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Lactobacillus plantarum - inoculated Napier grass silage on nutrient digestibility and rumen bacterial populations in sheep

Mark Anthony Tito MAÑA *[1], Richelle Arriesgado NIEPES [1], Julius Villar ABELA [2]

[1] College of Environment and Life Sciences, Mindanao State University at Naawan, Naawan, Misamis Oriental, Philippines.
[2] Visayas State University, Baybay City, Leyte, Philippiens.
*E-Smail: markanthony.mana@msunaawan.edu.ph

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ABSTRACT: This study aimed to address the optimization of fermentation and digestibility of Napier grass (*Pennisetum purpureum* Sch.) silage in sheep by evaluating the effects of varying levels (0%, 1%, 2%, 3%, and 4%) of *Lactobacillus plantarum* as a silage inoculant. A Completely Randomized Design (CRD) with six replications was employed. Sheep were housed in individual metabolic cages, with feed intake, water consumption, and waste output monitored. Rumen fluid and fecal samples were collected for microbial and proximate analysis. Results showed that dry matter digestibility (DMD) did not significantly differ among treatments (p = 0.451), although the 3% *L. plantarum* group (T3) exhibited the highest DMD (43.83%). Organic matter digestibility (OMD) was significantly affected (p = 0.004), with T3 recording the highest OMD (84.14%). Crude protein digestibility (CPD) showed highly significant differences (p < 0.000), with T3 and T1 achieving the highest CPD values (87.94% and 86.37%, respectively). Neutral detergent fiber digestibility (NDFD) also varied significantly (p = 0.003), with T3 again showing the highest NDFD (54.63%). In terms of rumen fermentation, *L. plantarum* levels significantly influenced rumen pH (p = 0.027). The initial rumen pH values were similar across groups; however, the final pH values decreased with increasing inoculant levels. The greatest reduction in pH was observed in the T4 (4%) group (9.26% decrease), followed closely by T3 (8.40%). These pH shifts corresponded to changes in rumen bacterial populations. Although initial bacterial counts were statistically similar (p = 0.462), significant reductions in final counts were observed (p = 0.001), with T3 showing the lowest population (2.36×10^7 cfu/mL), indicating a potential for improved microbial modulation at this level. The study concludes that moderate *supplementation with Lactobacillus plantarum*, particularly at 3%, optimally enhances silage digestibility, maintains beneficial rumen pH levels, and improves microbial balance. Excessive inclusion (4%) may lead to lower fermentation efficiency due to acidic rumen conditions.

Keywords: *Pennisetum purpureum* Sch.; fermentation; neutral detergent fiber; probiotics; ruminant nutrition; *Ovis aries* L.

Silagem de capim Napier inoculada com *Lactobacillus plantarum* sobre a digestibilidade de nutrientes e populações bacterianas no rúmen de ovinos

RESUMO: Este estudo teve como objetivo abordar a otimização da fermentação e digestibilidade da silagem de capim-Napier (*Pennisetum purpureum* Sch.) em ovinos, avaliando os efeitos de níveis variados (0%, 1%, 2%, 3% e 4%) de *Lactobacillus plantarum* como inoculante de silagem. Foi empregado um delineamento inteiramente casualizado (DIC) com seis repetições. Os ovinos foram alojados em gaiolas metabólicas individuais, com consumo de ração, consumo de água e produção de resíduos monitorados. Amostras de fluido ruminal e fezes foram coletadas para análise microbiana e centesimal. Os resultados mostraram que a digestibilidade da matéria seca (DMS) não diferiu significativamente entre os tratamentos (p = 0,451), embora o grupo com 3% de L. plantarum (T3) tenha apresentado a maior DMS (43,83%). A digestibilidade da matéria orgânica (DMO) foi significativamente afetada (p = 0,004), com T3 registrando a maior DMO (84,14%). A digestibilidade da proteína bruta (DPC) apresentou diferenças altamente significativas (p < 0,000), com T3 e T1 atingindo os maiores valores de DPC (87,94% e 86,37%, respectivamente). A digestibilidade da fibra em detergente neutro (DFDN) também variou significativamente (p = 0,003), com T3 novamente apresentando a maior DFDN (54,63%). Em termos de fermentação ruminal, os níveis de *L. plantarum* influenciaram significativamente o pH ruminal (p = 0,027). Os valores iniciais de pH ruminal foram semelhantes entre os grupos, mas os valores finais de pH diminuíram com o aumento dos níveis de inoculante. A maior redução no pH foi observada no grupo T4 (4%) (redução de 9,26%), seguida de perto por T3 (8,40%). Essas mudanças de pH corresponderam a mudanças nas populações bacterianas do rúmen. Embora as contagens bacterianas iniciais tenham sido estatisticamente semelhantes (p = 0,462), foram observadas reduções significativas nas contagens finais (p = 0,001), com o T3 apresentando a menor população ($2,36 \times 10^7$ UFC/mL), indicando um potencial para melhor modulação microbiana nesse nível. O estudo conclui que a suplementação moderada com *Lactobacillus*

plantarum, particularmente a 3%, melhora a digestibilidade da silagem, mantém níveis benéficos de pH ruminal e melhora o equilíbrio microbiano. A inclusão excessiva (4%) pode levar à menor eficiência da fermentação devido às condições ácidas do rúmen.
Palavras-chave: *Pennisetum purpureum* Sch.; fermentação; fibra em detergente neutro; probióticos; nutrição de ruminantes; *Ovis aries* L.

