

Effect of various incubation time on proximate component of fermentation process of ammoniated palm dregs using *Aspergillus niger* starter

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ABSTRACT

This study aimed to examine the effect of various fermentation times on proximate component of the fermentation process of ammoniated palm dregs using *Aspergillus niger* starter; conducted at Feed Technology Laboratory, and Feed Science Laboratory, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang. This study used ammoniated palm dregs (ammonia content was 5% DM substrate) and *A. niger* starter. This study employed a completely randomized design (CRD) with three treatments and five replications. The treatments applied were different fermentation times, including: T0 = 0 days fermentation time, T1 = 3 days fermentation time, T2 = 6 days fermentation time. Parameters observed were proximate components consisting of moisture, ash, crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen-free extract (NFE). Analysis of variance was used to examine the data, followed by Duncan's multiple range test at 95% significance level. Results showed that different fermentation times significantly affected ($P < 0.05$) all proximate components. Longer fermentation times decreased moisture, EE, CF, and NFE content, while increasing ash and CP content. The 6-day fermentation treatment resulted in the lowest moisture (50.37%), EE (0.65%), and CF (22.98%) content, and the highest ash (1.68%) and CP (20.04%) content. In conclusion, increasing the fermentation time to 6 days improved the nutritional value and quality of palm dregs by increasing ash and CP content and decreasing moisture, EE, CF, and NFE content, making it a suitable ruminant feed.

Introduction

High population growth in Indonesia has led to an increase in animal protein consumption, which requires an adequate supply of live-stock-derived food products. Feed is the material provided to livestock as the primary nutrient source (Utomo, 2015). Feed can consist of various ingredients, including forage, grains, and agricultural by-products. Efforts to improve production efficiency require the development of feed that is not only nutritious but also economical and sustainable. Feed is one of the most expensive components in livestock production (Agustono *et al.*, 2017). To address this, efforts are needed to find alternative, economical and readily available feed sources, one of which is the utilization of agro-industrial waste as animal feed, especially ruminants.

The solid agricultural waste known as palm dregs is produced when the sugar palm tree (*Arenga pinnata*) is used to make vermicelli or sago flour. It has excellent potential as a fermentation raw material and a rather high nutritional content. Palm waste is rich in carbohydrates and contains nutrient compounds that can support the growth of microorganisms. Palm waste has the potential to be used as feed, but there are obstacles in its use, namely the high crude fiber (CF) content that makes it difficult for livestock to digest and low protein content (Pamungkas *et al.*, 2014; Pranoto *et al.*, 2022).

Ammoniation-fermentation (amofer) is the process of processing organic materials using ammonia to increase nutrient content and quality of feed (Sundstøl and Owen, 1984). Ammonia treatment is usually carried out at room temperature. The novelty of this research was that the ammoniation process was carried out at a high temperature (60°C), which can shorten ammoniation time. Amofer technique can increase the efficiency of agricultural by-products because ammoniation process with urea can loosen the bonds of lignin with cellulose and hemicellulose, thereby increasing the effectiveness of fermentation by cellulolytic and hemicellulolytic microbes in breaking down CF in feed ingredients. In other

words, the fermentation process will be more effective if the ammoniation process is carried out beforehand (Riswandi *et al.*, 2014; Riswandi *et al.*, 2015; Samadi *et al.*, 2016).

The microbial fermentation process produces enzymes that convert complex compounds into simpler forms, and the microbes will synthesize proteins, a process known as protein enrichment. Fermentation utilized *Aspergillus niger*, a fungus from the phylum Ascomycetes that has septate hyphae and is commonly found in natural waste. *A. niger* can grow optimally at temperatures between 35–37°C and requires oxygen for its growth process (aerobic). This fungus is a source of single-cell protein that is rich in protein and non-toxic (Tampoebolon, 2009). The use of *A. niger* in fermentation can increase protein content and reduce fiber content (Nisak *et al.*, 2023).

Combining high temperature ammoniation with fermentation using *A. niger* in this study aimed to improve the quality of palm dregs in terms of proximate components. The benefit of this research is that the processed palm dregs will have an increased crude protein content and a decreased crude fiber content, making them suitable for use as a fiber feed source for ruminant livestock.

