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Effect of ensiling duration on nutritional composition and oxalate content in dwarf Napier grass silage

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Abstract. Napier grass (*Pennisetum purpureum*) is a common forage plant that is usually given to ruminant animals. Besides moderate nutritive values, this grass also contains anti-nutrients such as oxalate. Dietary soluble oxalate can combine with blood calcium (Ca) or magnesium (Mg) and form insoluble crystals, which can give negative effects on animal health. This study was conducted to assess the effects of ensiling durations on fermentation characteristics, nutritional composition and oxalate content of dwarf Napier grass silage. Plants were cultivated under standard level of fertiliser application, harvested at 45 days of plant maturity, and then chopped into 2-3 cm manually. Chopped grasses were mixed with 5% molasses (w/w), and filled in plastic bags, compressed and kept it in air-tight conditions in room temperature at five different ensiling durations (0, 15, 30, 60 and 90 days). Three replicates were made for each of the treatment durations. The fermentation characteristics, chemical composition and oxalate content, were evaluated. Silage at 30 day fermentation showed the highest lactic acid (10.02%) and NH₃-N (1.79%) contents, while silage at 90 d fermentation showed the lowest lactic acid (3.47%) and NH₃-N (1.50%) contents. The initial pH value (pH 5.01) and dry matter content (22.8%) declined to pH 4.36 and 20.2% at the end of 90 d ensiling period. Ensiling significantly improved the crude protein, ether extract and ash contents of Napier grass silage. The soluble, insoluble and total oxalate contents in silage decreased as ensiling durations increased. Ensiling also significantly improved the sodium contents of Napier grass silage, while no consistent trend was observed on Ca, Mg and potassium contents. The results suggest that at least 30 days of ensiling duration can be recommended for Napier grass silage to get the optimum nutrients and oxalate content.

1. Introduction

Shortage and inconsistent quality of forage are the factors of major constraints to the development of ruminant sector. Napier grass is high in dry matter (DM) production, has moderate nutritive value, and is easily established through stem propagation and can grow well in high-rainfall; however, it does not tolerate flooding. Because of these characteristics, it became a popular forage species for developing



countries including Malaysia. This grass is usually cut at 45-60 days interval to obtain higher DM and nutrient yields. It is fed to animals as fresh or silage.

The disadvantage of this grass is that it may contain soluble oxalate that is toxic to animals [14] since it can combine with serum calcium (Ca) or magnesium (Mg) to form insoluble crystals, which may result low serum Ca or Mg level and renal failure due to precipitation of these insoluble crystals in the kidneys. This may lead to lameness of the animals due to the demineralization of the bones which then become fibrotic and misshapen. It may also cause hypocalcaemia and hypomagnesaemia due to the high levels of oxalate. Limiting oxalate ingestion to <2.0% soluble oxalate would be suitable to avoid oxalate poisoning in ruminants [15]. Thus, it is required to reduce the soluble oxalate content in Napier grass using various methods such as ensiling it or cutting it at optimum age or applying Ca fertiliser to it when being cultivated before feeding to animals.

The methods that are undertaken to reduce the soluble oxalate content in Napier grass shall be further mentioned in this paragraph. Rahman et al. [16] encouraged applying Ca fertiliser to Napier grass to reduce the soluble oxalate content in it. Ensiling is another method which contributes to decreasing soluble oxalate content in Napier grass. In ensilation, bacteria may degrade the oxalate inside Napier grass, since oxalate-degrading bacteria in the rumen use the soluble oxalate as an energy source to produce harmless formic acid and carbon dioxide [15].

Although it is well known that fermentation process can enhance the nutritive quality of grass especially in terms of palatability and digestibility [11], there is little data about whether fermentation process can reduce oxalate level in Napier grass. Therefore, this study was undertaken to investigate the effects of ensiling duration on the fermentation characteristics, nutritional values and oxalate content in Napier grass silage.

2. Materials and Methods

2.1. Study area and plants

Dwarf Napier grass was grown at Agro Techno Park, Universiti Malaysia Kelantan, Jeli Campus, Kelantan, Malaysia. The re-growth plant was fertilised with standard level of NPK fertiliser (300 kg/ha/year) and harvested at 45 days of plant maturity. The harvested grass was chopped (about 2-3 cm) manually and dried for 2 h to reduce moisture of the plants. Wilted grasses were mixed with 5% molasses (w/w), and filled in plastic bags, compressed and kept it in air-tight conditions in room temperature at five different ensiling durations (0, 15, 30, 60 and 90 days). Three replicates were made for each of the treatment durations.

