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Fermentative characteristics and bromatological composition of pigeon pea (*Cajanus cajan*) silage at different silo opening times

[Características fermentativas e composição bromatológica da silagem de feijão guandu (*Cajanus cajan*) em diferentes períodos de abertura de silos]

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Abstract: Pigeon pea (*Cajanus cajan*) shows potential as a forage alternative for the Brazilian semiarid region due to its hardiness and high crude protein content. However, its ensiling process faces challenges related to its high buffering capacity, which compromises fermentative efficiency. This study evaluated the fermentative profile and bromatological composition of pigeon pea silage at different silo opening times. Fermentative parameters (pH, ammoniacal nitrogen/N-NH_3) and bromatological components (dry matter, crude protein, neutral detergent fiber – NDF, acid detergent fiber – ADF, hemicellulose, cellulose, and lignin) were analyzed. The results indicated stability in NDF, ADF, and cellulose contents over time, with significant variation in lignin, associated with the degradation of soluble fractions and oxidative processes. The fermentative profile revealed relatively high pH values and accumulation of N-NH_3, reflecting intense proteolysis and low efficiency of lactic fermentation. It is concluded that pigeon pea silage, although presenting relevant protein value, shows fermentative and nutritional limitations, especially due to the accumulation of non-protein nitrogen compounds and lignin content, factors that reduce fiber digestibility. These results reinforce the need for management strategies, such as the use of additives or associations with grasses, to optimize the fermentative process and expand the use of pigeon pea as conserved forage in semiarid regions.

Keywords: Cajanus cajan; bromatological composition; fermentation; semiarid; silage.

Resumo: O feijão guandu (*Cajanus cajan*) apresenta potencial como alternativa forrageira para o semiárido brasileiro devido à sua rusticidade e ao elevado teor de proteína bruta. No entanto, sua ensilagem enfrenta desafios relacionados à elevada capacidade tampão, que compromete a eficiência fermentativa. Este estudo avaliou o perfil fermentativo e a composição bromatológica da silagem de guandu em diferentes períodos de abertura dos silos. Foram analisados parâmetros fermentativos (pH, nitrogênio amoniacal/N-NH_3) e bromatológicos (matéria seca, proteína bruta, fibra em detergente neutro – FDN, fibra em detergente ácido – FDA, hemicelulose, celulose e lignina). Os resultados indicaram estabilidade nos teores de FDN, FDA e celulose ao longo do tempo, com variação

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significativa para lignina, associada à degradação de frações solúveis e processos oxidativos. O perfil fermentativo revelou pH relativamente elevado e acúmulo de N-NH_3, refletindo intensa proteólise e baixa eficiência da fermentação lática. Conclui-se que a silagem de guandu, embora apresente valor proteico relevante, possui limitações fermentativas e nutricionais, especialmente devido ao acúmulo de compostos nitrogenados não proteicos e ao teor de lignina, fatores que reduzem a digestibilidade da fibra. Tais resultados reforçam a necessidade de estratégias de manejo, como o uso de aditivos ou associações com gramíneas, para otimizar o processo fermentativo e ampliar a utilização do guandu como volumoso conservado no semiárido.

Palavras-chave: *Cajanus cajan*; composição bromatológica; fermentação; semiárido; silagem.

1. Introduction

Livestock production in the Brazilian semiarid region is constrained by structural limitations in forage production and availability, largely resulting from pronounced climatic variability. Although pasture growth is abundant during the rainy season, forage scarcity during prolonged dry periods severely restricts animal performance, reducing both milk and meat productivity while increasing reliance on costly supplementation strategies [1 - 3]. This scenario highlights the importance of forage conservation technologies capable of ensuring a consistent year-round supply of roughage. Among these technologies, silage production has emerged as one of the most effective strategies for mitigating seasonal feed shortages in semiarid livestock systems [4].

Of the forage species adapted to semiarid conditions, pigeon pea (*Cajanus cajan*) has gained considerable attention due to its resilience and multifunctional role. This perennial shrubby legume is well adapted to low-fertility soils, prolonged drought, and high temperatures, making it a valuable component of livestock production systems in water-limited environments [5]. In addition to its ecological benefits, including soil rehabilitation and green manure production, pigeon pea possesses valuable nutritional characteristics that enhance its suitability as a forage resource. Its crude protein concentration is considerably higher than that of most tropical grasses. Consequently, using pigeon pea in feeding systems can help reduce reliance on external protein supplements, which are often costly and difficult to access for livestock producers in semiarid regions [6, 7].

