

Food fortification of pangola grass (*Digitaria eriantha* Steud) silage to increase its digestibility

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ABSTRACT

Objective: To evaluate the effect of different urea concentrations (2%, 3%, and 5%) on the nutritional composition and digestibility of pangola grass (*Digitaria eriantha* Steud) silage.

Design/Methodology/Approach: The study was conducted at the Instituto Tecnológico Superior, located in the municipality of Juan Rodríguez Clara, Veracruz. A completely randomized design with three repetitions was established. Pangola grass was harvested at 45 days of regrowth and three types of microsilos were prepared with 2%, 3%, and 5% urea, along with a control without treatment. After 30 days of fermentation, subsamples were taken from the microsilos to perform proximate and Van Soest analyses.

Results: The use of microsilos treated with 5% urea increased crude protein (CP) content, significantly improved protein content, and reached the highest concentration level. Additionally, it reduced acid detergent fiber (ADF) and neutral detergent fiber (NDF).

Study Limitations/Implications: The risk of urea intoxication in animals was the main limitation resulting from the urea-treated microsilos technique. This risk is directly related to incorrect dosages and the distribution of urea in the silage.

Findings/Conclusions: Adding urea to silage is a practical and cost-effective strategy to optimize livestock production in tropical regions, because it enhances the efficient use of forage resources and meets the nutritional needs of animals.

Keywords: Silage, grass, urea, nutritional quality.



INTRODUCTION

Silos are an alternative for forage preservation in livestock production systems. This method helps to preserve the nutritional quality of forage resources during drought periods (Patiño-Pardo *et al.*, 2022). Meanwhile, alternatives food sources for cattle in tropical regions include pangola grass (*Digitaria eriantha* Steud), whose adaptability to adverse conditions and high productivity (Murphy *et al.*, 2019) make it a strategic option to develop silage from tropical perennial grasses (Piltz *et al.*, 2022). The lack of quality forage is one of the most important challenges faced by producers during the dry season in the tropics. On the one hand, this situation does not only impact livestock health and productivity, but it also limits its sustainability. On the other hand, pangola grass can provide an advantage for the implementation of such strategies as silage production (Antonio-Medina *et al.*, 2024). Additionally, when it is fortified with urea, the nutritional value of pangola grass as crude protein (CP) records significant improvements (Wiyabot 2018; Almeida *et al.*, 2018).

Adding urea to silage is a feasible alternative to enhance the nutritional value of forages. Additionally, it acts as a fungicide and improves aerobic stability, minimizing matter losses once the silo has been opened and silage has been exposed to oxygen (Araujo *et al.*, 2023). Given its low cost, feeding ruminants with this type of grass is economically viable. Likewise, rumen microorganisms can metabolize silage, contributing to the synthesis of microbial proteins and consequently increasing nutrient availability (Wahyono *et al.*, 2022). The addition of urea to silages can increase their crude protein content and improve their digestibility (Calixto *et al.*, 2017); however, the dose and application conditions can prevent the risk of ammonia toxicity, guaranteeing a high-quality silage (González-Padilla and Merino-Zuñiga, 1984). In order to achieve a balance between safety and nutritional quality, adding urea to the silage technique applied to pangola grass must be optimized. Therefore, this research evaluated the effect of including urea on the nutritional characteristics of the silage during the preparation stage. The aim was to understand its performance according to the different doses. The ultimate purpose was to improve nutritional tropical forages and to provide practical solutions for livestock systems where pangola grass is a prevailing resource. This experiment resulted in an increase in crude protein (CP), dry matter (DM), ether extract (EE), net energy for lactation (NEL), net energy for maintenance (NEM), and net energy for gain (NEG), as well as a decrease in crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF), increasing the digestibility and quality of pangola grass silage.

MATERIALS AND METHODS

Study Location

Pangola grass was sown at the Instituto Tecnológico Superior de Juan Rodríguez Clara, located in the municipality of Juan Rodríguez Clara, Veracruz (18° 00' 6.1" N and 95° 24' 1.7" W, 133 m.a.s.l.). According to the Köppen classification and the modifications thereof proposed by García (2004), the climate is warm subhumid (AW₀), with a mean temperature of 24.5° C, and a mean annual precipitation of 1,100 mm. The soil is dystric cambisol, with a sandy and crumbly texture and a highly acid pH (4.67) (Tosquy-Valle *et al.*, 2020).

Crop establishment

The collected material was established by hand on October 15, 2021, and the last general cutting was carried out on August 8, 2024. To prepare the microsilages, 45-cm tall mature grass was cut at 45 days after the last cutting.

