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# Nutritive value of Tifton 85 hay ammoniated with urea

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**ABSTRACT.** The effect of ammonization was evaluated with urea at doses of 1, 2, 3 and 4%, on the basis of dry matter (DM) on Tifton 85 hay harvested at an advanced stage of development in the periods of 30 and 45 days, through analysis of dry matter, ash, ether extract (EE), neutral (NDF) and acid (ADF) detergent fiber, NDF corrected for ash and protein (NDFap), cellulose, lignin, crude protein (CP), acid detergent insoluble nitrogen (ADIN), fractionation of carbohydrates and *in vitro* gas production (Parameters: $V_{f1}$, $k_1$, $V_{f2}$, $k_2$ and L). The variables DM, EE, NDF, ADF, lignin, cellulose, NDFap, fraction A + B1, fraction B2 and C of carbohydrates, and the $V_{f1}$ and $V_{f2}$ parameters were influenced by treatments ($p < 0.05$). While DM, NDF, NDFap, fraction A + B1, C, CP and $V_{f2}$ showed linear effects according to the dose, NIDA and hemicellulose presented quadratic effects. $V_{f1}$ and $V_{f2}$ were also influenced by the treatment period. The ammonization with 2.7% urea on the basis of DM promotes improvement of Tifton 85 hay treated for 45 days, observed after derivation of the regression equation and evaluation of the nutritional and kinetic degradation *in vitro*.

**Keywords:** chemical treatment; gas production; low quality forages.

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## Introduction

Haymaking is a process that consists of the dehydration of the forage, in order to conserve and use it during the period of pasture shortage. Some factors such as: forage species and climatic conditions directly interfere with dehydration efficiency, and the faster the dehydration, the greater the preservation of the nutrients of the plant. In Brazil, Tifton 85 grass (*Cynodon spp.*) is one of the most used forage plants for haymaking, and if harvested at the right time, it has a high nutritional value, providing a great amount of nutrients to the animals (Bow & Muir, 2010). In tropical conditions, the season of higher forage production is characterized by high rainfall. In this period, it is common to delay mowing the forage for haymaking due to adverse weather conditions, to avoid losses occurring through the leaching of nutrients promoted by rainfall. However, the nutritive value of forage decreases as the plant ages, due to the reduced crude protein content and increase in NDF, ADF and lignin contents (Dean, Adesogan, Krueger, & Littell, 2008).

There are several methods that optimize the use of low quality forages (Hassan, Nisa, Shahzad, & Sarwar, 2011), such as chemical treatments using urea, which have been used with the aim of improving the nutritional characteristics of forage (Nguyen, Wanapat, Khejornsart, & Kongmun, 2012), allowing the use of a plant harvested at advanced stages of development for use in animal feeding. In addition to the increase in the digestibility and dry matter intake (Nisa, Sarwar, Shahzad, & Hassan, 2007), the crude protein content also increases due to non-protein nitrogen (García-Martínez, Albarrán-Portillo, Castelán-Ortega, Espinoza-Ortega, & Arriaga-Jordán, 2009), caused by ammonia on the structure of the cell wall of the hay (Morais et al., 2017). Forage quality, dose of urea applied, moisture content and treatment duration are the main factors affecting the ammoniation process (Ferreira & Zanine, 2013). Thus, the determination of urea doses (Gunun, Wanapat, & Anantasook, 2013), as well as the treatment period, potentiate the use and reduces the cost of the technique.

This study was realized to evaluate the nutritional value of Tifton 85 hay harvested at an advanced stage of development, ammoniated with urea doses and two treatment periods.

## Material and methods

The study was conducted at the Department of Animal Nutrition and Pastures of the Institute of Animal Science, Federal Rural University of Rio de Janeiro (DNAP/IZ/UFRRJ), located in the city of Seropédica, State of Rio de Janeiro, Brazil. The experiment was approved by the Research Ethics Committee of UFRRJ, with the number 23083.010666/2014-11.

The treatments consisted of a combination of four urea doses (1, 2, 3 and 4%) in two treatment periods (30 and 45 days) and control (non-ammoniated hay). The experimental design was completely randomized in double factorial arrangement, with an additional treatment 4 x 2 + 1 (four doses of urea in two treatment periods and one control), with 4 replicates per treatment.