1. INTRODUCTION

Silage production is a strategy for preserving forage, particularly during periods when fresh grass is unavailable or when maintaining consistent feed quality throughout the year is essential. Among tropical forages, Napier grass (*Pennisetum purpureum*) stands out due to its high biomass yield, palatability, and adaptability to a wide range of agroecological conditions (MORAN, 2005; MALEKO et al., 2019). Its widespread use in tropical regions, such as the Philippines, underscores its importance in supporting ruminant productivity. However, seasonal fluctuations in forage availability, quality losses during storage, and poor fermentation processes often compromise the utility of Napier grass silage.

Ensiling is an anaerobic fermentation process driven primarily by lactic acid bacteria (LAB), which convert water-soluble carbohydrates into organic acids, mainly lactic acid, thereby lowering pH and inhibiting undesirable microbial activity (WEBSTER, 1992). The use of microbial inoculants such as *Lactobacillus plantarum* has been widely recommended to enhance the fermentation quality of silage (REN et al., 2020; LIU et al., 2024). *L. plantarum* is a homofermentative LAB known for rapidly lowering silage pH, improving aerobic stability, and enhancing preservation through effective suppression of spoilage organisms (MUCK et al., 2019). Moreover, its inoculation has been reported to improve the nutritive value of silage and increase its digestibility by enhancing fiber degradation and maintaining protein integrity.

While several studies have documented the benefits of *L. plantarum* in improving fermentation profiles, aerobic stability, and overall silage quality, fewer have explored its effects on nutrient digestibility and rumen microbial dynamics in small ruminants such as sheep, especially in tropical conditions (PENG et al., 2024; JIN et al., 2024). Understanding these effects is particularly relevant in tropical forage systems, where nutritional limitations often constrain ruminant productivity. Additionally, the relationship between microbial inoculation and rumen microbial ecology remains underexplored.

A recent study by Maña et al. (2024) investigated the impact of varying *L. plantarum* levels (0% to 4%) on feed intake and growth performance in sheep fed Napier grass silage. While feed intake did not differ significantly among treatments, the 2% *L. plantarum* group demonstrated the most improvements in growth rate and feed conversion efficiency, suggesting a potential threshold for effective inoculation. The primary objective of this study is to evaluate the effect of *Lactobacillus plantarum*-inoculated Napier grass silage on nutrient digestibility in sheep (*Ovis arie*s L.). Secondary objectives include assessing the impact of *L. plantarum* inoculation on rumen bacterial populations and diversity, and determining how shifts in microbial communities correlate with nutrient digestibility.

2. MATERIALS AND METHODS

2.1. Experimental Animals and Design

All methods adhered to the Animal Welfare Act of the Philippines (Republic Act No. 8485, as amended by RA 10631) and were conducted under the supervision of a licensed veterinarian. A total of thirty female Dorper sheep, weighing between 15 and 19 kg, were used in the study. The sheep were housed in separate cages to prevent cross-contamination among animals and to avoid re-infection of pastures or soil after treatment. Before housing the animals, the cages were thoroughly cleaned and disinfected. The sheep were dewormed before the commencement of the study. Using a Completely Randomized Design (CRD), the 30 growing female sheep were randomly assigned to five treatments, with six replications, each containing one animal. The sheep were individually housed in metabolic cages, which were cleaned three days before the animals' arrival. Each treatment consisted of a base diet of Napier silage supplemented with varying concentrations of *Lactobacillus plantarum*, administered twice daily. Fresh potable water was continuously available to the sheep throughout the experiment.

2.2. Viability Testing of *Lactobacillus plantarum* Inoculant

Before use, the *Lactobacillus plantarum* inoculant was tested for microbial presence. Nutrient agar (NA) was used to culture microorganisms, while potato dextrose agar (PDA) was employed to culture molds. Dilution plating was performed to estimate the probiotic concentration. A total of 9 cc of sterilized water was added to a test tube to dilute a 1 ml sample to 10^7. Dilutions were then pipetted into sterile Petri dishes, starting with 1 ml from each dilution tube. A 10-12 ml medium, cooled to approximately 45°C, was poured into each plate and gently mixed. Six replicate plates were prepared for each dilution. The plates were incubated at room temperature in the laboratory, and colony counting was performed after 3-4 days for bacterial growth. The cultural characteristics of the colonies were observed, and a microscopic examination was conducted to study the morphology of the microorganisms. Gram staining was performed to identify the isolates.

