# A STUDY ON THE EFFECTS OF DIFFERENT CONCENTRATION OF LACTIC ACID AND SOUR YOGURT ON NAPIER GRASS SILAGE



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## A STUDY ON THE EFFECTS OF DIFFERENT CONCENTRATION OF LACTIC ACID AND SOUR YOGURT ON NAPIER SILAGE GRASS



## A Production Report Submitted as per Approved Styles and contents

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Contents:	Page No
List of tables	iv
List of Figures	iv
Abstract	V
Chapter 1: Introduction	1-2
Chapter 2: Methods and Methodology	3-4
2.1 Study Area	3
2.2 Collection of sample	3
2.3 Preparation of Silage	3
2.4 Physical properties	3
2.5 Proximate analysis	4
2.6 Moisture	4
2.7 Crude Protein	4
2.8 Crude Fiber	4
2.9 Ash	4
Chapter 3: Result	5-7
Chapter 4: Discussion	8-9
Chapter 5: Conclusion	10
References	11-12
Acknowledgement	13
Biography of Author	14

## **Table of Contents**

# List Of Tables:

TABLE 1: Table 1: Physical Evaluation of Silage	05
TABLE 2: Chemical Evaluation of Silage by Proximate Analysis	06
TABLE 3: Effect of lactic acid and sour yogurt on the pH of th silage	07

# **List of Figures:**

Fig 1: Chemical Component of Silage	06
Fig 2: pH value Of Silage	07

# List of abbreviations

M=Moisture

## **CP=Crude Protein**

**CF=Crude Fiber** 

LAB= Lactic Acid Bacteria

#### Abstract

Napier grass (Pennisetum purpureum) silage production is a promising method to preserve and improve the quality of this forage for ruminants. This study aimed to evaluate the effect of two additives, including lactic acid and sour yogurt, on the fermentation profile and chemical composition of Napier grass silage. Napier grass samples were collected from a local farm and chopped into small pieces before being filled into containers with the respective additives at different concentrations including T1 (5% Lactic acid,5% molasses), T2 (10% Lactic Acid and 5% molasses), and T3 (5% sour yogurt and 5% molasses). The containers were sealed and stored for 30 days at room temperature. The physical and chemical characteristics of the silage were assessed. The chemical analysis revealed moisture percentages highest In T2 (78.04%) and lowest in T1 (74.58%). Ash content is highest in T3 (3.59%) and lowest in T1 (2.53%), crude protein highest in T3 (14.5%) and lowest in T2 (12.62%), and crude fiber content highest in T3 (28.40%) and lowest in T2 (21.7%). The pH values of the silages were below 4.5, indicating good acidification and preservation. Overall, the use of 5% lactic acid and 5% sour yogurt in Napier grass silage production could be helped especially with crude protein improvement, and also helped on the fermentation profile and chemical composition of the silage.