After the Napier grass silage reached their ensiling duration, fresh silage samples of each treatment were measured for pH value, lactic acid and ammonia-nitrogen contents. Furthermore, the samples from each treatment were dried at 60°C for 72 h, and then ground using blender which then was passed through a sieve (1-mm) to be stored for chemical analysis in plastic zipper bags.

2.2. Silage characteristics

About 10 g of silage sample was put in a beaker with 100 ml of distilled water at room temperature for half an hour and then the pH value was recorded using pH meter. Reading was repeated 3 times for each sample. Lactic acid content was determined following the method of Amin *et al.* [2]. Briefly describing the process, about 2 g of samples was placed into flask and 10 ml of distilled water was added. Sample was boiled to eliminate CO₂ and cooled. Then, 5 drops of 1% phenolphthalein were added to the sample. It was titrated with 0.1N NaOH until obtain light pink colour. Total acidity content was represented as percent of lactic acid. Ammonia nitrogen was determined by using Kjeldahl method which involved digestion, distillation and titration process.

2.3. Chemical analysis

Dried samples were analysed for DM, crude protein (CP), ether extract (EE) and ash according to the method of AOAC [3] while samples were analysed for soluble and total oxalates as described by

Rahman *et al.* [17]. Oxalate was measured using high performance liquid chromatography with a column Synergi 4 μm Hydro-RP 80 \AA , LC Column 250 \times 4.6 m, Ea (Phenomenex, USA). The insoluble oxalate was calculated by subtracting the soluble oxalate from total oxalate. Mineral composition (Ca, Mg, potassium and sodium) was determined using Atomic Absorption Spectroscopy as described by Cottenic [6]. Total carbohydrate was estimated using following formula:

$$\text{Total carbohydrate (\%)} = 100 - (\text{H}_2\text{O\%} + \text{CP\%} + \text{EE\%} + \text{ash\%})$$

2.4. Statistical analysis

The data for proximate components, silage characters, oxalate and mineral composition were analysed using one-way ANOVA following the procedure of SPSS (version 22.0, SPSS Inc., Chicago, IL, USA), while Duncan Multiple Range Test was applied to know the difference between treatment means at $p < 0.05$.

3. Results and Discussion

3.1. Silage quality

The pH value in Napier grass silage was affected by ensiling duration and it varied from pH 4.36 to pH 5.01. The pH value tended to decrease with every increasing of ensiling duration. Silage at 0 d fermentation showed the highest pH value (pH 5.01), whereas silage at 90 d fermentation showed the lowest pH value (pH 4.36). The pH values of experimental silage in this study are in line with the findings of Danso and Nartey [7] who stated that the pH value decreases with the increasing of the fermentation period. Achievement of low pH is one of the critical aspects for final stage of fermentation quality. The pH value usually gives a good indication for whether silage has a good fermentation and can be stored for greater duration.

Table 1. Effect of ensiling duration (days) on silage characteristics of ensiled dwarf Napier grass.

Parameter	Ensiling duration (days)					P-value
	0	15	30	60	90	
pH	5.01 ^d \pm 0.09	4.90 ^d \pm 0.08	4.72 ^c \pm 0.04	4.55 ^b \pm 0.06	4.36 ^a \pm 0.06	0.000
Lactic acid	6.06 ^c \pm 0.25	7.78 ^d \pm 0.70	10.02 ^e \pm 0.59	4.67 ^b \pm 0.60	3.47 ^a \pm 0.25	0.000
NH ₃ -N	1.52 ^a \pm 0.06	1.73 ^b \pm 0.02	1.79 ^b \pm 0.04	1.76 ^b \pm 0.02	1.50 ^a \pm 0.05	0.000

^{abcde}Means in a row with different superscripts differ significantly ($p < 0.05$).