2.3. Experimental Treatments

The *Lactobacillus plantarum* (*L. plantarum*) was used as a fermenting agent for Napier silage at various levels, with the following treatments:

Treatment 0: Napier Silage + 0% *L. plantarum* (Control)
Treatment 1: Napier Silage + 1% *L. plantarum*
Treatment 2: Napier Silage + 2% *L. plantarum*
Treatment 3: Napier Silage + 3% *L. plantarum*
Treatment 4: Napier Silage + 4% *L. plantarum*

Each treatment was designed to assess the effect of different concentrations of *L. plantarum* on the fermentation and microbial activity in Napier silage.

2.4. Preparation of Test Animals

The experimental animals were individually housed to prevent direct contact with pastures, soil, or other animals, thereby reducing the risk of re-infection after treatment. Before housing the sheep, the cages were thoroughly cleaned and disinfected to maintain a controlled environment (Van Soest, 1994). The sheep, with a body weight ranging from 15 to 19 kg, were dewormed to ensure they were free from intestinal parasites before the commencement of the study (Chartier and Paraud, 2012). Each sheep was assigned to a treatment group and fed a ration consisting of a basal diet of Napier silage supplemented with varying levels of *Lactobacillus plantarum* (*L. plantarum*) twice daily. Fresh drinking water was made available ad libitum throughout the experimental period to ensure proper hydration and support overall health (NRC, 2007).

2.5. Metabolic cages

The sheep were individually housed in metabolic cages, which were specifically designed to facilitate the accurate collection of data on feed intake, water consumption, and waste output (urine and feces). The cages were constructed from durable, non-corrosive materials and equipped with separate compartments for feeding and waste collection, preventing contamination between different samples. Each cage had recommended dimensions of approximately 1.2 meters (length) x 0.6 meters (width) x 0.8 meters (height), providing adequate space for the sheep to move comfortably while maintaining a controlled environment for data collection. The floor of each cage was slatted to allow for efficient drainage of urine, ensuring the animals remained dry and comfortable.

Each cage was equipped with a removable tray beneath the slatted floor to collect fecal matter, and the urine was directed into a separate container, enabling the precise measurement of excretory output. The cages were equipped with individual feeding and watering systems to ensure controlled administration of the experimental diet and water. Water consumption was also measured daily by recording the volume of water provided and the volume left after each day. The metabolic cages were regularly cleaned and disinfected to maintain a hygienic environment throughout the study. The sheep remained in the metabolic cages for the entire experimental period to minimize environmental variability and ensure consistent data collection.

2.6. Rumen Fluid Collection

Rumen fluid samples were collected to evaluate the effects of various treatments on rumen bacterial populations in sheep. Fluid collection occurred at two time points during the study: the initial stage, before the start of the feeding trial, and on the 60th day after the feeding trial.

To maintain animal welfare during sampling, a non-invasive method using oral stomach tubing was employed. A flexible rubber tube connected to a suction device was gently inserted through the mouth into the rumen to collect the fluid. Sheep were calmly and securely restrained to minimize stress, and the procedure was performed swiftly by trained personnel to reduce handling time. All procedures were conducted under the supervision of a licensed veterinarian. Animals were observed before, during, and after sampling for signs of discomfort, and care was taken to ensure that they remained in good health throughout the study.

2.6.1. Initial Collection (Pre-feeding Trial)

On the first day of the experiment, before initiating the treatments, approximately 100 mL of rumen fluid was collected from each sheep to establish baseline measurements of rumen bacterial populations. Fluid was collected using an oral tubing method, where a sterile, flexible tube was inserted into the sheep's mouth, passed through the esophagus, and into the rumen. Once positioned in the rumen, the fluid was gently aspirated into sterile containers. The procedure was performed with care to minimize contamination and stress on the animals. The collected rumen fluid was immediately transported to the laboratory and analyzed for bacterial populations.

2.6.2. Final Collection (Post-feeding Trial, 60th Day)

After the 60-day feeding trial, approximately 100 mL of rumen fluid was again collected from each sheep using the same oral tubing method as the initial collection. This allowed for a comparison with the baseline data to assess the effects of *Lactobacillus plantarum* supplementation on rumen bacterial populations throughout the study. The fluid was immediately stored in sterile containers and analyzed for bacterial populations.

Rumen fluid collection was conducted in the morning, before the animals were fed, to minimize variations due to feed ingestion. To minimize stress, the animals were handled gently, and the procedure was performed by trained personnel under the supervision of a veterinarian. The collected rumen fluid was immediately transported to the laboratory for analysis, where the bacterial populations were determined using culture-based methods.

2.7. Rumen pH measurement

Rumen pH was measured immediately after fluid collection to prevent post-sampling fermentation from affecting pH values. A portable digital pH meter (Model: Hanna Instruments HI 99163) equipped with a glass pH electrode was used for direct measurement, following the method described by Mould et al. (1983).

For each sampling point (Day 0 and Day 60), a 10 mL aliquot of freshly collected rumen fluid was transferred into a sterile container. The pH probe was inserted directly into the sample, and readings were recorded within 10 minutes of collection at ambient temperature. To ensure accuracy, the probe was rinsed with distilled water between samples, and each reading was performed in triplicate.

