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Fábio Júnior Araújo Silva,

Monaliza Mirella de Moraes Andrade Cordeiro

Chemical composition and digestibility of hays of three grasses ammonized with urea

 Rayanna Campos Ferreira ^[1]

 Francisco Bezerra Neto ^[2]

 Fabíola Carla de Almeida ^[3]

 Aderbal Marcos de Azevêdo Silva ^[4]

 Maiza Araújo Cordão ^[5]

 José Moraes Pereira Filho ^[6]

[1] rayannacf@gmail.com. Programa de Pós-Graduação em Fitotecnia, Universidade Federal Rural do Semi-Árido (UFERSA), Campus Mossoró, Brazil

[2] bezerra@ufersa.edu.br. Departamento de Ciências Agrônomicas e Florestais, Universidade Federal Rural do Semi-Árido (UFERSA), Campus Mossoró, Brazil

[3] fabiolavet9@gmail.com. Universidade Federal de Campina Grande (UFCG), Campus Patos, Brazil.

[4] aderbal@pq.cnpq.br

[6] jmoraais@cstr.ufcg.edu.br. Departamento de Medicina Veterinária, Universidade Federal de Campina Grande (UFCG), Campus Patos, Brazil

[5] maizacordao@hotmail.com. Departamento de Medicina Veterinária, Faculdade Nova Esperança (FAMENE), João Pessoa, Brazil

ABSTRACT: This study aimed to evaluate the effect of ammoniation with urea on the chemical composition and in vitro digestibility of *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses. The experimental design was completely randomized, with a 3×2 factorial treatment (hay of three species, treated with or without urea) and five replications. The contents of dry matter, crude protein, neutral detergent fiber, acid detergent fiber, mineral matter, lignin, total carbohydrates and digestibility were evaluated in the grass (*R. repens*, *A. setifolia* e *E. indica*). The treatment with urea affected ($p < 0.05$) most of the variables analyzed in grass hays. Hay treated with urea showed an increase in crude protein levels and the highest value was observed for the hay of *Eleusine indica* grass. *Rhynchelytrum repens* grass hay presented the highest fiber value in acid detergent, with and without urea. An increase in vitro dry matter digestibility was observed after the use of urea. It is concluded that ammoniation with 5% urea improves the nutritional values of hay in *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses, increasing the levels of protein, minerals and digestibility.

Keywords: *Aristida setifolia*; chemical treatment; *Eleusine indica*; hay production; *Rhynchelytrum repens*.

Composição química e digestibilidade de fenos de três gramíneas amonizadas com ureia

RESUMO: Este estudo objetivou avaliar o efeito da amonização com ureia na composição química e digestibilidade in vitro de fenos de gramíneas *Rhynchelytrum repens*, *Aristida setifolia* e *Eleusine indica*. O delineamento experimental foi inteiramente casualizado, com tratamento fatorial 3×2 (feno de três espécies, tratado com ou sem ureia) e cinco repetições. Foram avaliados os teores de matéria seca, proteína bruta, fibra em detergente neutro, fibra em detergente ácido, matéria mineral, lignina, carboidratos totais e digestibilidade das gramíneas (*R. repens*, *A. setifolia* e *E. indica*). O tratamento com ureia

afetou ($p < 0,05$) a maioria das variáveis analisadas nos fenos das gramíneas. Os fenos tratados com ureia apresentaram aumento nos níveis de proteína bruta, e o maior valor foi observado para o feno do capim *Eleusine indica*. O feno de capim *Rhynchelytrum repens* apresentou o maior valor de fibra em detergente ácido, com e sem ureia. Observou-se aumento na digestibilidade *in vitro* da matéria seca após o uso da ureia. Conclui-se que a amonização com 5% de ureia melhora os valores nutricionais do feno em gramíneas *Rhynchelytrum repens*, *Aristida setifolia* e *Eleusine indica*, aumentando os teores de proteína, minerais e digestibilidade.

Palavras-chave: *Aristida setifolia*; *Eleusine indica*; produção de feno; *Rhynchelytrum repens*; tratamento químico.

1 Introduction

The economic development of the semiarid region in the Brazilian northeast depends on the increase in livestock productivity levels, as the edaphoclimatic conditions in that region do not support an economy based on agriculture in almost the entire area being livestock the region's natural vocation. In this context, the agricultural sector in the semiarid region deserves special attention, due not only to its economic potential but also especially to its geographical expression.

The Northeast region occupies an area of 1,548,000 km², approximately 70% of which is considered semi-arid (900,000 km²), constituting the drought polygon (SILVA; ANDRADE, 2008). The Northeastern semi-arid is characterized by the heterogeneity of natural conditions, such as climate, soil, topography, vegetation and socioeconomic characteristics, being climate the striking feature, mainly due to the existence of a pluviometric regime separating two very distinct seasons: a short, rainy season of 3 to 5 months, called "winter", and a long, dry season called "summer", which lasts from 7 to 9 months.

Food production in the semi-arid region is difficult, as most of the time it needs irrigation, increasing production costs (CASTRO, 2018), and animal products it is the biggest challenge in semiarid regions, mainly due to the variability and uncertainties in climate. In ruminant exploration systems, it is essential to produce roughage during the rainy season and to use during the dry season.

