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Nutritional Profile and In Vitro Rumen Fermentation of Mixed Silage of Kume Grass and Moringa Leaves

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ABSTRACT

A study was conducted to determine the nutritional profile and in vitro fermentation characteristics of mixed silage of Kume grass and Moringa leaves in the feed chemistry laboratory, Department of Animal Science, Faculty of Animal Husbandry, Fisheries and Marine, Universitas Nusa Cendana. The materials used were Kume grass and Moringa leaves, with the addition of rice bran as a fermentation stimulant. The research used an experimental method with a completely randomised design consisting of four treatments and five replications. The treatments were the proportions of Kume grass and Moringa leaves, respectively: KK0 = 100% Kume grass + 0% Moringa leaves; KK25 = 75% Kume grass + 25% Moringa leaves; KK50 = 50% Kume grass + 50% Moringa leaves and KK75 = 25% Kume grass + 75% Moringa leaves. The parameters measured included organoleptic quality, silage nutrient content, in vitro digestibility, and rumen fermentation. The results showed that increasing the level of Moringa leaves resulted in good ensilage, indicated by the absence of mould and a sour aroma. Rising levels of moringa leaves had a statistically significant effect ($P<0.01$) on the nutrient content of silage, rumen fermentation, and in vitro digestibility of the silage. It can be concluded that ensiling technology using Moringa leaves offers a promising solution to address feed shortages.

Keywords: Nutrient content, mixed silage, Moringa leaves, Kume grass

INTRODUCTION

The decline in forage production and quality during the dry season is due to decreased water-soluble carbohydrates (WSC) and protein content, along with increased structural carbohydrates. This occurs because of increased solar radiation, which raises ambient temperatures. The rate of photosynthesis usually increases with rising temperatures but decreases after reaching an optimum point. When temperatures exceed the optimal level for photosynthesis, the photosynthetic process is disrupted, and net photosynthesis decreases.

With declining WSC and protein levels below the minimum nutritional requirements for livestock, growth decreases, marked by a reduction in animal weight. This happens because a significant portion of the consumed feed is excreted as faeces due to an increased intake of feed with high cell wall content.

In general, silage made solely from natural grasses has low quality. Therefore, it is necessary to find forage that is readily available and adaptable to the climatic conditions of East Nusa Tenggara. One such plant is *Moringa oleifera* (kelor).

Moringa oleifera is a plant that can grow naturally in tropical regions. Cultivation practices, including fertilisation, can significantly increase dry matter production. Dry matter production of Moringa oleifera can reach 27 tons/ha with nitrogen fertilisation of 521 kg/ha/year at a plant density of 167,000 plants/ha when P and K in the soil are always available (Mendieta-Araica et al., 2013). Furthermore, it was stated that the higher the level of nitrogen fertilisation, the higher the crude protein content. Nitrogen fertilisation levels of 521 kg/ha and 782 kg/ha resulted in crude protein contents of 79 g/kg and 93.7 g/kg dry matter, respectively.

Dry matter production of *Moringa oleifera* is relatively high at the first cutting. A dry matter yield of 616.4 kg/ha at the first cutting with a planting distance of 1.30 m x 1.30 m (Frederick Nuhu, 2010). Furthermore, it was stated that *Moringa oleifera* can be used as a component in rabbit feed to substitute soybean meal. (Ritu Paliwal, n.d.). The use of *Moringa oleifera* leaves can reduce cholesterol levels in rabbit meat and blood, indicating that its inclusion in rabbit diets tends to produce healthier meat. In general, *Moringa oleifera* has a high protein content ranging from 27.5% to 36.5% depending on the variety. (Roloff et al., 2008).

In addition, this plant contains a complete profile of both essential and non-essential amino acids. (Kleden et al., 2017). The high protein content in *Moringa oleifera* leaves will affect the protein content of silage when combined with grass forage. One type of natural grass that is abundant in NTT, especially in Kupang City, is Kume grass (*Andropogon timorensis*) (Kamlasi et al., 2014). This grass is commonly found during the rainy season. Its short growth cycle and seasonal rainfall changes cause the grass to enter the generative phase, producing flowers and seeds, which eventually dry out in the dry season.

The high forage production during the rainy season can be optimally utilised as silage, which can be mixed with other protein-rich forage such as *Moringa oleifera*. The chemical composition of the mixed silage can be evaluated through scientific research.