Fermentation period had a significant ($p < 0.05$) effect on the percentage of lactic acid content in Napier grass silage and this value varied from 3.5% to 10.1%. The silage at 30 d fermentation showed the highest lactic acid content (10.02%), whereas the silage at 90 d fermentation showed the lowest lactic acid content (3.47%). Bilal [4] stated that lactic acid content in silage increased due to inclusion of additives during fermentation. Besides, 3% molasses level and 35 d of fermentation period were observed optimum to make quality silage as the pH was minimal and lactic acid was maximum under these circumstances. Fermentation process during ensiling was highly affected by the availability of fermentable carbohydrates and pre-dominant bacteria. By addition of some additives, there was an increase in the availability of carbohydrates in plants because of the degradation of complex carbohydrates by microorganisms, which in turn created lactic acid as by-product which ultimately decreased the pH of the silage [4]. During this study, higher percentage of lactic acid was achieved in 30 days of ensilation compared to other treatment durations, which is in line with the result obtained by Bilal [4].

Table 1 shows that fermentation period had a significant ($p < 0.05$) effect on the NH₃-N content in Napier grass silage and it varied from 1.5 to 1.8 %. The silage at 15, 30 and 60 d fermentations showed significantly ($p > 0.05$) higher NH₃-N content compared to silage at 0 and 90 d fermentations. However, there were no differences on the percentage of NH₃-N content in the fermentations at the treatment periods of 15, 60 and 90 d. The overall NH₃-N content in this study was much lower than the findings

of Bureenok et al. [5] who observed that 5% molasses treated Napier grass silage showed 6.8% $\text{NH}_3\text{-N}$ at 45 d of fermentation period. Umana et al. [19] reported that $\text{NH}_3\text{-N}$ content in silage should not be more than 10.0%.

3.2. Proximate components

The result showed that fermentation period had a significant ($p < 0.05$) effect on the DM content of Napier grass silage and it varied from 20.2 to 22.8%. However, no significant ($p > 0.05$) differences were found on the DM content between silages at treatment periods of 15 and 30 d, and 60 and 90 d, respectively. Silage at 0 d fermentation showed the highest DM content (22.8%), while silage at 90 d fermentation showed the lowest DM content (20.2%). The DM content in this study decreased when the fermentation period increased. This result is in line with the findings of Weinberg and Muck [22] who reported that once fermentation becomes stable at very low pH, DM content in silage reduces. Dry matter content in this study is in line with the results of Yammuen *et al.* [23] who observed that the DM content in Napier grass silage was in a range of 20.48 to 22.18%.

Fermentation period had a significant ($p < 0.05$) effect on the CP content of Napier grass silage and this content varied from 10.4 to 12.4% among the treatments. However, no significant ($p > 0.05$) difference was found between silages at fermentation periods of 0 and 90 d. There were also no significant difference on the CP content among silages at fermentation periods of 15, 30 and 60 d. Silage at 60 d fermentation showed the highest CP content (12.4%), while silage at 90 d fermentation showed the lowest CP content (10.4%). Results showed that CP content increased with every increase in the treatment duration from 0 to 60 d; but the CP value declined at 90 d. The CP content increased during ensiling possibly due to proteolysis activity producing NH_3 during fermentation process, but after good fermentation and early stability of the silage, proteolysis might be stopped. The resulted NH_3 from proteolysis aids in getting the aerobic stability due to its fungicidal properties [8]. Because of good fermentation, preservation and stability of silage, bacteria present in the silage have no opportunity to perform their activity and become the part of medium in silage. It is known that these bacteria are protein in nature and contain more than 75% true protein [24].

Fermentation period had a significant ($p < 0.05$) effect on the EE content in Napier grass silage and this value varied from 1.5 to 4.2 % among the treatments. However, there were no significant ($p > 0.05$) differences on the EE content in silages at fermentation periods of 15, 30, 60 and 90 d. Silage at 30 d fermentation provided the highest EE value (4.2%), while silage at 0 d fermentation showed the lowest EE value (1.5%). The EE values of grass silage in this study are in line with the findings of Yan and Agnew [25] who reported that the ranges of EE value in grass silage were from 2.1 to 5.8%. Unlike carbohydrates, fats do not experience fermentation losses in the rumen; however, rumen functionality hinders if the fat content in diet is greater than 5% [20].

Table 2. Effect of ensiling duration (days) on chemical composition (% , dry matter) of ensiled dwarf Napier grass.