Hay made from grasses in the semi-arid region is alternative for feeding herds in the period of forage scarcity. They are foods with certain energy value, low protein value, reduced digestibility and low voluntary consumption, probably due to the high levels of the cell wall (BERCHIELLI; PIRES; OLIVEIRA, 2011).

Searching for solutions, the use of roughage of low nutritional value, submitted to some type of physical, chemical and biological treatment, can be a viable alternative to remedy the lack of good roughage during the drought period, considering a rational exploration in order to promote the sustainable development of the region, in order to ensure the productions indispensable for socioeconomic development. According to Cruz and Silva (2016), the chemical treatment of low digestibility roughage is viable, since it decreases the fibrous fraction of vegetables, increasing the consumption of the animals and improving their performance.

Among these bulky, three types of grass developed in the semiarid region are widely accepted by ruminants and deserve special attention, mainly for their drought resistance. *Rhynchelytrum repens* grass is originally from South Africa, from the *Poaceae* family, which presents both annual and perennial habits, grows well in sandy, poor and acidic soils, withstanding dry conditions well, and for many, it is considered a weed (ANASTÁCIO *et al.*, 2012). The *Aristida setifolia* grass has thin and erect stems, reaching up to 90 cm in height, narrow and delicate leaves, narrow and loose panicles and lumps provided with a tripartite border. Its presence in soils of better agricultural aptitude usually suggests a stage of sharp impoverishment of fertility (SILVA *et al.*, 2000). *Eleusine indica* grass is widespread in almost the entire Brazilian territory, it is a kind of annual cycle, autogamous, and can produce up to 40,000 seeds per plant (KISSMANN, 2007).

Given the production of dry matter from these grasses in a natural and spontaneous way, the study aimed to measure the effect of ammoniation with urea on the chemical composition and the *in vitro* digestibility of the dry matter of the hay from *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses.

2 Material and methods

The study was carried out at the Rural Health and Technology Center of the Federal University of Campina Grande, on the farm used by the Research Center for the Development of the Semi-Arid Tropic, belonging to the campus of Patos, Paraíba (PB) State, Brazil, and the laboratory analyzes were performed at the Animal Nutrition Laboratory, in the Rural Health and Technology Center. The region's climate is classified as hot and dry, with two well-defined seasons, one rainy, from January to May, and the other dry, from June to December, with the accumulated rainfall period in 2021 of 783.7 mm (AESA, 2022).

2.1 Grasses studied and ammoniation

The collection of herbaceous plants *R. repens*, *A. setifolia* and *E. indica* was carried out in a 12-hectare caatinga area, with a diversity of native plants in which they stood out, where they were collected in the reproductive phase. The cut was made above five cm from the ground level. All the cut material was taken to a shed, crushed in a mincer, spread on plastic canvas, exposed to the sun during the day and night, covered with canvas tarpaulin for two to four days, being turned every hour in the first six hours and every six hours in the remaining time, until it reaches the hay point. Upon reaching the hay point, the grasses were placed in nylon bags and stored in a dry and ventilated place, as the methodology described by Cóser and Pereira (2001).

The treatments consisted of hay from *R. repens*, *A. setifolia* and *E. indica* grasses ammoniated with urea and without urea (not treated – NT). For each experimental plot, with or without urea, seven kg of hay were used, and in the treatment with urea a concentration of 5.0% NH₃ (ammonia) (ROTH, 2008) in relation to dry matter was used and in the control treatment distilled water was applied, simulating the same experimental condition, both spray-applied.

2.2 Chemical treatment of grasses

In the preparation of liquid urea NH₃, 375 g of urea were weighed and diluted in 1125 mL of water, a ratio of one part of urea to three parts of water (1:3) and then applied over each type of hay by sprinkling, homogeneously, in order to ensure that all material had contact with the solution. Immediately after application, all the material of each hay was homogenized and placed in five polyethene bags (1.400 kg each, to facilitate the homogenization of the sample) and later closed with adhesive tape so that all the ammonia included remained inside the bag, which was stored and covered during 15 days. Subsequently, the bags were opened and aerated for six hours to allow the release of excess ammonia. It was observed that the hays treated with urea were brown in colour and visually no fungi were found.

2.3 Determination of chemical composition and in vitro digestibility of dry matter

For the determinations of chemical composition, samples of hay from each bag were collected, taken to the laboratory and then milled with a 20 mesh sieve placed in the appropriate recipient, subsequently being determined the dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude energy (CE), ether extract (EE), mineral matter (MM), lignin (LIG), following the methodology described by Detmann *et al.* (2012).

The in vitro digestibility of nutrients was performed by an indirect method to evaluate the disappearance of dry matter of grass hays, using the technique of residual residue from microbial digestion. It was performed by the production curve, making it possible to estimate the degradation rates of the soluble (soluble sugars and readily available starch) and insoluble (cellulose, hemicellulose) fractions, after this process, the samples are taken to the oven to pass the methods of analysis of protein, where it is indirectly determined from the value of total nitrogen (N), which is determined by a method that is based on three steps: digestion, distillation and titration.

