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ANALYSIS OF CYANIDE ACID (HCN), CRUDE PROTEIN AND GROSS ENERGY CONTENT IN WINPROB (OTW_PRO) POWDER FERMENTED WASTE AT DIFFERENT TIMES

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Abstract: *The aim of this research is to determine the best storage time for the fermentation process for the content of cyanide acid (HCN), crude protein and gross energy in fermented winprob (otw_pro) onggok in powder form. The hypothesis of this research is that the best storage time for the fermentation process can reduce and increase the content of cyanide acid (HCN) and increase crude protein and gross energy in fermented winprob (otw_pro) onggok in powder form. The materials used in the research were onggok, winprob (powder form) and molasses. Materials for chemical feed nutritional tests are H2SO4, NaOH, Acetone, Boric acid HCl and methyl red indicator. The tools used are plastic jars, buckets, knives, and scales. The tools used in nutritional chemistry tests are calorie meter bombs, thermometers, digestion tools, distillation tools, titration tools, and shoxlet tools. The research method used in the research was a non-factorial Completely Randomized Design (CRD) with 5 treatments and 4 replications. The treatments given were as follows: LP0: storage time 0 days (control), LP 1: storage time 1 week, LP2: storage time 2 weeks, LP3: storage time 3 weeks, and LP4: storage time 4 weeks. The parameters observed in this research were analysis of the content of cyanide acid (HCN), crude protein and gross energy in Winprob (otw_pro) fermented onggok in powder form. Proximate testing is carried out in the laboratory. The conclusion of this research is that onggok fermented with Win Prob probiotics in powder form with a storage period of 3 weeks is the best treatment which can reduce cyanide acid (HCN) content and increase crude protein and gross energy.*

INTRODUCTION

The agriculture and livestock sector is faced with great challenges to meet the ever-increasing demand for food as the global population grows (Lasminingrat & Efriza, 2020). Therefore, research and development of alternative feed ingredients is very important to support the sustainability of the livestock industry. One of the potential materials to be explored further is onggok, which is a by-product of the cassava processing industry (Wiraputra et al., 2019).

The following is another paraphrase of the statement: Onggok, which is often viewed as agricultural waste, is now emerging as a potential candidate for verification as a high-value alternative feed ingredient (Suharno, 2020). In the context of cassava farming, onggok is often considered a by-product that has not been utilized optimally. However, through proper processing, ongok can be transformed into a valuable source of nutrition. Through innovation and a fermentation process with commercial probiotics, onggok has great potential to become an alternative poultry feed that is nutrient-rich, sustainable and efficient. In this way, onggok can be utilized more optimally, not only as waste, but also as a source of high-value feed for the livestock industry. In summary, onggok, which was previously ignored, is now proven to have the potential to be developed into an alternative feed ingredient with high nutritional value through appropriate processing, such as fermentation with probiotics.

Fermentation with commercial probiotics is a biotechnological process that provides an innovative solution in optimizing the nutritional content of onggok (Utama & Christiyanto, 2021). Involving beneficial microorganisms such as lactic acid bacteria, probiotic fermentation can positively change the chemical composition of hemp. In this process, microorganisms break down the complex compounds in the hemp, producing a final product that is easier to digest and richer in nutrients.

Onggok that has undergone probiotic fermentation offers a number of significant advantages as poultry feed. First of all, fermentation can increase the protein content of onggok (Asngad, 2005). Higher protein is very important to support optimal growth and development in poultry (Sjofjan & Djunaidi, 2016). By producing protein-rich feed, it is hoped that overall livestock productivity will increase.

Probiotic fermentation can help break down the crude fiber in onggok (Fuad et al., 2018a). Crude fiber that is more easily digested can improve the digestive efficiency of poultry, which in turn can improve nutrient utilization. In other words, fermented onggok has the potential to have a positive impact on feed conversion efficiency and the general health of poultry (Kompiang, 2009).

In this context, fermentation with commercial probiotics not only aims to increase the nutritional value of onggok, but also to ensure the safety of animal feed which contains anti-nutritional substances. The content of cyanide acid (HCN) in onggok can be a potential health risk such as poisoning in livestock if not managed properly (Hidayat et al., 2018). Therefore, an in-depth analysis of changes in HCN content during the fermentation process is very important.

Fermentation with commercial probiotics using Winprob (OTW_PRO) in powder form as an inoculum is a promising approach to improve the quality of onggok. Winprob has the potential to change the chemical composition of fodder, increasing its nutritional value, and in turn, making a positive contribution to efficient and sustainable livestock production. Variation in fermentation time is considered a critical factor in onggok processing. Different fermentation periods can provide valuable information about how changes in onggok nutrition develop over time. With a deep understanding of these changes, optimal fermentation strategies can be developed to achieve the best nutritional quality of onggok.