Despite its potential as a forage resource, the ensiling of legumes such as pigeon pea has important fermentative challenges. The high concentrations of proteins and minerals characteristic of legumes confer a strong buffering capacity to the forage mass, limiting the rapid decline in pH required to inhibit undesirable microorganisms and promote adequate silage preservation [8, 9]. As a result, the efficiency of lactic acid fermentation may be reduced, creating favorable conditions for the proliferation of clostridial bacteria. The activity of these microorganisms results in the production of butyric acid and biogenic amines, which negatively affect both the nutritional quality and palatability of silage [10].

Therefore, characterizing the fermentation profile and nutritional quality of these silages is a fundamental step toward understanding the limitations of the conservation process and developing strategies for large-scale adoption. Analyzing parameters such as pH, N-NH_3, and chemical composition (including dry matter, crude protein, and fiber fractions) enables the evaluation of not only fermentation efficiency but also the stability and nutritional value of the conserved forage [11,12].

In this context, this study aimed to evaluate the fermentation profile and chemical composition of pigeon pea silage across different ensiling periods, seeking to elucidate its stability, limitations, and nutritional implications for livestock production systems in the Brazilian semiarid region.

2. Material and methods

2.1 Study site and plant materials

This study was conducted at the Brazilian Agricultural Research Corporation (Embrapa Goats and Sheep), located at 3°44'55"S, 40°21'35"W, and altitude of 80 m. According to the Köppen-Geiger classification, the region has a semi-arid climate [13]. The area experiences a distinct rainy season between February and June, with an average annual rainfall of 621 mm recorded at the experimental site in 2022. The soil is classified as Haplic Luvisol, with low acidity, moderate levels of organic matter, phosphorus, and potassium, and high concentrations of calcium and magnesium. The photoperiod at the site is relatively neutral, with daily sunshine duration ranging from approximately 11h45m in the winter to 12h15m in the summer [14].

Soil preparation consisted of plowing to a depth of 0.00–0.40 m, followed by leveling harrowing to uniform the area. A pre-emergent herbicide was subsequently applied for weed control. Furrowing was then performed with a 0.75 m spacing between rows. Basal fertilization was applied using an NPK 8-28-16 formulation based on a recommendation of 130 kg P_2O_5 ha^{-1}, totaling 464 kg of fertilizer per hectare.

The Guatã cultivar was utilized for the experiment. The study followed a randomized complete block design, with experimental plots consisting of two 3-m rows spaced 0.75 m apart, with an in-row plant spacing of 0.25 m.

2.2 Ensiling process

For the experiment, ten plants were harvested from each plot. The forage was chopped into small particles to improve compaction and preservation during the ensiling process. Experimental silos were constructed from PVC tubes measuring 10 cm in diameter and 50 cm in height and were hermetically sealed at both ends. Fresh forage was weighed using a digital scale with a 40-kg capacity and 1-g precision. Each silo was filled with 1,980 g of homogenized fresh forage.

The forage was manually compacted using a wooden rammer to minimize the amount of air trapped between particles. Immediately after filling, the silos were sealed with plastic caps to ensure anaerobic conditions and stored in a protected environment, away from direct sunlight and rainfall.

The experiment was conducted in a completely randomized design consisting of seven treatments corresponding to ensiling periods of 3, 7, 14, 21, 28, 60, and 90 days after ensiling, with four replicates per treatment.

2.3 Silo opening

The silos were opened sequentially according to the predetermined ensiling periods, beginning on day 3 and continuing until day 90 after ensiling. At opening, approximately 10 cm of material from the top layer was discarded to avoid edge effects and ensure sample representativeness. The remaining silage was thoroughly homogenized. After homogenization, the fresh weight of the silage was recorded, and subsamples were collected for laboratory analyses: 10 g for pH determination, 800 g for chemical composition analyses, and 500 g for ammoniacal nitrogen (NH_3-N) determination.