Key Words: Napier Silage, lactic acid, pH, proximate analysis,

#### **Chapter 1: Introduction**

Napier Grass is a high-yielding forage crop that can produce up to 40 tons of dry biomass per hectare per year. It is palatable and nutritious for cattle, with crude protein content ranging from 7-18% depending on the stage of growth and fertilization (Negawo et al., 2017; Heuzé et al., 2020). Napier grass is suitable for agro-climatic conditions of BD as it can grow well in warm, humid, and rainy areas. It can also tolerate drought and waterlogging to some extent Napier grass can be planted by stem cuttings or root slips, and harvested multiple times in a year (Kabirizi et al., 2015). Napier grass silage production in Bangladesh is a potential way to preserve and enhance the quality of Napier grass as a feed resource for ruminants. Silage making is a process of fermenting green forages under anaerobic conditions to retain their nutrients and palatability. Napier grass silage can be prepared by adding different additives, such as cellulase, urea, or formic acid, to improve the fermentation, aerobic stability, and digestibility of the silage (Sarker et al., 2015; Kaewpila C et al., 2020). According to some publishers, Ideal amount of Moisture content of Napier silage should be between 65-85%, CP content should be above 10%, CF content should be below 35%, PH should be below 4.5%, Lactic Acid content should be above 6% (Shinoda et al., 1998; Jamsawat V et al., 2019). Lactic acid in Napier grass silage is an important indicator of the quality and stability of the silage. Lactic acid is produced by lactic acid bacteria (LAB) that ferment the sugars in the grass under anaerobic conditions. Lactic acid lowers the pH of the silage and inhibits the growth of spoilage microorganisms, such as molds and yeasts (Peng et al., 2021; Guan et al., 2020). The amount and proportion of lactic acid in Napier grass silage depend on several factors, such as the type and amount of LAB present on the grass, the moisture content and sugar content of the grass, the additives used to enhance the fermentation, and the storage conditions of the silage (Guan et al., 2020; Hao et al., 2022; Chen D et al., 2022). Generally, Napier grass has a high fiber and low sugar content, which makes it difficult to achieve good fermentation and high lactic acid production. Moreover, the epiphytic LAB present in Napier grass is usually low in number and diversity, resulting in poor silage fermentation and low intake and digestibility (Gulfam et al., 2016). Therefore, it is recommended to use additives, such as cellulase, urea, or pyroligneous acid, to improve the quality of Napier grass silage by increasing the sugar availability, enhancing the LAB growth, and accelerating the lactic acid production. We hypothesize that using Lactic acid, molasses, and Source Yogurt (a type of yogurt that has a tangy, acidic taste due to the fermentation of lactose in milk by lactic acid bacteria (LAB) which enhances the fermentation profile Napier silage under tropical

conditions. Therefore, this study aimed to evaluate changes in the fermentation profile pH and chemical composition of Napier silages.

# **Objectives:**

To determine the optimum additive concentration for better silage production.

To evaluate the comparative between lactic acid and sour yogurt effects on Napier grass silage production.

## **Chapter 2: Materials And Methods**

#### 2.1.Study Area:

The Study was Performed in the Department of Animal Science And Nutrition Laboratory, Faculty of Veterinary Medicine at Chattogram Veterinary and Animal Sciences University (CVASU), Khulshi, Chattogram-4202, Bangladesh From April to May 2023.

#### **2.2.** Collection of Sample:

Napier grass was collected from a Napier fodder plant that is located in Eidgaon, Cox's Bazar, Chattogram at pre flowering stage.

#### **2.3.Preparation of Silage:**

Napier grass without stem is chopped into small pieces (1 inch). Chopped grass was filled into a container layer by layer which helped to remove air from the container. After filling the container sealed as early as possible which reduces the risk of fermentation. Then it was kept for 30 days at room temperature.

#### The type of silage prepared Using Following Composition:

**Silage-1(T1):** Napier grass with 5% lab grad LacticAcid (82%) (collected from Taj Scientific, Anderkilla, Chattogram) and 5% molasses. Then, mix up the ingredients properly and fill the container.

**Silage-2(T2):** Napier grass with 10% lab grad LacticAcid (82%) (collected from Taj Scientific, Anderkilla, Chattogram) and 5% molasses collected from the local market.

Silage-3 (T3): Napier grass with 5% Sour Yogurt (Cow Milk) and 5% molasses.

#### **Physical Characteristics:**

Appearance, color, odor, fungus, and water content were tested immediately after opening the container.

## **2.4.Proximate Analysis:**

Sample randomly taken from the container approximately about 200gm that kept into a trey. The sample was dried overnight at 60°C in the incubator and grind the dried sample finally.

### 2.5.Moisture:

10gm Sample kept in an oven at 105°C and record the loss weight until constant weight found (B. G. Kim et al., 2005).

## 2.6.Crude Protein (CP):

Crude protein is determined by the Micro Kjeldhal method. This method is done in 3 steps (digestion, distillation, and titration). For digestion, the 0.5gm sample was oxidized with Sulphuric Acid in the presence of a catalyst. Then digestible solute was distilled with 2% Boric Acid. Then the solution was titrated with standard alkali (NaOH) and the Nitrogen amount was calculated. The amount of Crude Protein (CP) is obtained by multiplying the Nitrogen value by 6.25.