Preparation of microsilages

At 45 days after the homogeneous cutting, microsilages were prepared in 20-L buckets. The grass was cut into 2-3 cm strips to make management and compacting easier. In addition to the amount of urea for the treatments (0, 2, 3, and 5%), the fresh weight of the grass was estimated for every dose (silos). Urea was uniformly, carefully, and homogeneously distributed. The resulting mix was placed in the silos and was then compacted. The air was expelled to create ideal anaerobic conditions. The silo was hermetically sealed to prevent the passage of air and to encourage lactic acid fermentation. After 35 days, the silos were opened to evaluate the quality of the silage (appearance, smell, and texture). Afterwards, the silage samples were collected, prepared, and sent to the Fogasa lab in Aguascalientes, where they were subjected to a proximate chemical analysis.

Nutritional composition

Dry matter (DM) and moisture (MOI)

Pangola grass was dried in an oven at 105 °C for 24 h to determine its DM and MOI. Both parameters were calculated based on the weight before and after the drying process (AOAC, 2005).

Crude protein (CP)

The CP content was analyzed through the Kjeldahl method. The sample was digested in sulfuric acid. Subsequently, it was neutralized and titrated to determine total nitrogen. The result was multiplied by a 6.25 factor to determine the amount of protein (Montegiove *et al.*, 2021).

Ether extract (EE)

The EE percentage was determined through the Soxhlet method. Hexane ether was used as solvent to extract lipids from the sample. Subsequently, the solvent was evaporated and the fat residue was weighted to estimate its ratio in the silage (AACC, 2009; Rybicka *et al.*, 2021).

Crude fiber (CF)

CF was determined through acid and alkaline digestion. Soluble components were eliminated and the fiber residue was measured (AOAC, 2019).

Digestibility

Neutral detergent fiber (NDF)

The method proposed by Van Soest *et al.* (1991) was used to measure the total fraction of the cell wall of the forage, including its cellulose, hemicellulose, and lignin. This

parameter was used to evaluate the amount of fiber which can be potentially digested by the rumen, because high levels can reduce the willing consumption of food.

Acid detergent fiber (ADF)

ADF was determined through digestion in an acid detergent solution. Hemicellulose was eliminated, leaving only cellulose and lignin. The efficiency with which ruminants digest silage was determined based on the less digestible fiber amount (Van Soest *et al.*, 1991).

Non-fiber carbohydrates (NFC)

NFCs are the energy fraction of soluble carbohydrates that is not available in food, excluding structural fiber. It was indirectly calculated based on the balance of dry matter components, using the following formula:

$$NFC = 100 - (CP + EE + NDF + Ashes)$$

Total digestible nutrients (TDN)

TDN was estimated based on the total energy contribution of the food (DM %). In the case of silage and forages, TDN was determined based on the ADF content, using the formula suggested by the NRC (2001).

Net energy for lactation (NEl) in Mcal/Kg

A predictive equation was applied to directly estimate NEl:

$$NEl = a + b \times CP + NDF$$

The coefficients (*a*, *b*) vary according to the type of feed and were determined for dairy cattle requirements (NRC, 2001).

Net Energy for Maintenance (NE_m) in Mcal/Kg

The following equation was used to determine NE_m:

$$NE_m = a + b \times CP + d \times NDF$$

The coefficients (*a*, *b*, *d*) were adjusted by the type of feed, using the NRC requirement tables for dairy cattle (NRC, 2001).

Net energy for gain (NE_g) in Mcal/Kg

The following equation was used to determine NE_g:

$$NE_g = a + b \times CP + d \times NDF$$

The coefficients (*a*, *b*, *d*) were adjusted by the type of feed, using the NRC cattle tables (NRC, 2001).

Statistical analysis

A completely randomized design (CRD) was used for the experiment, with four treatments (0%, 2%, 3%, and 5% urea) and three repetitions per treatment, resulting in 12 experimental units. The data were subjected to an analysis of variance (ANOVA) to evaluate the effect of the treatments on the nutritional and energy variables of silage. When significant differences ($p < 0.05$) were detected, Tukey's honest significant difference test was applied to identify contrasts between treatments. Additionally, the Pearson correlation coefficient was estimated to explore relations between bromatological and energy variables. SAS v. 9.4 (SAS, 2014) was used for all statistical analysis and the figures were developed using GraphPad Prism v. 8 (2019).

