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**Research Article**

# A Comparative Study on the In Vivo and In Situ Degradability of Napier (*Pennisetum purpureum*), Guinea (*Megathyrsus maximus*), and Paspalum (*Paspalum conjugatum*) as Forage Grasess

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**ABSTRACT**

This study evaluated the *in vivo* and *in situ* degradability of 3 local forage grasses: Napier sp., Guinea sp., and *Paspalum* sp. Three (3) rumen-cannulated cattle of similar age were used for the degradability assessments. The *in vivo* experiment followed a 3×3×3 Latin Square Design (LSD), while the *in situ* degradability study employed a 3×5 factorial in a randomized complete block design (RCBD). Dietary treatments consisted of A–Napier sp., B – Guinea sp., and C – *Paspalum* sp. In the *in vivo* digestibility trial, no differences were observed except for GE and NDF digestibility. As for the test diets, *in vivo* digestibility was comparable using local forages in the feed and nutrient digestibility assays ($p>0.05$). In contrast, no significant interactions were observed in the *in situ* ruminal degradability in feed, DMD, CPD, NDFD, and ADFD ($p>0.05$). However, main effects for Forage (factor A) showed a significant effect for both DMD ($p<0.0028$) and NDFD ($p<0.0385$). In addition, feed degradability was significant ($p<0.0189$). For the incubation time (Factor B), feed disappearance, DMD, and ADFD showed strong quadratic effects ($p<0.0018$, $p<0.0001$, and $p<0.0095$, respectively), suggesting that the breakdown process began rapidly but gradually slowed over time. In contrast, CPD and NDFD displayed a linear increase ($p<0.0001$).

***Keywords:*** *In situ degradability, In vivo digestibility, Rumen-cannulated cattle, Guinea, Napier, Paspalum*

## Introduction

Forage grasses are of critical significance to the diets of ruminant livestock, particularly in the tropics and subtropics where ruminant production relies highly on the quality and quantity of available feed resources (Rusdy, 2016). One of the most widely cultivated forage species is Napier sp. due to their adaptability, high biomass production, and nutritional quality for ruminant livestock (Islam et al. 2024).

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Also, Guinea sp., and *Paspalum* sp. are very common and recognized for its palatability and digestibility, contributing positively to ruminant diets. However, the quantity of forage that can be obtained from *Paspalum* sp. is very limited than Guinea sp. (Bestil et al. 2014). Nevertheless, the *Paspalum* sp. is the predominating forage grass species in extensive grazing system in the Philippines.

*In vivo* and *in situ* measurements of forage digestibility give a good understanding of digestive processes. The *in vivo* nature of the digestibility trials further supports the accuracy and relevance of the findings, as this method captures the complex interactions between feed, microbial activity, and host physiology more comprehensively than *in vitro* or *in situ* techniques (Ørskov & McDonald, 1979). The *in situ* method is used where the samples of feed are incubated within the rumen to quantify nutrient loss over time and thereby get the direct measurements of ruminal degradation kinetics (Bacorro et al. 2019). *In vivo* experiments, however, study the actual digestion and absorption processes within the animal and therefore represent the overall effect of the feed on the physiological processes of the animal.

Comparative studies do not exist on the *in vivo* and *in situ* degradability of Napier sp., Guinea sp., and *Paspalum* sp. These studies are crucial to establish species-specific variation in nutrient availability and to provide practical guidelines in ruminant forage management and ration formulation. This study addressed this gap by having a comparative evaluation of the *in situ* and *in vivo* digestibility of the three major local forage grasses. Also, with these 2 methods, digestibility of forages can be estimated and apply necessary measures and technologies to maximize its utilization.

## Materials and Methods

### Animal Research Ethics

All animal procedures were conducted in accordance with the guidelines and regulations approved by the Institutional Animal Care and Use Committee (IACUC).