Parameter	Ensiling duration (days)					P-value
	0	15	30	60	90	
Dry matter	22.8 ^c ± 0.4	21.7 ^b ± 0.5	21.5 ^b ± 0.1	20.4 ^a ± 0.3	20.2 ^a ± 0.1	0.000
Crude protein	11.0 ^a ± 0.4	12.1 ^b ± 0.1	12.3 ^b ± 0.3	12.4 ^b ± 0.3	10.4 ^a ± 0.3	0.000
Ether extract	1.5 ^a ± 0.0	3.0 ^b ± 0.2	4.2 ^b ± 0.4	4.1 ^b ± 1.0	4.0 ^b ± 0.8	0.002
Crude ash	10.0 ^a ± 0.2	12.4 ^b ± 0.4	13.0 ^b ± 1.2	13.4 ^b ± 0.4	12.3 ^b ± 1.4	0.007
Total carbohydrate	48.7 ± 2.0	47.9 ± 1.7	50.2 ± 2.3	45.9 ± 3.3	52.3 ± 2.0	0.063

^{abc}Means in a row with different superscripts differ significantly ($p < 0.05$).

Similarly with EE content, fermentation period had a significant ($p < 0.05$) effect on the crudash content in Napier grass silage. Silage at 0 d fermentation showed significantly ($p < 0.05$) lower crude ash content compared to silages at other fermentation periods, while silages at 15, 30, 60 and 90 d fermentation periods resulted no significant ($p > 0.05$) differences in crude ash content. Silages at 60 d

fermentation showed the highest crude ash content (13.4%), while silage at 0 d fermentation showed the lowest crude ash content (10.0%). Crude ash content in this study is in line with the findings of Mustafa et al. [10] who observed that the ash contents increases to some extent during ensiling at different fermentation periods. Excessive total ash content in animal ration can lead to unbalancing nutritional value that can affect animal's performance.

Ensiling duration had no significant ($p>0.05$) effect on the total carbohydrate content in Napier grass silage; it being within a range of 45.9 – 52.3%, which much lower than the findings of Rego et al. [12] who observed that ensiled Napier grass contained 79.6% total carbohydrates. This difference might be resulted due to various factors such as plant maturity, since nutritive values in plants are altered markedly by plant maturity.

3.3. Oxalate content

Table 3 shows that the ensiling duration had a significant ($p<0.05$) effect on the soluble oxalate content in Napier grass silage. The soluble oxalate content in silage decreased with increasing fermentation period. Silage at 0 d fermentation showed the highest soluble oxalate content (0.75%), while the silage at 90 d fermentation showed the lowest soluble oxalate content (0.51%). Rahman *et al.* [15] reported that if huge quantities of oxalate-rich plants are consumed, the rumen is overwhelmed and thus incapable to metabolize the oxalate, resulting to oxalate-poisoning in animals. Soluble oxalate can combine with Ca^{2+} or Mg^{2+} in the rumen or intestine, where then it forms insoluble crystals which then are either end through faeces or precipitate in the kidneys to form stones [1]. Therefore, the oxalate content in animal diets must be strictly supervised and applied in safe-levels to avoid poor/harmful diets given to the animal. In this study, it was observed that when dwarf Napier grass is fermented into silage, the soluble oxalate content is considerably reduced. This shows the possibility of including Napier grass silage in ruminants' diet without worrying about excess soluble oxalate affecting their health.

Table 3. Effect of ensiling duration (days) on oxalate content (% dry matter) in ensiled dwarf Napier grass.

Parameter	Ensiling duration (days)					P-value
	0	15	30	60	90	
Soluble oxalate	0.75 ^c ± 0.02	0.68 ^d ± 0.01	0.61 ^c ± 0.01	0.57 ^b ± 0.01	0.51 ^a ± 0.01	0.000
Insoluble oxalate	0.76 ^b ± 0.02	0.73 ^b ± 0.03	0.69 ^b ± 0.00	0.60 ^a ± 0.05	0.57 ^a ± 0.07	0.001
Total oxalate	1.51 ^c ± 0.01	1.41 ^d ± 0.02	1.30 ^c ± 0.01	1.17 ^b ± 0.06	1.08 ^a ± 0.07	0.000

^{abcde}Means in a row with different superscripts differ significantly ($p<0.05$).

Similarly with soluble oxalate content, the insoluble oxalate content in Napier grass silage decreased with increasing duration of fermentation. The silage at 60 and 90 d fermentation showed significantly ($p<0.05$) lower insoluble oxalate content than the silage fermented in 0, 15 and 30 d. Since insoluble oxalate can be excreted through faeces (and thus not being assimilated by animal's body), the animal is not harmed [18].

