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Quality of Chemically Processed Flying Fish (*Hyrundicthys oxycephalus*) Waste Silage

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ABSTRACT

Flying fish are the fish that are widely found in the waters of the Makassar Strait, especially in Majene Regency. During the harvest season, flying fish leave a lot of waste that pollutes the environment. This research aimed to evaluate flying fish waste silage's physical and chemical qualities. The design used was a completely randomized design (CRD) with four treatments and four replications groups. To process the sample, formic acid at concentrations of 0%, 2.5%, 3%, and 3.5% was used. Anova was applied for data analysis, followed by Duncan's test if the treatments differed significantly. Parameters observed were chemical quality, including crude protein and fat content, total bacteria count, and pH, while physical-chemical quality included aroma, color, and texture of flying fish waste silage. The results showed that the addition of formic acid to fly fish silage had a significant effect ($P < 0.05$) on protein, fat, pH, and total bacteria levels but had no significant effect ($P > 0.05$) on the aroma, color, and texture of the hay. This research concludes that chemically processing flying fish silage with the addition of 2.5% formic acid can increase the protein content to the optimum level in the P1 treatment resulting in the highest protein content of 48.81% and the fat content of 1.63%, pH 3.5, total bacteria 4 x 10^4, sour taste, gray color and liquid texture that can be safely used as raw feed.

Key words: formic acid, flying fish, physical, chemical quality, silage

INTRODUCTION

The livestock business production triangle includes genetics, feed, and management. One of the most important factors in poultry farming is feed quality, and this is because almost 60-70% of production costs come from a feed. Fish flour is one of the feed ingredients whose procurement still depends one hundred percent on foreign supplies, and the price is relatively higher when compared to other feed ingredients because of its function as a source of protein (Sugiyono et al., 2015). Imports of fish meal in 2021 were 24,465 tons, equivalent to 58.1% of total imports. The quality of fish flour produced domestically is still low because, for its production, fish used are not fresh, including parts like offal, fins, and heads. Flying fish are the alternative raw feed material that can be used as a source of animal protein.

The peak season for flying fish is May-November, many fishers' catches that are not sold are only made into smoked fish, and the rest turns into waste that pollutes the environment. The proximate test results stated that the nutrients in flying fish had a protein content of 19.04%, a fat content of 0.2%, an ash content of 0.78%, and water content of 2.21%. Flying fish is one of the fourth largest catches after skipjack, tuna, and scad and has a significant enough potential to be utilized, one of which is used as feed fermentation material to produce silage. Flying fish processing can be done by two methods, namely biologically and chemically. The addition of probiotics and Lactic Acid Bacteria (LAB) is generally used in biological silage processing, while chemical processing uses organic acids and mineral acids.

Chemical silage processing has several advantages, including a relatively low price so that the cost of feed is more efficient and the manufacturing process does not pollute the environment; according to (Subekti et al., 2019), formic acid added to silage is more practical because it can be used directly without needing to be air dried to pH 7. Kurniawan et al. (2019) stated that formic acid could accelerate the ensilage process as indicated by a rapid decrease in pH and an increase in enzyme activity so that the amino acids as constituents of proteins become shorter in composition.

Another advantage of fish silage is that its processing does not cause environmental pollution. No previous researchers have used flying fish with the addition of formic acid as fly fish silage. Feed raw materials are considered safe for livestock if they meet the quality requirements of the feed according to the Indonesian National Standard (SNI). SNI no 01-2715-1996 concerning quality standards of fish meal, namely minimum 45% crude protein, maximum 12% crude fat, maximum 3% crude fiber, 30% maximum ash, calcium 2.5-7, phosphorus 1.6-3,2 NaCl 2%, harmful *Salmonella* bacteria, and organoleptic test with a minimum score of 7. This research aims to know and evaluate the processing of flying fish silage in terms of physical characteristics, including color, aroma, and texture. In contrast, chemical elements include protein content, fat, pH, and the total number of microbes. This research hypothesizes that the higher the percentage of formic acid in flying fish silage, the better its physical and chemical qualities.

