

Research Article

Increasing Nutritional Quality from the Mixture of Wood Tuber Skin, and Fermented Soya Bean Instruction with Microorganisms in Probio-7

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Abstract

Cassava tuber skin (CTS) and soybean husk (SH) are wastes that can be an alternative feed for poultry because their utilization does not compete with human needs and has good nutritional content. Still, high fiber content causes limited use. Improving the nutritional quality of this waste can be done through fermentation technology using microorganisms in Probio-7. The aim is to obtain the effect of the substrate composition and fermentation time with microorganisms in the Probio-7 on nutrient quality from the fermented product. The research method used is experimental with a completely randomized design (CRD) with a factorial pattern 3 x 2 treatments with three replications. The treatment consisted of factor A (substrate composition) namely A1 = 90% CTS + 10% SH, A2 = 80% CTS + 20% SH, and A3 = 70% CTS + 30% SH. Factor B (fermentation time) is B1 = six days, and B2 = eight days. The observed variables were the increase in protein, nitrogen retention, cellulase enzyme activity, decreased fiber, and fiber digestibility. The study concludes that the interaction between the substrate composition of 70% CTS + 30% SH and 8 days of fermentation time of a mixture of CTS and SH fermented with microorganisms in Probio-7 is the best.

Keywords: Cassava peel; Fermentation; Nutritional quality; Probio-7; Soybean husk

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Preliminary

Background

Efforts to increase livestock productivity with obstacles to meeting feed needs have not been completed in terms of quality, quantity, and continuity. Feed is one factor that significantly affects the success of a livestock business because the most significant production costs come from feed costs. One of the efforts to reduce the cost of expensive feed is to make your feed, among others, by utilizing waste that is no longer useful and does not compete with human needs, such as cassava tuber skin and soybean husk.

The potential for cassava production in Indonesia is quite significant, and according to the Central Statistics Agency (2018), cassava production in Indonesia in 2018 was 19,341,233 tons. Cassava production in West Sumatra in 2018 was 201,833 tons. In West Sumatra, many cassava tubers are processed into various unique foods such as sweet potato chips, rendang, and others. In the processing process, waste is produced, such as cassava tuber skin (CTS) [1]. According to [2], every kilogram of cassava tubers can have 27.3%, CTS which can pollute the environment if disposed of. Based on the data above, the potential for CTS in Indonesia in 2018 is 5,280,156,609 tons, and the potential for CTS in West Sumatra is 55,100,409 tons [3].

Cassava tuber skin has the potential as an alternative feed for poultry because it contains nutrients, namely: dry matter 24.61%, protein rough 10.55%DM, high fiber that is 25.59% DM, crude fat content 1.29%, calcium 0.36%, phosphorus 0.11%, and metabolic energy 2,596.16 kcal/kg, lignin 7.2%, and cellulose 13.8%, and HCN of 109 ppm [4]. According to [5], Cassava peels can only be used up to 7% in broiler rations because of the high fiber content.

Soybean husks (SH) are waste from the tempe-making industry, which can be used as an alternative feed for poultry. According to the Central Statistics Agency (2018), soybean production in Indonesia in 2018 was 982,598 tons, while soybean production in West Sumatra in 2018 was 2,225.55 tons. 100 kg of soybeans produced 17.98 kg of SH or 17.96% [6]. Based on the data above, the potential for SH in Indonesia in 2018 estimate is 176,474.6 tons, and the potential for SH in West Sumatra is 399.7 tons.

Soybean epidermis (SH) contains nutrients, namely dry matter 23.62%, protein 18.35%DM, high fiber, which is 23.35%, the content of crude fat 3.04%, ash 3.15% [7], Ca 0.23%, P 0.58% [8], and metabolic energy 2648.30 kcal/kg. Because of the high fiber content, using soybean husks in broiler rations just up to 10% [9].

In this study, a mixture of CTS and SH was used as a fermentation substrate. CTS can be used as a carbon source, but the protein content is low, so it is mixed with SH, which contains higher protein (protein 18.35%DM) so that a suitable balance of carbon and nitrogen is obtained for the growth of microorganisms contained in Probio-7. According to [10] the success of fermentation is influenced by the optimum conditions such as substrate composition, substrate thickness, inoculum dose, and fermentation time.