For ether extract, extraction was performed with solvents such as ether in a Soxhlet-type device, with the capacity for six tests (model MA487/6/250), followed by removal by evaporation or distillation of the solvent used. The resulting fractions are called soluble in a neutral detergent and are composed of protein, non-protein nitrogen (NPN), lipids, pigments, sugars, organic acids and pectin, and NDF (comprised basically of cellulose), fiber-bound N, hemicellulose and lignin. The ADF is the least digestible portion of the forage cell wall by the rumen microorganisms, consisting almost entirely of lignocellulose, that is, lignin and cellulose. Therefore, the proportion of hemicellulose is determined by the difference between NDF and ADF.

Values of the content of digestible total nutrients (*DTN*) were estimated from the chemical composition of the hays using the equation of Weiss, Conrad and St. Pierre (1992), where:

$$DTN = DNFC + DCP + DEE + DNDF - 7 \quad (1)$$

$$DNDF = 0.98 \times (100 - (NDFa + CP + EE + Ashes)) \quad (2)$$

$$DCP = CP \times e^{(-1.2(NIAD/CP))} \quad (3)$$

$$DEE = (EE - 1) \quad (4)$$

$$DNDF = 0.75 \times (NDFa - LIG) \times \left(\frac{LIG}{NDFa} \right)^{0.667} \quad (5)$$

which refer to *DNFC*: digestible non-fibrous carbohydrates); *DCP*: digestible crude protein; *DEE*: digestible ether extract; *DNDF*: digestible neutral detergent fiber; *LIG*: lignin; *NIAD*: nitrogen insoluble in acid detergent; *NDFa*: neutral detergent fiber adjusted for ashes. And the fractions nitrogen insoluble in neutral detergent (*NIND*), indigestible protein in neutral detergent (*IPND*) and indigestible protein in acid detergent (*IPAD*), following the methodology described by Detmann *et al.* (2012).

In vitro digestibility of dry mass of the hays from treated grasses were carried out according to the methodology of Tilley and Terry (1963), adapted for the artificial incubator Daisy II Model D200 (ANKOM® Technology Corp. Fairport, New York, USA), in the Animal Nutrition Laboratory from Rural Health and Technology Center of the Federal University of Campina Grande, in Patos (PB).

To obtain ruminal fluid, two Santa Inês sheep, castrated males, with approximately one year of age and an average weight of 40 kg, fistulated in the rumen, were used. The animals were adapted to the diet with hay from grasses treated with urea and untreated grasses, for 15 days. For the collection of ruminal fluid samples, an 18-hours liquid and solid fasting was performed. With the aid of a vacuum pump, 600 mL of fluid were collected, placed in a thermos, identified and heated with water at 39-40 °C, maintaining the appropriate temperature for the rumen microorganisms. Then, the fluid was immediately taken to the laboratory, where incubation was started in the rumen fermenter jars. In each jar, 20 bags made of non-woven fabric -100 g/m² were placed, each with samples weighing 1 g of grass hay, with the buffer solution, for 48 hours.

Subsequently, for 24 hours, the samples were subjected to acid digestion, by administering the pepsin jars in 6N HCl (hydrochloric acid). After this period, the fermentation bags were removed and washed with cold water until it was clear. Then, the bags with the residue were placed in the forced ventilation oven at 55 °C, for 24 hours.

Rumen-degraded protein, non-rumen-degraded protein and non-degradable rumen-digestible protein were also evaluated, which were calculated according to the Agricultural and Food Research Council (AFRC, 1993). Ruminal protein degradation is often described by a first-order mass action model. An important feature of this model is that it considers that the crude protein (*CP*) of foods is made up of multiple fractions, which differ greatly in terms of degradation rates, and that the ruminal disappearance of the protein is the result of two simultaneous activities: degradation and passage (NRC, 2001), performed by the *in vitro* technique on the equipment artificial incubator Daisy II Model D200 (ANKOM® Technology Corp. Fairport, New York, EUA).

The *PDR* and *PNDR* values can be calculated directly through the association of the *CP* fractions obtained, with their respective passage rates (*Kd*) and digestion. In this way, the *PDR* (%PB) can be calculated as:

$$A + B1 (KdB1 / [KdB1 + Kp]) + B2 (KdB2 / [KdB2 + Kp]) + B3 (KdB3 / [KdB3 + Kp]) e a PNDR = 1 - PDR \quad (6)$$

where: *A*: soluble fraction-NPN; *B1*: rapidly degraded fraction in the rumen; *B2*: insoluble fraction, with intermediate degradation rate in the rumen; *B3*: insoluble fraction slowly degraded in the rumen.

2.4 Statistical analysis and design

A completely randomized design was used, in a factorial arrangement 3×2, with three hays of forage species submitted or not to ammoniation with urea, with five replications. The data obtained were subjected to analysis of variance; for the significant sources of variation, the Tukey test was applied at the 5% of probability ($p < 0.05$), using the software SAS (SAS INSTITUTE, 2004).

3 Results

This section presents the results obtained from the research carried out.