Based on the description above, the researcher wants to conduct research on analysis of cyanide content (HCN), crude protein and gross energy in Winprob fermented onggok (OTW_PRO) powder at different times.

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RESEARCH METHODS

This research was carried out at the Panca Budi Development University Laboratory, Building C. The research period is from December 2023.

The materials used in the research were onggok, winprob (powder form) and molasses. Materials for chemical feed nutrition tests are H_2SO_4, NaOH, Acetone, Boric acid HCl and methyl red indicator. The tools used are plastic jars, buckets, knives, and scales. The tools used in nutritional chemistry tests are calorie meter bombs, thermometers, digestion tools, distillation tools, titration tools, and shoxlet tools.

The research method used in the research was a non-factorial Completely Randomized Design (CRD) with 5 treatments and 4 replications. The treatment given is as follows: LP0: storage time 0 days (control)

LP 1: storage time 1 week
LP2: storage time 2 weeks
LP3: storage time 3 weeks
LP4: storage time 4 weeks

RESEARCH METHODE

Research Implementation

Manufacturing Procedure

After preparing each component according to its proportion of usage, weigh them all at once. Pour all the components into the container from above, beginning with the substance that is used the most in the bottom layer (onggok) for probiotics (winprob powder). After dissolving 15% of the weight of onggok in molasses-mixed water (using ± 30% of the total weight of the pile), the mixture of water, molasses, and winprob is poured over the ingredient pile and mixed with your hands until it becomes homogenous (even). Next, transfer it into a plastic jar, push it down, seal the jar firmly, and ferment for a lengthy period of time in accordance with anaerobic treatment.

Figure 1. Winprob Probiotic Powder for Fermentation

Analysis Sampling

Samples for chemical analysis of nutritional content were taken randomly based on treatment. Sampling started from the beginning of the onggok production before fermentation (LP0), 1 week after fermentation (LP1), 2 weeks after fermentation (LP2), 3 weeks after fermentation (LP3) and 4 weeks after fermentation (LP4). The samples that have been taken are immediately dried (drying in the sun/in an oven at 60 degrees Celsius), then the samples are weighed and ground with a blender for later analysis in the laboratory.

Research Parameters

The parameters observed in this research were, analysis of the nutritional content of crude protein, crude fiber and gross energy in fermented onggok using Win Prob (OTW_PRO) in liquid form. Proximate testing is carried out in the laboratory.

1) Analysis of Cyanide Acid (HCN) content, carried out using the procedure according to Arianto et al. (2014) as follows:
- Weigh 20 grams of liquid Win Prob fermentation hemp sample, fill the Erlenmeyer flask with 100 ml of distilled water, and let sit for two hours.
- Distill with steam after adding an additional 100 ml of distilled water. Twenty milliliters of 2.5% NaOH were added to an Erlenmeyer flask before the distillate was collected.
- The distillation process is terminated when the volume of the distillation (contained in an Erlenmeyer flask) reaches 150 ml. 8 ml of NH4OH and 5 ml of 5% KI were added to the distillation. Up to turbidity, the distillate mixture was titrated using a 0.02 N AgNO3 solution.
- Next, use the formula to get the cyanide acid content.:

$$\boxed{HCN = \frac{\text{ml AgNO3} \times 0{,}54}{\text{Berat bahan}} \times 1000 \text{ mg/kg}}$$

2) Analysis of Crude Protein Content

⇒ Destruction Stage

This step involves weighing the sample, placing it in a Kjeldahl flask, adding concentrated sulfuric acid (H_2SO_4) and a catalyst, and constantly digesting the mixture at 410°C until it becomes clear. Finally, the mixture is let to cool. Following this phase, the results will go on to the distillation phase.

⇒ Distillation Stage

Following the step of digestion, the solution is introduced to a distillation flask along with NaOH and distilled water. After that, the solution was collected using an Erlenmayer that held a typical acid solution.

⇒ Titration Stage

Using HCl solution, the distillation stage solution is titrated until it undergoes a color change. The formula is used to determine the crude protein content.:

$$\text{Crude protein content (\%)} = \frac{(VA - VB) \text{ x N HCl s } 14{,}007 \text{ x } 6{,}25}{\text{sample weight (g) x } 1000} \text{ x } 100\%$$

Note: VA = milliliter titration for sample
VB = military titration for blank
N = HCl concentration used
14.007 = atomic weight of nitrogen
6.26 = Conversion factor

3) Gross Energy Analysis

⇒ The calorific value or gross energy of feedstuffs is measured using a bomb calorimeter

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by joining the electrode tip to the burner wire.