2.8. Fecal collection

During the experimental period, fecal samples were collected from each sheep housed in metabolic cages. The cages were specifically designed to facilitate the. Fecal samples were collected directly from the trays beneath the cages at regular intervals during the experimental period. The collected feces were transferred to sterile containers to prevent contamination and immediately transported to the laboratory for analysis.

2.9. Proximate analyses.

The proximate composition of the feed (Napier silage) and fecal samples was analyzed for key components, including dry matter (DM), organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF). The samples were sent to the Department of Animal Science, College of Agriculture and Food Science, Visca, Baybay City, Leyte, where the analysis was performed using the methods outlined by the AOAC (1990).

2.10. Data gathered

All samples, including the treatments, are first subjected to an oven at 100°C for 24 hours to obtain their dry matter value, which can be used for the following computations. Dry matter digestibility of the basal diet, which is the Napier and different levels of probiotics silage, was determined using the following formula:

$$DMD\% = \frac{\text{Dry Matter intake} - \text{Dr matter excreted}}{\text{Dry matter Intake}} \times 100 \quad (01)$$

where: DM excreted = fecal output, kg x % DM of feces.

The same formula was applied to determine the digestibility of other nutrients, with slight modifications to reflect the specific nutrient being assessed (e.g., Crude Protein (CP), Organic Matter (OM), Neutral Detergent Fiber (NDF)). The nutrient composition of the basal diet, which included Napier grass and silage with varying levels of probiotics, was evaluated for the following: %DM (Dry Matter), %CP (Crude Protein), %OM (Organic Matter), and %NDF (Neutral Detergent Fiber).

For each nutrient, the corresponding digestibility was calculated using the same approach as outlined in equation (1), substituting the appropriate nutrient values in place of dry matter.

2.11. Rumen bacterial count

To assess rumen bacterial count, rumen fluid samples were collected from experimental animals using a stomach tube or a rumen cannula, depending on the experimental design and ethical considerations (HUNGATE, 1966; ØRSKOV & MCDONALD, 1979). The collected rumen fluid was filtered through four layers of cheesecloth to remove large particulate matter, and the filtrate was then used for microbial enumeration. Serial dilutions of the filtrate were prepared using sterile phosphate-buffered saline (PBS), followed by inoculation onto suitable growth media, such as roll tubes containing anaerobic media (BRYANT; BURKEY, 1953). The cultures were incubated anaerobically at 39°C for 48–72 hours. After incubation, bacterial colonies were counted, and the results were expressed as colony-forming units per milliliter (CFU/mL) of rumen fluid. In some cases, total bacterial counts were further confirmed using direct microscopic counts with a hemocytometer after staining with a fluorescent dye such as DAPI (4',6-diamidino-2-phenylindole), as described by Hobson; Stewart (1997). This combination of culturing and microscopic techniques provided a comprehensive evaluation of the bacterial population in the rumen.

2.12. Statistical analysis

The collected data were analyzed using analysis of variance (ANOVA) through the General Linear Model (GLM) procedures of the Statistical Package for Social Sciences (SPSS) Version 25. Treatment means were compared using Tukey's Honestly Significant Difference (HSD) Test.

3. RESULTS

The nutrient digestibility of sheep fed Napier grass silage treated with varying levels of Lactobacillus plantarum was evaluated to determine the effect of microbial inoculation on feed utilization. Parameters assessed included dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and neutral detergent fiber digestibility (NDFD). The results are summarized in Table 1.

Table 1. Effects of *Lactobacillus plantarum* - inoculated Napier grass (*Pennisetum purpureum* Sch.) silage on nutrient digestibility in sheep (*Ovis aries* L.).
Tabela 1. Efeitos da silagem de capim-elefante (*Pennisetum purpureum* Sch.) inoculada com *Lactobacillus plantarum* na digestibilidade de nutrientes em ovinos (*Ovis aries* L.).

Treatmen	DMD, %	OMD,%	CPD, %	NDFD, %
T0	41.76	70.21^{c}	65.10^{c}	38.35^{c}
T1	41.74	78.87^{ab}	86.37^{a}	51.35^{ab}
T2	38.26	73.75^{bc}	77.48^{b}	46.81^{b}
T3	43.83	84.14^{a}	87.94^{a}	54.63^{a}
T4	34.72	71.72^{bc}	73.36^{b}	48.03^{ab}
p-value	0.451	0.004	<0.000**	0.003**

*Means with different superscripts within a column differ ($p < 0.05$). ns = not significant; *$p < 0.05$; **$p < 0.01$; T0–T4: Napier silage + 0, 1, 2, 3, 4%. *Lactobacillus plantarum.*
*Médias com sobrescritos diferentes dentro de uma coluna diferem ($p < 0,05$). ns = não significativo; *$p < 0,05$; **$p < 0,01$; T0–T4: silagem Napier + 0, 1, 2, 3, 4%. *Lactobacillus plantarum.*

3.1. Dry matter digestibility

The dry matter digestibility (DMD) of Napier silage-based diets with varying levels of *Lactobacillus plantarum* did not show statistically significant differences among treatments (p = 0.451). However, a closer examination of the mean values reveals that the treatment with 3% *L. plantarum (*T3) achieved the highest DMD at 43.83%, followed closely by the control group (T0, 0% *L. plantarum*) at 41.76% and the 1% level (T1) at 41.74%.