MATERIALS AND METHODS

Research Materials

The materials used in this study were Kume grass and Moringa forage harvested from the university campus area, jars as silos, and rice bran (finely ground) as a fermentation stimulant. The available forage was chopped, wilted, and then mixed with rice bran at 5% (w/w) of the forage weight. It was then compacted into the silos and left to ferment for three weeks.

Research Methods

This study used an experimental method with a completely randomised design (CRD) consisting of four treatments and five replications. The treatments tested were different proportions of Kume grass and Moringa leaves: KK0: 100% Kume grass + 0% Moringa leaves; KK25: 75% Kume grass + 25% Moringa leaves; KK50: 50% Kume grass + 50% Moringa leaves; KK75: 25% Kume grass + 75% Moringa leaves. All treatments received 5% (w/w) rice bran as a fermentation stimulant. Parameters measured: The parameters observed in this study included: 1. Organoleptic quality (smell, presence or absence of mould, texture, and colour) 2. Nutrient content: proximate analysis of silage 3. Total Digestible Nutrients (TDN) 4. In vitro digestibility of dry matter (DM) and organic matter (OM) 5. Fermentation characteristics: volatile fatty acids (VFA), ammonia (NH_3), pH, and in vitro methane production of silage concentration.

Research Procedure

Kume grass and Moringa leaves were chopped into pieces of 3-5 cm and wilted. The forages were then weighed according to the treatment proportions. After that, 5% (w/w) rice bran was added, and the mixture was compacted into 2-kg jar silos, which were sealed and stored for 21 days (ensiling). After that ensiling period, the silos were opened, and organoleptic changes were observed. Samples were then collected for laboratory analysis, including in vitro digestibility testing. Organoleptic evaluation was conducted using self-assessment criteria as presented in Table 1.

Table 1. Organoleptic Assessment Score

No	Paremeter	Assessment score							
1	Aroma	1	Rotten	2	Slightly sour	3	Sour	4	Very sour
2	Color	1	Black	2	Dark brown	3	Brown is green	4	Bright green
3	Mold	1	Very much	2	Much	3	Somewhat much	4	Little
4	Texture	1	Very rough	2	Rough	3	Slightly soft	4	Soft

Notes : Remarks: 1 = Poor; 2 = Fairly good; 3 = Good; 4 = Very good

RESULTS AND DISCUSSION

Forage Quality before Ensiling

Forage provided to livestock in tropical regions, including East Nusa Tenggara, is generally fresh. The common problem is that, in addition to limited quantity, freshly harvested forage quickly undergoes nutrient degradation. If left for too long, the quality deteriorates significantly. The appropriate technology to extend the shelf life of forage is ensiling. Changes in silage quality are highly dependent on the initial quality of the forage. The initial quality usually refers to the characteristics of the fresh forage before it undergoes ensiling or other preservation methods. The initial nutritional composition can be evaluated through laboratory analysis. Dietary changes during the ensiling process are essential to anticipate, as they occur frequently and can affect silage quality. Therefore, proper handling is needed both before and during ensiling to maintain quality. During the ensiling process, several biological and chemical reactions occur, resulting in nutritional changes compared to the original fresh forage. (Santos & Kung Jr., 2016). Data on nutrient content before ensiling are shown in Table 2.

Table 2. Nutrient Content of Kume Grass, Moringa Leaves, and a Mixture of Kume Grass and Moringa Leaves before Ensiling

Parameter	Treatment			
	KK0	KK25	KK50	KK75
Dry Matter (%)	38.85	37.75	36.65	35.75
Organic Matter (%)	88.89	87.09	86.45	84.59
Crude Protein (%)	9.66	11.49	14.71	15.32
Crude Fibre (%)	26.99	21.59	19.23	17.78
Carbohydrate (%)	75.68	70.47	66.85	62.84
Nitrogen-Free Extract (%)	48.69	48.88	47.62	46.06
Dry Matter Digestibility (%)	26.42	37.11	42.58	50.10
Organic Matter Digestibility (%)	24.52	34.26	39.39	47.65
Volatil Fatty Acid (mM)	59.31	64.33	69.09	73.94
NH_3 (mM)	2.93	4.46	5.99	7.56
pH	7.2	7.1	7.2	7.3

Data in Table 2 indicates that when Kume grass and Moringa leaves were mixed before the ensiling process, the nutrient content was relatively high. As the level of Moringa leaves increased, the resulting nutrient content, particularly crude protein, also increased significantly. This increase was due to the difference in protein levels between Kume grass and Moringa leaves. The crude protein content in moringa leaves in this study was 21.52%, while that of Kume grass was 8.69%.