Fermentation with microorganisms requires a substrate containing carbon, nitrogen, minerals, and vitamins for maximum growth and development. According to [11], the substrate's composition, especially the C: N balance is essential for the growth of fungi and bacteria. According to [10] for the development of fungi and molds, a C: N balance ranging from 13:1 to 18:1. According to [12], a C: N balance for bacterial growth, namely 7:1 to 10:1.

The mixture of CTS waste with the addition of SH still contains high fiber, namely: Fiber content in treatment A1 (90% CTS + 10% SH) was 25.35% DM, in treatment A2 (80% CTS + 20% SH) was 25.15%, and in treatment A3 (70% CTS + 30% SH) which is 24.92% DM. Reducing the fiber content can be done through fermentation technology, one of which uses microorganisms contained in Probio-7. Fermentation is a process of chemical change in the substrate in a biochemical catalyst, namely an enzyme produced by microbes [13, 14].

Probio-7 contains seven probiotic microorganisms [15]. The organisms in Probio-7 *Bacillus subtilis* produces protease, amylase, glucanase, xylanase, chitinase, lipase, and cellulase [16]. *Lactobacillus acidophilus* produces protease [17, 4] and cellulase [18] *Saccharomyces cerevisiae* produces amylase, protease [19], and cellulase [20]. *Aspergillus oryzae* produces protease, cellulase, amylase, glutamine, and lipase [21]. *Rhodopseudomonas* produces cellulase and hemi-cellulase [22], *Actinomyces* produces lipase, cellulase, xylanase, chitinase, and protease enzymes [23] and *Nitrobacter* is a nitrifying bacterium capable of converting nitrate to nitrite [24]. According to [25] that in probiotics, there are bacteria that can produce several enzymes for feed digestion, such as protease, amylase, lipase, and cellulase, which can hydrolyze complex molecules into simpler ones to facilitate the process of digestion, and absorption of nutrients in the digestive tract.

Study that fermented cassava peel using 0.3% Natura and an incubation period of 11 days obtained an increase in the protein of 42.15%, a decrease in the fiber of 40.87%, and received fiber digestibility of 50.11%. [26,27] Reported that soybean husk fermentation using microorganisms in Effective Mikroorganisme 4 (EM4) for six days increased the protein by 18.85%, and a decrease in the fiber of 6.86%. According to [28], fermented soybean husk with *Aspergillus niger* resulted in a reduction of 36.32% fiber and 3350 kcal/kg metabolic energy.

Results fermentation research using commercial probiotics containing *Lactobacter*, *Acetobacter*, and yeast bacteria. In surimi waste by [29] with a commercial probiotic dose of 9% and seven days of fermentation can reduce the water content from 11.45% to 9.61% and increase protein from 56.09% to 69.97% (increased protein 19.83%). According to [4], who reported that pineapple peel fermentation with Probio-7 for eight days, protein increased by 46.48%, and nitrogen retention was 56.73%.

Substrate composition and optimum fermentation time with microorganisms in Probio-7 need to be studied because it will affect the increase in protein, cellulase enzyme activity, and decreased fiber from a mixture of CTS and SH. The increase in protein after fermentation does not necessarily increase the protein quality, so it is necessary to study the protein quality of fermented products by measuring nitrogen retention in broilers. The longer the fermentation caused, the higher the activity of the cellulase enzyme and the more overhauled cellulose, resulting in a decrease in the fiber content. Low fiber will affect the digestibility of fiber.

Materials and Methods

Materials

The raw materials used are cassava peels obtained from the potato chip manufacturing site in East Padang District and soybean husks obtained from the tempe processing industry in East Padang District, Padang City, West Sumatra. The microorganisms in Probio-7 were obtained from a poultry shop. Other materials used consist of chemicals for proximate analysis (protein). The experimental cattle used to test nitrogen retention and fiber digestibility were 20 broilers (eight chickens for treatment and two chickens for endogenous N) aged six weeks with a weight of $\pm 1,500$ grams. The equipment used in this study consisted of an analytical balance, autoclave, oven, a set of equipment for protein analysis, fiber, cellulase enzyme activity, and metabolic cage and equipment.