3.1 Chemical composition

There was no interaction effect ($p > 0.05$) between nutrients and studied grasses. The chemical composition of *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses with and without ammoniation showed significant differences ($p < 0.05$) for the variables dry matter (DM), crude protein (CP), mineral matter (MM), neutral detergent fiber adjusted for ash and protein (NDFap), digestible neutral detergent fiber (DNDF), acid detergent fiber (ADF), digestible crude protein (DCP) and digestible total carbohydrates (DTC) (Table 1).

Table 1 ►
Average values (g/kg) of chemical composition (Part 1) of grass hays *Rhynchelytrum repens*, *Aristida setifolia* e *Eleusine indica*, not treated (NT) and treated with urea (5% DM).
Source: research data (1989)

Nutrients*		Hays (g/kg)			CV (%)
		Grasses			
		<i>Rhynchelytrum repens</i>	<i>Aristida setifolia</i>	<i>Eleusine indica</i>	
DM	NT	928.50Aa**	925.2Aba	919.2Ba	0.35
	Urea	900.90Bb	877.00Cb	914.40Aa	
CP	NT	55.90Bb	45.80Bb	102.80Ab	2.91
	Urea	135.10Ca	157.70Ba	290.00Aa	
MM	NT	80.30Bb	40.80Ca	110.00Ab	5.21
	Urea	95.50Ba	58.70Ca	306.20Aa	
NDFap	NT	715.65Aba	779.65Aa	677.45Ba	3.03
	Urea	739.30Aba	787.20Aa	565.45Cb	
DNDF	NT	378.90Aa	465.50Aa	383.50Ab	6.89
	Urea	398.90Aa	432.20Aa	233.00Ba	
ADF	NT	549.90Aba	500.80Bb	434.20Cb	1.91
	Urea	563.10Aa	535.80BCa	521.00Ca	
DCP	NT	54.00Bb	44.70Bb	100.00Ab	2.94
	Urea	132.70Ca	156.00Ba	286.70Aa	
DTC	NT	471.10Aba	523.55Aa	441.20BCa	5.96
	Urea	379.55Ab	387.30Ab	167.80Bb	

* DM (dry matter), CP (crude protein), MM (mineral matter), NDFap (neutral detergent fiber adjusted for ash and protein), DNDF (digestible neutral detergent fiber), ADF (acid detergent fiber), DCP (digestible crude protein), DTC (digestible total carbohydrates). Averages followed by the same letters suggest they do not differ, by the Tukey test at 5% probability, with $n = 5$. The lower-case letters compare the averages between the columns and the upper-case letters compare them between the lines.

It was observed that the highest DM value was obtained in *Rhynchelytrum repens* hay not treated with urea, 928.50 g, and the lowest, 877.00 g, was obtained with *Aristida setifolia* hay treated with urea (ammonized).

As for the CP content, it is observed that the effect of the factors was dependent and there were differences ($p < 0.05$) between treated and untreated with urea for the three hays, verifying that the CP values of the urea-treated hays were superior to those obtained in untreated hays.

For MM, there was an interaction effect with differences between treated and untreated hays ($p < 0.05$) for *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses, the highest value observed for *Eleusine indica* grass hay, treated and untreated.

The NDFap of hays showed an effect ($p < 0.05$) of ammoniation and was dependent. The *Aristida setifolia* grass hay had the highest NDFap value, with and without urea, and the lowest value occurred for *Eleusine indica* grass hay. After the treatment, *Aristida setifolia* and *Rhynchelytrum repens* grasses showed an increase of 4.6 and 27.9 g, respectively, and a reduction of 150 g for *Eleusine indica* hay.

The DNDF showed interaction effect between factors ($p < 0.05$). The *Aristida setifolia* grass hay had the highest DNDF value, with and without urea, and the lowest value was for *Eleusine indica* grass hay.

It is observed that the ADF showed an effect ($p < 0.05$) on the treatments and was dependent. The hay of *Rhynchelytrum repens* grass presented the highest value of ADF, with and without urea, and the lowest value occurred for the hay of *Eleusine indica* grass. After treatment, it was observed among the hays that the *Aristida setifolia* and *Eleusine indica* grasses increased ($p < 0.05$) by 3.5 and 8.68%, respectively, while the *Rhynchelytrum repens* grass hay did not vary, maintaining up, however, with the highest average.

The DCP showed an effect ($p < 0.05$) on the treatments and was dependent. The hay of *Eleusine indica* grass showed the highest DCP value, with and without urea, and the lowest value occurred for *Rhynchelytrum repens* grass hay (Table 1). After treatment, it was observed that the hay of *Eleusine indica*, *Aristida setifolia* and *Rhynchelytrum repens* grasses increased ($p < 0.05$) by 18.66; 11.13 and 7.87%, respectively.

The DTC had an effect ($p < 0.05$) on the treatments and was dependent. The hay from *Aristida setifolia* grass showed the highest value, with and without urea, and the hay from *Eleusine indica* grass had the lowest average. After treatment, it was observed among the hays that the grass *Aristida setifolia* and *Eleusine indica* decreased ($p < 0.05$), with a difference of 13.62 and 27.34%, respectively.

