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Nutritional quality and microbiological evaluation of ensiled guinea grass, *Panicum maximum* Jacq. (Poales: Poaceae) and mulberry foliage, *Morus* spp. (Rosales: Moraceae) mixtures

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Abstract

Silage has been proven to be an effective and alternative source of nutrients for livestock and to provide high-quality feeds, especially during the dry season. However, the ratio of ensiling mixed silages of guinea grass (*Panicum maximum* Jacq.) and mulberry leaves (*Morus* spp.) needs to be evaluated, given their nutritional value as livestock feeds. Hence, this paper is to determine the best ratio of these forage materials as an alternative feed for livestock. The experiment was laid out using the Completely Randomized Design (CRD) with six treatments: T1 (100% guinea grass), T2 (100% mulberry leaves), T3 (50% GG + 50% ML), T4 (70% GG + 30% ML), T5 (30% GG + 70% ML), and T6 (56% GG + 44% ML) replicated three times. Treatments were evaluated with their crude protein, crude fat, crude fiber, ash, moisture, dry matter, ADF, NDF, lactic acid, pH, yeasts, molds, and total coliforms. Findings show that silage mixed with a 70% GG + 30% ML ratio produced the highest composition of nutrients (ADF, NDF, DM, moisture, ash, crude fiber, and crude fat) and microbial composition (lactic acid), making it the best-mixed silage for livestock. Therefore, the study recommends 70% guinea grass and 30% mulberry foliage silage mixtures due to their high nutritional value and microbial content. Moreover, the materials for silage production are readily available within the community. Therefore, this practice can be adopted by livestock farmers.

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Introduction

The quality of nutrition and management primarily determines animal performance. Nutrition is often a limiting factor in the productivity of animals selected for high genetic value, whether measured in milk production, prolificacy, growth rate, or disease resistance. Ruminant animals rely more on pasture than any other feed source for their nutrition requirements. Assuring forage quality is one of the most effective management tools, where a sufficient supply of nutrients is critical to the animal's maximal performance and from the economic viewpoint of the farmer. However, one of the significant challenges that livestock raisers face during the long dry season is meeting the dietary requirements of their ruminant animals due to the scarcity and seasonality of forage and fodder crops.

In the Philippines, cool, dry to hot, dry seasons start typically in December and last until May (PAGASA, 2021). During this period, good-quality pasture and feed sources for livestock animals are limited. During times of drought, silage production is paramount to avoid feed shortages. Forage preservation is essential, especially in seasons when forage materials are scarce. According to Semina: Ciencias Agrarias (2020), during dry periods when roughage is scarce, silage has proven to be an effective alternative to feed availability, providing high-quality feed that is commonly employed in ruminant feeding.

Silage is any crop collected green and stored in a moist state by partial fermentation in a more-or-less airtight container, such as a silo, by decreasing the pH within the minimum fermentation period. Nutrients obtained in silage are higher than hay and are used in conjunction with grass to supplement and enrich rations, particularly in summer and rainy periods (MacDonald and Reitmeier, 2017). The success of the silage can be achieved when the number of lactic acid bacteria (LAB) is dominant. During the natural ensiling process, the epiphytic LAB is the essential microflora for spontaneous silage fermentation. The epiphytic LAB converts water-soluble carbohydrates (WSC) into organic acids, mainly lactic acid, under anaerobic conditions. As a result, the pH is reduced, inhibiting the activity of undesirable microorganisms, especially clostridium (Yang *et al.*, 2004), which produces high concentrations of butyric acid and ammonia-N and nutrient loss (Ellis *et al.*, 2016), resulting in poor silage quality.

Silage has been proven for fodder maize, giant pearl millet, and multi-cut fodder sorghum, among other crops (Srinivasa Rao *et al.*, 2016). In the Philippines, guinea grass (*Panicum maximum*) is available anywhere. It is a fast-growing and leafy grass that is palatable to livestock and has good nutritional value, whether as fodder, hay, or silage. However, guinea grass is generally used to supplement protein-rich feed materials to meet dietary requirements and improve the animal's performance (Heuzé and Tran, 2020).

Mulberry (*Morus spp.*) is characterized by a high degree of adaptability (Sugiyama *et al.*, 2016) and a high protein level (Sánchez-Salcedo *et al.*, 2017; Thaipitakwong *et al.*, 2018) and has long been regarded as a promising fodder tree, particularly as a source of protein (Sugiyama *et al.*, 2016). Mulberry provides a highly palatable forage suitable for most farm animals (Martin *et al.*, 2017), and according to Dong *et al.* (2016), Trabi *et al.* (2017), and Neto *et al.* (2019), mulberry leaves can be used to make silage, and adding silage additives to it can improve its quality.