2.4 Laboratory analyses

Analyses were conducted at the Laboratory of Animal Nutrition and Forage Quality of Embrapa Goats and Sheep. The pH was determined according to the methodology described by Detmann et al. [15], and ammoniacal nitrogen was quantified following standard protocols [16, 17]. Dry matter (DM), organic matter (OM), and crude protein (CP) contents were determined according

to the procedures described by Silva and Queiroz [18]. The neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, and lignin fractions were evaluated in accordance with Van Soest et al. [19].

2.5 Statistical analysis

Treatment means were subjected to the Shapiro-Wilk normality test, at a 5 % significance level. All statistical analyses were performed using R software.

3. Results and discussion

The pH assessment of pigeon pea silage revealed statistically significant variations ($p < 0.01$) across the treatments, demonstrating substantial changes in fermentation dynamics as a function of ensiling periods. On the third day post-ensiling, a high pH of 6.73 was observed (Figure 1), indicating that the fermentation process had not yet reached stabilization. From day 7 to day 28, a gradual but limited decline in pH occurred, with values remaining elevated between 6.51 and 6.19. This slow acidification and prolonged high pH can be attributed to the high buffering capacity of the forage, which typically results from the high concentrations of proteins and minerals characteristic of pigeon pea.

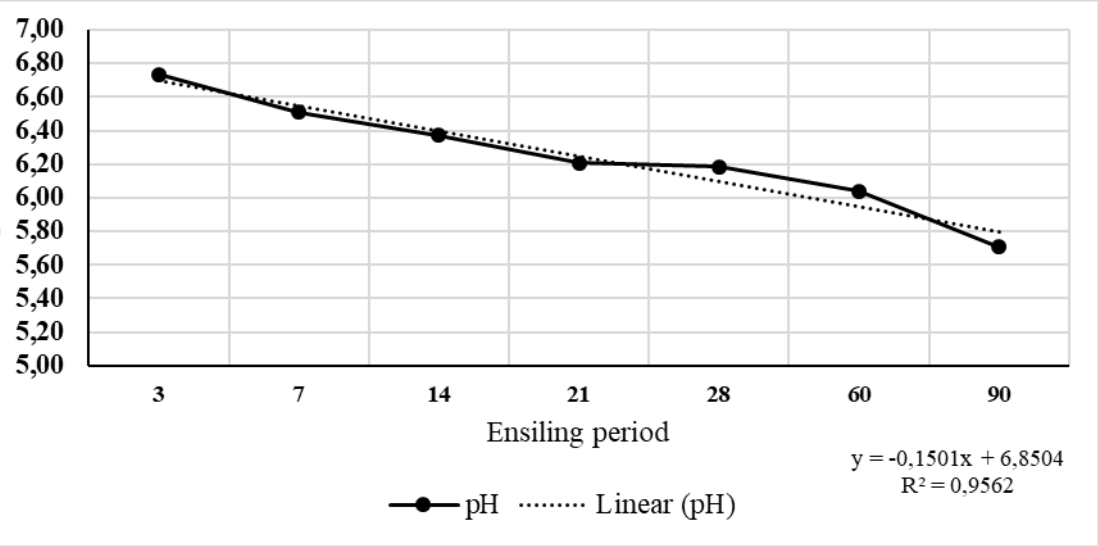

Figure 1. Variation of pH in pigeon pea silage at different opening periods.

By day 60, silage pH had declined to 6.04 and reached its lowest value of 5.71 on day 90, indicating a gradual progression toward fermentation stabilization. Despite this reduction, the final pH remained substantially above the range considered optimal for well-preserved silage, suggesting that lactic acid fermentation was insufficient throughout the ensiling period. Such elevated pH values create favorable conditions for the proliferation of undesirable microorganisms, particularly *Clostridium* spp., whose metabolic activity is associated with the production of butyric acid and biogenic amines. These compounds negatively affect aerobic stability and palatability while promoting losses in nutritional quality [9, 20].

The progressive decline in pH from 6.73 to 5.71, with an overall mean of 6.25, reflects the typical fermentation pattern observed in legume monoculture silages. The inherently high buffering capacity of legumes restricts rapid acidification, thereby limiting the establishment of a desirable lactic acid fermentation and allowing competing anaerobic microorganisms to remain active during the early and intermediate stages of storage [9, 20].