Research methods

Experimental design

This study used an experimental method with an experimental design, namely a completely randomized design (CRD) with 3 x 2 factorial patterns with three replications. The treatment factors given are:

Factor A is the composition of the substrate

A1 = 90% cassava root skin + 10% soybean husk

A2 = 80% cassava root skin + 20% soybean husk

A3 = 70% cassava root skin + 30% soybean husk

Factor B is the length of fermentation

B1 = 6 days fermentation time

B2 = 8 days of fermentation

Observed variables: Increase in Protein (%DM), Nitrogen Retention (%DM), Cellulase Enzyme Activity (U/ml), Decrease in Fiber (%DM), and Fiber Digestibility (% DM).

Fermentation with microorganisms in Probio-7

The substrate used was 500 grams fresh according to the treatment, namely: A1 = 90% CTS + 10% SH, A2 = 80% CTS + 20% SH, and A3 = 70% CTS + 30% SH. The substrate is inserted into the plastic and then homogenized. Furthermore, the substrate was sterilized using an autoclave (121°C, 15 minutes), then allowed to cool down to room temperature (25-30°C). The substrate was inoculated with 1% Probio-7, stirred until evenly distributed and incubated according to the treatment (6 days and 8 days) in facultative anaerobes. After the incubation is complete, the product is weighted to determine its fresh weight. The fermented product was taken as much as 10 grams per sample to analyze cellulase enzyme activity and stored in the freezer before use. Then put into the oven (80°C, 2 hours) to kill the microbes, dried in the oven (60°C, 10 hours), then ground, and analyzed for protein and fiber.

Data analysis

The data were analyzed statistically by analysis of variance according to a completely randomized design with 3 x 2 factorial patterns with three replications. Differences between treatments were tested using Duncan's Multiple Range Test (DMRT) according to [30].

Results

Effect of Substrate Composition and Duration of Fermentation with Microorganisms in Probio-7 on the Nutrient Content of Mixed Cassava Peel and Soybean Husk Skin

Effect of Substrate Composition and Fermentation Time with Microorganisms in Probio-7 on the increase in protein shown in Table 1, on nitrogen retention in Table 2, on cellulase enzyme activity in Table 3, on a decrease in fiber in Table 4, and fiber digestibility in Table 5 of a mixture of cassava tuber skin, and soybean husk.

Factor A	Factor B (Long Fermentation)		Average
Substrate Composition (CTS: SH)	B1 (6 days)	B2 (8 days)	
A1 (90:10)	46.39d	69.38b	57.89b
A2 (80:20)	47.05c	70.99a	59.02a
A3 (70:30)	47.32c	71.14a	59.23a
Average	46.92b	70.50a	
SE	0.13		

Table 1: Increase in protein from a mixture of cassava tuber skin and soybean husk fermented with microorganisms in Probio-7.

Note: ** = Very significant effect (P<0.01)

CTS = cassava tuber skin

SH = Soybean husk

Protein content before fermentation in treatment A1 was 11.33%, in treatment A2 was 12.11%, and in A3 was 12.89%. Fermentation with microorganisms in Probio-7 increases protein from the fermented product, shown in Table 1.

The most significant increase in protein in treatment A3B2 (mixture of 70% CTS + 30% SH with a fermentation time of eight days), which was 71.14% DM, and the lowest increase in protein in treatment A1B1 (mixture of 90% CTS + 10% SH with fermentation time) 6 days) which is 46.39% DM.

Factor A	Factor B (Long Fermentation)		Average
Substrate Composition (CTS: SH)	B1 (6 days)	B2 (8 days)	
A1 (90:10)	53.35d	57.11b	55.23b
A2 (80:20)	54.02c	58.32a	58.17a
A3 (70:30)	54.11c	58.57a	56.34a
Average	53.83b	58.00a	
SE	0.09		

Table 2: Nitrogen retention from a mixture of cassava tuber skin and soybean husk fermented with microorganisms in Probio-7.

Note: ** = Very significant effect (P<0.01)

The highest nitrogen retention in the A3B2 treatment (substrate composition of 70% CTS + 30% SH, and fermentation time of eight days), which was 58.57%, and the lowest nitrogen retention in treatment A1B1 (substrate composition of 90% CTS + 10% SH, and long fermentation six days) that is 53.35%.

The decrease in fiber in the A3B2 treatment (substrate composition of 70% CTS + 30% SH, and fermentation time of eight days) was high, 47.01%, and the lowest decrease in fiber was found in treatment

Factor A	Factor B (Long Fermentation)		Average
Substrate Composition (CTS: SH)	B1 (6 days)	B2 (8days)	
A1 (90:10)	0.25d	1.54b	0.90a
A2 (80:20)	0.73c	1.78a	1.26a
A3 (70:30)	0.88c	1.89a	1.39b
Average	0.62a	1.74b	
SE	0.056		

Table 3: Cellulase enzyme activity of a mixture of cassava tuber skin and soybean husk fermented with microorganisms in Probio-7.