Mulberry and guinea are silage materials that provide high-quality nutrients to ruminants, so their combination as silage could be employed as a dry-season alternative feed material for ruminants. However, because there are few references on the nutritional quality, microbiological population, and silage characteristics of ensiling mixed guinea grass and mulberry leaves as feed material for ruminant animals during the long dry season, it is vital to figure out the best proportion of the two forage materials that works best for ruminant nutrition.

Therefore, this study aimed to evaluate the nutritional quality and microbiological population of ensiling grass-legumes forage mixtures (guinea grass and mulberry leaves). Specifically, to determine the nutritional composition and microbial population of the mulberry-guinea blend silage and evaluate silage quality by determining pH level and quantifying lactic acid bacteria after 21 days of fermentation.

Materials and methods

Experimental site, design, and treatments

The study was conducted at the College of Agriculture, Cagayan State University, Lal-Lo Campus. The Completely Randomized Design (CRD) was employed and replicated three times. The treatments evaluated were as follows: T1 – 100% Guinea grass; T2 – 100% Mulberry leaves; T3 – 50% Guinea grass + 50% Mulberry leaves; T4 – 70% Guinea grass + 30% Mulberry leaves; T5 – 30% Guinea grass + 70% Mulberry leaves; and T6 – 56% Guinea grass + 44% Mulberry leaves.

Forage materials for ensiling

Ten weeks' regrowth of guinea grass and mature mulberry leaves were harvested using sickle and shear and brought to the experimental area for processing. The harvested silage materials were cleaned, air dried for two hours, and cut into 2-3 inches' lengths using a forage chopper.

Ensiling and harvesting procedures

Polypropylene bags with a size of 13×18×0.0016 inches were used in ensiling harvested forages. A kilogram of chopped forage materials was mixed with 50 grams of molasses. Silage materials were properly compacted and fermented for 21 days at a temperature ranging from 30-35 degrees Celsius. After 21 days of fermentation, silage was harvested.

Physico-chemical analysis and microbial population evaluation

The physicochemical analysis and microbiological evaluation of the harvested silages were done at the Regional Feed Chemical Analysis Laboratory, Department of Agriculture Regional Field Office No. 02, to determine the percentage content of moisture, crude protein, crude fat, crude fiber, ash, dry matter, acid detergent fiber, and neutral detergent fiber. Parameters tested under microbial evaluation were yeast, molds, and total coliform.

Determination of silage quality

The silage quality was assessed by determining the silage's pH and lactic acid (ppm) content. Immediately after the arrival of samples at RFCAL, DA RFO 02, samples were opened. Sub-samples from the different points and depths were taken and mixed together for pH determination using the potentiometric analysis method. With the pH known, the lactic acid concentration of the silages was determined using the acid dissociation constant, K_a, of lactic acid.

Statistical tool

The data were analyzed using the Analysis of Variance (ANOVA) and the Statistical Tool for Agricultural Research (STAR Stat) software.

Results and Discussion

Crude protein (%)

Table 1 shows the silage's crude protein (%) level in the different treatments. The highest crude protein was recorded in T_3 with 10.37%. However, no significant difference was observed when compared to T_2, with a corresponding mean of 9.77. Highly significant differences were observed when compared to T_4, T_5, and T_6, with corresponding means of 7.84, 8.94, and 8.11. The lowest crude protein was observed in T_1, with a mean of 4.37. The analysis of variance shows a highly significant difference among the treatments tested. The result was due to the inclusion of mulberry leaves in different proportions with guinea grass. These results coincide with the findings of Cardenas *et al.* (2013) who found that by increasing the ratio of mulberry to grass *(Pennisetum purpureum),* the crude protein content significantly increased. Evangelista *et al.* (2009) also reported that crude protein content would increase as silage additives were added to silages.

