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The Nutrition Quality of Cassava Leaf Silage with Different Fermentation Lengths

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Abstract. This study aimed to examine the nutrient contents (crude protein/CP, crude fiber/CF, crude fat/CFat, and nitrogen-free extract/NFE) of cassava leaf silage using rice bran as the source of carbohydrates and starter EM-4 as inoculants under different fermentation times. A Completely Randomized Design was applied to four treatments (fermentation times) with five replicates at each time duration. The treatments were P0 = 0-day fermentation, P1 = 7-day fermentation, P2 = 14-day fermentation, and P3 = 21-day fermentation. Samples of silage were taken and analyzed, and the data was subjected to Analysis of Variance (ANOVA). The results showed that the duration of fermentation significantly affected the nutrient content of cassava leaf silage. The post hoc DMRT indicated different levels of CP, CFat, and CF across treatments, while R0 and R1 shared equal NFE. Differences and similarities in nutritional quality between treatments are caused by differences in fermentation lengths. The results of the orthogonal polynomial test for all contents show cubic equations. The lowest crude fiber content based on the cubic equation is for a fermentation time of 12 days, namely 12.11%, while crude protein is 24%, crude fat is 9.05%, and NFE is 38.64%. The lowest crude fiber content is the basis for determining the length of fermentation because the fiber content determines the nutrient quality.

Keywords: Fermentation, Cassava leaf, Rice bran, EM-4

Abstrak. Penelitian ini bertujuan untuk mengetahui kandungan nutrisi (protein kasar/PK, serat kasar/SK, lemak kasar/LK, dan bahan ekstrak tanpa nitrogen/BETN) silase daun singkong menggunakan dedak padi sebagai sumber karbohidrat dan starter EM-4 sebagai sumber inokulan pada waktu fermentasi yang berbeda. Rancangan Acak Lengkap diterapkan pada empat perlakuan (waktu fermentasi) dengan lima ulangan pada setiap jangka waktu. Perlakuan yang diberikan adalah P0 = fermentasi 0 hari, P1 = fermentasi 7 hari, P2 = fermentasi 14 hari, dan P3 = fermentasi 21 hari. Sampel silase diambil dan dianalisis dan data dilakukan Analisis Varians (ANOVA). Hasil penelitian menunjukkan bahwa lama fermentasi berpengaruh nyata terhadap kandungan nutrisi silase daun singkong. Uji beda DMRT menunjukkan tingkat SK, PK dan LK yang berbeda antar perlakuan, sedangkan R0 dan R1 memiliki BETN yang sama. Perbedaan dan persamaan kualitas nutrisi antar perlakuan disebabkan oleh perbedaan lamanya fermentasi. Hasil uji ortogonal polinomial seluruh kandungan nutrien menunjukkan persamaan kubik. Kadar serat kasar terendah silase daun singkong berdasarkan persamaan kubik Nampak pada lama fermentasi 12 hari yaitu sebesar 12,11%, sedangkan protein kasar 24%, lemak kasar 9,05% dan BETN 38,64%. Kandungan serat kasar yang paling rendah menjadi dasar penentuan lama fermentasi karena kandungan serat menentukan kualitas nutrisi.

Kata kunci: Fermentasi, Daun singkong, Dedak padi, EM-4

Introduction

Agricultural waste carries immense potential as livestock feed but also, unfortunately, low nutrient contents, potentially dangerous anti-nutrition substances when consumed by the animals, and a short shelf life. One of the agricultural wastes that has a relatively high potential for livestock feed is cassava leaf. Based on data from Indonesia Statistics (BPS, 2019), the production of cassava in Banyumas Regency in 2018 was 41,453 tons, while the fresh cassava foliage represented 10–40%, which equals 4.15–16.62 tons of the entire cassava plant. This abundant cassava leaf can be converted into animal feed, particularly for ruminant cattle (Sirait & Simanihuruk, 2010).