Note: ** = Very significant effect (P<0.01)

Factor A	Factor B (Long Fermentation)		Average
Substrate Composition	B1 (8 days)	B2 (8days)	
A1 (90:10)	21.92d	43.48b	32.70a
A2 (80:20)	26.94c	46.04a	36.49a
A3 (70:30)	27.93c	47.01a	37.47b
Average	25.60a	45.51b	
SE	0.377		

Table 4: Decrease in fiber from a mixture of cassava tuber skin and soybean husk fermented with microorganisms in Probio-7.

Note: ** = Very significant effect (P<0.01)

A1B1 (substrate composition of 90% CTS + 10% SH, and long fermentation six days) that is 21.92%.

Factor A	Factor B (Long Fermentation)		Average
Substrate Composition (CTS: SH)	B1 (6 days)	B2 (8days)	
A1 (90:10)	42.70d	53.02b	47.86a
A2 (80:20)	45.82c	59.36a	52.59a
A3 (70:30)	46.76c	60.35a	53.56b
Average	45.09a	57.58a	

Table 5: Digestibility of fiber from a mixture of cassava tuber skin and soybean husk fermented with microorganisms in Probio-7.

Description: ** = very significant effect (P>0.01)

CTS = cassava tuber skin

SH = soybean husk

The highest fiber digestibility was found in the A3B2 treatment (substrate composition of 70% CTS + 30% SH, and fermentation time of eight days), which was 60.35%, and the lowest digestibility of fiber was found in treatment A1B1 (substrate composition of 90% CTS + 10% SH, and long fermentation six days) that is 42.70%.

Discussion

Effect of Treatment on Increase in Protein (%DM)

In the A3B2 and A2B2 treatments, increased protein compared to other treatments. The substrate composition was caused by increasing the addition of SH as a nitrogen source and a longer fermentation time (8 days). More microorganisms grew and thrived on the substrate, which was indicated by the high total colonies on the substrate. Microorganisms use food substances on the substrate as an energy source to grow, develop, and produce enzymes. To break down food

substances from complex molecules into simpler molecules. Carbohydrates are broken down into glucose, proteins into amino acids, and fats into fatty acids. The resulting molecules water so that the water content increases and the dry matter decrease [31]. The low dry matter content causes the protein percentage to increase. According to [32] that the low dry matter content due to the breakdown of food substances by enzymes contained in Probio-7 causes the protein quality of fermented products to increase.

In the A3B2 and A2B2 treatments, the growth was Fertil. It is influenced by the balance of carbon and nitrogen elements in the substrate. In the A3B2 treatment, the balance of C: N was 13.82:1, and in the A2B2 treatment, it was 14.97:1. According to [11], the substrate's composition, especially the C: N balance is essential for microbial growth because it can be a limiting factor in microbial metabolism if it is not balanced. According to [10] for the development of molds and fungi, a C: N balance is needed, ranging from 13:1 to 18:1. According to [12], a C: N balance is required for bacterial growth, namely 7:1 to 10:1.

During fermentation, the microorganisms contained in Probio-7 produce protease enzymes, namely *B. subtilis* [16], *L. acidophilus* [17, 4], *S. cerevisiae* [19], *A. oryzae* [21], and *Actinomyces* [23]. Protease enzymes can remodel protein ingredients into amino acids, which the animal's body will later absorb. [13] Stated that the increase in protein during the fermentation process is due to enzymes produced by microbes. The higher the number of microbes in the fermentation process, the more enzymes produced. According to [33] that enzymes are catalysts and are classified as proteins.

The increase in protein is caused by the contribution of protein sources from the microbial body due to more growth. According to [7], there is additional protein donated from microbial cells due to their development, which produces single cell protein products (PST) or cell biomass containing about 31-51% protein of fermented products increases. Added by [34], who stated that informing PST, the protein from microbes and the substrate protein could not be separated because it affects the product's protein content.