Table 1. Summary of statistical analysis of the nutritional quality of guinea grass and mulberry leaves silages at different proportions after a 21-day fermentation period

Treatments	Crude protein (%)	Crude fiber (%)	Crude fat (%)	Ash (%)	Dry matter (%)	Moisture (%)	ADF (%)	NDF (%)
T1-100% Guinea grass	4.37^{e}	11.47a	0.75^{c}	3.83^{b}	36.45^{c}	63.55^{a}	14.74^{a}	15.30^{a}
T2-100% Mulberry leaves	9.77^{ab}	5.39^{e}	0.96^{b}	4.40^{a}	41.41^{b}	58.59^{b}	7.84^{e}	9.79^{e}
T3-50% Guinea grass + 50% Mulberry leaves	10.37^{a}	8.15^{c}	1.21^{a}	4.62^{a}	43.36^{a}	53.64^{c}	10.75^{c}	1277^{c}
T4-70% Guinea grass + 30% Mulberry leaves	7.84^{d}	9.80^{b}	1.34^{a}	4.41^{a}	43.12^{ab}	56.88^{bc}	12.79^{b}	14.21^{b}
T5-30% Guinea grass + 70% Mulberry leaves	8.94^{bc}	6.76^{d}	0.72^{c}	4.40^{a}	41.28^{b}	58.72^{b}	9.40^{d}	11.17^{d}
T6-56% Guinea grass + 44% Mulberry leaves	8.11^{cd}	8.58^{c}	0.86^{bc}	4.41^{a}	40.84^{b}	59.16^{b}	11.42^{c}	12.99^{c}
Result	**	**	**	**	**	**	**	**
CV (%)	4.62	4.67	6.03	4.35	3.61	2.57	4.19	2.86

** Highly significant at 5%

Crude fiber (%)

As reflected in Table 1, the crude fiber content of pure Guinea grass (T_1) silage outranked all other treatments with a mean of 11.47. This was followed by the 70:30 guinea grass-mulberry leaf proportions with 9.80% crude fiber. The ranking was also followed by T_3, T_6, and T_5, with corresponding means of 8.15, 8.58, and 6.76 however, no significant difference was observed between T_3 and T_6. Analysis of variance reveals highly significant differences among the treatments tested. Based on the results, pure grass had the highest level of crude fiber, followed by treatments with increasing proportions of grasses, and this was backed up by research done by Stallcup (1958,) which claimed that fiber was higher in grasses than in legumes. Moreover, Ganovsk *et al.* (1982) and Kung Jr. (2014), described the importance of crude fiber in ruminants' diets, stating that crude fiber promotes excellent digestion and favors feeding to ruminant animals while maintaining optimal rumen function.

Crude fat (%)

Table 1 reveals the crude fat value of silages after 21 days of fermentation. The result shows no significant difference between T_3 and T_4, despite recording the highest crude fat values of 1.21% and 1.34%, respectively. On the other hand, T_6, T_5, and T_1 obtained the lowest crude fat, ranging from 0.72% to 0.86%. Analysis of variance reveals highly significant differences existed among the treatments tested. This was due to the addition of mulberry leaves, which contain a high crude fat value compared to guinea grass. Similarly, Cardenas *et al.* (2002) obtained the same result.

Crude ash (%)

The highest crude ash was obtained in Treatments 2, 3, 4, 5, and 6, with means ranging from 4.4 to 4.62. The significance of the study can only be observed when the above treatments are compared to a pure guinea grass treatment with the lowest crude ash of 3.83%. Based on the results of the study, this could be attributed to the addition of mulberry to guinea grass. This result could be explained by Ba *et al.* (2005), who found that mulberry leaf silage has significant ash content, especially when molasses is added at a rate of 5%. It is also concluded that silages produced are free from soil contaminants. Undersander and Hoffman (2005) discussed that silages with less than 10% ash content are unlikely to be contaminated with soil.

Dry matter (%)

The percent dry matter of the silages is shown in Table 1. The highest dry matter was recorded in T_3 with a mean of 46.36 but was not significantly different from T_4 with a mean of 43.12. The analysis of variance shows that significant differences existed among the treatments tested. This could be attributed to the wilting of forage materials before ensiling, producing high dry matter and improving ensiling.

This could be explained by Ojeda and Lopez (2006). Dry matter is the indicator that interacts the most with preservation processes because of its limiting influence on the development of microorganisms. In addition, when the dry matter is below 25%, effluents are formed in the silages, causing significant nutritional losses. From the results, it was concluded that the dry matter of silage produced is acceptable. As described by Devaney (2017) and Goordon *et al.* (1964), the higher the dry matter, the higher the potential intake for silage and dry matter contents, up to 63%, resulting in efficient preservation.