⇒ After the sample has been weighed, it is placed on the electrode support and within the combustion bowl. Tightly fasten the bomb cap onto the container until it is securely fastened.

⇒ By activating the Fill menu on the device monitor, oxygen gas was pumped into the bomb vessel for a duration of one minute.

⇒ The bomb vessel is placed within a water vessel that has been partially filled with up to two liters of distilled water. After that, the water vessel was placed into the jacket container and fastened securely with the bomb bucket.

⇒ After that, connect the electrode cable to a 23 V power source and hit the Start button. Hold off until the stirring is done, which should take around five minutes. The temperature was taken using code t1 at the sixth minute.

⇒ In order to cause combustion within the bomb, the power supply button is activated. Once the temperature stabilizes once again, observe the temperature change and record it once more, coding it as t2.

⇒ The formula below is used to calculate gross energy content.:

Description : VA = milliliter titration for sample
VB = military titration for blank
N = HCl concentration used
14.007 = Atomic weight of nitrogen
6.26 = Conversion factor

RESULTS AND DISCUSSION

The recapitulation of the nutritional content (crude protein, crude fiber, gross energy) in onggok fermented with Win Prob (otw_pro) in liquid form is presented in Table 1.

Table 1. Recapitulation of average nutritional content (crude protein, crude fiber, gross energy) in onggok fermented with Win Prob (otw_pro) in liquid form.

Treatment	Parameters		
	Gross Energy (cal/100g)	Crude Fiber (%)	Cyanide Acid (ppm)
LP_0	3121,6003[a]	2,5051[a]	109,0438[b]
LP_1	3240,3899[a]	3,3300[a]	104,9659[b]
LP_2	3312,8660[a]	4,3877[a]	102,0318[b]
LP_3	3402,2388[b]	6,3281[b]	99,3822[a]
LP_4	3266,9648[a]	4,9000[a]	100,6416[b]

Notes: Different superscripts in the same column indicate significantly different results (p>0.05).

Gross Energy

Based on the results of the analysis of variance, it shows that onggok fermented with Win Prob in powder form with a fermentation time of 0 days to 4 weeks gives significantly different results ($P<0.05$) in the gross energy content of onggok. The average gross energy content of each LP_0, LP_1, LP_2, LP_3 and LP_4 is 3121.6003cal/100g; 3240.3899cal/100g; 3312.8660cal/100g; 3402.2388cal/100g; and 3266.9648cal/100g. Based on Table 1, in the onggok treatment fermented with Win Prob in powder form with a fermentation time of 3 weeks, the highest average gross energy content was obtained, namely

3402.2388cal/100g in the LP_3 treatment, and the lowest was 3121.6003cal/100g in the LP0 treatment (onggok fermented with Win Prob in powder form with a fermentation time of 0 weeks). In the results from the first week to the first week of fermentation, there was an increase in gross energy content from 3121.6003cal/100g to 3240.3899cal/100g. The probiotic microbes in Win Prob, which start to break down the complex ingredients in onggok, such fiber and starch, into simpler and easier-to-digest substances, are what's responsible for this boost. Additionally, probiotic microbes generate enzymes that aid in this breakdown process, increasing the material's energy content (Ezraneti et al., 2018). In the third week, the gross energy content increased to 3402.2388cal/100g. The results of this research are not much different from the results of research (Pratama & Siregar, 2024) that onggok fermented with Win Prob in liquid form with a fermentation time of 4 weeks gave results of a gross energy content of 3465.57cal/100g. During the period At this time, the activity of probiotic microorganisms reaches its peak. The breakdown of fiber and starch is more intensive, producing more simple compounds such as glucose and organic acids (Yani & Akbar, 2019). The energy content of these compounds is larger than that of the original component, leading to a significant increase in gross energy. Even though the fermentation process continued and microorganisms continued to break down the remaining components, the gross energy content decreased in the fourth week to 3266.9648cal/100g. The majority of the complex's components had been converted into simpler forms, but because the majority of the primary substrate had already been fermented, the rate of energy increase began to slow.