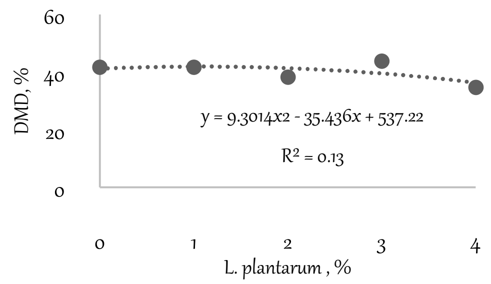

Figure 1. Regression curve showing dry matter digestibility (DMD) of Napier silage-based diets supplemented with varying levels of *Lactobacillus plantarum*. Values are fitted with a second-degree polynomial regression ($R^2 = 0.13$).
Figura 1. Curva de regressão mostrando a digestibilidade da matéria seca (DMS) de dietas à base de silagem Napier suplementadas com níveis variados de *Lactobacillus plantarum*. Os valores são ajustados por uma regressão polinomial de segundo grau ($R^2 = 0,13$).

Despite the lack of statistical significance, this numerical trend suggests a potential improvement in dry matter digestibility at the 3% inoculant level. This may indicate that a moderate level of inoculation can enhance fermentation quality and microbial activity, thereby improving the breakdown and utilization of fibrous components in the diet The lowest DMD value was observed in T4 (4% *L. plantarum*) at 34.72%, which could imply that excessive inoculant levels may not favor further improvements and might even negatively affect silage quality or palatability, potentially due to over-fermentation or production of undesirable metabolites. Interestingly, the DMD of T2 (2%) was also lower than that of the control, which reinforces the observation that the relationship between *L. plantarum* level and DMD is not linear (Figure 1).

3.2. Organic matter digestibility

Organic matter digestibility (OMD) was significantly influenced by the application of different levels of *Lactobacillus plantarum* as a silage inoculant in Napier silage-based diets for sheep, with a p-value of 0.004 indicating a statistically significant difference among treatments (Figure 2). The highest OMD value was observed in T3, where 3% *L. plantarum* was applied, yielding a value of 84.14%. T1, which contained 1% *L. plantarum*, also showed a significantly high OMD value at 78.87%, statistically comparable to T3. This implies that even at a lower inoculant level, *L. plantarum* could stimulate favorable microbial activity and promote efficient breakdown of organic matter in the rumen.

In contrast, the control group (T0), which did not receive any inoculant, had the lowest OMD at 70.21%, indicating poor fermentation quality and possibly a higher presence of indigestible compounds or spoilage. Meanwhile, treatments with 2% and 4% *L. plantarum* (T2 and T4) showed intermediate OMD values of 73.75% and 71.72%, respectively, which were not significantly different from the control.

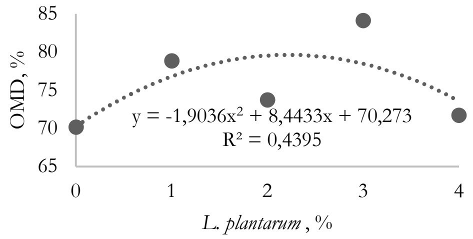

Figure 2. Regression curve showing organic matter digestibility (OMD) of Napier silage-based diets supplemented with varying levels of *Lactobacillus plantarum*. Values are fitted with a second-degree polynomial regression ($R^2 = 0.4395$).
Figura 2. Curva de regressão mostrando a digestibilidade da matéria orgânica (DMO) de dietas à base de silagem Napier suplementadas com níveis variados de *Lactobacillus plantarum*. Os valores são ajustados por uma regressão polinomial de segundo grau ($R^2 = 0,4395$).

3.3. Crude protein digestibility

Crude protein digestibility (CPD) was significantly influenced by the inclusion of *Lactobacillus plantarum* as a silage inoculant in Napier silage-based diets for sheep, with a highly significant p-value of less than 0.000 ($p < 0.000$), indicating strong treatment effects (Figure 3). Among the different treatment levels, the highest CPD was observed in T3, where 3% *L. plantarum* was applied, with a value of 87.94%. This was closely followed by T1 (1% inoculant) at 86.37%. Both T3 and T1 were statistically similar and significantly higher than the control group (T0), which recorded the lowest CPD at 65.10%.

This suggests that *L. plantarum* inoculation, especially at 1% and 3% levels, substantially improved the digestibility of protein in Napier silage. In contrast, treatments T2 (2%) and T4 (4%) recorded CPD values of 77.48% and 73.36%, respectively. Although higher than the control, these values were significantly lower than those observed in T1 and T3 (Figure 3). This indicates that while inoculant use improved CPD to some extent, excessively high or suboptimal levels of *L. plantarum* may not provide the same degree of benefit, potentially due to changes in microbial balance or suboptimal fermentation profiles at those levels.