Ammoniation did not affect ($p > 0.05$) the levels of NDF, ADFap, NIND, LIG, EE, DEE and CE from the hay of *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses when treated with urea (Table 2). For the variables digestible non-fibrous carbohydrates (DNFC), nitrogen insoluble in acid detergent (NIAD), indigestible protein in acid detergent (IPAD) and digestible total nitrogen (DTC), there were differences when treated with urea ($p < 0.05$).

Table 2 ▶

Average values (g/kg) of the chemical constituents (Part 2) hays of not treated (NT) *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica*, treated with urea (5% DM).
Source: research data (1989)

Nutrients*	Hays (g/kg)			Treatments averages	
	Grasses			NT	Urea
	<i>Rhynchelytrum repens</i>	<i>Aristida setifolia</i>	<i>Eleusine indica</i>		
NDF	812.00A	809.30A	742.50B	784.40A	791.50A
DNFC	23.10A	-1.30A	-25.90A	59.10A	-61.90B
ADFap	495.50A	431.70AB	334.20B	412.20A	428.80A
NIND	3.10B	3.50B	7.00A	4.40A	4.70A
NIAD	1.80B	1.10C	2.60A	1.60B	2.10A
IPND	19.50B	21.60B	44.20A	27.60A	29.20A
IPAD	11.30B	7.10C	16.20A	10.10B	12.90A
LIG	69.30A	60.40AB	46.80B	57.70A	60.00A
EE	62.70A	62.20A	59.30A	63.90A	58.80A
DEE	58.10A	56.60A	51.40A	58.90A	51.70A
CE	41.90 A	45.30A	40.90A	44.60A	40.70A
DTN	506.80AB	537.20A	484.50B	533.80A	485.10B

* NDF (neutral detergent fiber), DNFC (digestible non-fibrous carbohydrates), ADFap (acid detergent fiber adjusted for ash and protein), NIND (nitrogen insoluble in neutral detergent), NIAD (nitrogen insoluble in acid detergent), IPND (indigestible protein in neutral detergent), IPAD (indigestible protein in acid detergent), LIG (lignin), EE (ether extract), DEE (digestible ether extract), CE (crude energy), DTN (digestible total nitrogen). Averages followed by the same letters suggest they do not differ, by the Tukey test at 5% probability, with $n = 5$. The lower-case letters compare the averages between the columns and the upper-case letters compare them between the lines.

The values of EE, DEE and CE did not affect the main independent factors, not differing ($p > 0.05$) among the treatments, showing the hay treatment of *Rhynchelytrum repens*, and *Aristida setifolia* and *Eleusine indica* indicate grasses with urea did not influence the values of these variables.

As for the NDF, there was no interaction effect ($p > 0.05$) and the 5% urea dose was not enough to promote a reduction in NDF levels, in comparison to *in natura* hay.

There was no interaction with DNFC and the effect of the main species and the treatment were independent. Analyzing the effect of ammoniation, a significant difference is observed ($p < 0.05$), with the use of urea decreasing the DNFC. The hay of the grasses treated with urea obtained an average of -61.90 g and the untreated grass hay obtained 59.10 g.

Regarding to ADFap, the effects of the species and treatment were independent, observing that the ammoniation did not interfere with the concentration of ADFap. Analyzing the effect of the treatment, it is observed no differences ($p > 0.05$); however, observing the grass hays *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica*, it is verified that the content of ADFap of the grass *Rhynchelytrum repens* was greater than that of *Aristida setifolia* and *Eleusine indica*, and these ones were similar.

Regarding the nitrogen insoluble in neutral detergent (NIND), the effects of species and treatment were independent. Analyzing the effect of the treatment, it is observed that there was no significant difference ($p > 0.05$), however, the evaluated grass hay certifies that the NIND of the grass hay *Aristida setifolia* and *Rhynchelytrum repens* was similar and these showed NIND content inferior to that of *Eleusine indica*.

Regarding the NIND, the effects of the species and the treatment were also independent. There was an effect of ammoniation on the content of NIND ($p < 0.05$), hay from grasses treated with urea obtained an average of 2.10 g and the untreated obtained 1.60 g (Table 2). As for forages, the *Eleusine indica* grass is differentiated, showing the highest content of NIND ($p < 0.05$), followed by *Rhynchelytrum repens* grass.

The effects of species and the treatments of IPND and IPAD were independent. Analyzing the effect of the treatments, with respect to IPND, it is observed that they did not show a significant difference ($p > 0.05$); however, observing the hays (ammoniated or not) of the three species of grasses, it is verified that the content of IPND of the grass *Eleusine indica* was greater (44.20 g) ($p < 0.05$) than that of the *Aristida setifolia* (21.60 g) and *Rhynchelytrum repens* (19.50 g), which were similar to each other ($p > 0.05$).

Checking the effect of treatments, with respect to IPAD, it is observed a significant difference ($p < 0.05$), obtaining greater averages when the grasses are treated with urea. Likewise, considering only the species of the three types of grass, there were differences ($p < 0.05$), showing that the IPAD of the grass hay *Eleusine indica* was greater (16.20%) than that of *Rhynchelytrum repens* (11.30%) and *Aristida setifolia* (7.10%) grasses.