Materials and methods

Studying the effect of various fermentation times on proximate component of the fermentation process of ammoniated palm dregs using *Aspergillus niger* starter conducted at Feed Technology Laboratory, and Feed Science Laboratory, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang.

Research materials

Materials used for the study were unmilled palm dregs, urea, *A. niger* starter, molasses, distilled water, 70% alcohol, half-cooked rice, wax,

and rubbing alcohol. Reagents used for proximate analysis included 0.3 N H₂SO₄, 1.5 N NaOH, acetone, N-hexane, catalyst (a mixture of selenium, potassium sulphate, and cupric sulphate), HBO₄, methyl red indicator, methyl blue indicator, boric acid, 45% NaOH, 0.1 N HCl, and distilled water.

The equipment used in the study were tarpaulin, plastic jar, hygrometer, large tray, small tray, Bunsen, plastic clip, plastic cling, measuring cup, pipette, Erlenmeyer flask, beaker glass, plastic bucket, tape, scissors, pH paper, analytical balance (accuracy 0.0001 gram), digital scale, desiccator, water bath, porcelain crucible, Buchner funnel, ash-free filter paper/Whatman, filter flask, Kjeldahl flask, burette, Soxhlet, upright cooler, glass incubator, autoclave, oven, stove, blender, magic com, spoon, tissue, label, and latex gloves.

Research design

A completely randomized design (CRD) including three treatments and five replications was employed in this study. The treatments included different fermentation periods i.e. 0, 3, and 6 days. Fermentation was carried out using 1% molasses and 1.5% *A. niger*.

The experimental design given was as follows:

T0: Ammoniated palm dregs + 1% molasses + 1.5% *A. niger*, 0 days fermentation time.

T1: Ammoniated palm dregs + 1% molasses + 1.5% *A. niger*, 3 days fermentation time.

T2: Ammoniated palm dregs + 1% molasses + 1.5% *A. niger*, 6 days fermentation time.

Research methods

The research methods consisted of preparation stage, *A. niger* enrichment, ammoniation, fermentation, and laboratory analysis. The preparation stage began with the preparation of the necessary equipments and materials. Unmilled palm dregs were dried in the sun for approximately 7 days to determine their air-dry weight. Moisture content assay was then performed before proceeding with the ammoniation process.

A. niger cultivation stage was carried out sterilely using half-cooked rice placed on a tray and covered with plastic wrap by making small holes so that oxygen could enter and the spore conidia of *A. niger* can grow optimally. Successful cultivation was indicated by the appearance of black spore conidia which indicates that *A. niger* had grown. *Aspergillus niger* which had successfully grown perfectly was then oven-dried for 2 weeks at 40°C. The dried *A. niger* was then blended until smooth and put into a plastic clip according to the calculated dosage for each treatment, then a total plate count (TPC) test was carried out on *A. niger* and resulted 109/gram, so it can be declared as a potential starter because the requirements for a good starter for fermentation is at least contain a minimum of 107/gram of microbes.

Ammoniation stage was carried out using a wet method using 5% DM ammonia. Palm dregs were mixed with the urea solution, then placed in a heat-resistant jar and placed in an oven at 60°C for 7 days. The 60°C temperature results in optimum urease enzyme activity, so the ammoniation process can occur more quickly (Utomo, 2015). After undergoing ammoniation process, the palm dregs were then harvested and air-dried to remove the ammonia odor, then the moisture content assay was carried out after ammoniation.

Fermentation stage was carried out by weighing 100 grams of ammoniated palm dregs, then each sample was added with distilled water (moisture content for fermentation was 65% DM) and 1% DM molasses, then sample was put into a heat-resistant nylon plastic and tightly closed, then sterilized in an autoclave at 121°C and an air pressure of 2 atm for 20 minutes. Sterilized samples were then cooled to room temperature, then placed on a tray according to the treatment and replication, then sprinkled with 1.5% *A. niger* starter. Fermentation process was carried out according to the incubation period of the treatment. Samples with a fermentation time of 0 days (T0) were immediately harvested and subjected to laboratory assays. Samples with a fermentation time of 3 days (T1) and a fermentation time of 6 days (T2) were harvested when they had undergone an incubation time according to the treatment. Sample preparation was oven-dried and ground, then placed in plastic clips labeled according to the treatment and replication, then continued with laboratory assays (moisture, ash, CP, EE, CF and NFE) according to AOAC (2005).