When both forages were combined at various ratios, a clear difference in nutrient composition, especially crude protein content, was observed. The protein values found in this study differed from those reported by (Kleden et al., 2017), likely due to differences in environmental growing conditions for the Moringa plants.

Crude protein is often used as a key indicator of feed quality. Higher protein levels typically contribute to a better supply of amino acids to the body. The results obtained in this study suggest that the protein requirements of livestock, especially ruminants, can be met through this type of silage.

Crude protein is often used as a key indicator of feed quality. Higher protein levels typically contribute to a better amino acid supply to the animal's body. The results obtained in this study suggest that the protein requirements of livestock, especially ruminants, can be met through this type of silage.

In dryland areas such as East Nusa Tenggara (NTT), meeting the nutritional needs of ruminant livestock is a significant challenge due to the lack of consistent forage availability.

This is mainly due to the region's limited and short-duration rainfall. Ensiling technology can help overcome this challenge by preserving forage for more extended periods. Through silage production, the long-term feed needs of ruminant livestock can potentially be met more effectively.

Effect of Treatment on Organoleptic Quality

Organoleptic assessment is often one of the criteria for silage quality. Organoleptic assessment includes aroma, colour, presence or absence of mould, and texture. The results of the organoleptic evaluation are presented in Table 3.

Table 3. Organoleptic Assessment of Mixed Silage of Kume Grass and Moringa Leaves

Parameter	Treatment			
	KK0	KK25	KK50	KK75
Aroma	2.8	2.83	3.33	3.53
Mold	4	4	4	4
Color	3	3.27	3.1	2.8
Texture	2.5	2.5	3	4

Data in Table 3 shows that the organoleptic evaluation, particularly regarding mould presence, indicates that the mixed silages produced in various ratios performed very well.

The silage produced showed very little to no mould growth. This suggests that compaction was adequate, minimising air pockets. Air infiltration allows mould to develop, which can lead to nutrient losses. Any mould observed was minimal and generally restricted to the top of the silo cover. The aroma of the mixed silage ranged from slightly acidic to acidic, with an average score of 3.12. This indicates that the acidity of the aroma falls within acceptable criteria. This is attributed to lactic acid bacteria fermenting available organic compounds during ensiling and producing lactic acid, which gives silage its characteristic odour.

Effect of Treatment on Silage Quality

One indicator of silage quality is the absence of mould. Changes in pH during ensiling affect the nutritional value for livestock. The ensiling mechanism, being compacted in a silo, will undergo changes that ultimately affect the nutrient content of the silage. The nutrient content of the silage obtained in this study is presented in Table 4.

Table 4. Nutrient Content of Silage

Parameter	Treatment				P-Value
	KK0	KK25	KK50	KK75	
DM (%)	36.34±1.85a	33.99±1.11b	32.88±0.91bc	32.03±1.77c	0,001
OM (%)	89.65±1.32	89.38±0.78	90.29±0.73	88.14±1.41	0.069
CP (%)	5.98±0.51a	8.64±0.71b	12.67±0.28c	14.14±0.52d	<0.0001
CF (%)	33.62±0.78a	28.34±1.63b	23.08±0.72c	17.45±2.59d	<0.0001
CHO (%)	79.51±1.57a	71.18±0.79b	64.65±1.45c	58.20±2.12d	<0.0001
NFE (%)	45.88±1.42a	42.85±2.03b	41.37±1.99b	40.76±1.36b	0.003
GE (Kcal/kg)	3958.76±67.26a	4206.67±58.74b	4455.68±58.56c	4496.36±59.76c	<0.0001

Note: Different superscripts within the same row indicate a significant difference ($P<0.05$). DM=dry matter, OM=organic matter, CP=crude protein, CF=crude fiber, CHO=carbohydrate, NFE=nitrogent-free extract, GE=gross energi.

As seen in Table 4, the dry matter (DM) content of the silage tends to decrease. This is due to the varying water content in the different materials used. During the ensiling process, microbial activity, particularly by lactic acid bacteria, produces organic acids like lactic acid.

