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RESEARCH ARTICLE

Proximate analysis on animal feed granules composed of raw material from fish innards wastes

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Abstract

Introduction: Fish innards contain 14.01% protein, 20% lipid, 4.75% ash, and 60.62% water. Fish innards are formulated into granules for practicality in their application as animal feed. **Aim:** This research on the proximate analysis of animal feed granules composed of raw material from fish innards wastes used a descriptive quantitative method. **Results:** The result indicated that the water content measured using the thermogravimetric method was 6.62%, the ash content observed using the dry ashing method was 10.25%, the protein content checked using the biuret method was 37.03%, fat content using the soxhlet method was 6.13%, and carbohydrate content measured using phenol sulfate method was 26.14%. **Conclusion:** These findings show that nutrient contents in the composition of animal feed granules of raw material from fish innards wastes fulfill the regulation of animal feed content based on SNI-8509-2018.

Introduction

Fish is one of the food sources with many nutritional benefits. It is rich in essential amino acids, unsaturated fats, vitamins, and minerals and is easily digested (Wibowo & Darmanto, 2014). Besides producing useful products to fulfil food, industry, and daily needs, fish production also generates wastes, reaching up to 500,000 tons each year (Harianti, 2012). Wastes resulting from the fish industry consist of fish that are no longer good to consume or process, fish innards, and other non-commercial parts. These wastes pile up every day due to the lack of skills of the people handling them (Komariyanti & Surachman, 2018). These unutilized fish wastes can cause environmental pollution (Hildawianti, Vanny & Abram, 2017) as they become ideal media for microbes to grow, causing unpleasant odours (Jayanti, Herpandi & Lestari, 2018).

Fish innards are wastes resulting from the fish industry that, if not used, will cause harm to the environment, health, and economy (La Apu, 2017). In Indonesia, several types of research were conducted on the use of fish innards, one of which is to transform fish processing wastes into animal feed (Komariyanti & Surachman, 2018). Other research explored the utilization of fish waste as animal feed raw material (Sihite, 2013).

The formulation of artificial animal feed is based on the producer's considerations. Animal feed production should acknowledge animal nutritional needs, sources, raw material quality, and economic value (Niode & Nasriani & Irdja, 2017). Based on SNI-8509-2018, content requirements of quality animal feed are as follows: 12.00% water (max), 14.00% ash (max), 16.00% crude protein (min), and 14.00% carbohydrates (min) (Standar Nasional Indonesia, 2018). Based on several studies in Indonesia, which found protein and fat contents in fish innards and used them as animal feed, this research aims to do a proximate analysis on animal feed granules composed of raw material from fish innards wastes.

Method

The material used was fish innards, thick H_2SO_4, H_2SO_4 52%, aqua dest, alcohol 95%, petroleum ether, glucose, phenol, biuret reactor, and BSA (Bovine Serum Albumin). Formula modification of other researchers entitled: "Optimization of Tapioca Flour and Molasses Flour in Cat Food and Dog Food Pellets From Fish Innards Wastes Raw Material with the Factorial Design Method".

Ash content determination

The working cup was firstly dried for 30 minutes in the oven at 100-105°C or until the fixed weight was obtained. It was then cooled in a desiccator for 30 minutes and then weighed (B1). Five grams of sample were put into the formerly weighed cup and burned on a Bunsen burner, a smokeless stove. It was then put into an ashing furnace to be burned at 400°C until greyish ash was obtained or the sample had fixed weight. Then the furnace temperature was increased to 550°C for 12-24 hours. The sample was then cooled in a desiccator for 30 minutes and weighed (B2) (Hafiludin, 2011). Ash content can be calculated as follows:

$$\text{Ash content (\%)} = \frac{\text{B2}-\text{B1}}{\text{sample weigh}} \text{ x } 100\%$$

Water content determination

The working cup was firstly dried for 30 minutes in the oven at 100-105°C or until the fixed weight was obtained. It was then cooled in a desiccator for 30 minutes and then weighed. Five grams of sample (B1) were weighed on the cup and then dried in an oven at 100-105°C until the fixed weight was obtained (8-12 hours). The sample was then cooled in a desiccator for 30 minutes and then weighed (B2) (Hafiludin, 2011). Water content can be calculated as follows:

$$\text{Water content (\%)} = \frac{\text{B1}-\text{B2}}{\text{Sample weight}} \text{x } 100\%$$

Protein content determination

Standard solution preparation

One gram of Bovine Serum Albumin (BSA) was dissolved in distilled water in a volumetric flask 10 mL up to the designated mark to obtain a standard solution of 10% W/V.