Processing cassava leaf into feed is imperative to extend its shelf life because fresh cassava leaf is highly degradable. According to Saenab (2010), the multivarious benefits of feed technology include improving the nutritional

quality of waste-based feed and extending the shelf life for a relatively long time. Among the methods of processing high-moisture forage is ensiling or fermentation, which results in silage. Ensiling forages results in feed with a stable, more digestible dry matter content than fresh forage (Kung et al., 2018). Additionally, silage can reduce the level of cyanide acids in fresh cassava leaves. It has been reported that storing cassava leaf in the form of silage can maintain its condition, quality, and palatability for a relatively long time and decrease the HCN level up to 60–70%, thus inducing safe feeding for the livestock (Ly et al., 2005) because cattle can only tolerate no more than 50 ppm of HCN. Feed processing like ammoniation and fermentation can increase the levels of protein and digestibility and reduce HCN levels in cassava (Hanifah et al., 2010). The obstacle in the fermentation process is related to its duration; the longer the fermentation, the lower the feed nutrient, which eventually leads to the degradation of feed nutrients, particularly protein, by proteolytic bacteria (Setiyawan & Thiasari, 2017). Three-week fermentation was involved in making banana stem-based complete feed (Dhalika et al., 2011). (Setiyawan & Thiasari, 2017) reported that microbial effectiveness occurred on the seventh day in making a total mixed ration based on sugarcane tops, where crude fiber decreased by 5% at pH 4 and dry matter increased by 39.91%. The objective of this study was to determine the optimum fermentation time for cassava leaf supplemented with EM-4 in terms of crude protein, crude fiber, crude fat, and nitrogen-free extract**.**

Materials and Methods

Location of Study

The study was conducted from April to September 2022 in an experimental farm in Karangsalam Kidul Village, RT 4 RW 5, Kedungbanteng District, Banyumas Regency, and the Laboratory of Nutrition and Livestock Feed, Faculty of Animal Science, Universitas Jenderal Soedirman, Purwokerto.

Experimental Design and Fermentation Process

The design of this experiment was the method of Completely Randomized Design. There were four (4) treatments at different fermentation times, namely R0 (0 days), R1 (7 days), R2 (14 days), and R3 (21 days). Each treatment was repeated five times. Samples were taken and fermentation lengths ended after 21 days, 14 days, 7 days, and without fermentation. The variables measured were the crude fiber, crude protein, crude fat, and NFE contents. The materials used for the fermentation process were the following: (1) mature cassava (Manihot esculenta Crantz) leaf and its stem (foliage), the one with the red stem; (2) rice bran; (3) EM-4; (4) water; and (5) regensia analysis of nutrient content. The mature cassava leaves (Manihot esculenta Crantz) and the stems used were also analyzed for their nutrient content. The results of the proximate analysis were as follows (%): dry matter 21.97, organic matter 78.03, crude protein 16.79, crude fat 18.28, crude fiber 27.83, ash 5.67, and BETN 31.43. The analysis of the crude protein, crude fat, crude fiber, and NFE contents of cassava leaf silage was conducted according to the proximate analysis (AOAC, 2005). The process of making cassava leaf silage was as follows: a) The mature cassava leaves (not fit for human food) at its stem were chopped to a size of 3–5 cm, followed by wilting at room temperature for 24 hours; b) 5 kg of the chopped cassava leaf was mixed with 10% rice bran (0.5 kg) and 4% EM-4 (v/b) or 200 ml; c) the mixture was placed inside a plastic bag, pressed tightly to remove all air from the bag, followed by sealing the bags and tying them closed with a polypropylene raffia ribbon; and d) stored in a safe place according to each treatment (0, 7, 14, and 21 days durations) that was previously randomized.

Table 1. The average nutrition content of Cassava leaf silage with different fermentation times

Nutrient Contents	Treatments			
	R0	R1	R2	R3
Crude protein (%)	18.08[d]	24.81[a]	23.17[c]	21.14[b]
Crude fat (%)	5.12[d]	7.42[b]	6.62[c]	9.30[a]
Crude fibre (%)	23.29[a]	14.34[c]	12.59[b]	21.58[d]
Nitrogen-free extract (%)	41.77[b]	41.99[c]	37.93[b]	44.78[a]

Note: Values bearing different superscript within column show significant difference by 5%

Data Analysis

The obtained data were subjected to Analysis of Variance (ANOVA), and the results of the ANOVA were explored for differences using the post hoc Duncan's Multiple Range Test and orthogonal polynomial tests. Orthogonal polynomial tests were carried out to determine the relationship between the variables measured and the fermentation lengths.