Ammonia nitrogen (N-NH_3) concentration was also significantly affected by ensiling period ($p < 0.01$). The response was adequately described by a quadratic regression model ($R^2 = 0.8632$), indicating that 86.32 % of the variation in N-NH_3 accumulation was explained by ensiling period (Figure 2). This variable is widely recognized as a key indicator of silage fermentation quality because it reflects the extent of proteolysis and amino acid deamination occurring during ensiling, processes that are often intensified under clostridial fermentation [21].

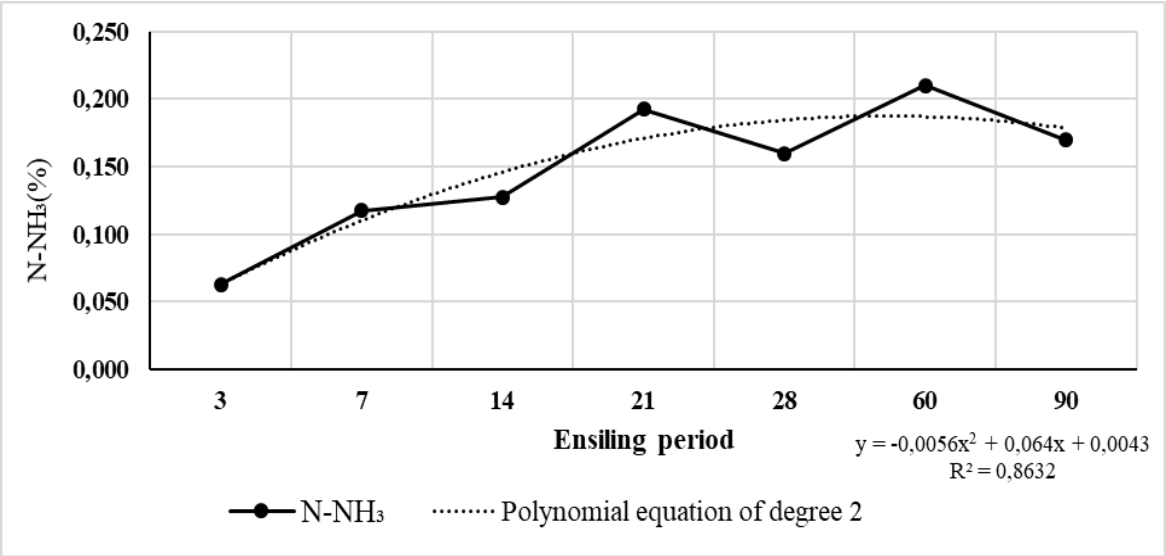

Figure 2. Variation of ammoniacal nitrogen (N-NH_3) in pigeon pea silage at different ensiling periods.

Ammonia nitrogen (N-NH_3) concentrations increased throughout the ensiling period, rising from 0.063 % on day 3 to a maximum of 0.210 % on day 60, before declining slightly to 0.170 % on day 90. This trend reflects a progressive increase in proteolytic activity during ensiling, likely associated with the action of proteolytic microorganisms and the limited efficiency of fermentation in preserving the protein fraction [18, 23].

The low N-NH_3 concentration observed after 3 days of ensiling indicates minimal protein degradation during the early stages of fermentation, suggesting a greater preservation of nitrogen-containing compounds at the onset of the ensiling process [10, 22]. However, the subsequent increase in N-NH_3 concentrations between 21 and 60 days demonstrates continued proteolysis and deamination, resulting in the accumulation of ammonia and other nitrogenous metabolites. This pattern is characteristic of suboptimal fermentation conditions and may be associated with the activity of undesirable microorganisms, including clostridia, that contribute to the deterioration of silage quality and reduce the efficiency of nitrogen utilization by ruminants [8, 33].

The highest N-NH_3 concentration recorded at 60 days (0.210 %) corresponds to the period of greatest proteolytic activity, indicating increased fermentative instability and greater protein losses. Although these values remained below the thresholds typically associated with severe silage deterioration, the consistent rise in N-NH_3 over time highlights the persistence of protein degradation throughout the ensiling process [24]. The slight decline observed at 90 days may reflect the depletion of readily degradable protein substrates or shifts in the microbial population as fermentation progressed. Nevertheless, N-NH_3 concentrations at this stage remained considerably higher than those measured at the beginning of ensiling, confirming that proteolytic processes continued throughout the ensiling period [25, 26].