RESULTS AND DISCUSSION

Nutrient composition

Figure 1a shows the DM and MOI behavior, reporting an increase in dry matter content with the 5% urea treatment. CP recorded the same behavior, unlike EE (Figure 1b). Finally, Figure 1c shows that the fiber values (CF, NDF, and ADF) significantly diminish as the urea level increases. This phenomenon could be related to a higher degradation of the cell wall under treatments with more urea.

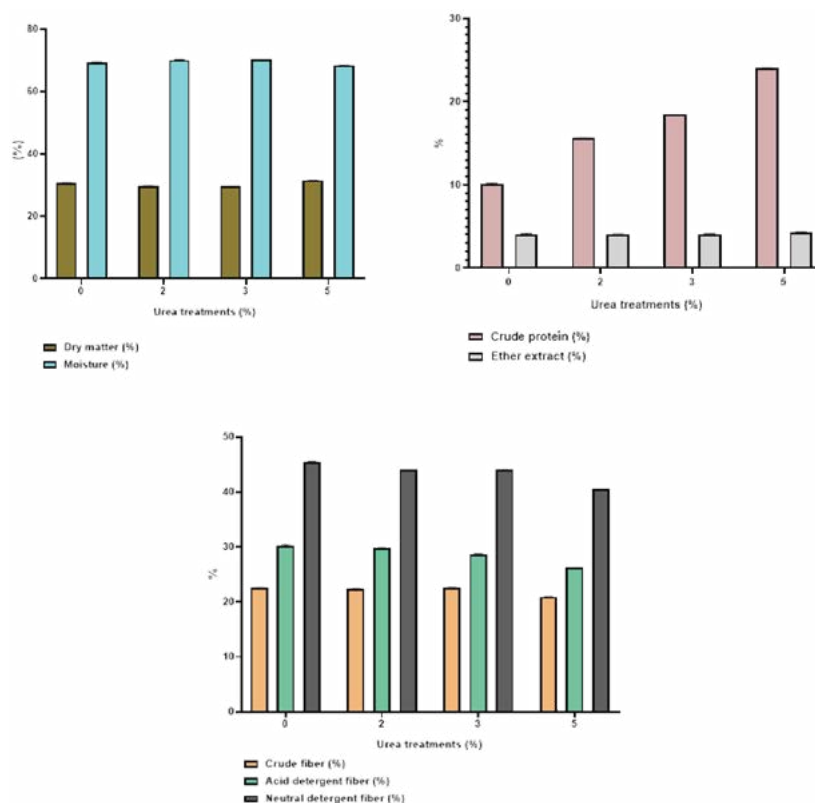


Figure 1. Behavior of the nutrient composition of pangola grass microsilos. a) Dry matter and moisture behavior (%). b) Crude protein and ether extract behavior (%). c) Crude fiber, neutral detergent fiber, and acid detergent fiber behavior (%), in pangola grass microsilages with the addition of various urea levels (0, 2, 3, and 5%).

The interpretation of the nutritional content of pangola grass microsílages treated with 2, 3, and 5% urea shows significant differences in all the evaluated variables ($p < 0.05$ or $p < 0.01$) (Table 1). In conclusion, the urea level impacts the nutritional parameters.

Figure 2 (NFC and TDN behavior) shows that the NFC content diminishes as the urea dose increases. Meanwhile, TDN had a slightly increasing trend, which reached its peak

Table 1. Analysis of the nutritional content of pangola grass microsílages, treated with 2, 3, and 5 % urea.

Variable	Control	Urea treatments			EEM
		2	3	5	
MS	30.7 ^b	29.8 ^c	29.5 ^c	31.6 ^a	0.02
HUM	69.3 ^b	70.1 ^a	70.4 ^a	68.4 ^c	0.02
PC	10.1 ^d	15.6 ^c	18.4 ^b	24 ^a	0.02
EE	4.1 ^b	4.0 ^b	4.0 ^b	4.3 ^a	0.01
FC	22.5 ^{ab}	22.4 ^b	22.6 ^a	20.9 ^c	0.01
FDA	30.2 ^a	29.8 ^a	28.7 ^b	26.2 ^c	0.05
FDN	45.5 ^a	44 ^b	44 ^b	40 ^c	0.01
CNF	32.3 ^a	28.3 ^b	25.4 ^c	23.0 ^a	0.05
TDN	85.05 ^c	85.2 ^c	86.0 ^b	87.6 ^a	0.03
ENI	1.9 ^c	1.9 ^c	1.9 ^b	2.0 ^a	0.00
ENm	2.1 ^d	2.1 ^c	2.1 ^b	2.2 ^a	0.00
ENg	0.8 ^c	0.9 ^c	1.01 ^b	1.19 ^a	0.00