### Study 1: *In Vivo* Forage Digestibility

Animal Selection and Acclimatization

Three female Brahman heifers were randomly selected and underwent a seven days acclimatization before the *in vivo* digestibility trial. An individual feeding stall was constructed to minimize stress, featuring secured stalls with smooth cement floors, plastic waterers, and feeding troughs. The acclimatization phase allowed the cattle to adjust to their environment and diets, promoting microbial adaptation in the rumen. This process allows establishment of specific microbial populations that will be crucial in nutrient breakdown. Following the acclimatization period, the *in vivo* forage digestibility trial commenced.

Dietary Treatments and Design

The experiment evaluated the *in vivo* digestibility of Napier sp., Guinea sp., and *Paspalum* sp. using three (3) Brahman female species of similar age. It was laid out in a 3×3×3 Latin Square Design with animal (block), type of forage (treatment), and batch (period) as variables.

Feeding Trial

A consistent supply of high-quality forage was maintained throughout the study. Three (3) tropical grasses namely: Guinea sp., Napier sp., and *Paspalum* sp. were selected for *in vivo* digestibility trials. To optimize feed intake and digestion, the grasses were manually chopped about 1-2 inches to enhance microbial attachment and digestion. After acclimatization, 36h fasting period was observed prior to *in vivo* digestibility trial. A known amount of the 3 types of forages were given after a fasting period for 5d.

Fecal Collection and Drying

Fecal collection and drying were conducted daily for 5d. A specialized spade was used for fecal collection and placed in a plastic container for storage. To protect the samples from flies, the collected fecal matter was immediately sun dried, then, oven dried at 105$^{o}$C until constant weight. The dried excreta were then weighed and analyzed for chemical composition.

Chemical Assays of Forage and Feces

Forage and fecal samples were analyzed and determined for dry matter (DM), crude protein (CP) using standard AOAC (2006)

methods. Gross energy (GE) was analyzed using Bomb Calorimeter, Parr Instrument (IL, USA). Moreover, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed using Van Soest et al. (1991) method.

*Table 1. Chemical Composition of Napier sp., Guinea sp., and Paspalum sp. Grasses*

| ANALYSIS | Napier sp. | Guinea sp. | *Paspalum* sp. |
|---|---|---|---|
| Dry Matter, % | 11.87 | 31.38 | 19.01 |
| Crude Protein, % | 3.59 | 2.85 | 2.22 |
| NDF, % | 63.98 | 66.38 | 66.13 |
| ADF, % | 35.60 | 40.23 | 33.41 |
| Gross Energy, cal/g | 3573 | 3774 | 3770 |

Statistical Analysis

Data collected were run in analysis of variance (ANOVA) following 3×3×3 LSD using Proc General Linear Model of SAS v9.4 M8. Assumptions of normality and homogeneity of variances were tested. The Shapiro-Wilk test was used to assess the normality of residuals, while Levene's test was applied to verify homogeneity across treatment groups. Tukey's Honest Significant Difference (HSD) test was used to compare treatment means and was declared significant when $p<0.05$.

**Study 2: *In Situ* Forage Degradability**

Dietary Treatments and Design

Three tropical forage grasses were evaluated for rumen *in situ* degradability as follows: a) Napier sp. b) Guinea sp., and c) *Paspalum* sp. in three (3) cannulated Brahman heifers. It was laid out in a 3×5 factorial in RCBD with Factor A as forage grasses and Factor B as incubation time (0h, 12h, 24h, 36h, and 48h). Each cannulated cattle served as block.

Rumen Incubation of forages

One-hundred thirty-five nylon bags (5x10 cm, ±53-micron pore size Bar Diamond Lane, Parma, ID, USA) were used in rumen *in situ* degradability representing 3 forage grass species, 5 incubation time points, and 3 subsamples per treatment. The bags were oven-dried at 65°C for 30 min. and were weighed before being filled with 5g of the ground forages as test samples. A lingerie bag was added with weights to prevent the samples from floating. Samples were sequentially incubated in the rumen for 12h, 24h, 36h, and 48h, except for the 0h. After incubation, the nylon bags were washed under running water until cleared. Thereafter, these were oven-dried at 65°C for 48h until constant weight to determine nutrient degradability (Osuji et al. 1993).