MATERIALS AND METHODS

Materials

This research uses the following tools: a Miyako brand blender, 1 kg capacity scale, jar, knife, cutting board, wooden stirrer, pH meter, measuring cup, plastic bucket, stove, measuring cup, test tube, kjehdal tube, oven, and distillatory. This study used materials such as 12 kg flying fish waste, 300 ml formic acid concentration of 85%, aqua dest, $CuSO_4$, H_2SO_4, NaOH, BH_3O_3, HCL, hexane solvent, and water.

Figure 1: Flying fish

Flying Fish Silage Making

The flying fish are washed thoroughly using running water, then sliced thinly and blended until the fish becomes mush, then the fish pulp is put into a 1 kg jar. Formic acid was added according to the treatment P1 = 2.5%, P2 = 3% and P3 = 3.5%. The silage was stirred from day 1 to day 4 four times together with measuring pH, temperature, and physical changes of the silage, then stirring on days 5 to 7 was carried out once. On days 8 to 14, the silage was left in anaerobic condition. On the 14th day, the silage was harvested and then tested for crude protein, crude fat, and a total number of microbes at the Animal Food Chemistry Laboratory, Hasanuddin University. In contrast, organoleptic tests were carried out at the University of West Sulawesi.

Physical and Chemical Quality Testing of Flying Fish Silage

Implementation of testing at the Integrated Laboratory of the University of West Sulawesi involved 30 panelists in assessing the quality of color, aroma, and texture of flying fish waste silage. Crude protein content testing was carried out using the Kjeldahl method. The destruction stage was done by taking a sample weighing 1g, then placing it in a Petri dish by adding 2g of $CuSO_4$ solution and 25ml of concentrated H_2SO_4 solution, then heated using spirit. The next step was to cool the sample; then, it was diluted in 10 ml of distilled water and placed in a distiller for 10 minutes. As a container, 10 ml of 2% BH_3O_3 solution and five drops of pp indicator were added. The titration step was carried out by adding a 0.01 N HCl solution and then observing it until the color of the solution changed to clear (Hafiludin, 2011).

Analysis of protein data using the formula:

$$\%\ \mathrm{N} = \frac{(\mathrm{A} - \mathrm{B})\mathrm{x\ NHCl\ x\ 14}}{\mathrm{mg\ sample}} \mathrm{x\ 100\%}$$

Protein = %N x

A = Titration of sample

B = Titration of blank of convertion factor= 6,25

The crude fat content test was carried out using the Soxhlet method. The sample of as much as 2 g was weighed and then put into a beaker, followed by adding 20 ml of water and 30 ml of 10 N HCl solution. Then the sample was boiled for 2 minutes, and while it was still hot, the model was filtered until it did not react with acid by washing it with hot water. The next step was to dry the filter paper and its contents at a temperature of 100 - 105°C. 50 ml of hexane extract was mixed into filtered water and waited for 3 hours at a temperature of 80°C. The hexane extract was then distilled, and the fat extract was dried at a temperature of 100-105°C and then cooled before weighing (Ramlah et al., 2016).

Fat data analysis using the formula:

$$\text{Fat level} = \frac{(pumpkin\ weight\ +\ fat\ extract)\ -\ empty\ pumpkin\ weight}{Sample\ weight}\ \text{x 100\%}$$

Measurement of pH

A pH meter can measure the degree of acidity used to determine whether a solution is acidic or basic. The scale on the pH meter indicates a solution is acidic if it < 7, alkaline > 7, or neutral if it shows the number 7 (Ihsanto and Hidayat, 2014).

Figure 2. Measurement of pH of Flying Fish silage pH

The measure of Total Bacterial Count

Allaily et al. (2011) stated that the total number of bacteria was measured using the Total Plate Count (TPC) method. The silage sample weighing 2 ml was added with 1 ml aqua dest, then diluted seven times using 0.5 ml of sample solution put into 0.5 ml aqua dest. Samples were taken at 1 ml at dilutions 6 and 7 then the specimens were plated and incubated for three days with MRS agar media. Colonies formed are slightly yellowish in color and round in shape.