DM (MS): dry matter (%). MOI (HUM): moisture (%). CP (PC): crude protein (%). EE: ether extract (%). CF (FC): crude fiber (%). ADF (DFA): acid detergent fiber (%). NDF (FDN): neutral detergent fiber (%). NFC (CNF): non-fiber carbohydrates. TDN: total digestible nutrients. NEI (ENI): net energy for lactation (Mcal Kg). NEm (ENm): net energy for maintenance (Mcal Kg). NEg (ENg): net energy for lactation (Mcal Kg). SEM (EEM): standard error of the mean. Tukey ($p = 0.05$).

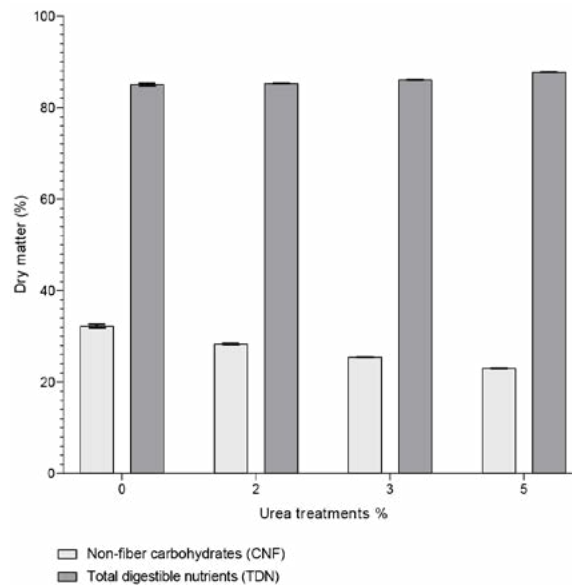


Figure 2. Behavior of non-fiber carbohydrate (NFC) and total non-digestible nutrients (TNDF) in pangola grass microsílages, with the addition of different urea levels (0, 2, 3, and 5%).

with the 5% urea treatment. Data suggest that urea modifies the energy composition of silage, reducing soluble sugars, while improving the total digestibility of forage.

Net energies (NEI, NEm, and NEg)

Figure 3 (net energy behavior) shows that NEI, NEm, and NEg consistently increase in microsilages as the urea concentration increases, reaching its peak with the 5% urea treatment. This behavior reflects an improvement on the energy value of microsilages using that treatment.

The correlation coefficients for the bromatological and energy variables of the microsilages (Table 2) show positive correlations between CP and the following energy fractions: NEI ($r=0.97$), NEm ($r=0.97$), and NEg ($r=0.91$). These results suggest that an increase in CP directly improves the energy available for the silage. Likewise, CP had negative correlations with ADF ($r=-0.93$), NDF ($r=-0.94$), and NFC ($r=-0.99$); therefore, adding urea reduces the fiber fraction and increases the CP quality of the forage. The fiber fractions (ADF) and energy (TDN) variables likewise had negative relationships ($r=-1.00$)—an inverse relationship that was to be expected. Correlations were statistically significant ($*p<0.05$ and $**p<0.01$).

Nutritional content

Table 1 shows that the nutritional content of microsilages treated with different urea levels (0, 2, 3, and 5%) (Table 1) point out significant changes in the evaluated variables with regard to the control. Unlike the 2% and 3% urea treatments, the 5% treatment caused a significant increase in DM. Given its high hygroscopic nature, urea tends to combine with the free water found in silages, consequently reducing moisture (Figure 1a) (Gutiérrez *et al.*, 2022).

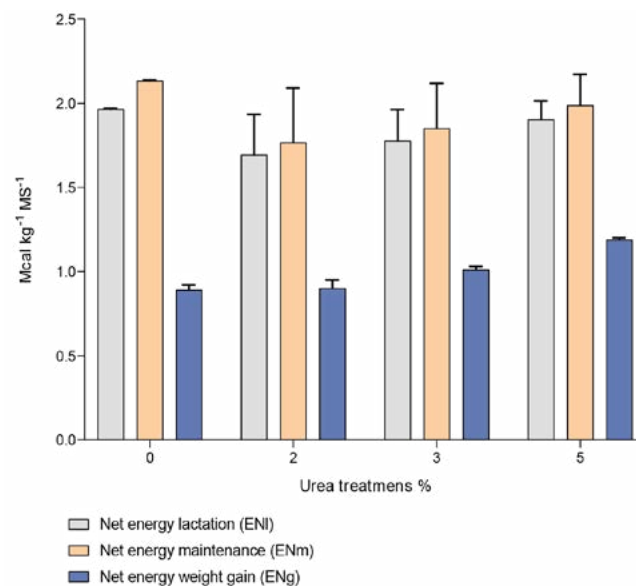


Figure 3. Behavior of net energy for lactation (NEI), net energy for maintenance (NEm), and net energy for weight gain (NEg), in pangola grass microsilages with the addition of different urea levels (0, 2, 3, and 5%).