Results and Discussion

Crude Fibre (CF) Content

The CF contents of cassava leaf silage are illustrated in Table 1. The analysis of variance showed that the fermentation length significantly affected (P > 0.05) the CF content of cassava leaf silage. The post hoc DMRT demonstrated a significant difference (P > 0.05) across treatments. CF content in this study, from the lowest to the highest, was identified at R0 (23.29%), R2 (21.57%), R1 (14.35%), and R3 (12.59%).

At R0, the highest observed crude fiber (CF) content suggests that prolonging the fermentation period could enhance silage quality. This is due to microbial activities breaking down substrates and producing enzymes that contribute to the degradation of complex bindings, resulting in a less intricate structure. Rohmawati et al. (2015) mentioned the presence of cellulose, lignin, and hemicellulose in crude fiber that are collectively utilized by microbes for their growth. In other words, the longer the microbial fermentation, the longer it takes to decompose substances, which eventually help decrease nutritional biomass (Zheng et al., 2011). The 14-day fermentation had less CF than the 7-day fermentation. This might be because the anaerobic phase was reached on the 14th day, and the lactic acid-producing bacteria (Lactobacillus) in EM-4 could use the rice bran as an accelerator to lower the pH so that yeast and bacteria that break down food could not grow. One of the purposes of incorporating an accelerator in the silage process, according to McDonald (1981), is to inhibit the growth of particular bacteria. Chrysostomus et al. (2020) stated crude fiber in silage is lowered because the need for microorganisms' energy has been met (due to the accelerator and additive substances), and thus, the performance has improved through cellulolytic activities during fermentation to reduce the substrate's crude fiber. The results of orthogonal polynomials in this study demonstrated linear, quadratic, and cubic responses (P<0.05). The linear equation was Y = 18,98 - 0.10 X, where r^2 = 2,8%; the quadratic equation was Y = 23.47 - 2.02 X + $0.09X^2$, where r^2 = 99,15%; and the cubic equation was Y = 23.29 -1.62 X + 0.04 X^2 + 0.002 X^3, where r^2 = 99,91%. The full illustrations are presented in Figure 1. Based on the cubic equation, it appears that the lowest content is at a fermentation length of 12 days. The lowest crude fiber content is used as the best standard for fermentation. Low crude fiber content is the most important standard for determining the best quality of feed fermentation.

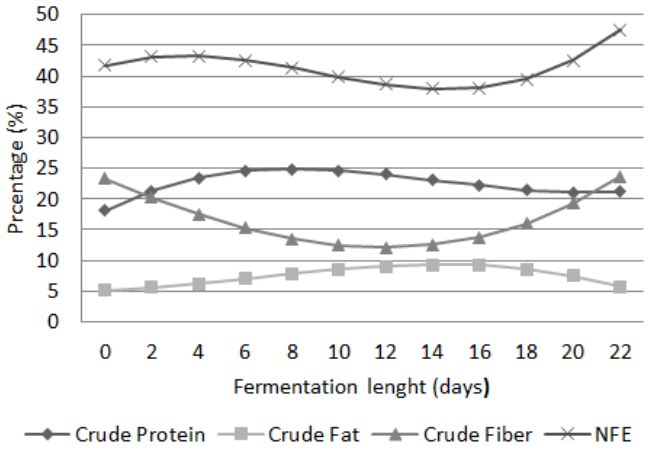

Figure 1. Crude protein, crude fat, crude fiber dan nitrogen free extract content of cassava leaf silage

Crude Protein Content

The CP contents of cassava leaf silage with different lengths of fermentation are illustrated in Table 1. It was found that the fermentation times significantly affected (P<0.05) the CP content. The highest CP content (24.82%) was obtained for treatment R1 (seven days), followed by treatment R3 (23.16%; 21 days) and R2 (21.14%; 14 days), while the lowest CP was at R0 (18.08%; 0-day fermentation). The results of post hoc DMRT showed significant differences (P<0.05) between the control treatment and the other treatments. Cassava leaf silage supplemented with EM-4 contained bacteria, which contributed to the increased protein contents compared to those in the control treatment. (Aro, 2008) stated that fermentation can increase crude protein in a food material through increased microbial populations. Also, Ohmomo et al. (2002) reported that the protein content is affected by the storage time, water content, and the quality and level of protein in the raw materials.