As for the content of LIG, the effects of the species and treatment were independent. Analyzing the treatment effect, it is observed that there was no significant difference ($p > 0.05$). However, among the species, for the grass hays, it is observed that hay of *Rhynchelytrum repens* grass obtained greater content than that of *Eleusine indica* ($p < 0.05$), which was similar to the content of LIG from the *Aristida setifolia* grass ($p > 0.05$).

Regarding DTN, the effects of the species and treatment were independent and it is observed that there was an effect of ammoniation of hays, and the hays treated with urea obtained an average of 485.10 g and the not treated obtained 533.80 g.

3.2 In vitro digestibility of grass hay

In vitro digestibility was influenced in a dependent and independent way, observing an increase ($p < 0.05$) in the levels of degraded protein in the rumen and of digested undegraded protein in the rumen (Table 3).

Table 3 ►
Average values (%) of DPR and of DUPR from the grass hays of *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* treated and not treated (NT) with urea, *in vitro*.
Source: research data (1989)

Nutrients*		HAYS		
		Grasses		
		<i>Rhynchelytrum repens</i>	<i>Aristida setifolia</i>	<i>Eleusine indica</i>
DPR	NT	66.47Bb	45.21Cb	70.10Ab
	Urea	80.47Aa	84.09Aa	91.91Aa
DUPR	NT	5.46Ba	24.22Aa	3.83Ba
	Urea	0.38Aa	0.08Ab	0.47Aa

* DPR (degraded protein in the rumen), DUPR (digested undegraded protein in the rumen). Averages followed by equal letters indicate that they do not differ from each other, by the Tukey test at 5% probability, with $n = 5$. The lower-case letters compare the averages between the columns and the upper-case letters compare the average between the lines.

Regarding the DPR content of hays, it is observed effect of interaction among the species and the treatments, where the greatest value, the treated and the untreated, occurred for the hay of *Eleusine indica* grass, with a content of 91.91%. Ammoniation increased DPR of the three hays assessed, with no differences between them, and the greatest effect of ammoniation on DPR was observed in *Aristida setifolia* grass, with an increase of 46.24%. For DUPR, there was a difference ($p < 0.05$) among the untreated grass hays, and the *Aristida setifolia* showed the greatest value, while the hays of the grasses treated with urea did not show differences ($p > 0.05$).

The hays of the grasses *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* obtained greater averages ($p < 0.05$) of degradability of ruminal dry matter (DRDM), *in vitro* digestibility of dry matter (IVDDM), *in vitro* digested protein (IVDP), *in vitro* digestibility of neutral detergent fiber (IVDNDF), *in vitro* digestibility of acid detergent fiber (IVDADF) than untreated ones (Table 4).

Table 4 ►
Average values (%) of DRDM, IVDDM, IVDP, IVDNDF and IVDADF for the hays of *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses treated and not treated (NT) with urea.
Source: research data (1989)

Nutrients*	HAYS			TREATMENT AVERAGES	
	Grasses			NT	Urea
	<i>Rhynchelytrum repens</i>	<i>Aristida setifolia</i>	<i>Eleusine indica</i>		
DRDM	51.24B	41.12C	61.70A	44.85B	57.83A
IVDDM	53.42B	42.46C	60.44A	46.70B	57.52A
IVDP	75.87B	76.97AB	82.51A	71.66B	85.23A
IVDNDF	46.30B	34.52C	56.01A	37.90B	53.33A
IVDADF	46.24B	34.53C	52.54A	36.14B	52.73A

* DRDM (degradability of ruminal dry matter), IVDDM (*in vitro* digestibility of dry matter), IVDP (*in vitro* digestibility protein), IVDNDF (*in vitro* digestibility of neutral detergent fiber), IVDADF (*in vitro* digestibility of acid detergent fiber). Averages followed by the same letters in each factor suggest they do differ, by Tukey test at 5% probability, with $n = 5$.
Source: research data

The average content of DRDM, IVDDM, IVDP, IVDNDF and IVDADF of the hays from *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses did not show interaction effect, being the effects of species and treatment independent. Analyzing the effect of ammoniation on the hay species of *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses, it is verified a significant difference, and the average content of DRDM, IVDP, IVDDM, IVDNDF and IVDADF from the grass *Eleusine indica* was greatest and the that of the grass hay was the smallest.

When comparing the effect of treatments with and without urea, it is verified that DRDM treated with urea was 57.83% and not treated obtained 44.85%. A significant increase which allows saying the use of urea increases the rumen degradability. There is a significant increase in the levels of fiber digestibility when ammoniation with urea in hays is performed.

4 Discussion

This section presents the discussions of the results obtained in the research as indicated in section 3.

4.1 Chemical composition of grasses

The results obtained in the chemical treatment with urea suggested that ammoniation improves the chemical composition of the grass hays, which was also related by Garcez *et al.* (2020) when observed that the use of chemical treatments may improve the quality of native forage, an alternative to optimizing its use in ruminant feeding systems. These results show that the three types of grass studied can be used for hay production, reducing losses in storage, caused by excess moisture, and preventing the proliferation of fungi.