Table 2. Correlation coefficients of the nutritional content of pangola grass microsilage, at the Instituto Tecnológico Superior de Juan Rodríguez Clara (spring-summer cycle 2023).

	HUM	PC	EE	FC	FDA	FDN	CNF	TDN	ENL	ENM	ENG
MS	-1.00	0.36	0.74	-0.85	-0.65	-0.63	-0.23	0.65	0.62	0.62	0.61
	<.0001	0.24	0.01	0.00	0.02	0.03	0.47	0.02	0.03	0.03	0.03
HUM		-0.36	-0.74	0.85	0.65	0.63	0.23	-0.65	-0.62	-0.62	-0.61
		0.24	0.01	0.00	0.02	0.03	0.47	0.02	0.03	0.03	0.03
PC			0.63	-0.79	-0.93	-0.94	-0.99	0.93	0.94	0.94	0.91
			0.03	0.00	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
EE				-0.80	-0.77	-0.73	-0.58	0.77	0.77	0.77	0.77
				0.00	0.00	0.01	0.05	0.00	0.00	0.00	0.00
FC					0.91	0.94	0.69	-0.91	-0.90	-0.90	-0.87
					<.0001	<.0001	0.01	<.0001	<.0001	<.0001	0.00
FDA						0.96	0.88	-1.00	-1.00	-1.00	-0.97
						<.0001	0.00	<.0001	<.0001	<.0001	<.0001
FDN							0.88	-0.96	-0.96	-0.96	-0.92
							0.00	<.0001	<.0001	<.0001	<.0001
CNF								-0.88	-0.90	-0.90	-0.87
								0.00	<.0001	<.0001	0.00
TDN									1.00	1.00	0.97
									<.0001	<.0001	<.0001
ENL										1.00	0.97
										<.0001	<.0001
ENM											0.97
											<.0001

DM (MS): dry matter (%). MOI (HUM): moisture (%). CP (PC): crude protein (%). EE: ether extract (%). CF (FC): crude fiber (CF%). ADF (FDA): acid detergent fiber (%). NDF (FDN): neutral detergent fiber (%). NFC (CNF): non-fiber carbohydrate (%). TDN: total digestible nutrients (%). NEI (ENL): net energy for lactation (Mcal Kg). NEM (ENM): net energy for maintenance (Mcal Kg). NEg (ENG): net energy for gain (Mcal Kg)
 ** Highly significant (p<0.01). * Significant (p<0.05).

The urea increase resulted in a significant CP increase, reaching the highest level with the 5% treatment (24%). In contrast, control recorded a 10.1% increase (Figure 1b). This increase is caused by the direct contribution of non-protein nitrogen (NPN) made by urea, which favors microbial growth and the conversion of NPN into protein (Wesseh and Ayantunde, 2021). According to Rodríguez-Romero (2004), lipids found in the soil are structural compounds which are not significantly influenced in any way by the addition of NPN. However, the slight advantage recorded by the 5% urea treatment is attributed to the indirect effect of an enhanced preservation of nutrients in the silage, resulting from a more efficient control of the fermentation process (Figure 1b). Likewise, a greater urea dose resulted in a reduction of fiber fractions (ADF and NDF), indicating an improvement in digestibility. For their part, Rashid *et al.* (2025) researched the impact of wheat straw treated with the urea of Azi-Kheli buffalos and recorded significant improvements in CF digestibility. This phenomenon is caused by the action of urea in the cell wall of plants. The decomposition of urea into ammonia causes a partial split of the lignocellulosic bonds,

reducing the structural fiber content of forage. This effect is more noticeable with higher urea levels (*e.g.*, 5%): a higher decrease in NDF and ADF enhances the digestibility of silage (Figure 1c) (Nascimento *et al.*, 2023). Net energies increased in proportion with the urea level, reaching the highest levels with the 5% urea treatment (Figure 3). Therefore, the energy availability of the feed improves as a result of a greater solubilization of the fiber components and the increase in CP—key factors for animal performance (NRC, 2001; Elizondo-Salazar 2020).