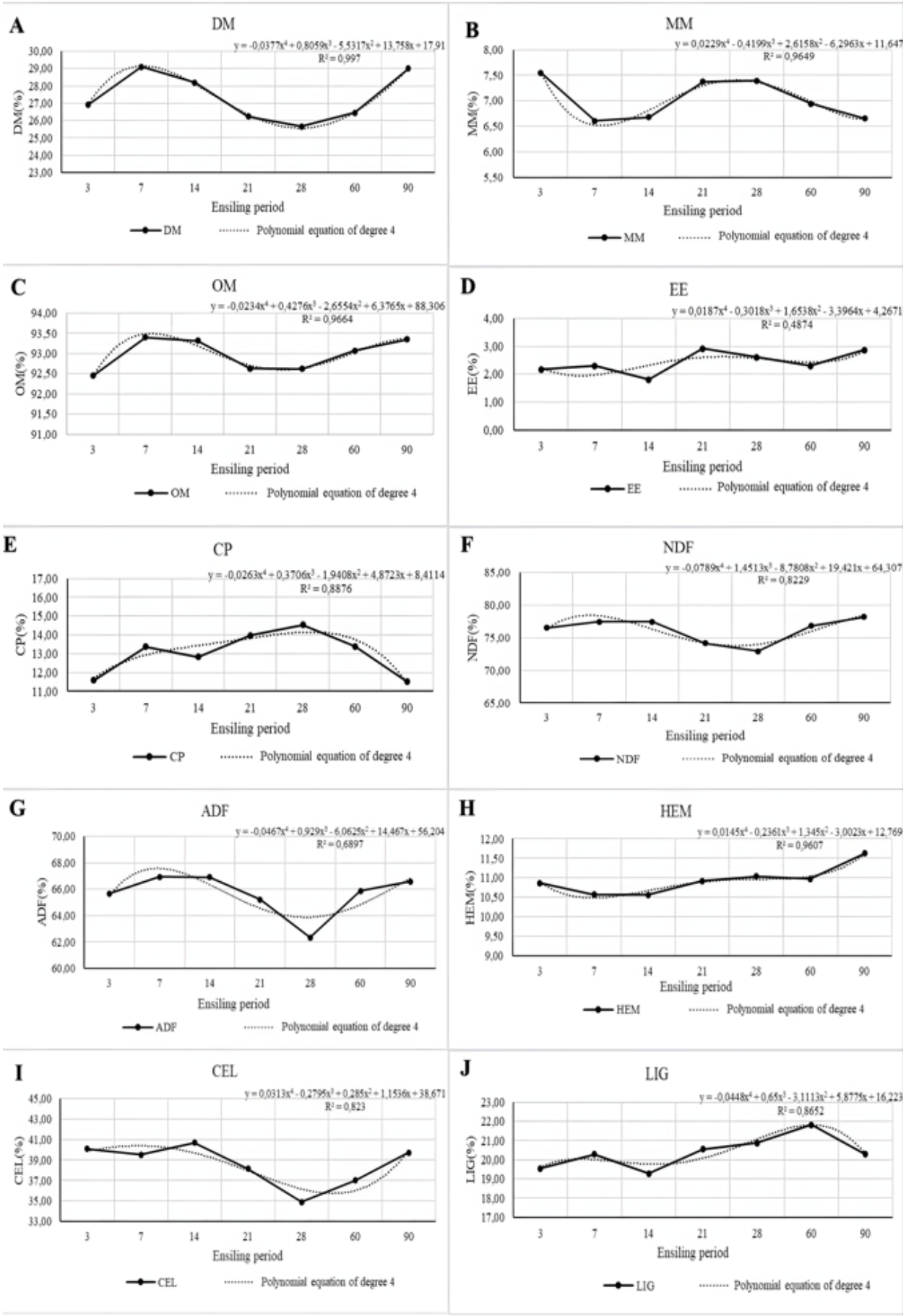

Figure 3. Chemical composition of pigeon pea (*Cajanus cajan*) silage at different ensiling periods. DM: dry matter; Ash: mineral matter; OM: organic matter; EE: ether extract; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; HEM: hemicellulose; CEL: cellulose; and LIG: lignin. Dashed lines represent the fitted fourth-degree polynomial regression models.