Moisture content (%)

Based on the result (Table 1), the silage with the highest moisture content was recorded in T_1 with a mean of 63.55. This was followed by T_2, T_4, T_5, and T_6 with corresponding means of 58.59, 56.88, 58.72, and 59.16, respectively, with no significant differences. On the other hand, the lowest moisture value was recorded in T_3 at 53.64%. Analysis of variance reveals highly significant differences existed among the treatments tested. As described by Bernhart (2018), optimum silage moisture should range from 55 to 60 percent during harvesting. Bauder (2019) also claimed that silage with over 70% moisture should not be harvested. Therefore, the moisture content recorded during harvesting is acceptable and fit for animal consumption.

Acid detergent fiber

The average ADF value of silages in the different treatments is presented in Table 1. Based on the recorded mean ADF values, T_1 recorded the highest ADF, with 14.74%. T_4 followed this with 12.79%, while the lowest ADF value was recorded in T_2 with 7.84%. Analysis of variance reveals that highly significant differences existed among the treatments tested. The study's result indicates that all silages in the different treatments are considered good-quality feed. This result could be explained by Extension (2013), who found that higher ADF values within a feed suggest a lower-quality feed. ADF of <35% in either grass or legume indicates that the feed is of high quality. According to Lardy (2018), the forage becomes less digestible as ADF increases. In addition, based on the findings of Scott (2019) on corn silage, lower ADF values have high energy content and are ideal for animal feeding.

Neutral detergent fiber

The Neutral Detergent Fiber value of silages in the different treatments is presented in Table 1. Based on the recorded mean NDF values, T_1 recorded the highest NDF with 15.3%. T_4 followed this with 14.21%, while the lowest NDF value was recorded in T_2 with 9.79%. Analysis of variance reveals that highly significant differences existed among the treatments tested. From the results of the study, it was concluded that silages in all treatments demonstrate good silage quality. As discussed by Scott (2019), Extension (2013), silage with low NDF (%) is considered desirable, grasses with an NDF value of <50% would be regarded as high quality, and legumes with below 40% NDF would be considered good quality. According to Mertens (1992), the ideal NDF level in the diet is not fixed, and it varies according to the net energy requirements of the animals; the animals need to consume more to meet their energy requirements.

pH level of silages

The pH concentration of the guinea grass and mulberry leaf silages at different proportions after 21 days of fermentation is presented in Table 2. Analysis of variance revealed no significant differences among the treatments tested.

Table 2. pH and Lactic Acid (ppm) concentration of silages at different proportions of guinea grass and mulberry leaves after 21-days fermentation.

Treatment	pH	Lactic acid (ppm)
1	6.13	0.0733
2	5.77	0.1800
3	5.81	0.2133
4	6.50	0.0300
5	6.25	0.0533
6	6.51	0.0267
Statistical result	NS	NS

Lactic acid concentration (ppm)

As shown in Table 2, the highest lactic acid was obtained in T_3 with 0.2133 ppm and was closely followed by T_2 with 0.1800 ppm. The lowest lactic acid was observed in T_6, with 0.0267 ppm. Despite numerical variances, no significant differences were observed among the treatments tested.

Table 3. Microbial population (CFU/g) of silages utilizing different proportions of guinea grass and mulberry foliage during the 21-days Fermentation Period

Treatment	Total coliform CFU/g	Yeast CFU/g	Molds CFU/g
T_1	<1.0 × 10^3	>1.0 × 10^4	<1.0 × 10^3
T_2	<1.0 × 10^3	>1.0 × 10^4	<1.0 × 10^3
T_3	<1.0 × 10^3	>1.0 × 10^4	<1.0 × 10^3
T_4	<1.0 × 10^3	>1.0 × 10^4	<1.0 × 10^3
T_5	<1.0 × 10^3	>1.0 × 10^4	<1.0 × 10^3
T_6	<1.0 × 10^3	>1.0 × 10^4	<1.0 × 10^3

Microbiological evaluation of silages after 21-days fermentation

Table 3 shows the microbial population of silages using different proportions of guinea grass and mulberry foliage at 21-day fermentation. Based on the results, yeast were the most effective aerobic microorganisms identified, recording about 10,000 colonies forming units/g. The total coliform and mold population was minimal and can be degraded in the rumen of animals. Analysis of variance revealed that no significant difference existed among the treatments tested.

Conclusion

Based on findings, it was found that the 70% guinea grass + 30% mulberry leaf silage mixtures recorded the highest value in terms of ADF, NDF, DM, moisture, ash, crude fiber, crude fat, and lactic acid concentration, making it as the best nutrient composition for farm animals, especially livestock. Results of the study reveal that the use of 70% guinea grass + 30% mulberry leaf mixtures can be adopted by livestock raisers due to their high nutritional value, and the materials used in the production of silage are readily available within the community

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