Figure 2 shows that the gradual increase of CP results in a decrease of non-fiber carbohydrates (NFC). These changes in the chemical composition are associated with a better digestibility of the silage and, therefore, a significant increase of the net energy value for lactation (NEI), maintenance (NEM), and gain (NEG). With 5% urea, NEI, NEM, and NEG reached 2.0, 2.2, and 1.19 Mcal/kg, respectively (Table 1). Villalba *et al.* (2021) pointed out that a lower NFC content, combined with a better balance of fiber and protein, optimizes ruminal fermentation and improves the efficient use of nitrogen in sustainable livestock systems. Meanwhile, López-Herrera *et al.* (2017) mentioned that grass and banana silages combined with nitrogen additives recorded a higher CP content and a lower fibrous fraction, favoring the fermentation and energy of the forage. For their part, Rodríguez-Chacón *et al.* (2014) reported that adding urea and treacle not just increased CP content, it also favored NFC and increased the total digestible nutrients (TDN), positively impacting the energy content of the silage (Table 2). Meanwhile, negative correlations between ADF, NDF, and NEI, NEM, and NEG indicate that the reduction of structural fiber significantly contributes to a better energy use in the forage and strengthens its nutritional value.

The results indicated that adding urea to the pangola grass silage significantly modified its chemical and energy composition (Table 2). Adding 5% urea increased CP and reduced the ADF and NDF levels, potentially improving the nutritional quality of the forage. Sánchez-Santillán *et al.* (2022) reported a CP increase and a NDF reduction in papaya waste and hay treated with urea and treacle, resulting in a higher silage digestibility.

Regarding the nutritional variables, a negative ratio was found between MOI and DM (Table 2), indicating that, when MOI decreases, DM increases. This factor is fundamental to preserve microsilages. Likewise, Krüger *et al.* (2020) pointed out that a higher DM content is associated with lower NDF and ADF content, which favors the nutritional quality of silage. Meanwhile, a positive and highly significant correlation between CP and energy parameters was recorded—*i.e.*, a higher CP content improves the efficiency of energy production, which is fundamental for lactating and fattening animals. These results match the findings of Calixto Junior *et al.* (2017) and Pineda-Cordero *et al.* (2016), who reported improvements in the nutritional and fermentation quality of grass silages combined with different treacle and urea additives.

These correlations strengthen the trend: CP had a negative correlation with ADF ($r = -0.9302$) and NDF ($r = -0.94246$). These results are similar to those reported by Krüger *et al.* (2020). Meanwhile, Juárez *et al.* (2009) recorded that a decrease in the fibrous fraction of tropical grasses had a positive association with the increase of energy and digestibility. In addition, CP and the energy fractions of the silage had a positive correlation: NEI 0.94%, NEM 0.94%, and NEG 0.91%. These results indicate an improvement of the protein content

and a better use of the energy available for the animals. Araújo *et al.* (2023) and Zamir *et al.* (2020) reported similar results: digestibility and energy significantly increased in the silages treated with urea and treacle. A positive ratio between CP and ADF ($r=0.91005$) and NDF ($r=0.94434$) indicates that these fractions are closely linked in the forage. These results match the findings of Pineda *et al.* (2016), who pointed out that a high CF content usually involves a higher ratio of structural fibers, which reduces the digestibility of the silage. Meanwhile, a negative correlation between NDF and the energy fractions (NEI, NEm, and NEg) suggests a lower fiber content, associated with a higher availability of energy in the feeding. These results also match the findings of Medina *et al.* (2008), who evaluated a supplementation for milking cows prepared with millet silage, urea, and other additives. Overall, adding of urea is a viable and economic strategy to improve the quality of pangola grass silage, particularly in tropical regions where forages have high levels of fiber and low levels of proteins.

CONCLUSIONS

Adding urea to pangola grass during silage is a highly effective strategy to improve the nutrient content of forages, because it increases crude protein and net energy content, optimizing food quality. Meanwhile, the positive impact of adding 5% urea on the silage nutritional profile requires further research. The said impact should be reflected on the performance of lactating and fattening animals. Livestock should be fed with a balanced and energetic diet, where urea plays a key role to reduce dependency on expensive protein supplements, during food shortage. In addition, urea could help producers in tropical areas to obtain a high-quality forage for their livestock.

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