**2.7.Crude Fiber (CF):** About 2gm sample was hydrolyzed with 1.25% H2SO4 and 1.25% NaOH to estimate Crude Fiber by applying the methods of Maynard.

**2.8.Ash:** For Determining Ash, 5gm samples were taken that were incinerated in the Muffale Furnace at 600°C for 24 hours. The Residue was weighed and calculated as ash percentage.

## **Chapter 3: Result**

Traits	<b>T</b> <sub>1</sub>	<b>T</b> <sub>2</sub>	<b>T</b> <sub>3</sub>
Color	Light Brown	Yellowish Brown	Greenish Yellow
Odor	Sweetish	Sour	Sweetish
Fungus	No	No	No
Water	No	No	No

## **Table 1: Physical Evaluation of Silage.**

The physical examination of the silage showed that Light brown color in T1, a Yellowish brown color in T2, and a Greenish yellow color in T3. In the case of odor sweetish smell is found in T1 and T3, and a sour odor is found in T2. There was no fungus and water among T1, T2, and T3.

Traits	T1	T2	Т3
Moisture	74.58%	78.04%	75.68%
Ash	2.53%	2.97%	3.59%
Crude Protein	14%	12.62%	14.5%
Crude Fiber	22.89%	21.7%	28.40%

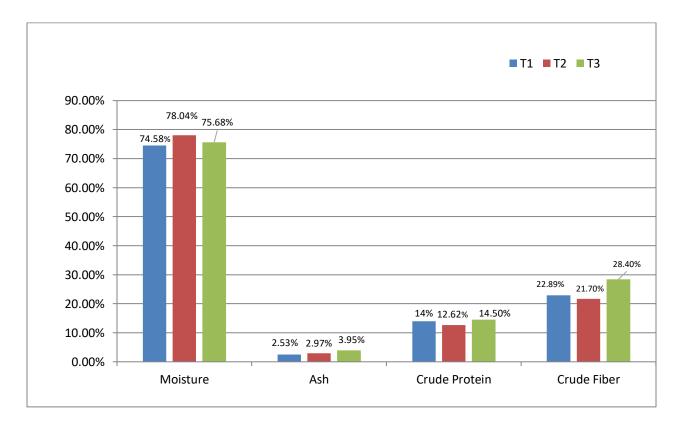


Fig 1: Chemical Component of Silage

## Table 3: Effect of lactic acid and sour yogurt on the pH of the silage.

Traits	T1	T2	Т3
p <sup>H</sup>	1.91	1.65	2.43

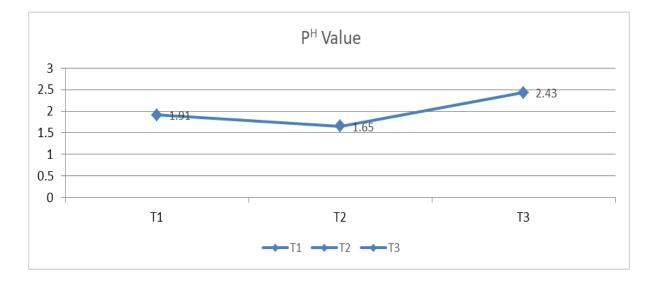


Fig 2: pH value of silage

#### **Chapter 4: Discussion**

#### **Physical Quality Characteristics of Silage:**

Silage color is the most important indicator for determining the silage quality (Jaelani et al.,2014). The color of the silage should be light green or similar to the original grass color. Here we found Light brown color in T1, Yellowish brown in T2, and Greenish yellow in T3. Greenish yellow in T3 is closely related to the normal Napier silage color. The color is different from the original color due to lower quality silage.