Data analysis

This study used a completely randomized design (CRD) with 4 treatments and 4 replications, the factors studied were the level of formic acid mixture P0 = 0%, P1 = 2.5%, P2 = 3%, and P3 = 3.5%. This study used analysis of variance, which was processed using the SPSS version 25 program. If the results between treatments had a significant effect, the analysis was continued using Duncan's test.

RESULTS AND DISCUSSION

Physical Quality of Flying Fish Silage

Physical quality testing of flying fish silage includes color (gray, brown, and blackish brown; aroma (sour, aromatic acid, slightly acidic and not harmful; texture (liquid, soft or hard)—thirty panelists consisting of 10 panelists experts and 20 panelists from students.

Table 1. Physical quality of Flying Fish waste silage

Treatment	Color			aroma				Texture		
	Gray	Brown	Dark brown	Acid	Stinging acid	Slightly sour	Not acid	Liquid	Mushy	Hard
P0	51.66	21.66	26.67	42.55	41.66	10.80	5.00	86.67	15.83	2.5
P1	71.66	6.66	21.66	40.00	25.83	26.67	7.50	64.16	33.33	2.5
P2	72.50	9.16	18.33	41.66	22.50	20.00	15.83	62.50	27.50	10.0
P3	78.33	4.16	17.50	45.00	20.00	27.50	7.50	55.00	41.66	3.3

Description: P0= unfermented flying fish
P1= flying fish + 2.5% formic acid
P2= flying fish + 3% formic acid
P3= flying fish + 3.5% formic acid

Color

The physical quality of good fish silage has the same color as before being fermented or resembles fresh fish. So the color is the same as before the fermentation or incubation process. The place for the organoleptic test of flying fish silage at the Integrated Laboratory of the University of West Sulawesi consisted of 30 people as panelists. The data obtained from 30 panelists were tabulated in percentages to determine the silage color value by averaging each treatment. The color assessment of flying fish silage was carried out at the Integrated Laboratory of the University of West Sulawesi, with a total of 30 respondents. The data obtained from the 30 people were expressed in percentage (%) and averaged to determine the value of the silage color. The addition of formic acid in flying fish silage made the color of the resulting silage appear gray, as stated by respondents of 51.66%, 71.66%, 72.5%, and 78.33%. During the fermentation process of flying fish silage, there was no significant change in color; no addition of other materials into the silage. The fermentation process results in an increase in heat in the silage, this increase in heat causes the silage to change color to blackish brown. According to (Reniawati et al., 2016), the activity of microorganisms that occur enzymatically through a biochemical process is called the fermentation process. (Handajani, 2014) increased enzyme activity during fermentation causes color changes in feed raw materials. In contrast, color differences can occur in aroma, shape, heat, and other physical and chemical properties.

Aroma

In **t**he treatment of fish silage added with formic acid 2.5%, 3%, and 3.5%, respondents assessed that the silage's aroma was sour with 42.25%, 40%, 41.66%, and 45%—aroma acid produced due to the addition of formic acid. According to (Subekti et al., 2019), fat is degraded into free fatty acids such as palmitic, stearic, oleic, linoleic, and linolenic acids during the fermentation process, with the highest content being linolenic acid. Handajani (2014) stated that molds use about 40% linolenic acid during fermentation, which causes the fat content to decrease while crude protein is denatured into aromatic amino acid groups such as threonine valine, lysine, and tryptophan. The resulting flying fish silage is expected to have a fresh aroma, not rancid or rotten. Suppose there is a foul or sour odor. In that case, it indicates that the material has been damaged by feed ingredients such as the presence of material overgrown with fungi or other aflatoxins. Daud et al. (2020) stated that foul-smelling or rancid materials in fish feed indicate the presence of aflatoxins that can cause disease, especially inflammation of the liver and intestines.