The protein (CP) content of the silage increases as the amount of Moringa leaves is increased. Statistical analysis confirms that the treatments significantly increased ($P<0.01$) the crude protein content. This is because Moringa leaves have a higher protein content compared to guinea grass. The high crude protein content, along with a complete amino acid profile, is beneficial for both humans and livestock as a food or feed source. (Culver et al., 2012)

The crude fibre (CF) content decreased significantly. The statistical analysis shows that as the amount of moringa leaves increases, the CF content decreases. This indicates that the initial CF content of the feed material affects the final CF content of the silage. The lower the CF content, the higher the nutrient digestibility when fed to livestock. This is due to the easier degradation of nutrients in the rumen by microorganisms. Higher CF content tends to decrease nutrient digestibility. (Van Soest, 2019).

The silage energy content also follows a similar pattern, increasing with higher levels of Moringa leaves used in the silage. High energy content will fulfil the energy requirements of rumen microbes and simultaneously meet the energy needs of livestock when used as a feed source.

When compared to the data in Table 1, a decrease in nutrient content, especially crude protein, is evident. In each treatment, the crude protein content that could be retained was 61.91% (KK0), 75.16% (KK25), 86.13% (KK50), and 92.29% (KK75), with a general average of 78.87%. The value obtained in this study is in line with that stated by (McDonald, P., A. R. Henderson, 1991), which states that through the ensilage process, the crude protein content can be maintained at 70-90%. Nevertheless, through the application of ensilage technology, most of the crude protein content can be preserved.

The loss of protein content during ensilage is highly dependent on various factors such as the initial protein content before ensilage, the type of forage (grass vs. legume), and the level of compaction during silage making. The silage-making process can be divided into four phases: 1) the aerobic phase in the silo immediately after harvest, 2) the fermentation phase, 3) the stable phase during storage in the silo, and 4) the opening phase after the silo is opened (Borreani et al., 2018). In this process, a decrease in pure protein content occurs, and an increase in soluble protein occurs due to proteolytic activity (Winters et al., 2001).

Nutrient loss during ensilage depends on the type of forage used. Proteolytic activity in corn silage is related to the degradation of prolamins, resulting in increased starch digestibility in the rumen. (Emmanuel & Zaku, 2011). In addition, changes that occur during ensilage also include changes in the forage structure, such as the loss of hemicellulose in alfalfa and orchardgrass after 56 days of ensilage. (Yahaya et al., 2001).

The results of the statistical analysis showed that the treatment level of Moringa leaves in the mixed silage of Kume grass and Moringa leaves had a very significant effect on all observed nutritional components. (Hartati et al., 2013). This indicates that Moringa leaves are an essential component that can be used as a feed source through ensiling technology. The results obtained also explain that the ensiling process proceeded well.

The results obtained in this study differed from those reported by (Kleden et al., 2024), especially in terms of protein and crude fibre. The protein content in this study increased with the increasing level of Moringa leaves used, with an overall average of 10.36%, while the data reported by (Kleden et al., 2024) Was 9.27%. This difference occurred due to the difference in the types of forage used, namely, grass versus sorghum. (Takamitsu All, 1976). When linked to crude fibre content, the research results showed a decrease in crude fibre as the level of Moringa leaves used increased. This fact indicates that Moringa leaves have excellent potential in influencing the crude fibre content of silage. (Whiter & Kung, 2001).

Many factors affect the quality of silage, particularly dry matter loss. Dry matter loss is related to fermentation in the silo, especially the production of carbon dioxide. Dry matter loss ranges from 2-4% (Zimmer, 1980). Dry matter loss depends on the dominant microbial species and the substrate being fermented. The group of microbes that carry out fermentation are lactic acid bacteria, enterobacteria, clostridia, and fungi. (Pahlow et al., 2003).

Effect of Treatment on In Vitro Digestibility

The quality of forage can be evaluated both chemically**,** based on the results of laboratory analysis and biologically. Biological testing can be conducted directly on animals or simulate what happens in the animal's body through in vitro methods. In vitro evaluation provides an overview of how a feed material influences animal growth. Data on in vitro digestibility are presented in Table 5.