Data analysis

To ascertain if the treatment had a significant impact on the parameters, the acquired data were subsequently subjected to analysis of variance at a 95% significance level. The difference in mean values between the treatments at the 95% significance level was assessed using Duncan's multiple range test to see if there was a significant effect (Steel and Torrie, 1991). The following is the additive linear model for a completely randomized design (CRD):

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Statistical analysis

The proposed statistical hypothesis is as follows:

H₀ = $\tau_0 = \tau_1 = \tau_2 = 0$; there is no effect of various incubation times in the fermentation process of ammoniated palm dregs using the *A. niger* starter on moisture, ash, CP, EE, CF, and NFE content.

H₁ = there is at least one $\tau_i \neq 0$ ($i=1,2,3$); there is at least one effect of various incubation times in the fermentation process of ammoniated palm dregs using the *A. niger* starter on moisture, ash, CP, EE, CF, and NFE content.

Results

Table 1 displays the findings of the proximate analysis of moisture, ash, CP, EE, CF, and NFE of fermentation of ammoniated palm dregs employing *A. niger*. The Duncan test result showed that the moisture content of ammoniated palm dregs in 6 days of fermentation time was significantly ($P < 0.05$) lower than in 3 days and 0 day. The average percentage of ash content based on 100% DM at 0 days of fermentation (T0), 3 days of fermentation (T1), and 6 days of fermentation (T2) were 1.36%, 1.45%, and 1.68%, respectively. The average percentage of CP content based on 100% DM on T0 was 13.43%, T1 was 17.57% and T2 was 20.04%. The average percentage of CP content based on 100% DM on T0 was 13.43%, T1 was 17.57% and T2 was 20.04%. The average percentage of EE content based on 100% DM on T0, T1, T2 were 0.98%, 0.93%, and 0.65%, respectively. The average percentage of CF content based on 100% DM on T0, T1, and T2 were 28.06%, 27.18%, and 22.98%, respectively. The average percentage of NFE content based on T0, T1, and T2 were 55.63%, 52.26%,

Table 1. Proximate Components of Various Fermentation Time of Ammoniated Palm Dregs.

Treatments	Moisture	Ash	CP	EE	CF	NFE
T0	60.25±0.55 ^a	1.36±0.11 ^c	13.43±1.41 ^c	0.98±0.04 ^a	28.06±0.47 ^a	55.63±1.20 ^a
T1	54.97±0.69 ^b	1.45±0.05 ^b	17.57±1.49 ^b	0.93±0.04 ^b	27.18±0.19 ^b	52.26±1.49 ^c
T2	50.37 ±1.00 ^c	1.68±0.22 ^a	20.04±0.05 ^a	0.65±0.42 ^c	22.98±0.37 ^c	54.05±0.88 ^b

Different superscripts in the same column indicate significant differences ($P < 0.05$)

and 54.05%, respectively (Table 1).

Discussion

Various fermentation times of ammoniated palm dregs significantly ($P < 0.05$) reduced the moisture content of treated palm dregs. Longer fermentation times caused a decrease in moisture content in treated palm dregs, because molds used moisture (water) in feed ingredients. Fitria *et al.* (2019) explained that changes in moisture content of the material in ammoniation-fermentation treatment can occur due to the presence of microorganisms that work to break down the substrate in the fermentation process. Microorganisms utilized water for metabolism, producing heat energy that also accelerates evaporation. Water is necessary for the fermentation process and is crucial because it influences the activity of microorganisms, the fermentation's success, and the product's ultimate quality. Haryanto *et al.* (2020) stated that water affects the temperature and osmotic pressure during fermentation, which play an important role in maintaining the stability of enzymes and metabolites produced.