Crude Protein

Based on the results of the analysis of variance, it shows that onggok fermented with Win Prob in powder form with a fermentation time of 0 days to 4 weeks gave significantly different results ($P<0.05$) to the crude protein content of onggok. The average crude protein content of each LP_0, LP_1, LP_2, LP_3 and LP_4 was 2.5051%; 3.3300%; 4.3877%; 6.3281%; and 4.9000%. Based on Table 1, in the onggok treatment fermented with Win Prob in powder form with a fermentation time of 3 weeks, the highest average crude protein content was obtained, namely 6.3281% in the LP_3 treatment, and the lowest was 2.5051% in the LP0 treatment (the onggok containing fermented with Win Prob in powder form with a fermentation time of 0 weeks). In the results from the first week to the first week of fermentation, there was an increase in crude protein content from 2.5051% to 6.3281%. This increase is caused by the activity of probiotic microorganisms in Win Prob which begin to break down the complex components in the hemp, such as fiber and starch, and utilize existing nutrients for their growth and reproduction. This process results in an increase in the amount of microbial biomass, which naturally contains high protein, thus increasing the crude protein content in onggok (Utomo et al., 2013). In the second week of fermentation, the crude protein content increased to 6.3281%, where the crude protein content is the highest compared to other treatments. During this period, the activity of probiotic microorganisms reaches its peak Microorganisms also break down complex proteins into simpler amino acids and peptides, which are easier to measure as crude protein (Fuad et al., 2018b).

The results of this research show that fermenting onggok with Win Prob can increase the crude protein content significantly. Increasing the crude protein content is

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very important in the use of onggok as animal feed, because it can increase the nutritional value and efficiency of feed use. The increase in crude protein content shows that the probiotic microorganisms in Win Prob are effective in modifying the nutritional content of onggok through the fermentation process. This has the potential to improve the quality of animal feed and better support animal growth and health. In the LP_4 treatment, namely onggok fermented with Win Prob for 4 weeks, the crude protein content reached 4.9000%. Even though there was a decrease in the third week, there was an increase in the second week and other treatments. This is because probiotic microorganisms continue to carry out the protein biosynthesis process. At this stage, most of the complex components have been converted into simpler forms, and protein-rich microbial biomass becomes dominant in the fermentation mixture.

Cyanide Acid (HCN)

One of the purposes of this research is to determine the effect of long fermentation time using Win Prob in powder form on the content of cyanide acid (HCN) in onggok. Variations in fermentation time used were 0, 1, 2, 3 and 4 weeks. The results showed that there was a decrease in the cyanide acid content in onggok fermented with Win Prob as the fermentation time increased. Cyanide acid (HCN) is an anti-nutritional substance that is poisonous if consumed by livestock. The presence of these anti-nutritional substances creates obstacles in the use of onggok as animal feed, because high concentrations of cyanide acid can have fatal consequences for livestock. According to (Yuningsih, 2012), reducing the cyanide content can be done through physical, chemical or biological treatment, such as fermentation, so that the onggok can be used as animal feed. Cyanide acid levels in onggok are usually measured by cyanide acid content analysis. This analysis aims to determine the amount of cyanide acid in feed ingredients. The highest reduction in HCN content in this study was in the LP3 treatment (fermented onions with Win Prob for 4 weeks), namely 99.3822 ppm. This decrease was caused by the initial activity of probiotic microorganisms in Win Prob which began to break down anti-nutritional components, including cyanide acid. These microorganisms produce enzymes that can hydrolyze cyanogenic glycosides into less toxic compounds, so that the cyanide acid content is reduced.

There was a decrease in the HCN content because the microorganisms used for fermentation could excrete the extracellular enzyme linamarase. HCN levels decrease with increasing fermentation time, the longer the fermentation time, the greater the enzyme's ability to degrade linamarin into harmless compounds (Stephanie Dan Purwadaria, 2013). (Sari & Astili, 2018) wrote that the reduction in HCN was due to an increase in the concentration of microorganisms during the fermentation process, thereby accelerating the destruction of cyanogenic glycosides. The decrease in cyanide acid levels is due to an increase in the activity of the B-glucosidase enzyme, resulting in the breakdown of cyanogenic glucosides. The process of breaking down cyanogenic glucosides contained in the substrate by the B-glucosidase enzyme into glucose and acetone cyanohydrin compounds then releasing cyanide acid and acetone (Sari & Astili, 2018) . This is also supported by the results of research by (Oboh, 2006), that there was a decrease in cyanogenic levels by 86.1% after fermentation. In research by (Adamafio et al., 2010) they succeeded in reducing the cyanide acid content by up to 86.2%. Cyanide acid is a toxic substance that is classified as strong and works very quickly (Murni et al., 2008).

CONCLUSION

Onggok have its crude protein and gross energy content increased and its cyanide acid (HCN) concentration decreased when it is fermented for three weeks with Win Prob probiotics in powder form.

Suggestion

It is recommended for future researchers to conduct further studies on onggok by increasing the percentage of Win Prob usage correctly in order to obtain the best findings so that they can be recommended to the public to obtain more accurate results.

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