The values of crude protein improved after the treatment with ammoniation, which is important, as after the treatment with urea the three hays studied showed values of crude protein above 7%, which is suitable for the rumen microorganisms to act, justifying the beneficial effect derived from the addition of urea to the hays (FARIA *et al.*, 2008; BRANDÃO *et al.*, 2011). This is very important for the semi-arid regions to find alternatives to increase the contents of protein of low quality bulky, therefore being a great advantage of the treatment, since protein is one of the main nutrients in the constitution of animal feed.

This increase is due to the addition of non-protein nitrogen, via ammoniation, which is in agreement with Brandão *et al.* (2011) and Faria *et al.* (2008). As noted by Garcez *et al.* (2020), who found that ammoniation contributed to an increase in CP levels in cutting heights, with an average increase of 20% in protein content with each increase in urea doses, due to the incorporation of non-protein nitrogen to the material. This effect is significant for the treated grasses and makes them totally viable for handling and subsequent consumption by the animals, increasing their performance.

The grass hay *Eleusine indica* obtained a higher value of mineral matter (MM), where possibly the ammoniation caused a greater breakdown in hemicellulose, reducing the NDF content and increasing the MM content, as ammoniation benefits the nutritional value through the action of alkaline hydrolysis, improves the nutritional value of bulky by providing nitrogen and reducing the fiber content (GARCEZ *et al.*, 2014). This result is quite positive for the *Eleusine indica* grass, as it is one of the grasses that most develops in semi-arid areas, integrating a large part of forage, mainly for ruminants.

The fiber content was influenced by the addition of urea, increasing the amount in the grasses. According to Garcez *et al.* (2020), ammoniation with urea improves the use of the fibrous fraction by hydrolyzing the ester-like covalent bonds between the lignin and the cell wall, in addition to providing non-protein nitrogen in the form of ammonia. An increase in the levels of ADF is not desirable because it decreases the passage rate in the rumen as it is an indigestible fiber. Faria *et al.* (2008), evaluating the sisal mucilage under different levels of ammoniation, found levels of ADF close to 32.5% with 4% urea and elevation of ADF after four weeks of storage. The acid detergent fiber is considered poorly digestible, therefore the stability of *Rhynchelytrum repens* grass is considered a positive effect for this grass.

Energy values are not influenced by ammoniation, however, it is very important for protein and fiber, in agreement with the results obtained by Silva *et al.* (2008) and disagreeing with those observed by Brandão *et al.* (2011). This result was already expected because ammoniation does not interfere with the energy ingredient. According to Buxton and Redfearn (1997), most of the digestible energy comes from grass fiber, which can be justified in this work because the NDF content has not been modified either.

The neutral detergent fiber (NDF) was not altered, an unexpected fact, however, this may have occurred because the percentage of urea inclusion was not sufficient. Decreases

in the NDF contents of ammoniated materials have been observed by several authors (OLIVEIRA *et al.*, 2009).

This was an unexpected effect since normally ammoniated materials should have lower cell wall contents, due to the solubilization of their components, however, this decrease in the NDF contents may be assigned to the partial solubilization of the hemicellulose fraction of the cell wall (VAN SOEST; FERREIRA; HARTLEY, 1984). However, Reis *et al.* (2003) reported that the addition of urea did not alter this forage component, corroborating the results obtained in the present experiment. According to Silva *et al.* (2016), the use of chemical treatments in roughage improves the composition of food, mainly in the levels of fiber and protein, making it an option for the use of foods with high fiber content.

The negative values of DNFC, for urea-treated grass hays, probably occurred due to the ammonia volatilization, during the EE, NDF and MM analyses, overestimating the concentration of these nutrients in the sample, according to Detmann *et al.* (2012) and Oliveira Júnior (2017). Another possibility is that the elevation of the mineral matter, previously discussed, contributed to the reduction of the carbohydrates content, since the mineral matter is subtracted from the carbohydrates calculations, a fact also observed by Romão *et al.* (2013), when they studied the fractionation of carbohydrates and ruminal degradability of sugarcane treated with calcium oxide. It is important to consider that responses to the ammoniation of bulky vary depending on several factors, such as the moisture content of the treated material and the chemical characteristics of the plant (VAN SOEST, 1994).

The increase in the levels of NIAD in treated hays, according to Buettner *et al.* (1982), may be due to the aminolysis reaction, since the dosed nitrogen was retained in the insoluble portion in acid detergent (cellulose and lignin). According to Fernandes *et al.* (2009), both the values of NIAD and IPND tend to increase with the addition of ammonia or urea, which would be harmful to animals, since these fractions are not degraded or are very slowly degraded in the rumen, however, these authors found no significant difference in the treatments of this study.