The Duncan test result showed that the moisture content of ammoniated palm dregs in 6 days of fermentation time (50.37%) was significantly ($P < 0.05$) lower than in 3 days (54.97%) and 0 days (60.25%). The longer the fermentation process, the more enzymatic activity occurs, which causes the water content in the substrate to tend to decrease. This resulted in a significant decrease in moisture content at T2 compared to T1 and T0, because T2 had a longer fermentation time (6 days). Haryanto *et al.* (2020) found that a longer fermentation time can lower moisture content of the material because water was used as a medium for enzymatic reactions and experiences evaporation due to the exothermic process.

Various fermentation time of ammoniated palm dregs had a significant effect ($P < 0.05$) on increasing the ash content in treated palm dregs. Because mold utilized organic matter (OM), a longer fermentation period increased the amount of ash produced. This led to a drop in OM and an increase in inorganic matter (ash). According to Mawarni *et al.* (2017), the fermentation process breaks down complex molecules into simpler ones that microbes need to flourish. The OM content and the percentage of ash content are directly correlated. This observation supports the assertion made by Mawarni *et al.* (2017) that the fermentation process will only have an impact on the amount of organic matter in feed because microbes will break down complicated molecules into simpler ones that they can consume for growth and development. The study's findings were also corroborated by Hastuti *et al.* (2011) and also supported by the opinion of Arif and Lamid (2014) who stated that if OM increases then the ash content decreases and if OM decreases then the ash content will increase.

Result of Duncan's test showed that the ash content in fermentation for 6 days (1.68%) was higher than that of 3 days (1.45%) and 0 days (1.36%). Fermentation process can increase the availability of minerals from the substrate through enzymatic activity that breaks down mineral bonds with organic compounds, making them more easily detected in proximate analysis as ash (Haryanto *et al.*, 2020). OM will be broken down (degraded) by the fermentation process until equilibrium is achieved. This is consistent with the findings of Soejono *et al.* (1988), who claimed that an increase in ash content is the result of the loss of OM, particularly neutral detergent soluble (NDS). Because T2 has a longer fermentation period (6 days), the ash concentration at T2 is significantly higher than at T1 and T0.

Various fermentation time of ammoniated palm dregs had a significant effect ($P < 0.05$) on increasing CP content of treated palm dregs. Longer fermentation duration increases CP content because *A. niger* produced exo-glucanase and cellobiohydrolase enzymes, which contributed to extracellular proteins. Cellobiohydrolase enzyme is the most abundant enzyme component produced by molds and plays a role as a contributor to extracellular proteins (Hilakore *et al.*, 2013). In 6 days of fermentation time, *A. niger* entered the exponential phase, so mycelial growth

increases, in accordance to Lie *et al.* (2015) who explained that longer fermentation times increase mold activity and the number of mycelium, so that total nitrogen also increases. The activity of *A. niger* causes the number of microbes to increase, which is counted as protein, so that CP content in the palm dregs increased. Glucose will be reused by molds in the formation of protein-containing biomass (Ngaji *et al.*, 2016). Molds also use inorganic nitrogen from ammoniation, converting it into organic nitrogen, which improves the protein quality of the material (Sukaryana *et al.*, 2011).

Result of Duncan test showed that the CP content in 6 days of fermentation period (20.04%) was considerably ($P < 0.05$) greater than those in 3 days (17.57%) and 0 days (13.43%). The increase in CP content was due to the longer fermentation process, the more microorganisms developed and the higher the microbial protein synthesis that occurred. This caused a significant increase in CP content in T2 compared to T1 and T0, because T2 had a longer fermentation time (6 days). According to Haryanto *et al.* (2020), the breakdown of other organic components and the contribution of microbial proteins are the main causes of the rise in protein content of fermented materials.

The reduction of EE content in treated palm dregs was significantly impacted ($P < 0.05$) by various fermentation time of ammoniated palm dregs. The longer the fermentation period, the lower the EE content was because the mold used fat as an energy source due to the increased free fat content. An active fermentation process can reduce the fat content in material (Hastuti *et al.*, 2011). The growth of *A. niger* became more active and required more nutrients as the fermentation time increase.