Texture

The silage treatment added with formic acid showed that the texture of the fly fish silage produced was liquid, and the percentage of respondents was 86.67%, 64, 16%, 62.5%, and 55%, respectively. The fluid texture of the fish added with formic acid decreased the water content; the higher the level given, the water content decreased. The hydrolysis process that occurs during fermentation requires a lot of water so that the water content reduces while harvesting silage. (Noviana et al., 2012) added that when hydrolysis takes place, it requires water (H_2O). It causes hydrogen ions to increase, which results in a rapid water content decrease. Winarni et al. (2011) stated that silage is a raw material that contains a lot of water and is sTable Adding formic acid causes the pH to drop quickly and is more optimal for proteolytic enzymes to hydrolyze proteins into simpler ones and shorten amino acid groups.

Chemical Quality of Flying Fish Silage

Chemical quality testing includes crude protein content, crude fat, pH, and total bacteria. Testing of crude protein and fat was carried out at the Animal Biochemistry Laboratory of Hasanuddin University; pH measurements were carried out at the Integrated Laboratory of the University of West Sulawesi. In contrast, the total bacteria test was carried out at the Laboratory of Mathematics and Natural Sciences, Hasanuddin University.

Table 3. Average chemical quality of fly fish waste silage

Treatment	Crude protein (%)	Crude fat (%)	pH	Total bacteria
P0	19.82 ± 0.08^a	0.14 ± 0.01^a	6.5^a	$(3.8 \times 10^{12})^c \pm 0.98$
P1	48.81 ± 0.08^b	1.63 ± 0.06^b	3.5^b	$(4 \times 10^4)^a \pm 1.73$
P2	42.92 ± 1.24^c	0.89 ± 0.00^c	3.5^b	$(2.5 \times 10^5)^a \pm 1.04$
P3	38.73 ± 0.49^d	0.87 ± 0.12^c	3.5^b	$(3.3 \times 10^6)^c \pm 1.52$

Description: Superscripts in the same column showed significantly different treatments (P<0.05),
P0= unfermented flying fish
P1= flying fish + 2.5% formic acid
P2= flying fish + 3% formic acid
P3= flying fish + 3.5% formic acid

Crude Protein

The analysis of variance showed that chemical processing of flying fish silage with different formic acid levels showed a significant effect ($P < 0.05$) on the crude protein content of flying fish silage. The value of crude protein content in flying fish ranged from 19.82% to 48.81%. The fish meal quality is said to be good if it has a crude protein content of at least 45%. Based on the results of research that meets the SNI standard is the P1 treatment with the addition of 2.5% formic acid with a protein content of 48.81%, One of the factors that caused the P1 treatment to produce the highest protein was because the protein fermentation process composed of complex amino acid groups succeeded in breaking down long chain peptide bonds into shorter amino acids due to the addition of formic acid at the right level so that the levels of proteolytic bacteria increased. At P1, it has the safest Total Bacteria value ranging from 4 x 10^4 and is classified as grade A so that it can help improve the protein content.

Reniawati et al. (2016) stated that adding formic acid to silage effectively increased proteolytic enzyme activity to increase the development of proteolytic bacteria, which functioned to help break down complex peptide bonds into simpler ones so that amino acid groups would be more easily absorbed. It is not much different from the results of the treatment of P2, as much as 42.92% with the addition of formic acid as much as 3% and P3 as much as 38.73% with the addition of formic acid as much as 3.5%. The value of crude protein content in adding 2.5% formic acid meets the SNI value for the crude protein in feed, where the SNI for the crude protein in a fish meal is at least 45.00% (BSN, 2016).

Crude Fat

The analysis of variance showed that the chemical processing of fly fish silage with a mixture of formic acid had a significant effect ($P < 0.05$) on the crude fat content of flying fish silage. The average crude fat content ranges from 0.14% to 1.63%. Based on the SNI standard, the crude fat content produced in all treatments met the quality of fish meal with a maximum fat content of 8%. One of the uses of formic acid is a bacteria repellent that occurs during fermentation. On the eighth day of fermentation, fat has begun to be degraded into two parts, fat and fatty acids. During fermentation, complex molecules change into simpler molecules converted into short-chain fatty acids; this makes fat easily soluble, decreasing fat content. Bioprocesses can increase cell biomass, primary and secondary metabolic enzymes, and vitamins, which causes changes in complex chemical compounds into simpler molecules due to enzyme activity (Abun et al., 2012).