During fermentation, microbes also donate nucleic acids from their bodies which are products of NPN (Non-Protein Nitrogen) which can cause the protein to increase. According to [35], protein consists of pure protein (a combination of amino acids linked by peptide bonds) and NPN. According to [36] that fungi (molds and mushrooms) contain 31-50% protein, and 9-14% nucleic acid, bacteria contain 72-78% protein, and 8-16% nucleic acid, yeast contains 47-53% protein, and 5-9.5% nucleic acids, and algae containing 47-63% protein, and 8-10% nucleic acids.

The increase in protein in the A1B1 treatment (substrate composition of 90% CTS + 10% SH with a fermentation time of six days) was low. It was caused by the substrate with the addition of less SH as a nitrogen source and shorter fermentation time so that the microorganisms that grew and developed were slightly marked with a low total colony in the A1B1 treatment, which was 3.7×10^{18} CFU/g. Less growth of microorganisms is also caused by the high C:N balance. In the A1B1 treatment, the C: N balance was obtained, which was 16.27:1. Visually, the biomass of microorganisms growing in the A1B1 treatment was less so that the protein contributed by the microbes was also small, resulting in a lower increase in protein.

The increase in protein in the A3B2 treatment (substrate composition of 70% CTS + 30% SH with eight days of fermentation)

was 71.14% DM. This result is higher than Burhan's (2016) study that fermented cassava peel with 0.3% Natura organic decomposer with an incubation period of 11 days, an increase in the protein of 47.24%. Soybean skin fermentation with EM4 for six days received an increase in the protein of 18.85% [27].

Effect of Treatment on Nitrogen Retention (%DM)

The high nitrogen retention in A3B2 and A2B2 treatments was caused by the high protein content of fermented products in A3B2, and A2B2 treatments, namely 22.06% and 20.71%, which resulted in high protein consumption, namely 2.13 g/head and 2.09 g/head. The high content of protein in the A3B2 and A2B2 treatments was caused by the suitable substrate composition between CTS and SH (with the addition of COA as a nitrogen source) and the longer fermentation time so that more microorganisms grew and grew (total colonies were higher in the A3B2, and A3B2 treatments). A2B2 are 3.9×10^{22} CFU/g, and 3.7×10^{22} CFU/g, respectively). The high nitrogen retention is influenced by feed consumption, especially protein consumption. If the protein quality is low, the nitrogen retention produced is also common [13]. Added by [37] states that rations are containing good quality protein cause high palatability so that high ration consumption results in increased protein consumption. Consumption of high protein causes the amount of nitrogen absorbed by livestock to release less nitrogen. According to [13], if the nitrogen consumed is more significant than that excreted through the feces, a positive balance is achieved, meaning that the chicken body can absorb nitrogen so that the livestock body utilizes more nitrogen.

The nitrogen retention in the A1B1 treatment (substrate composition of 90% CTS + 10% SH with six days of fermentation) was low. Caused the addition of less SH as a nitrogen source and shorter fermentation time so that fewer microorganisms grew on the substrate. It was so protein-reduced. Donation is also lower, so that protein consumption is also low. Protein content in A1B1 treatment was 16.59% DM with a lower protein consumption of 1.9 eight g/head. It causes the nitrogen retained by livestock to be also lower.

According to [38], if the protein quality is low or one of the amino acids is missing, the nitrogen retention produced will be below. [39] State that low protein quality results in low protein consumption. The absorbed amino acids are also expected, and nitrogen retention is more deficient. According to [40], feeds with low protein content will move more quickly from the digestive tract than feeds that contain high protein, so that more nitrogen comes out than is left in the body. Nitrogen retention in the A3B2 treatment (substrate composition 70% CTS + 30% SH with eight days of fermentation) was 5 eight.57%. This result is higher than that obtained by Putri (201 six), who stated that the fermentation of cassava tuber skin flour with Effective Microorganisms 4 (EM4) for 11 days obtained nitrogen retention of 2 six.41%. The A3B2 treatment was the best and continued testing the content of other food substances, namely fiber 13.21%, crude fat 3.92%, Ca 0.24%, total P 1.33%, and metabolic energy 3265.32 kcal/kg.