1. Optimum wavelength determination
   Five per cent BSA Standard solution was put in a test tube by sampling 2.5mL of BSA d with 0,8 mL biuret reactor, and distilled water was added to make a total of a 5 mL solution. The solution was allowed to sit and react for ± 10 minutes; the absorption was then measured at a wavelength of 450-600 nm. The maximum absorption of the wavelength was recorded.
2. Standard curve making
   Six test tubes were prepared. The first one was filled with a blank solution (solvent), while the five others were filled with BSA standard solution at concentrations of 1, 2, 3, 4, and 5%, and completed with 0.8mL aqua dest to a total volume of 5 mL. The solution was allowed to sit for 10 minutes, and then each absorption was measured using a UV-VIS spectrophotometer at the maximum wavelength.
3. Measurement of the protein content of the sample
   Each sample weighing 25 grams was put into a beaker added with 250 mL of distilled water, smoothly ground, and filtered with a filter paper.

Protein content measurement was carried out as follows: 2.5 mL of protein sample was added to 0.8 mL of biuret reactor and completed with distilled water to a total of 5 mL solution. The solution was then vortexed let sit for 30 minutes to make a perfect purple. The maximum absorption of the wavelength was measured and recorded (Keppy & Allen, 2016).

Protein content calculation was obtained by:

$$\text{Protein content} : \frac{\textbf{Protein Weight}}{\textbf{Sample Weight}} \textbf{ x } \mathbf{100}\%$$

Protein weight: Sample volume x Protein concentration of a sample

Fat Content Determination

A round bottom flask was made fat-free using alcohol 95%, and 3 grams were wrapped with a filter paper and put into a Soxhlet tool. Then 200 mL of petroleum ether was put into the round bottom flask, and the Soxhlet toolset was connected to continue with sample filtering for 8 hours until the sample became clear. The solvent in the round bottom flask was evaporated until almost dry. Then it was put into an oven at 100°C for 30 minutes, then cooled in a desiccator for 30 minutes. The fat was then weighed (Suriani, 2015).

The fat content calculation was obtained by:

$$\text{Fat content (\%)} = \frac{\textbf{(B}-\textbf{A)}}{\textbf{Sample weight}} \textbf{x } \mathbf{100}$$

Carbohydrate content determination

Sample preparation

One gram of animal feed pellet sample was added to 10 mL of aqua dest while stirring, then 13 mL H_2SO_4 52% were added while stirring for 20 minutes using a magnetic stirrer and put into a test tube. Aluminium foil was used as a lid to cover the tube. One hundred mL of aqua dest were added and filtered into a 250 mL volumetric flask then aqua dest was added to the volumetric flask up to the designated mark.

Carbohydrate content measurement

Standard glucose solution was prepared at concentrations of 0, 100, 200, 300, 400, and 500 ppm. Each solution measuring 0.5 mL was put into separate flasks, then soaked in water, then 0.5 mL of phenol 5% and 2.5 ml of thick H_2SO_4 were added carefully and slowly along the wall of the flasks. Those solutions were allowed to sit for 10 minutes, then vortexed before being allowed to sit for 20 minutes. The absorption was then measured using a spectrophotometer at the wavelength of 490 nm. The linear equation was then made as a standard curve. Sample measuring was done by putting 0.5 mL of sample solution into a flask, then soaking it in the water, then adding 0.5 mL of phenol 5% and 2.5 mL H_2SO_4 slowly. The following process was the same as it was with a standard glucose solution, then the measurement value was plotted on the standard curve (Bintang, 2018). Carbohydrate content calculation was obtained by:

$$\text{Percent of glucose (\%)} = (G)/W \times 100\%$$

G = glucose concentration (g); W = sample weigh (g)

Results

In this research, the average contents were as follows: 10.25% ash, 6.62% water, 37.30% protein, 6.13% fat, and 26.14% carbohydrate (Table I). The samples of ash, water, protein, fat, and carbohydrate are shown in Figures 1-5.

Table 1: Proximate analysis result of animal feed granules composition

Content	Average (%)
Ash	10,25
Water	6,62
Protein	37,30
Fat	6,13
Carbohydrate	26,14

Figure 1: Sample of ash content

Figure 2: Sample of water content

Figure 3: Sample of protein

Figure 4: Sample of fat

Figure 5: Sample of carbohydrate

Based on the standard curve solution absorption, linear regression equation was y = 0.148 x + 0.088 (Figure 6).

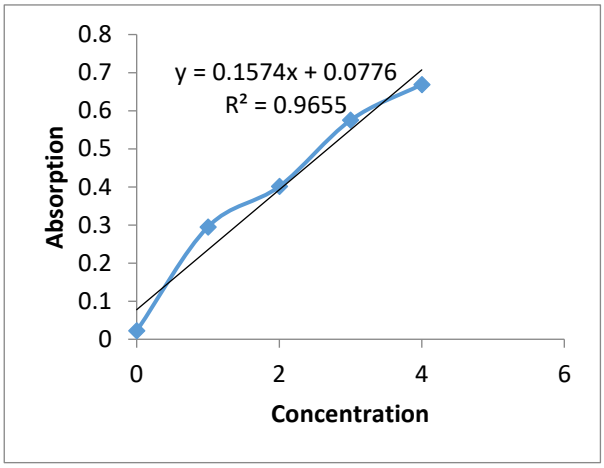

Figure 6: BSA Standard curve

Based on absorption measurement of glucose standard solution, the regression value obtained was y = 0.006x + (-0.066) (Figure 7).