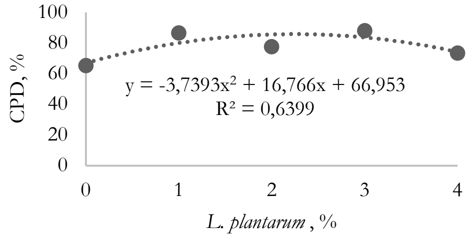

Figure 3. Regression curve showing crude protein digestibility (CPD) of Napier silage-based diets supplemented with varying levels of *Lactobacillus plantarum*. Values are fitted with a second-degree polynomial regression ($R^2 = 0.6399$).
Figura 3. Curva de regressão mostrando a digestibilidade da proteína bruta (DBP) de dietas à base de silagem Napier suplementadas com níveis variados de *Lactobacillus plantarum*. Os valores são ajustados por uma regressão polinomial de segundo grau ($R^2 = 0,6399$).

3.4. Neutral detergent fiber digestibility

Figure 4 illustrates the effect of varying levels of *Lactobacillus plantarum* on the neutral detergent fiber digestibility (NDFD) in Napier silage-based diets. The relationship is modeled with a second-degree polynomial regression ($R^2 = 0.6867$), showing a statistically significant trend ($p = 0.003$). Neutral detergent fiber digestibility (NDFD) was significantly affected by the addition of *Lactobacillus plantarum* in Napier silage-based diets, as shown by the highly significant p-value of 0.003 ($p < 0.01$). The highest NDFD was observed in T3 (3% *L. plantarum*) at 54.63%, indicating a marked improvement in the digestibility of fibrous components compared to the other treatments. This was followed by T1 (1%) at 51.35%, which was statistically similar to T3.

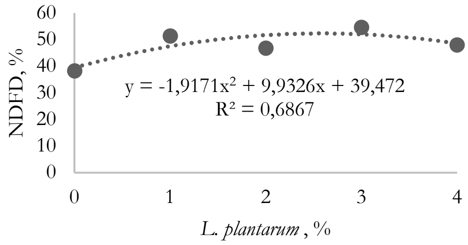

Figure 4. Regression curve showing neutral detergent fiber digestibility (NDFD) of Napier silage-based diets supplemented with varying levels of *Lactobacillus plantarum*. Values are fitted with a second-degree polynomial regression ($R^2 = 0.6867$).
Figura 4. Curva de regressão mostrando a digestibilidade da fibra em detergente neutro (FDDN) de dietas à base de silagem Napier suplementadas com níveis variados de *Lactobacillus plantarum*. Os valores são ajustados por uma regressão polinomial de segundo grau ($R^2 = 0,6867$).

The control group (T0, no inoculant) exhibited the lowest NDFD at 38.35%, which was significantly lower than that of T1 and T3. This indicates that in the absence of an inoculant, fiber digestibility is limited, possibly due to less favorable fermentation conditions, which may result in the preservation of more lignified, less digestible fiber fractions. Treatment T2 (2% *L. plantarum*) had a moderate NDFD value of 46.81%, while T4 (4%) recorded 48.03%. Both values were numerically higher than the control but statistically comparable to T1 and T3, reflecting some improvement, albeit not as consistently high.

3.5. Rumen pH and rumen bacterial population

Table 2 shows the initial and final rumen pH values, as well as the percentage reduction in pH of sheep fed Napier grass silage treated with varying levels of *Lactobacillus plantarum*. Initial rumen pH values were statistically similar across all treatments (p = 0.872), indicating a uniform baseline before feeding. However, significant differences were observed in the final rumen pH values (p = 0.027), with the lowest pH recorded in T4 (6.18) and T3 (6.21), which correspond to 4% and 3% *L. plantarum* inclusion, respectively. These reductions suggest increased fermentation activity associated with higher inoculant levels. Furthermore, the percentage reduction in rumen pH was significantly affected by treatment (p = 0.015), with T4 and T3 showing the greatest declines (9.26% and 8.40%, respectively), while the control (T0) and T1 exhibited the lowest reductions.

Table 2. Initial and Final Rumen pH and Percent Reduction in Sheep Fed Napier Grass (*Pennisetum purpureum* Sch.) Silage Inoculated with Varying Levels of *Lactobacillus plantarum*.
Tabela 2. pH inicial e final do rúmen e redução percentual em silagem de capim-elefante (*Pennisetum purpureum* Sch.) alimentada com ovelhas inoculadas com níveis variáveis de *Lactobacillus plantarum*.

Treatment	Initial pH	Final pH	% Reduction
T0	6.82	6.52[a]	4.40[c]
T1	6.79	6.48[ab]	4.57[c]
T2	6.80	6.35[b]	6.62[b]
T3	6.78	6.21[c]	8.40[a]
T4	6.81	6.18[c]	9.26[a]
p-value	0.872[ns]	0.027*	0.015*

*Means with different superscripts within a column differ (p < 0.05). ns = not significant; *p < 0.05; **p < 0.01. T0–T4: Napier silage + 0, 1, 2, 3, 4 % *Lactobacillus plantarum*.
*Médias com sobrescritos diferentes dentro de uma coluna diferem (p < 0,05). ns = não significativo; *p < 0,05; **p < 0,01. T0–T4: Silagem Napier + 0, 1, 2, 3, 4% *Lactobacillus plantarum*.