Effect of Treatment on Cellulase Enzyme Activity (U/ml)

The activity of cellulase enzymes in A3B2 and A2B2 treatments was high because of the increasing addition of SH as a nitrogen source and the longer fermentation time (8 days). Many microorganisms grew, and cellulase enzyme activity increased. According to [41], the longer the fermentation, the higher the cellulase enzyme activity produced. The increased activity of the cellulase enzyme was

related to the number of microorganisms that grew more with an entire colony of A3B2 3.9×10^{22} CFU/g, and A2B2, i.e., 3.7×10^{22} CFU/g. In Probio-7, some organisms produce cellulase enzymes, namely: *Bacillus subtilis*, *Lactobacillus acidophilus*, *Actinomycetes*, *Aspergillus*, *oryzae*, *Saccharomyces cerevisiae*, and *Rhodopseudomonas*.

According to [42], if the cellulase enzyme activity is high, more enzymes will enter the fiber network. According to [10] factors that affect cellulase enzyme activity are substrate concentration, incubation time, temperature, and pH. Cellulase enzymes can degrade cellulose through a catalytic process to release sugar (glucose) [43]. According to [10] that complete enzymatic hydrolysis requires a synergistic action of 3 types of cellulase enzymes. First, endo-1, 4- β -D-glucanase (endocellulose, carboxymethyl cellulose, or CMCase), which breaks down cellulose polymers randomly at -1,4-glycoside internal bonds to produce oligo-dextrins with varying chain lengths. Second, Exo-1,4- β -D-glucanase (cellobiohydrolase) breaks cellulose to produce cellobiose and glucose. Third, -glucosidase (cellobiose) breaks down cellobiose to produce glucose.

The activity of the cellulase enzyme in the A1B1 is low. Treatment (Substrate composition of 90% CTS + 10% SH with a fermentation time of eight days) which is 0.25 U/ml, is caused by the design of the cassava tuber skin substrate is more than soybean husk with a short fermentation time (6 days). With the decreasing length of fermentation, the microbes also decreased, indicated by a small total colony of 3.7×10^{18} CFU/g. In line with that, the cellulase enzyme activity is also getting lower. Shorter fermentation time causes fewer microorganisms to grow and develop, producing fewer enzymes. According to [44], the length of fermentation is related to the time for microbes to grow and reproduce and affect the cellulase enzyme activity.

In this study, the A3B2 treatment (substrate composition 70% CTS + 30% SH with a fermentation time of eight days), fermented with microorganisms in Probio-7, obtained a cellulase enzyme activity of 1.89 U/ml. The results of this study are higher than those obtained by [19]. The cellulase enzyme activity of pineapple peel fermented 8 days with Probio-7 received cellulase enzyme activity of 1.78 U/ml.

Effect of Treatment on Decrease in Fiber (% DM)

The fiber content of the mixture of cassava tuber skin and soybean husk before fermentation in treatment A1 was 25.37% BK, treatment A2 was 25.15% DM, and treatment A3 was 24.92% BK. After fermentation, there was a decrease in fiber, which can be seen in table 4. The reduction in fiber high was found in the A3B2 treatment (70% CTS + 30% SH substrate composition with eight days of fermentation), which was 47.01% DM, and the lowest was in the treatment A1B1 (substrate composition 90:10, and fermentation time six days) is 21.92%BK.

The high decrease in fiber in the A3B2 and A2B2 treatments compared to other treatments was due to the suitable substrate composition with the increased use of SH as a nitrogen source and the longer fermentation time (eight days) more microorganisms grew and reproduced on the substrate. The growth of more fertile microorganisms in the A3B2 and A2B2 treatments was influenced by the balance of C and N on the substrate. In the A3B2 treatment, the C: N balance was obtained, namely 13.8:1, and in the A2B2 treatment, which was 14.97:1, this balance was lower than the other treatments.

The addition of carbon (C) or nitrogen (N) sources in the substrate significantly affects the metabolic activity of microbes. According to [45], carbon and nitrogen sources in the correct ratio are needed to

propagate and produce enzymes. It was added by [46] that nitrogen sources have always been an essential nutrient for fungal growth and enzyme production. According to [10] For the growth of molds, and fungi, a C: N balance is needed, ranging from 13:1 to 18:1. According to [11], the substrate's composition, especially the C: N balance, is essential for microbial growth because it can be a limiting factor in microbial metabolism if it is not balanced.