The chemical composition of pigeon pea silage (Figure 3) varied throughout the ensiling period, with significant changes observed in some nutritional parameters, whereas others remained relatively stable. The effects of ensiling period on dry matter (DM), mineral matter (MM), organic matter (OM), ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEM), cellulose (CEL), and lignin (LIG) were described by fourth-degree polynomial regression models. In general, the models exhibited high R^2, indicating a good fit to the observed data and effectively capturing the temporal patterns of variation.

Dry matter content was significantly affected by ensiling period ($p < 0.01$), ranging from 25.66 % at 28 days to 29.11 % at 7 days. The highest DM concentrations were observed at 7 days (29.11 %), followed by 90 days (29.00 %) and 14 days (28.19 %). These results suggest that DM preservation was favored during both the early and late stages of storage, potentially reflecting distinct processes. In the initial phases, rapid fermentation and the accumulation of organic acids may have contributed to reducing fermentative losses, whereas the higher values observed at later stages likely indicate greater stabilization of the ensiled mass [10, 27].

At 3 days after ensiling, DM content was 26.91 %, reflecting the onset of the fermentation process, when intense microbial activity and residual respiration may still occur. The lowest DM values were recorded at 21 and 28 days (26.23 % and 25.66 %, respectively), suggesting increased fermentative losses during the intermediate stages of storage. This reduction may be associated with secondary fermentations and greater nutrient losses, processes commonly linked to reduced silage quality and stability [8].

A slight increase in DM content was observed at 60 days (26.45 %), indicating the progression toward fermentative stabilization. This response is consistent with the findings of Muck et al. [28], who reported that the accumulation of organic acids during ensiling suppresses undesirable microorganisms and promotes greater preservation of the stored forage. The recovery of DM content observed at 90 days may reflect the completion of fermentation and stabilization of the silage mass. Although moisture losses during prolonged ensiling cannot be ruled out, particularly under suboptimal sealing conditions, the relatively high DM concentration at this stage suggests improved conservation compared with the intermediate storage periods.

Mineral matter (MM) and organic matter (OM) contents did not show statistically significant variations between the different ensiling periods, with average values ranging from 6.61 % (7 days) to 7.55 % (3 days) for MM, and from 92.45 % (3 days) to 93.40 % (7 days) for OM. These results are consistent with findings in the literature [29, 30], which highlight the stability of these parameters as indicative of an efficient fermentation process. Similarly, ether extract (EE) content, a representative fraction of the lipid content, ranged from 1.81 % (14 days) to 2.92 % (21 days), also without significant differences between treatments, suggesting effective preservation of the forage's energy constituents, as reported by the same authors.

Crude protein (CP) varied significantly ($p < 0.05$) across the different ensiling periods, with values ranging from 11.51 % (on day 90) to 14.54 % (on day 28). The lowest early-stage CP content (11.58 %) was observed during the first 3 days post-ensiling, which can be attributed to intense proteolytic activity driven by endogenous plant enzymes that break down true proteins into peptides, amino acids, and subsequently ammonia. This initial dynamic aligns with findings by Alves [11], who highlighted that plant protease activity during the early phases of fermentation is a determining factor in reducing the usable protein fraction.

From day 7 to day 28, a gradual increase in CP content was observed, peaking at 14.54 % on day 28. This upward trend likely reflects a relative concentration effect caused by the loss of soluble DM fractions, such as soluble sugars and organic acids, which proportionally increased CP content within the remaining DM. Similar results were reported by Coelho and Araújo [31], who observed an apparent increase in CP content during fermentation, attributing it to nutrient concentration driven by fermentative dry matter losses.

During the longer ensiling periods (60 and 90 days), CP contents declined slightly, reaching their lowest point on day 90 (11.51 %). This decrease may be linked to the conversion of protein into volatile nitrogenous compounds, such as ammonia, which are poorly utilized by ruminants and signal a decline in the nutritional quality of the silage. This process underscores the observations of Zambom et al. [32], who emphasized the impact of microbial proteolytic activity and the subsequent formation of non-protein nitrogen compounds during prolonged fermentation cycles.