The data presented in Table 3 shows the effects of varying levels *of Lactobacillus plantarum* on the rumen bacterial populations in sheep fed Napier silage. The initial bacterial counts, measured at 1x10^7 cfu/mL, showed some variation across the treatments, but no statistically significant differences were found (p-value = 0.462). This suggests that at the beginning of the experiment, the bacterial populations in the rumen of sheep were similar, regardless of the treatment. The range of initial counts between 7.28 and 7.86 x 10^7 cfu/mL indicates that the sheep were in a relatively uniform microbial state before the introduction of *Lactobacillus plantarum*.

The final bacterial counts, measured after administering Napier silage treated with different levels of *Lactobacillus plantarum*, revealed significant variations (p-value = 0.001). These results indicate that the presence of *Lactobacillus plantarum* had a notable effect on the bacterial populations in the rumen. The final counts were lowest in the treatment with 3% *Lactobacillus plantarum* (T3), which recorded a final bacterial count of 2.36 x 10^7 cfu/mL, suggesting that this level of supplementation most effectively reduced the overall rumen bacterial population. In contrast, the treatment with 1% *Lactobacillus plantarum* (T1) had the highest final bacterial count (7.26 x 10^7 cfu/mL), indicating a less pronounced effect on bacterial reduction. The other treatments, T2 (2% *Lactobacillus plantarum*) and T4 (4% *Lactobacillus plantarum*), had intermediate final bacterial counts (3.76 x 10^7 and 3.36 x 10^7 cfu/mL, respectively), suggesting that the 2% and 4% levels also influenced the bacterial populations, but to a lesser extent than the 3% level.

Table 3. Changes in Rumen Bacterial Populations in Sheep Fed Napier Silage Treated with Varying Levels of *Lactobacillus plantarum*.
Tabela 3. Alterações nas populações bacterianas do rúmen em silagem Napier de ovelhas alimentadas e tratadas com níveis variáveis de *Lactobacillus plantarum*.

Treatments	Initial $1x10^7$ cfu/mL	Final $1x10^7$ cfu/mL	% Reduction
T0	8.35	7.26[a]	13.05[c]
T1	7.32	3.18[bc]	56.57[ab]
T2	7.86	3.76[b]	52.16[b]
T3	7.38	2.36[c]	68.02[a]
T4	7.28	3.36[bc]	53.85[b]
p- value	0.462[ns]	0.001*	.001**

*Means with different superscripts within a column differ (p < 0.05). ns = not significant; *p < 0.05; **p < 0.01. T0–T4: Napier silage + 0, 1, 2, 3, 4 % *Lactobacillus plantarum*.
*Médias com sobrescritos diferentes dentro de uma coluna diferem (p < 0,05). ns = não significativo; *p < 0,05; **p < 0,01. T0–T4: Silagem Napier + 0, 1, 2, 3, 4% *Lactobacillus plantarum*.

4. DISCUSSION

The application of *Lactobacillus plantarum* as a silage inoculant has been shown to influence nutrient digestibility in ruminant diets in a variable manner. In this study, increasing levels of *L. plantarum* significantly affected organic matter digestibility (OMD), crude protein digestibility (CPD), and neutral detergent fiber digestibility (NDFD). In contrast, dry matter digestibility (DMD) did not differ statistically across treatments (p = 0.451).

Dry Matter Digestibility (DMD), which reflects the proportion of feed digested and absorbed (Al-arif et al., 2017), showed the highest numerical value in the T3 treatment (43.83%). Although not statistically significant, this trend is consistent with the findings of Liu et al. (2016) and Jin et al. (2024), who reported enhanced in vitro dry matter digestibility with *L. plantarum*-inoculated silage. This improvement may be attributed to enhanced fermentation efficiency and improved preservation of nutrients, as L. plantarum rapidly lowers the pH and inhibits the growth of spoilage microorganisms (OKOYE et al., 2022). Additionally, *L. plantarum* may improve rumen microbial balance by promoting the growth of fiber-degrading bacteria (Matthews et al., 2019), which indirectly supports better dry matter utilization. Organic Matter Digestibility (OMD) was significantly improved by *L. plantarum* (p = 0.004), with the highest value observed at 3% inoculant level (T3, 84.14%). This supports the idea that optimal fermentation preserves more digestible organic compounds by reducing nutrient losses due to proteolysis or spoilage (KUNG et al., 2018). Efficient fermentation enhances the breakdown of plant cell wall components, especially cellulose, hemicellulose, and lignin, which are typically resistant to digestion (HOLLAND et al., 2020). The improved OMD seen in this study reflects the positive impact of *L. plantarum* on both the preservation of digestible nutrients and the enhancement of microbial accessibility to complex plant structures.