The fertile microbial growth in the A3B2 and A2B2 treatments was indicated by the high total colonies in the A3B2 treatment, which was 3.9×10^{22} CFU/g, and the whole colonies in the A2B2 treatment 3.7×10^{22} CFU/g compared to other treatments. Microorganisms that grow a lot will cause higher cellulase enzyme activity successively. Participated in the A3B2, and A2B2 treatments, namely 1.89 U/ml, and 1.78 U/ml. As a result, the fiber content was lower. [47] stated that the higher the cellulase activity, the more cellulose could be degraded into glucose. At the end of the fermentation, the amount of fiber decreases. In A3B2 and A2B2 treatments (eight days fermentation time), the microorganism found are *Actinomycetes*, *Saccharomyces cerevisiae*, *Aspergillus oryzae*, *Rhodopseudomonas*, and *Lactobacillus acidophilus*. The cellulase enzyme found in Probio-7 will use the food substances in the substrate and degrade them into simple sugars to support their life needs.

The low decrease in fiber in the A1B1 treatment (substrate composition of 90% CTS + 10% SH with a fermentation time of six days), which was 22.16% DM, was caused by the design of the substrate with the addition of SH as a nitrogen source which was less. The fermentation time was shorter so that microbial growth was not optimal, indicated by the low total colony in the A1B1 treatment, which was 3.7×10^{18} CFU/g. Microorganisms that grow a little also cause the cellulase enzyme activity to be lower at 0.25 U/ml. The intense activity of the resulting cellulase enzyme causes only a small amount of cellulose to be broken down so that the decrease in fiber is low [48]. The decrease in selected fiber was found in the A3B2 treatment (substrate composition 70% CTS + 30% SH with eight days of fermentation) of 47.01%. The results of this study were higher than those obtained by Burhan (2016) that in cassava peel fermented using Natura 0.3%, and incubation time of 11 days, there was a decrease in the fiber of 40.87%. This result is also higher than [28] that fermented soybean husk with *Aspergillus niger* decreased fiber by 36.32%.

Effect of Treatment on Digestibility of Fiber (% DM)

The high fiber digestibility in the A3B2 and A2B2 treatments resulted from a higher fiber decrease. This is related to the higher activity of the cellulase enzyme in both treatments to break down cellulose into glucose; as a result, the fiber content is low so that the digestibility of fiber is higher. Digestibility of fiber has a negative relationship with fiber [40, 10] Stated that the digestibility of fiber depends on fiber in feed ingredients. the higher the fiber content, the lower the digestibility of fiber due to the limitations of poultry to digest fiber. Conversely, the lower the fiber content, the higher the digestibility of fiber.

The low digestibility of fiber in the A1B1 treatment was caused by the decrease in fiber, which was also lower (the fiber content was still high) in the A1B1 treatment. The high fiber was associated with low cellulase enzyme activity in A1B1 treatment; to break down cellulose into glucose so that the digestibility of fiber was lower. According to [49], fiber content in feed ingredients, fiber composition, and microorganism activity can affect fiber digestibility. According to [50] that

the high fiber content in the ration causes the birds to feel full quickly because the fiber is bulky (thick) as a result of the consumption of the ration decreases while the need for nutrients has not been met; as a result, the performance of the livestock decreases.

The digestibility of the selected fiber was found in the A3B2 treatment (70% CTS + 30% SH substrate composition and eight days of fermentation), which was six0.35%. The results of this study are higher than the digestibility of fiber obtained by [51-62] that cassava peel fermentation using *Bacillus amyloliquefaciens* bacteria with Inoculum dose of 3%, and fermentation time of 4 days obtained fiber digestibility of 44.45%. This result is also higher than Burhan's (2016) that in cassava peel fermented using Natura 0.3%, and incubation time of 11 days obtained fiber digestibility of 50.11%. In treatment, A3B2 was the chosen treatment, and continued testing the content of other substances, namely protein 22.06%, crude fat 3.92%, Ca 0.24%, total P 1.33%, and metabolic energy 3265, 14 kcal/kg.

Conclusion

From the study results, it can be concluded that the interaction between the substrate composition of 70% CTS + 30% SH and 8 days of fermentation with microorganisms in Probio-7 is the best treatment.

Declarations

Author's contribution

Nuraini contributed to designing the experiment (fermentation), analyzing data, writing this article. M. Ikhsan Rias: analyzing data and review the article. Puja Wati Sukma and Nadya Khairiyah contributed checking data and reporting the article. All authors confirmed the final revised form of the article for publishing in this journal.

Competing interests

The authors declared that they have no competing interests.

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