The odor of the silage should be sour or acidic, indicating a good lactic acid fermentation (Wati et al., 2015). Here we find in the experiment a sweetish odor in T1 and T3 and, a sour odor in T2 that indicates the good quality of silage. In the study, there is no fungus produced in the silage due to the air-tight condition of the container and the effect of the PH from the silage.

#### **Chemical Quality Characteristics of Silage:**

The Moisture content of the silage should be between 65% to 85%, indicating a suitable water content for fermentation and preservation (Jamsawat et al., 2019). In the Study the moisture percentage of the silage is 74.58% in T1,78.04% in T2,75.68% in T3 these indicate the good moisture percentage in the Napier silage (table 3). According to one study ( Jamsawat et al., 2019), the ash percentage in super Napier grass silage was 6.77% on a dry matter basis. The study found ash percentages are 2.53%, 2.97%, and 3.59% respectively in T1, T2, and T3 silage. These ash percentages are below the normal value. However, the ash content of forages can vary depending on the soil contamination, harvesting methods, and storage conditions (Kidder, 1945). The CP content of the silage should be above 10%, indicating a high protein value and low fiber content (Jamsawat et al., 2019). In this study, the found CP are 14%,12.62%, and 14.5% respectively in T1, T2, and T3. Among them, T3 contains the highest protein. Immature grass tends to have higher protein (Duane E,1997). The CF content of the silage should be below 35%, indicating a low fiber content and high digestibility. In this study, the founded CF are 22.89%, 21.7%, and 28.40% respectively T1, T2, and T3. Low fiber content increases high digestibility (Jamsawat et al., 2019). The pH of the silage should be below 4.5, indicating good acidification and preservation. In this study, the founded PH values are 1.91, 1.65, 2.43 respectively in T1, T2, and T3. Those indicate the

good quality of silage. In our present experiment, T3 was the best quality of silage because of yogurt. Yogurt is a good source of lactic acid-producing bacteria which lowers the pH and makes a sweetish odor of the silage. In comparison to T1 and T2, T1 is closely related to normal characteristics and composition. The overall findings of the study are that T3 is best and T1 is better than T2.

## **Chapter 5: Conclusion**

Overall, this study provides valuable insights into the production of high-quality Napier grass silage, highlighting the importance of proper fermentation techniques and the strategic use of additives for optimizing silage preservation and livestock feeding practices.

In this study comparing the effects of 5% lactic acid and sour yogurt on Napier silage, it's evident that both treatments led to improved results. However, when evaluating the choice between 5% lactic acid and sour yogurt, several factors should be considered. While both treatments show promise, preference could depend on practical considerations such as cost, availability, and ease of application. Additionally, analyzing the specific nutritional and microbial changes brought about by each treatment could provide insights into their long-term impact on silage quality. Further research could delve into optimizing lactic acid and sour yogurt dosages to achieve the best results.