Evaluation of the fibrous components demonstrated overall stability for most fractions, with the sole exception of lignin, which varied significantly ($p < 0.05$). Neutral detergent fiber (NDF), which represents the cell wall fraction composed of cellulose, hemicellulose, and lignin, remained stable across all opening intervals, with values ranging from 72.95 % (on day 28) to 78.21 % (on day 90). This structural consistency suggests that the fermentation process did not compromise the integrity of the cell wall fibers, which is beneficial for maintaining the physical effectiveness of the diet and stimulating ruminal motility. Borreani et al. [8] highlight that maintaining NDF stability during silage storage is crucial for preserving ruminal health and optimizing feed efficiency, reinforcing that maintaining strict anaerobic conditions prevents the premature degradation of this structural fraction.

Acid detergent fiber (ADF), which comprises the less digestible components of the cell wall, primarily cellulose and lignin, was not significantly affected by ensiling period ($p > 0.05$), with values ranging from 62.34 % at 28 days to 66.92 % at 7 days. Likewise, hemicellulose content, estimated as the difference between NDF and ADF, remained stable throughout the ensiling period, ranging from 10.56 % at 14 days to 11.63 % at 90 days. The absence of significant changes in these fiber fractions suggests limited degradation of structural carbohydrates during storage. Given that hemicellulose is generally more susceptible to microbial degradation than cellulose and lignin, its stability may indicate that anaerobic conditions were sufficiently maintained to restrict the activity of undesirable cellulolytic and hemicellulolytic microorganisms throughout the fermentation process [8, 33].

Cellulose concentrations also remained unchanged ($p > 0.05$), ranging from 37.00 % to 40.68 % across the evaluated storage periods. In contrast, lignin was the only fiber component significantly influenced by ensiling time ($p < 0.05$), increasing from 19.54 % at 3 days to 21.83 % at 60 days. Because lignin is not degraded during ensiling, this increase likely reflects a relative concentration effect resulting from the loss of soluble constituents and other fermentable fractions during storage rather than an actual accumulation of lignin within the silage mass [33 - 35].

The increase in lignin concentration has important nutritional implications, as lignin forms strong associations with structural polysaccharides and acts as a physical barrier to microbial colonization and enzymatic degradation of the cell wall. Consequently, higher lignin contents are generally associated with reduced fiber digestibility and lower forage utilization by ruminants. Consistent with this interpretation, Cao et al. [36] reported that elevated lignin concentrations significantly reduced fiber degradability in tropical silages. Therefore, strategies aimed at improving cell-wall degradability, including the use of biological additives with ligninolytic activity, may enhance the nutritional value of pigeon pea silage.

4. Conclusion

Pigeon pea silage showed potential as a forage resource for livestock production in semi-arid regions due to its relatively high crude protein content. However, the elevated pH, progressive accumulation of ammonia nitrogen ($N\text{-}NH_3$), and evidence of ongoing proteolysis observed during storage indicate limitations in the fermentation and preservation process. Although most fiber fractions remained stable, the increase in lignin concentration may negatively affect forage digestibility. Therefore, the use of pigeon pea as a sole silage crop should be approached with caution, and strategies such as the use of additives or co-ensiling with grasses and other carbohydrate-rich forages are recommended to improve fermentation quality, reduce nutrient losses, and enhance the nutritional value of the conserved forage.

Conflict of interest statement

The authors declare that there are no conflicts of interest related to conducting this study, interpreting the results, or publishing this manuscript.

Data availability statement

The data generated and analyzed during this study are available upon request to the corresponding author.

Author contributions

Conceptualization: Guedes, F.L. Data curation: Vasconcelos, F.J.O., Alves, C.B., Sousa, V.A. and Moraes, I.C.L. Formal analysis: Vasconcelos, F.J.O., Alves, C.B., Sousa, V.A. and Tonucci, R.G. Methodology: Guedes, F.L. Supervision: Guedes, F.L. and Bezerra, J.W.T. Visualization: Alves, C.B., Moraes, I.C.L. and Tonucci, R.G. Investigation: Vasconcelos, F.J.O., Sousa, V.A. and Moraes, I.C.L Writing (original draft): Vasconcelos, F.J.O., Alves, C.B., Sousa, V.A., Moraes, I.C.L., Guedes, F.L., Bezerra, J.W.T. and Tonucci , R.G. Writing (revision and editing): Alves, C.B., Sousa, V.A., Moraes, I.C.L., Guedes, F.L. and Tonucci , R.G.

Generative AI use statement

The authors did not use generative artificial intelligence tools or technologies in creating or editing any part of this manuscript.

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