The ensiling duration had a significant ($p<0.05$) effect on the percentage of total oxalate content in Napier grass silage, the content varying from 1.08-1.51% in the treatments. The silage at 90 d fermentation showed the lowest total oxalate content (1.08%), while the silage at 0 d fermentation showed the highest total oxalate content (1.51%). Overall, the soluble, insoluble and total oxalate contents of Napier grass silage at 0 d fermentation were 0.75, 0.76 and 1.51% respectively, much lower than the values found in the previous study [14]. This variation might be occurred due to several factors such as rainfall, soil fertility and agronomic management as reported by Raman and Kawamura [13].

3.4. Macro minerals

Data on macro mineral content in Napier grass silage is shown in Table 4. The findings showed that the ensiling duration had a significant ($p < 0.05$) effect on the percentage of Ca content in Napier grass silage; this content varying from 0.06 to 0.23% in the treatments. The Ca content in Napier grass silage increased with increasing ensiling duration until 30 d, while this content declined gradually at ensiling duration from 30 d to 90 d. The silages at 15 d and 30 d fermentation contained significantly ($p < 0.05$) higher Ca content than the other treatments. Silages in this study contained adequate Ca, which is considered enough for ruminants as mentioned by McDowell and Arthington [9]. Rahman and Kawamura [13] reported that there was a positive correlation between oxalate and mineral contents in Napier grass. The amount of inorganic ions in plant tissues can be differed by changing the nutritional conditions and the ions maintaining ionic balance in plants can change the amount of carboxylic acid including oxalate. Generally, most forage has sufficient amount of Ca required for animal production; however, its compound, Ca-oxalate, has an insoluble nature which affects Ca bioavailability. Therefore, Ca is poorly available to grazing animals, which is instead excreted in faeces [21]. Oxalate-rich plants containing abundant Ca is safe to feed despite its high oxalate content because sufficient Ca can be absorbed by animal's body.

The potassium (K) content in the silages were not affected ($p > 0.05$) by ensiling durations, except for the 30 d silage where the K content was significantly ($p < 0.05$) lower than the ones from the other treatments. The K content in the silages was higher than the recommended K content (0.6–0.8%) for ruminants [25]. The Mg content in silages decreased ($p < 0.05$) with every increase of the ensiling duration. Silage at 0 d fermentation showed the highest Mg content (0.20%) while the silage at 90 d fermentation showed the lowest value (0.12%). However, no significant ($p < 0.05$) differences were observed on Mg content in silages from 15, 30 and 60 d fermentations. According to McDowell and Arthington [20], the recommended Mg content (0.2%) in forages for ruminants is higher than the Mg content of the Napier grass silages observed in this study. The ensiling duration had a significant ($p < 0.05$) effect on the percentage of sodium (Na) content in Napier grass silage and this content varied from 0.01 to 0.04% in the treatments. The Na content in the Napier grass silages increased with every increase of the ensiling periods until 60 d, while the Na content declined from 60 d to 90 d fermentation periods. In this study, all the Napier grass silages showed deficient amounts of Na for ruminants [25].

Table 4. Effect of ensiling duration on macro mineral content (% , dry matter) of ensiled Napier grass.

Parameter	Ensiling duration (days)					P-value
	0	15	30	60	90	
Calcium	0.18 ^b ± 0.03	0.22 ^c ± 0.01	0.23 ^c ± 0.01	0.17 ^b ± 0.02	0.06 ^a ± 0.02	0.000
Potassium	2.68 ^b ± 0.05	2.41 ^{ab} ± 0.41	1.99 ^a ± 0.14	2.84 ^b ± 0.42	2.70 ^b ± 0.19	0.028
Magnesium	0.20 ^c ± 0.03	0.16 ^{ab} ± 0.01	0.16 ^b ± 0.01	0.17 ^b ± 0.02	0.12 ^a ± 0.05	0.007
Sodium	0.02 ^a ± 0.01	0.03 ^b ± 0.00	0.04 ^c ± 0.00	0.04 ^c ± 0.01	0.03 ^b ± 0.01	0.012

^{abc}Means in a row with different superscripts differ significantly ($p < 0.05$).

4. Conclusion

In conclusion, the best ensiling duration was the 30 d fermentation as it resulted in better nutrients and fermentation characteristics when compared with the other ensiling durations. It was also observed that the soluble, insoluble and total oxalate contents in Napier grass silage decreased with every increase of the ensiling durations. In short, ensiling is an efficient and effective method that can be used to improve nutritional composition and reduce the oxalate content in Napier grass and therefore be safely fed to ruminants.

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