Chemical Assays of Forage Residues

Forage residues were analyzed and determined for DM, CP (AOAC, 2006); and NDF and ADF (Van Soest et al., 1991).

Statistical Analysis

Data were analyzed in 3×5 factorial in RCBD using Proc General Linear Model of SAS v9.4 M8. Assumptions of normality and homogeneity of variances were tested. The Shapiro-Wilk test was used to assess the normality of residuals, while Levene's test was applied to verify homogeneity across treatment groups. Interaction effects and main effect of the treatment means were tested using Tukey's Honesty Difference (HSD) and trend analysis for the incubation time. Significance difference was declared when $p<0.05$.

## Results and Discussion

### *In Vivo* Forage Digestibility

Results in Table 2 shows no significant differences in the blocking (animals) at $p>0.05$. However, period of treatment implementation showed significant difference in GE ($p<0.0288$) and NDF ($p<0.0495$). As for the test diets, *in vivo* digestibility was comparable using the three local forages across all nutrients of interest ($p>0.05$).

*Table 2. In vivo digestibility of Napier sp., Guinea sp., and Paspalum sp. Grasses*

| Item | *In Vivo* Digestibility, % | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|
| | Napier | Guinea | *Paspalum* | | Animal | Period | Forage |
| Feed | 71.74 | 66.00 | 67.06 | 3.66 | 0.0850 | 0.7708 | 0.5897 |
| DM | 71.93 | 68.17 | 70.01 | 4.30 | 0.0833 | 0.6191 | 0.8394 |
| CP | 81.78 | 79.24 | 85.77 | 4.66 | 0.5766 | 0.1105 | 0.6673 |
| GE | 71.01 | 73.78 | 73.38 | 3.60 | 0.2924 | 0.0288 | 0.8532 |
| NDF | 78.02 | 78.28 | 79.81 | 3.76 | 0.3971 | 0.0495 | 0.9385 |
| ADF | 72.52 | 60.62 | 59.96 | 10.09 | 0.8642 | 0.1197 | 0.6711 |

DM- dry matter, CP- crude protein, GE- gross energy, NDF- neutral detergent fiber, ADF- acid detergent fiber;

* In the row, different letters mean statistical difference at ($p<0.05$)

In terms of DM digestibility, all the selected forage grasses; Napier sp., Guinea sp., and *Paspalum* sp. were observed to be highly succulent, a characteristic that was evident from their relatively low DM content at harvest 11.87%, 31.38%, and 19.01%, respectively (Table 1). This succulence typically reflects a high moisture content and is often associated with forages at an early stage of physiological maturity (McDonald et al., 2011). This translates to a DM digestibility of 71.93% for Napier sp., 68.17% in Guinea sp., and 70.01% for *Paspalum* sp. The lower DM content implies reduced concentrations of structural carbohydrates, which was confirmed by the relatively low values of NDF and ADF as presented in Table 1. Since NDF and ADF are indicative of the fibrous cell wall components of plant tissue which are largely composed of hemicellulose, cellulose, and lignin. A lower concentration of these components is indicative of increased digestibility, as these fiber fractions are less accessible to rumen microbial enzymes (Van Soest, 1994; Mertens, 2013). The high DM digestibility observed among the three grasses can primarily be attributed to their early harvest stage, which is a well-documented factor in forage quality. Immature forages typically contain a higher proportion of rapidly fermentable cellular contents such as sugars, starches, and soluble proteins, and a lower proportion of lignified cell walls (Minson, 1990; Jung & Allen, 1995). As plants mature, lignification increases, especially in the vascular tissues, which reduces the accessibility of microbial enzymes and limits degradation in the rumen (Buxton & Redfearn, 1997). Furthermore, CP digestibility was notably high (between 79% to 85%) across all three grasses, suggesting not only elevated protein concentrations but also greater solubility and ruminal degradability of the protein fractions. This may be due to the low proportion of bound or fiber-associated nitrogen, which typically increases with forage maturity and is less degradable in the rumen (Broderick et al., 1991). The softness and less lignified texture of the grasses further contributed to this observation, supporting Minson's (1990) assertion that softer plant tissues facilitate higher CP digestibility due to better microbial access. Additionally, the results may reflect the adaptability of the rumen microbial population to these specific forages. Napier sp., Guinea sp., and *Paspalum* sp. are commonly included in tropical ruminant diets, particularly for cattle. Rumen microbes in such systems may be well adapted to their specific carbohydrate and protein profiles (Makkar & Becker, 1999).