Table 5. In vitro digestibility and rumen fermentation

Parameter	Treatment				P-Value
	KK0	KK25	KK50	KK75	
DMD (%)	35.35±1.28a	40.64±1.96b	51.87±0.94c	58.97±0.96d	0.000
OMD (%)	34.19±0.82a	39.54±1.66b	50.28±0.64c	58.19±0.39d	0.000
VFA (mM)	57.49±1.39a	67.90±4.59b	82.66±3.25c	101.76±6.71d	0.000
NH3 (mM)	2.93±0.08a	4.97±0.36b	7.63±0.42c	9.01±059d	0.000
pH	7.18±0.04a	7.19±0.07a	7.25±0.05ab	7.36±0.11b	0.021
TDN (%)	56.22±2.36	56.62±1.71	58.63±1.71	58.70±1.61	0.153

Note: Different superscripts within the same row indicate a significant difference (P<0.05). DMD=dry matter digestibility, OMD=organic matter digestibility, VFA=volatile fatty acid, NH3=ammonia, TDN=total digestible nutrient.

Table 5 data shows that the digestibility of dry matter and organic matter, as well as rumen fermentation, increased with the increasing amount of Moringa leaves used. This increase occurred due to an increase in crude protein content. Protein plays a vital role in meeting the nitrogen (N) requirements of microbes, thus increasing their number and activity. The increase in the number and activity of microbes is closely related to the rise in fermentation rate, which enhances digestibility.

The increase in moringa leaf level also affected the increase in soluble carbohydrates. The increase in soluble carbohydrates tends to increase microbial protein synthesis. (Hoover & Stokes, 1991). An increase also followed the rise in Moringa leaf level in crude protein content. Crude protein in feed serves as a source of protein that ruminants can metabolise by providing rumen-degradable protein for microbial protein synthesis and undegraded rumen protein. Microbial protein synthesis depends on the appropriate sources of N and carbohydrates. (Pathak, 2008).

The digestibility of dry matter and organic matter obtained in this study is relatively similar to that reported by (Mayulu et al., 2020). Many factors affect the digestibility of feed, such as animal species, animal age, feed composition, feed maturity stage, feeding level, processing, and feeding frequency. (M. K. Patil, 2024).

Ammonia concentration is a byproduct of protein fermentation. Research results show that ammonia concentration increases with increasing levels of Moringa leaves in silage. This is due to the high crude protein content in Moringa leaves. The protein present during ensiling undergoes fermentation, resulting in ammonia production. This product is used as a precursor for the synthesis of amino acids and microbial protein. The increase in microbial population and activity leads to a rise in the rate of feed fermentation, including grass.

Ammonia concentration in the rumen of ruminants varies depending on the feed used. (Mehrez et al., 1977). An increase in the degradation rate in the rumen of sheep fed barley was accompanied by a rise in N-NH3 concentration up to 23.5 mg/dl. Reported that the rate of ammonia utilisation by rumen microbes in vitro varied between 1.6 and 26.7 mg/dl (J. Nikolic, M. Jovanovic, 1974).

Statistical analysis showed that the treatment had a very significant effect ($P<0.01$) on ammonia concentration. This proves that the protein content of silage is positively correlated with an increase in ammonia concentration. This fact demonstrates that using Moringa leaves as silage and a feed source for livestock will help improve protein fulfilment for ruminants. One of the determining factors for meeting their protein needs is the contribution of rumen microbes.

The concentration of VFAs obtained in this study showed that harvest age had a significant effect on VFA concentration. In general, the higher the level of Moringa leaves, the higher the VFA concentration produced. This occurs because the epidermal layer of Moringa leaves is thinner and more easily fermented by enzymes produced by microbes.

High VFA concentration will serve as an energy source for livestock when silage is used as feed. Meeting the energy needs derived from VFAs will provide opportunities for higher livestock growth. The results obtained in this study, especially for leaves, both VFA and NH3 concentrations, differ from those reported by (Mayulu et al., 2020). The differences exist due to the variations in feed materials used with different fermentation inoculants.

CONCLUSION

Based on the research results, it can be concluded that Moringa leaves can be used as a material in making mixed silage with grass, which can improve the quality and digestibility of nutrients. Nutrient loss, particularly of crude protein, after ensiling remains within a low limit, making ensiling an effective solution to overcome feed shortages during the dry season. Based on the conclusion, it is recommended to encourage the use of ensiling technology within the community to maintain feed continuity by utilising moringa leaf resources.

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