#### References

- Ahn, J. Y., Kil, D. Y., Kong, C., & Kim, B. G. (2014). Comparison of Oven-drying Methods for Determination of Moisture Content in Feed Ingredients. Asian-Australasian Journal of Animal Sciences, 27(11), 1615-1622. https://doi.org/10.5713/ajas.2014.14305
- Chen, D., Zheng, M., Zhou, Y., Gao, L., Zhou, W., Wang, M., ... & Xu, W. (2022). Improving the quality of napier grass silage with pyroligneous acid: Fermentation, aerobic stability, and microbial communities. Frontiers in Microbiology, 13, 1034198.
- Guan, H., Li, H., Gan, L., Chen, S., Yan, Y., Jia, Z., ... & Zhou, Q. (2023). The effects of native lactic acid bacteria on the microbiome, fermentation profile, and nutritive value of Napier grass silage prepared with different legume ratios. Frontiers in Microbiology, 13, 1112058.
- Gulfam, A., Guo, G., Tajebe, S., Chen, L., Liu, Q., Yuan, X., ... & Saho, T. (2017). Characteristics of lactic acid bacteria isolates and their effect on the fermentation quality of Napier grass silage at three high temperatures. Journal of the Science of Food and Agriculture, 97(6), 1931-1938.
- Hao Guan, Yang Shuai, Qifan Ran, Yanhong Yan, Xia Wang, Dandan Li, Yimin Cai, Xinquan Zhang, The microbiome and metabolome of Napier grass silages prepared with screened lactic acid bacteria during ensiling and aerobic exposure, Animal Feed Science and Technology
- Heuzé V., Tran G., Giger-Reverdin S., Lebas F., 2020. Elephant grass (Pennisetum purpureum). Feedipedia, a program by INRAE, CIRAD, AFZ, and FAO. https://www.feedipedia.org/node/395 Last updated on October 5, 2020, 10:34
- Jaelani A, Djaya S and Rostini T 2014 Int. J. Biosci 5 144 150
- Jamsawat V, et al. Study on Chemical Composition of Super Napier Grass Silage Treated with Lactobacillus Buchneri and Lactobacillus Plantarum.Mycol Mycological Sci 2019, 2(2): 000109.
- Kabirizi, J.; Muyekho, F.; Mulaa, M; Msangi, R.; Pallangyo, B.; Kawube, G.; Zziwa, E.; Mugerwa, S.; Ajanga,S.; Lukwago, G.; Wamalwa N.I. E; Kariuki, I.; Mwesigwa, R.;

Nannyeenya-Ntege, W.; Atuhairwe, A.; Awalla, J.; Namazzi, C.; Nampijja, Z. 2015. Napier grass feed resource: production, constraints, and implications for smallholder farmers in Eastern and Central Africa. ISBN: 978-9970-9269-1-6

- Kaewpila C, Khota W, Gunun P, Kesorn P, Cherdthong A. Strategic Addition of Different Additives to Improve Silage Fermentation, Aerobic Stability and In Vitro Digestibility of Napier Grasses at Late Maturity Stage. Agriculture. 2020; 10(7):262.
- Kidder, R. W. (1945). COMPOSITION AND DIGESTIBLE NUTRIENT CONTENT. Journal of Agricultural Research, 70, 89.
- Negawo AT, Teshome A, Kumar A, Hanson J, Jones CS. Opportunities for Napier Grass (Pennisetum purpureum) Improvement Using Molecular Genetics. Agronomy. 2017; 7(2):28.
- Peng, C., Sun, W., Dong, X. et al. Isolation, identification, and utilization of lactic acid bacteria from silage in a warm and humid climate area. Sci Rep 11, 12586 (2021).
- Poster 8.3: Quality and nutritive value of Napier grass silage at different growth stages and chopped or unchopped in northeast Thailand Mitsuru Shinoda, Tomoyuki Kawashima, Pimpaporn Pholsen and Taweesak Chuenpreecha.
- Sarker, Nathu & Yeasmin, Dilruba & Tabassum, Farah & Amin, Md & Habib, Md. (2019). Comparative Study on Biomass Yield, Morphology, Silage Quality of Hybrid Napier and Pakchong and Their Utilization in Bull Calves. Journal of Agricultural Science and Technology. 9. 166-176. 10.17265/2161-6256/2019.03.004.
- Wati W, Mashudi S dan Irsyammawati A 2018 J. Nutr. Ternak Trop. 1 45–53

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## **Biography of Author**

This is Imran Khan Emo, the first child of Md. Abdul Mazid Khan and Saba Kunnahr, doing his graduation with a Doctor of Veterinary Medicine (DVM) at Chattogram Veterinary and Animal Sciences University under the Faculty of Veterinary Medicine. He passed the Secondary School Certificate Examination (SSC) in 2014 from Eidgah Model High School, Cox's Bazar and got a GPA of 5.00, and then the Higher Secondary Certificate Examination (HSC) in 2016 from Govt. Haji Mohammad Mohsin College, Chattogram and got a GPA of 5.00 out of 5.00. Currently, he is doing his yearlong internship. He has great enthusiasm in his study area to develop day-one skills and gain more practical knowledge to be prepared for the modern era of science.