Fernandes *et al.* (2002), evaluating the changes in chemical composition and *in vitro* digestibility of dry matter of *Brachiaria decumbens* Stapf hay treated with anhydrous ammonia or urea, observed values of NIAD and IPND, respectively, were 0.28% and 0.34% in the untreated *Brachiaria decumbens* hay, 0.33% and 0.45% in the *Brachiaria decumbens* hay treated with 3% anhydrous ammonia and 0.29% and 0.39% in the *Brachiaria decumbens* hay treated with 5% urea, being close to those obtained in this experiment. The values of NIAD and NIND have been used by some researchers as indicators of the number of ammoniacal nitrogen compounds, covalently bound to the compounds of the fibrous fraction of forages treated with ammonia.

Lignin is an indigestible component, however, with ammoniation it can be solubilized, making it available for use in digestion (SILVA *et al.*, 2016). Ammoniation contributes to improving the chemical composition, providing more nutrients for animal consumption. Garcez *et al.* (2014), studying the effect of alkaline treatments by ammoniation with urea and by NaOH (sodium hydroxide) and Ca(OH)₂ (calcium hydroxide) of the leaflets hay from babassu pindoba, observed that the alkaline treatments improve the chemical composition, with a reduction in the contents of hemicellulose, lignin and insoluble nitrogen and an increase in the protein content, proving that the ammoniation in hays presents favorable results for its use.

The hays of *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses treated with urea obtained higher averages of DRDM, IVDP, IVDDM, IVDNDF and

IVDADF than not treated ones, and it is probably due to the addition of urea. According to Tonucci (2006), the chemical treatment with urea, as a source of ammonia, it provides an increase in the digestibility of fibrous materials, due to the increase in the total nitrogen content of the material, to its effect on the cell wall, breaking the ester bonds between the components of the cell wall and phenolic acids and the depolymerization of lignin. It should also be considered that ammoniation promotes an increase in the content of fermentable carbohydrates, which results in increased digestibility and consumption of treated fibrous materials (VAN SOEST, 1994).

The fiber contents are determinant for the quality of food, mainly for ruminants, constituting part of the growth of ruminal microorganisms. Other than that it is important these aliments contain more non-fibrous carbohydrates, as they are represented by the more quickly degraded fractions in the digestive tract (BERCHIELLI; PIRES; OLIVEIRA, 2011). A degree of importance is established for the *Aristida setifolia* grass, which is extremely important for the region, insofar as it is resistant to the critical periods of the year and one of those that are present at these times to contribute to the food supply.

4.2 *In vitro* digestibility of dry matter

The degradability of dry matter in the rumen, *in vitro* digestibility of dry matter and *in vitro* digestibility of protein increased in significant numbers through the use of urea in grasses, in comparison with untreated grasses, suggesting that ammoniation increases the efficiency of ruminal microorganisms, which leads to high degradability. According to Cruz and Silva (2016), the use of low-quality roughages treated with urea increases the total consumption of digestible organic matter, so that the proper relationship between the degradable protein in the rumen and the digestible organic matter, present in the diet of the animal, allows increasing consumption and digestibility. This increase in consumption may be associated with the supply of nitrogen in adequate amounts to maintain microbial activity, due to changes in the rumen environment, which result in better productive and reproductive performance of the animals.

According to Silva *et al.* (2016), ammoniation is a way to take advantage of bulky food in an advanced stage or to improve the conditions of hay and silage, constituting alternatives to optimize livestock production systems, with chemical treatments being a way out, as is the case of ammoniation and hydrolyzation, which improve fiber degradability, making it more digestible.

In addition, there is a possibility that ammonia promotes the breakdown of hydrogen bonds between cellulose molecules, promoting their partial solubilization (VAN SOEST, 1994), as well as hydrogen bonds between cellulose and hemicellulose molecules, allowing faster hydration and efficient cell wall, facilitating the access of ruminal microorganisms and increasing digestion.

The increase in *in vitro* dry matter digestibility of ammoniated material is due to the action of ammonia on the constituents of the cell wall. Ammonia can act on hemicellulose molecules, promoting the breaking of bonds and partial solubilization of this component, facilitating the action of ruminal microorganisms, improving the use of grasses with higher fiber values and providing more nutrients. This percentage of increase in the digestibility of fiber in neutral detergent (53.33%) compared to not treated with urea (37.90%) in grasses is extremely important, as it indicates that more nutrients will be absorbed, promoting greater performance and, consequently, positive values for animal production.

According to Fernandes *et al.* (2002), the observed increase of in vitro dry matter digestibility is probably due to changes in the chemical composition of the fibrous fraction, occurring a decrease in the content of NDF, providing a greater supply of readily digestible carbohydrates for rumen microorganisms. Associated with these changes, the increase in available nitrogen provides better development conditions for rumen bacteria, which also increases the digestibility of forage, but no decrease in NDF contents was observed in this experiment.

5 Conclusion

Ammoniation with 5% urea provides an improvement in the nutritive values of the hay of *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses, increasing the levels of protein, minerals and digestibility. It is recommended to add 5% NH₃ (based on DM), in the treatment of hay from *Rhynchelytrum repens*, *Aristida setifolia* and *Eleusine indica* grasses, with positive effects on the chemical composition of native herbaceous plants and forage digestibility for sheep.

More research should be carried out with different proportions of urea, for ammoniation in the hay of native herbaceous plants, observing consumption and digestibility, which may reflect in positive performances for ruminants.

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