### *In Situ* Forage Degradability

Of the Three (3) local forages fed to Brahman heifers, no interaction effects were observed in feed, DMD, CPD, NDFD, and ADFD ($p>0.05$).

In table 3, for the forage type (Factor A) feed degradability was observed to be higher in Napier sp. (36.13%) and Guinea sp. (35.75%) compared to *Paspalum* sp. (31.09%) at $p<0.0189$. Then, for the nutrient degradability both DMD ($p<0.0198$), and NDFD ($p<0.0385$) were significantly higher for both Guinea sp. and Napier sp. compared to *Paspalum* sp.

*Table 3. Rumen In Situ Nutrient Degradability on Forage (Main Effect A)*

| Item | Forage | | | SEM | P-value |
|---|---|---|---|---|---|
| | Napier sp. | Guinea sp. | *Paspalum* sp. | | |
| Feed, % | 36.13[a] | 35.75[a] | 31.09[b] | 4.9071 | 0.0189* |
| DMD, % | 55.48[a] | 54.15[ab] | 47.60[b] | 7.2902 | 0.0198* |
| CPD, % | 55.51 | 50.40 | 47.50 | 8.3039 | 0.5156 |
| NDFD, % | 33.43[a] | 30.78[ab] | 27.20[b] | 4.4114 | 0.0385* |
| ADFD, % | 34.97 | 33.34 | 32.04 | 5.0225 | 0.7783 |

DMD- dry matter degradability, CP- crude protein degradability, NDF- neutral detergent fiber degradability, ADF- acid detergent fiber degradability;

*In the row, different letters mean statistical difference at (p<0.05)

In table 4, for the incubation time (Factor B), Feed disappearance, DMD, and ADFD showed strong quadratic effects (p<0.0018, p<0.0001, and p<0.0095, respectively), suggesting that the breakdown process began rapidly but gradually slowed over time. In contrast, CPD and NDFD displayed linear increase (p<0.0001). DM and CP were degraded as much as 51-58% after 24h and 78-82% after 48h compared to NDF and ADF at 32-34% and 49-51%, respectively.

*Table 4. Rumen In Situ Nutrient Degradability on Incubation Time (Main Effect B)*

| Nutrient | Incubation Time | | | | | SEM | *P-value* | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 12 | 24 | 36 | 48 | | Nutrient | Linear | Quadratic |
| Feed, % | 5.51 | 27.16 | 36.14 | 49.36 | 54.23 | 4.3194 | <.0001 | <.0001 | 0.0018 |
| DMD, % | 4.89 | 46.05 | 58.53 | 73.90 | 78.66 | 2.4163 | <.0001 | <.0001 | <.0001 |
| CPD, % | 9.81 | 38.86 | 51.26 | 74.28 | 81.46 | 5.2347 | <.0001 | <.0001 | 0.1698 |
| NDFD, % | 7.01 | 21.49 | 32.02 | 42.38 | 49.05 | 2.1382 | <.0001 | <.0001 | 0.1094 |
| ADFD, % | 4.74 | 31.13 | 36.09 | 44.49 | 50.79 | 3.4648 | <.0001 | <.0001 | 0.0095 |

