digestible proteins than those derived from chemical silage. Proteases and lactic acid, which are released by the LAB, help to accelerate the process. Lactic fermentation helps to stabilise the fat in fish silage, making it more suitable for use in animal feeding (Ramírez et al., 2013).

2.5 The Tilapia fish

According to Popma and Masser (1999), the form of a tilapia is quite similar to that of a crappie or a sunfish, nonetheless, tilapia is readily distinguished from other members of the Cichlid family of fishes due to the presence of an interrupted lateral line. Tilapias are deep-bodied fish with large dorsal fins, and they have a laterally compressed body shape (Popma and Masser, 1999). In addition to this, the front section of their dorsal fin is strongly spined. According to Popma and Masser (1999), the spines may also be found in the pelvis and the anal fins of the fish.

There are different types of banding patterns and coloration of tilapia depending on their species. Tilapis has a diversity habitat which is one of the major factors behind the wide distribution of Tilapia. Tilapias are able to tolerate extraordinarily varied environmental conditions to survive. Tilapia which came from genera Oreochromis was probably introduced unintentionally outside Africa. After the discovery, the species of Oreochromis mossambicus was further introduced to many other countries in South and South-East Asia.

All members of Oreochromis are omnivores.Tilapia are able to thrive in aquaculture because to the fact that they feed on food that is lower on the food chain. Tilapia are known to consume a broad range of natural food items, such as plankton, certain aquatic macrophytes, planktonic and benthic aquatic invertebrates, larval fish, detritus, and organic debris that is decaying, as mentioned by Popma and Masser (1999). Tilapias are omnivores, however their diet of choice is often composed of algae and other forms of aquatic debris. As stated by DeSombre and Barkin (2011), this omnivorousness makes the species both easy to establish in new habitats, and useful for cleaning up freshwater sites of excess plant and other undesirable materials. As such, Tilapia can be used to purify and control vegetation in water even when not being farmed.

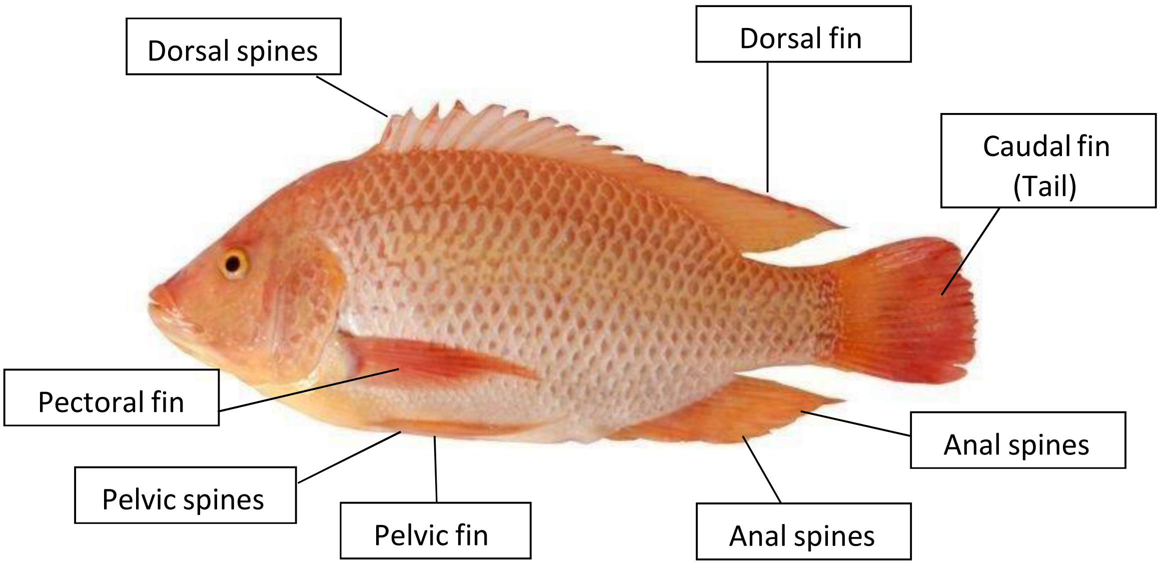

Figure 3 : The morphology of red hybrid tilapia (Popma & Masser, 1999).

In Malaysia, tilapia is a hardy and an ideal fish for farming because of the relatively short culture period of about 6 months. It has good tolerance to high stocking density, high productivity rates and is adaptable to a wide range of culture systems. They grow rapidly on formulated feeds with low protein content and can tolerate high carbohydrate levels.

These qualities translate to a relatively inexpensive product as compared to marine fish. In Malaysia, tilapia aquaculture refers to red hybrid tilapia (*Oreochromis* spp.) and black tilapia species, including the genetically improved farmed tilapia (GIFT) (*Oreochromis niloticus*) (Hashim, n.d.).

Department of Agricultural Sarawak reported that the production of farmed tilapia in Batang Ai reservoir kept on increasing as the demands from the market is getting higher by years. The department also reported that there will be an increment in the cages number in the years more to come as the current number of the cages will not meet the huge demand from their customer especially from Singapore (News Desk, 2020).

Chapter 3

Materials and Methods

3.1 Materials

The material used in this research was fish wastes from local market, molasses, sodium benzoate, *L. plantarum,* semi-automated digestion machine (Gerhardt Kjeldahltherm), Turbosog, Soxhlet extraction apparatus, Gerhardt Vapodest, Boric acid, and acetone.

3.2 Sample collection and processing

Raw samples of fish wastes were obtained from Stutong market and was stored in an icebox. The samples were sent to the FSTS laboratory for preservation. After 24 hours being frozen, the samples were thawed at room temperature and minced through a mincer by using an industrial grinder to pass through a fine sieve and the minced samples were used silage preparations (Ramírez et al., 2013).

3.2.1 Preparation of *Lactobacillus plantarum*

The LAB strains were used in this experiment was taken from the collection of Molecular Genetic Laboratory, Faculty of Resource Science and Technology, UNIMAS. *L. plantarum* were used as a starter. *L. plantarum* was cultured on Man Rogosa and Sharpe agar (MRS) at 30 °C for 1 day in the dark. The culture plate was shaken gently until the *L. plantarum* culture was obtained. The *L. plantarum* concentration that was used in this study was in the range 1×10^9 cfu/ml (Ramírez et al., 2013).