According to Duncan's test results, the EE concentration was lower ($P < 0.05$) after 6 days of fermentation (0.65%) than after 3 days (0.93%) and 0 days (0.98%). A significant decrease in EE content occurred from day 0 to day 6. The ongoing fermentation process caused fat contained in the substrate being broken down and utilized as an energy source by microbes for growth and metabolism. Fat can be oxidized or decomposed due to increased biochemical activity during the fermentation process. Intensive microbial activity on the 6 days of fermentation time caused more fat to be broken down, resulting in a lower remaining EE content (Haryanto *et al.*, 2020). This decrease was supported by Arief *et al.* (2018), who stated that the longer the fermentation process lasts, the higher the enzymatic activity towards nutrient components, including fat.

The reduction of crude fiber (CF) content in treated palm dregs was significantly impacted ($P < 0.05$) by the various fermentation durations of ammoniated palm dregs. Longer fermentation duration resulted a decrease of CF content because the increasingly active growth of *A. niger* increased the production of cellulase enzymes, which are effective in degrading CF. Increasing fermentation time provides an opportunity for microorganisms to degrade more material nutrients (Hastuti *et al.*, 2011). *A. niger* is a cellulolytic fungus that is able to produce cellulase enzymes, thus facilitating the breakdown of CF.) Optimal cellulase enzyme production occurs when the mold reaches the exponential phase, which continues to reduce CF until it enters the stationary phase (Lie *et al.*, 2015).

Result of Duncan's test showed that CF content in 6 days of fermentation (22.98%) was lower ($P < 0.05$) compared to 3 days (27.18%) and 0 days (28.06%). The decrease in CF content during fermentation was caused by the activity of enzymes produced by *A. niger*, such as cellulase, hemicellulase, and xylanase. These enzymes are able to degrade the structural components of CF such as cellulose, hemicellulose, and lignin into simpler and more soluble compounds (Arief *et al.*, 2018). The degradation process became more intensive as the fermentation time increased, so that the CF content in the material decreased. During fermentation, microbes utilize fibrous compounds as a carbon source for growth, especially when the starch or sugar content in the material begins to decrease (Sutardi, 2009). This fact showed on 6 days of fermentation time (T2), the utilization of fiber components by microorganisms reached its highest level, where there was a significant decrease in CF compared to other treatments.

The reduction of NFE content in treated palm dregs was not linear, but it was significantly impacted ($P < 0.05$) by various fermentation time of ammoniated palm dregs. Longer fermentation durations caused a decrease in NFE content. The availability of both readily easily digestible carbohydrates and nutritional value of the feed ingredients had an impact on this decline. NFE content was influenced by ash, CP, EE, and CF (Sari *et al.*, 2015). Carbohydrate availability depended on nutrient content (Pakpahan *et al.*, 2015). NFE content decreased because microbes used NFE as an energy source and carbon skeleton in their growth. The microbial fermentation process requires a certain amount of energy for growth and reproduction which will be obtained through the breakdown of nutrients in the substrate.

According to Duncan's test results, the NFE content was substantially lower ($P < 0.05$) after 6 days of fermentation (54.05%) than after 3 days (52.26%) and 0 days (55.63%). The decrease in NFE content during fermentation occurred because these compounds were utilized as the main energy source by microorganisms, especially in the early and middle phases of fermentation (Arief *et al.*, 2018). The activity of *A. niger* in fermenting these components caused NFE content to decrease, as seen on day 3 (T1) of fermentation time. On day 6 (T2) of fermentation time, there was a slight increase in NFE content. This increase was caused by the degradation of complex compounds such as cellulose or hemicellulose which produced simple sugars included in NFE fraction (Sutardi, 2009).

Conclusion

Treatment of increasing the fermentation time to 6 days can increase the nutritional value and quality of palm dregs, including the increasing of ash and CP content and decreasing moisture, EE, CF and NFE content, so that it can overcome the obstacles in utilizing palm dregs as ruminant feed.

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Conflict of interest

The authors have no conflict of interest to declare.

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