*DMD- dry matter degradability, CP- crude protein degradability, NDF- neutral detergent fiber degradability, ADF- acid detergent fiber degradability;*

**In the row, different letters mean statistical difference at (p<0.05)*

Napier sp. (36.13%) and Guinea sp. (35.75%) exhibited significantly higher feed degradability compared to *Paspalum* sp. (31.09%). The observed variation in feed degradability across 3 forage grasses can be attributed to differences in chemical composition, particularly fiber content and lignification levels which was shown in NDF and ADF content (Table 1). Napier sp. and Guinea sp. are known to have relatively lower lignin concentrations and higher cell wall digestibility compared to *Paspalum* sp. when harvested at similar stages of maturity (Van Soest, 1994). The superior DMD of Napier sp. and Guinea sp. can be attributed to their greater content of readily fermentable carbohydrates, lower structural fiber fractions, and higher tissue succulence. All of which facilitate more efficient microbial colonization and enzymatic hydrolysis within the rumen. These findings align with previous reports by Minson (1990) and McDonald et al. (2011), which underscored the importance of forage maturity in digestibility; grasses harvested at earlier stages generally possess less lignified tissue and a higher proportion of digestible cell soluble. Conversely, the significantly lower DMD observed in *Paspalum* sp. may be related to its higher concentration of indigestible components, such as lignin, anatomical characteristics which limit microbial access (Jung & Allen, 1995). Additionally, *Paspalum* sp. was harvested at a greater height (8–10 inches) than what the animals were typically accustomed to, as they usually graze this species at a shorter height. This increased height is indicative of a more advanced maturity stage, which is generally associated with less favorable morphological characteristics, such as a denser cell wall structure and potentially higher fiber-to-leaf ratios. Moreover, observational data from

the study indicated that ruminants consistently preferred Napier sp. and Guinea sp., consuming them first, suggesting higher palatability, which often correlates with digestibility. A similar trend was observed for NDFD ($p<0.0385$) where Napier sp. (33.43%) showed significantly greater fiber degradation than *Paspalum* sp. (27.20%), while Guinea sp. (30.78%) was intermediate and did not differ significantly from either. These results reflect species-level differences in fiber composition, and cell wall architecture; all of which are critical determinants of NDFD. The superior NDFD of Napier sp. likely results from its open vascular structure and less lignified parenchyma, which improve microbial access to hemicellulose and cellulose (Buxton & Redfearn, 1997; Van Soest, 1994). In contrast, *Paspalum* sp. lower NDFD may indicate a greater proportion of fiber tightly bound within a lignified matrix, along with potential inhibitory effects from secondary plant compounds, such as phenolic acids or silica, which can impede microbial fiber degradation (Makkar & Becker, 1999). In addition, in the study of Giordano et al (2014) where *Paspalum* sp. was reported to exhibit lower degradability due to its high lignin content composition. This is especially evident in their stolon, which are thick and lignified, resembling sticks. The lignin in these grasses acts as a physical barrier, limiting microbial access to cell wall polysaccharides and thereby reducing digestibility. Moreover, lignin is not degraded in the rumen. CPD values were comparable among forages ($p>0.5156$), suggesting no differences in the level of protein solubility and degradability across species. Potentially CP content was relatively low in these grasses (2-3 % CP) and the fact that all forages were harvested at relatively early growth stages. Early-harvested forages typically exhibit proteins that are less associated with fiber matrices, making them more accessible to ruminal proteolysis (Broderick et al., 1991). Similarly, no significant differences were observed in ADFD across 3 grass species ($p>0.6907$), indicating that the recalcitrant portions of the cell wall (cellulose and lignin) were equally resistant to ruminal degradation across the forages. ADFD values tend to exhibit less variation than NDFD due to the highly indigestible nature of lignified tissues, which are not easily broken down by rumen microbes (Van Soest, 1994).