Crude Protein Digestibility (CPD) was also significantly affected ($p < 0.001$), with the T3 and T1 treatments showing the highest values (87.94% and 86.37%, respectively). These results align with those of Pranoto et al. (2013), who reported a 92% improvement in protein digestibility of sorghum flour following *L. plantarum* fermentation. The bacterium likely enhances CPD by lowering silage pH, thereby limiting protein degradation into non-utilizable forms such as ammonia (CAMMACK et al., 2018). Effective acidification helps preserve true protein, which remains more available for rumen microbial synthesis and subsequent absorption (BACH et al., 2005). The superior CPD in this study suggests a beneficial preservation of nitrogenous compounds and improved nutrient uptake.

Neutral Detergent Fiber Digestibility (NDFD) was likewise significantly influenced by *L. plantarum* ($p = 0.003$), with T3 again showing the highest digestibility (54.63%). Fiber digestibility is crucial in ruminant diets, as it determines how efficiently animals can utilize fibrous plant cell walls for energy. *L. plantarum* is known to stimulate rumen cellulolytic bacteria, facilitating the breakdown of cellulose and hemicellulose (PEREZ et al., 2024). These effects contribute to greater fiber utilization and energy extraction from fibrous feed components (AN et al., 2022). However, the decline in NDFD at the highest inoculant level (T4, 48.03%) suggests a potential threshold beyond which benefits decline. This may be due to over-fermentation, leading to excessive lactic acid accumulation, a decrease in rumen pH, or the inhibition of fiber-degrading microbes (WEIMER, 2022).

The results presented in Tables 2 and 3 reveal that increasing levels of *Lactobacillus plantarum* (LP) inoculation in Napier grass silage significantly influenced rumen pH and bacterial populations in sheep. The final rumen pH decreased progressively with higher levels of LP, with the 4% treatment (T4) showing the lowest final pH (6.18) and the highest percent reduction (9.26%), compared to the control group (T0), which had only a 4.40% reduction. This trend aligns with the findings of Zhu et al. (2022), who observed that higher LP concentrations in *Pennisetum* silage increased lactic acid production and resulted in a reduced rumen pH. Similarly, Popova-Krumova et al. (2024) reported that LP supplementation stimulated lactobacilli proliferation, leading to increased lactate accumulation and subsequently lowered pH, particularly during lactic acidosis induction.

For the rumen bacterial population, the supplementation of *Lactobacillus plantarum* in sheep fed Napier silage showed significant effects, particularly in reducing bacterial counts as the level of LP increased. This reduction was most pronounced at the 3% LP level (T3), which exhibited a 68.02% decrease in bacterial population, suggesting that this concentration achieved an optimal balance between promoting beneficial microbes and suppressing harmful ones. One of the primary mechanisms through which *Lactobacillus plantarum* exerts this effect is via lactic acid production, which acidifies the rumen environment and inhibits the growth of bacteria less tolerant to low pH (KADAM et al., 2024). This shift favors acid-tolerant and beneficial microbial populations while suppressing undesirable microbes.

In addition to acidification, *Lactobacillus plantarum* likely reduced rumen bacterial populations through competitive exclusion, a process in which it competes with other microbes for nutrients and attachment sites within the rumen. As noted by Astuti et al. (2022), this competition effectively limits the proliferation of less efficient or potentially pathogenic bacteria, helping to maintain a more stable and health-promoting microbial ecosystem. However, at 4% LP (T4), no further significant bacterial reduction was observed, indicating a possible threshold beyond which increased dosing yields diminishing returns. This suggests that while LP is effective in modulating the microbial population, its application should be optimized to avoid unnecessary overuse that may disrupt microbial equilibrium.

However, the current study did not identify or characterize specific microbial species affected by *Lactobacillus plantarum* supplementation. Unlike studies that utilized molecular techniques such as 16S rRNA sequencing or DGGE to describe shifts in microbial community structure (e.g., Si et al., 2018; Lettat et al., 2012), the present findings are limited to changes in total bacterial counts. Thus, while significant microbial modulation was observed, future studies are recommended to use molecular approaches to determine which specific microbial taxa are being suppressed or promoted by LP in the rumen environment.

5. CONCLUSIONS

This study demonstrated that the addition of *Lactobacillus plantarum* to Napier silage significantly influenced the digestibility of key nutrients in sheep. The results revealed that a 3% inoculant level of *Lactobacillus plantarum* (T3) generally led to the highest digestibility values for dry matter, organic matter, crude protein, and neutral detergent fiber. However, the improvements were not always statistically significant for all parameters. In contrast, excessively high levels of *Lactobacillus plantarum* (such as the 4% treatment) had a detrimental effect, reducing digestibility and indicating that higher inoculant levels may negatively affect fermentation quality.

Furthermore, the study showed that the 3% level of *Lactobacillus plantarum* also resulted in the most substantial reduction in rumen bacterial populations, suggesting a potential benefit in controlling microbial balance. The findings support the use of moderate levels of *Lactobacillus plantarum* as an effective silage inoculant for improving the fermentation and digestibility of Napier silage in sheep, while cautioning against the use of excessive levels, which could be counterproductive.

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