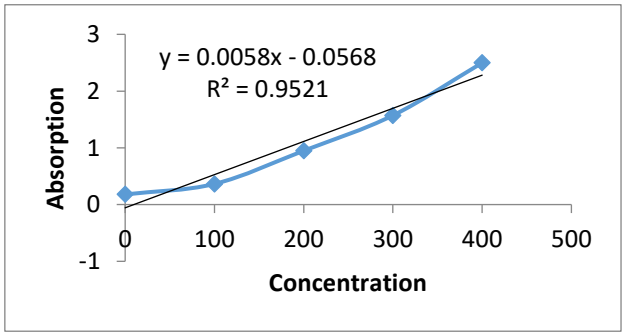

Figure 7: Glucose Standard curve

Discussion

Ash content determination of animal feed granules was done through the dry ashing method using a furnace to determine how good a process was in animal feed production (Widarta, 2015) and find the nutrient value of animal feed granules composition. In this research, the average amount of ash content with three replications was 10.25%. Referring to SNI-8509-2018, this amount should not exceed 14%. Hence, our results complied with SNI-8509-2018.

Water content determination of animal feed granules was done through the thermogravimetric method to find the nutrient content of the animal feed and check whether it meets the standards (Widarta, 2015). Water content determination is crucial because excess water can affect the appearance, texture, and taste of animal feed and support the growth of bacteria, mold, and yeast, resulting in the change of the animal feed. In this research, the average amount of water content with three replications was 6.62%. According to SNI-8509-2018, it should not be higher than 12%. Thus, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

The protein content of animal feed granules was determined through the biuret method, based on radiation absorption, using a spectrophotometer to measure protein content quantitatively, find the nutrient content in the sample, and verify whether it meets the standards Niode & Nasriani & Irdja, 2017). The biuret reactor forms complexes, which help identify the substances. The $CuSO_4$ produced by heating urea had a similar structure to that of the peptide bond of protein. The principle of the biuret reactor is based on the existence of a reaction between copper sulfate and alkalis with other compounds resulting in a distinct purple-blue (Machin, 2012) or red-violet or blue-violet (Purnama & Retnaningsih & Aprianti, 2019) solution. In this research, the average amount of protein content was 37.30%. Referring to SNI-8509-2018, this amount should not be any less than 16%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

The fat content of animal feed granules was determined using a Soxhlet, which extracted fat using petroleum ether solvent, to calculate calories in animal feed (Pargiyanti, 2019). In this research, the average amount of fat content with three replications was 6.13%. According to SNI-8509-2018, this amount should not be any less than 2%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

Carbohydrate content determination of animal feed granules used the phenol sulfate method. The principle of this method is that simple sugar and oligosaccharides

can react with phenol resulting in a stable yellowish-orange or orange colour. The standard carbohydrate used in this study was glucose. Based on absorption measurement, the regression value obtained was y = 0.006x + (-0.066). What follows is the glucose identification reaction by adding phenol and sulfate acid, which form the colour complex. In this research, the colour complex formed was orange. Based on Figure 8, the reaction shows that glucose was hydrated by thick sulfate acid to form hydroxymethyl-furfural. The colours obtained by hydroxymethyl furfural were various, ranging from orange-green to brown and purple, depending on the glucose concentration of the sample. In this research, the average amount of carbohydrate content with three replications was 26.14%. Referring to SNI-8509-2018, the amount should not be any less than 14%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

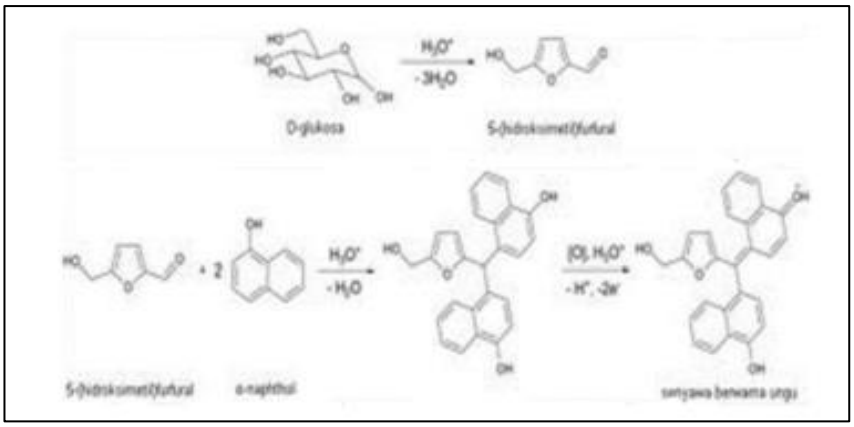

Figure 8: Glucose identification reaction with phenol-sulfuric acid (Wiyantoko, Rusitasari, Putri, dan Muhaimin, 2017)

Conclusion

The findings of this study show that nutrient contents in the composition of animal feed granules of raw material from fish innards wastes fulfil the regulation of animal feed content based on SNI-8509-2018.

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