One energy source in feed is fat, and its use in poultry feed is limited to a maximum of 2-5%, while good quality fish meal has a maximum crude fat content of 8%. The use of fat in feed is limited because the provision of fat in large or excessive amounts in the body of livestock will only be stored in adipose tissue, which can then increase blood fat which is characterized by triglycerides, cholesterol, and LDL in the blood above normal. The crude fat content of flying fish silage in this study met the SNI value for fish meal in feed, where the SNI for crude fat in a fish meal was a maximum of 8% (BSN, 2016). Sulistyoningsi (2015) stated that the administration of excessive fat would cause diarrhea in livestock because the fat source feeds quickly, rot, and smells rancid.

pH

The analysis of variance showed that among treatment groups, there was a significant difference ($P <0.05$) in the pH value of flying fish silage. In the P0 treatment, the pH was 6.5; at P1, P2, and P3, the pH was 3.5. Using formic acid 2.5% to 3.5% effectively lowers the pH value within 8 hours from the initial position of 6.5 to 3.5 and is stable until the silage is harvested. This study showed a better result than (Shabani et al., 2018) with the addition of probiotics to fish waste silage; the pH before silage was 6.04, and after fermentation was pH 4.5. It shows that formic acid can degrade complex bonds, especially peptide bonds, into simpler ones and is proven to increase the protein value of fish silage. Adding formic acid with a 2.5% to 3.5% reduced the pH from 6.5 before fermentation to 3.5.

The addition of formic acid can increase the activity of enzymes that accelerate the ensilage process, characterized by a drastic decrease in pH. It is supported by research (Noviana et al., 2012) which states that the hydrogen ion concentration will increase during the fermentation process, causing protein degradation due to the presence of the acid reaction caused by silage. The presence of formic acid added to silage will accelerate the protein degradation process into shorter molecules (Noviana et al., 2012). During fermentation, the silage becomes hot, pH extremes, an attraction between particles, freezing, heavy metals, and organic solvents cause the pH to drop drastically, resulting in denaturation.

Total Number of Microbes

The results of the analysis of variance (ANOVA) showed that the addition of formic acid in flying fish silage had a significant effect ($P<0.05$) on the total number of microbes. The total value of bacteria in flying fish silage was $4x10^4$ to 3.8 x 10^{12}. The average number of total bacteria at P0 ranged from 3.8 x 10^{12}. It was classified as grade C, where flying fish without adding acid was unsafe to feed because the number of bacteria was high, and the protein value obtained was only 19.82%. In P1 treatment, the total bacteria ranged from 4 x 10^4

including grade A, and P2 with a total bacteria ranged from 2.5 x 10^5 including grade A, while P3 total bacteria ranged from 3.3 x 10^6 included in grade C.

The addition of 2.5% and 3% formic acid produced silage, a safe feed ingredient because the total bacteria content was below 105 and proved efficient and able to increase the protein value from 19.82% to 48.81%. Adding 3.5% formic acid proved inefficient in reducing total bacteria and increasing protein. The low actual microbe in fish silage was caused by a drastic decrease in pH so that the bacteria *Salmonella* sp., *E. coli,* and *Clostridium* sp. cannot grow in acidic conditions which have a normal pH range of 4-9ppt (Sofia et al., 2021)

CONCLUSION

Chemical processing of flying fish waste silage using formic acid as much as 2.5% with a fermentation time of 14 days is the best fish silage with a crude protein content of 48.81%, crude fat content of 1.63, pH value of 3.5, aroma acid, liquid texture and total bacteria 4 x 10^4. So, it is very safe to use as animal feed ingredients.

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