Rumen fermentation principles explained that extended exposure to the ruminal environment promotes sequential microbial colonization, enzymatic hydrolysis, and solubilization of both soluble and structural feed fractions (Ørskov & McDonald, 1979). Feed degradability increased progressively with longer incubation times: from 5.51% at 0h to 54.23% at 48h, with significant differences observed between most time intervals. As incubation progresses, ruminal bacteria, protozoa, and fungi colonize the feed and secrete enzymes, breaking down structural carbohydrates like cellulose and hemicellulose (Mould et al., 1983; Van Soest, 1994). This enzymatic action increases with time, peaking around 36 to 48h. Similarly, a pronounced increase in DMD was observed throughout the incubation period, beginning at 4.89% at 0h to 46.05% by 12h, and reaching a plateau at 78.66% by 48h. The rapidly degraded fractions were observed between 0-12h likely reflects the disappearance of soluble carbohydrates and readily fermentable cell contents. Beyond this point, the rate of increase in DMD slows, indicative of a shift toward the fermentation of more recalcitrant and potentially degradable fiber components (Van Soest, 1994). The continued degradative activity between 36 and 48h suggests that microbial fermentation of resistant carbohydrates, such as hemicellulose and cellulose, persisted at a diminished rate. CPD followed a comparable trend, increasing from 9.81% at 0h to 81.46% at 48h. The steep rise in CPD up to 36h (74.28%) highlights the rapid utilization of soluble and non-protein nitrogen (NPN) fractions by ruminal microorganisms. Additionally, proteolytic activity targeting true protein within plant cellular matrices likely contributed to continued CPD increases over time (Broderick et al., 1991). The consistently high CPD values observed in later incubation periods may also be attributed to the low lignification of the forages, which enhances microbial access to protein trapped within fiber matrices (McDonald et al., 2011). NDFD increased significantly with time, ranging from 7.01% at 0h to 49.05% at 48h. This pattern underscores the relatively

slower fermentation kinetics of cell wall constituents, particularly hemicellulose and cellulose, which require prolonged ruminal retention to allow effective microbial attachment and enzymatic breakdown (Jung & Allen, 1995). The sharp increases between 12h and 36h, followed by further gains at 48h, highlight the progressive adaptation of the rumen microbiota and the time-dependent disassembly of the plant cell wall (Weimer, 1996). Among all nutrient fractions, ADFD exhibited the most limited degradability, progressing from 4.74% at 0h to 50.79% after 48h. As ADF primarily consists of cellulose and lignin-bound hemicellulose, its resistance to degradation is well-documented and largely attributable to the presence of lignin, which physically impedes microbial colonization and enzymatic access (Van Soest, 1994). The relatively moderate increases in ADFD beyond 24h reflect the inherent recalcitrance of these structural polysaccharides.

## Conclusion

The chemical composition shows that Napier sp. had the highest CP but lowest DM and energy content. Guinea sp. and *Paspalum* sp. had higher fiber and GE, suggesting greater energy density but potentially reduced digestibility and intake due to elevated NDF and ADF levels.

In the *in vivo* assays, all grasses showed high digestibility with no significant differences among treatments, suggesting the early-harvested forages generally support favorable nutrient utilization by ruminants. However, in the *in-situ* trial, significant differences were observed in the main effects A (forage) for feed, DMD and NDFD, with Napier sp. and Guinea sp. outperforming *Paspalum* sp. This reflects the finer resolution of *in situ* methodology in detecting species-level differences under controlled ruminal incubation conditions. The data also emphasized the role of incubation time, with degradability values of feed disappearance, DMD, and ADFD showed strong quadratic effects, suggesting that the breakdown process began rapidly but gradually slowed over time. In contrast, CPD and NDFD displayed linear increase.

These findings support the use of Napier and Guinea grasses in feeding programs aimed at maximizing ruminal digestibility, especially under tropical grazing systems.

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