The highest increase in crude protein was identified at 7-day fermentation, where microorganism reproduction occurred at an increasing rate, which degraded more nutrition and converted nutrition components, eventually increasing crude protein. Agustono & Paramita (2010) stated that during the fermentation process, microbial biomass increased and, in turn, elevated the crude protein level. However, the CP level slightly decreased on the 14-day fermentation and then declined by the 21st day because the microbes contained in the substrate had entered the stationary phase or zero growth phase. According to Fardiaz (1992), after the exponential phase, the microbial growth rate will continue to decline up to zero (the stationary phase), where the number of living cells and dead cells is equal or constant. Furthermore, Hamdat (2010) stated that although growth did not occur at the stationary phase, cell functions like metabolism remained in effect, and the microbes that remained alive would feed on substances in the substrate for their growth, and therefore, the food substance was depleting gradually. Niesen (2017) reported that silage quality can be measured by analyzing its nutritional content. Some proteins from the forage undergo degradation (proteolysis) by either the plant's protease enzyme or microbes and are converted into NPN compounds (non-protein nitrogen), particularly amino acids and ammonia.

In addition to the contributing factors above, microbial activities during the 7-day fermentation were assumedly influenced by the availability of a carbohydrate source (WSC) from the rice bran that was sufficient for the optimum growth of EM-4 bacteria. The nutritional content of silage can be maintained by incorporating additive substances, including water-soluble carbohydrate sources (McDonald et al., 1991; Hidayat, 2014). The increase in nutrient content in making silage can be attributed to additive

substances (like rice bran) acting as accelerators and eventually stimulating the fermentation activities, as reported by Santi et al. (2012). In the present study, the increase in CP level in treatment R1 was due partly to the rapid growth of microbes supplemented into the treatment through EM4 solution. Energy supply from rice bran can be utilized by lactic acid bacteria derived from the EM-4 so that optimum growth is reached and new microbial cells are formed to provide a source of proteins that will increase the CP level. The increased CP is assumed to have derived from the contribution of microbial protein, particularly lactic acid bacteria (Sumarsih et al., 2009), which stimulate the formation of microbial cells during the silage process (Jenie & Rahayu, 1995), or because lactic acid bacteria act directly as the source of protein (Widodo, 2002; Balo et al., 2022). Kieliszek et al. (2021) also stated that LAB is a group of microorganisms that have the ability to ferment sugar into lactic acid and produce proteolytic enzymes, which play an important role in supplying cells with nitrogen compounds for their growth. Additionally, fermentation plays an essential role in the process of increasing protein levels because the microbes in the fermentation help improve the crude protein content of the silage. Another cause of the increased CP level in the fermented ingredient was the enzyme activities of microbes contained in the EM4 solution, like cellulase, that could release the protein-bound lignin onto the cassava leaf. Nevertheless, the protein content in all treatments in this study was higher than that of mature cassava leaf (16,7895%) (Laboratory of Nutrition and Cattle Feed Fapet Unsoed, 2022). In other words, fermentation technology in the form of silage from cassava leaf is proven to be effective.

The outcomes from the orthogonal polynomial test on crude protein (CP) levels in cassava leaf silage, varying in fermentation durations, revealed linear, quadratic, and cubic responses. The linear contrast obtained from the equation $Y = 20.67 + 0.11 X$ and r^2 was 11,18%; the quadratic contrast from the equation $Y = 18.48 + 1.05 X - 0.05 X^2$ and r^2 was 86,85%; and the cubic contrast obtained from the equation $Y = 18.08 + 1.95 X - 0.17 X2 + 0.004 X^3$ and r^2 was 99,63%. The complete illustration is presented in Figure 1. Based on the cubic equation, the highest CP content was at a fermentation length of 8 days, namely 24.90%, while at a curing time of 12 days it was 24%, decreasing by 0.9%.

Crude Fat content (CFat)

CFat contents of cassava leaf silage with different fermentation lengths are presented in Table 1. It was evident that the fermentation lengths significantly affected ($P<0.05$) the CFat content. CFat contents from the highest to the lowest in this study were identified in R3 (9.30%), R1 (7.42%), R2 (6.62%), and R0 (5.12%). The results of DMRT showed a significant difference ($P < 0.05$) in average scores across treatments. CFat content was fluctuating with the fermentation length. Crude fat tended to increase in the 14-day fermentation, then decrease in the 21-day fermentation. The highest crude fat was observed on the 14-day fermentation, and the lowest was at the 0-day fermentation. It was assumed that during the 14-day fermentation, the microbes had a more optimum activity to degrade organic matter (like carbohydrates) into a less complex bound and then utilized the degraded matter for their reproduction. The degradation would produce fatty acids, thus increasing the crude fat contained in the fermented feed, and also because the fatty acids were synthesized in the substrate. Hafizh et al. (2016) reported that the increase of CFat in the complete feed sourced from sago waste was attributed to the synthesized fatty acids, while Yunianta & Hartatik (2015) stated that the increase of CFat was due to the metabolic activities of microorganisms contained in the substrate. According to Muchtadi (1989) and Wulandari et al. (2021), lipolytic bacteria (bacteria capable of

degrading fat into fatty acids or glycerol) also need fat for their growth. Lipolytic bacteria, such as Pseudomonas, Alcaligenes, Serratia, and Micrococcus (Fardiaz, 1992), are contained in EM-4. The results of the orthogonal polynomial test of the treatment response were demonstrated in linear, quadratic, or cubic contrasts. The linear contrast was Y = 0.52 + 0.04 X where r^2 = 22,34; the quadratic contrast was Y = 4.91 + 0.63 X - 0.025 X^2 where r^2 = 90,53%; and the cubic contrast was Y = 5.12 + 0.16 X + 0.038 X^2 - 0.0020 X^3 where r^2 = 99,89%. The full illustrations are presented in Figure 1. The highest fat content based on the cubic equation was obtained at a fermentation length of 14 days, namely 9.32%, while at a curing time of 12 days it was 9.06%, a decrease of 0.26%.

Nitrogen-free extract (NFE) Content

The NFE contents of cassava leaf silage is illustrated in Table 1. The analysis of variance showed that the fermentation lengths significantly affected the NFE of cassava leaf silage. The NFE contents from the highest to the lowest were identified in R2 (37.93%), R0 (41.77%), R1 (41.99%), and R3 (44.78%). The post hoc DMRT showed a significant difference (P > 0.05) between R2 and R3, R1 and R3, and R0 and R1. It was because the fermentation length is closely related to the microbe's life cycle during fermentation and the ability of lactic acid bacteria to provide the energy source for their growth. As a result, each treatment received a different effect, and NFE production was less because microbes utilized much of the NFE. Lactic acid bacteria are capable of fermenting sugar into lactic acid (Santi et al., 2012), and the optimum growth of LAB is the cause of changes in nutrient content in silage (Superianto et al., 2018).

The same NFE content in fermentation for 0 and 7 days was probably because microbial activities on 0-day fermentation were still low or at the lag phase. The lag phase is a growth phase where adjustment to the new environment occurs, and the number of bacteria is only a few. Consequently, the capacity of lactic acid bacteria to utilize the source of energy for their growth on days 0 and 7 is equal, so no significant difference is observed.

The lowest amount of NFE was found after 14 days of fermentation, which was the exponential or peak phase. During this time, the microbes were able to break down the NFE components, like carbohydrates, for food. Pakpahan et al. (2019) state during fermentation, microorganisms will digest such as carbohydrates which is main component contained in NFE, to become food. Fermentation for 21 days produced the highest NFE because, at this stage, the stationary phase (or zero growth) occurred, the number of microbes was depleting, and less energy was used. Collectively, these led to a high level of NFE. Fardiaz (1992) reported that after reaching the exponential phase, the growth rate of the microbe would keep declining to zero (the stationary phase). This is the phase where the number of living and dead cells is equal or constant. Furthermore, Hamdat (2010) stated in stationary phase the living microbes can use the food substances remaining in the substrate for their growth, thus depleting the food substances.

The orthogonal polynomial test in this study showed linear, quadratic, and cubic responses (P < 0.05). The linear equation was Y = 40.8719 + 0.07106 X, where r2 = 5.14%; the quadratic equation was Y = 42.53 - 0.64 X + 0.034 X^2, where r^2 = 50.90%; and the cubic equation was Y = 41.77 + 1.06 X - 0.20 X^2 + 0.007 X^3, where r^2 = 98.68%. The complete illustration is presented in Figure 1. The lowest BETN content appeared at a fermentation length of 14 days, namely 37.93%, while at 12 days it was 38.64%, only a difference of 0.71%.

Conclusions

Best fermentation time is 12 days because at this time the lowest CF content (12.11%), protein

(24%), CFat (9.06%), and NFE (38.64%) were obtained.

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