Tifton 85 hay harvested at an advanced stage of maturity (60 days of growth) was used. The urea dose for each treatment was diluted in 180 mL water to raise the moisture content of the forage up to 30% and applied to 1 kg hay packed in a polyethylene bag. After the application of urea, the bags were hermetically sealed and stored in the Forage Science Laboratory of the DNAP, for the established periods (30 and 45 days).

After opening the bags, a period of 2 days was waited for elimination of the excess of ammonia not incorporated into the material. Then, a sample of each replicate was collected and taken to a forced air oven at 55°C for 72h. After the pre-drying, the hay was processed in a Wiley mill with a 1 mm sieve and sent for analysis of chemical composition and in vitro gas production.

Dry matter, ash and ether extract were determined by the Goldfish extraction method with ether and total nitrogen by the Kjeldahl method, as described in Association Official Analytical Chemist (AOAC, 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose and acid detergent insoluble nitrogen (ADIN) were determined from the ADF residue according to Van Soest, Robertson, and Lewis (1991). Hemicellulose was determined by the difference between NDF and ADF. Carbohydrate fractionation was performed according to the methodology proposed by Sniffen, O'Connor, Van Soest, Fox, and Russell (1992), in which the fractions A + B1 are represented by non-fiber carbohydrates, as follows: $\mathrm{NFC} = 100 - (\%\mathrm{CP} + \%\mathrm{EE} + \%\mathrm{NDFap} + \%\mathrm{Ash})$, and total carbohydrates are: $\mathrm{TC} = 100 - (\%\mathrm{CP} + \%\mathrm{EE} + \%\mathrm{Ash})$. The fraction C was determined by the percentage of lignin multiplied by the factor 2.4 and the fraction B2 was obtained by the difference between NDFap minus Fraction C, where NDFap corresponds to the neutral detergent insoluble fiber corrected for ash and protein.

*In vitro* incubations were performed according to the methodology described by Goering and Van Soest (1970), where approximately 0.5 g sample was used in 40 mL reduced culture medium and 10 mL ruminal *inoculum*. The culture medium and the reducing solution were prepared as described by Hall and Mertens (2008). The ruminal inoculum was obtained from six adult castrated male sheep with ruminal fistula fed on corn silage and soybean meal. The pressure and volume measurements were performed at times 0; 2; 4; 6; 8; 10; 12; 16; 20; 30; 36; 48; 72 and 96 hours after addition of the ruminal *inoculum*, and cumulative gas production profiles were obtained using a non-automated device as described by Abreu et al. (2014). It was used the biphasic model composed by the monomolecular and GNG1 models (Vieira, Tedeschi, & Cannas, 2008), according to the equation:

$$V_t = V_{f1}x\,(1 - \exp\,(-k_1 t)) + V_{f2}x(1 - (\delta^N\ \exp(-\mathrm{k}_2\mathrm{t}) + \exp\,(-\lambda\mathrm{t}) \sum_{\mathrm{i}=1}^{\mathrm{N}-1} \frac{(1 - \delta^{\mathrm{N}-1})(\lambda\mathrm{t})^{\mathrm{i}}}{\mathrm{i}!}$$

In which: $V_t$ is cumulative gas production over time (t, h); $V_{f1}$ and $V_{f2}$: are gas productions of fast and slow digestion substrates, respectively (mL/0.1 g DM); $k_1$ and $k_2$: are mean digestion times of fast and slow digestion substrates, respectively (% $h^{-1}$); N: is a integer positive number that represents the order of time dependency; $\lambda$ is the asymptote of the rate of preparation for digestion (% $h^{-1}$), and $\delta = \lambda/(\lambda - k)$ is a constant. The models used to estimate the kinetics of gas production were adjusted to the degradation profiles using the NLMIXED procedure of Statistical Analysis System (SAS, 2013) (version 9.4). Data were tested by analysis of variance, and in case of significance, a regression analysis was performed for urea doses and the means were compared by Tukey's test for treatment periods using the ExpDes.pt package from the software R (Ferreira, Cavalcanti, & Nogueira, 2014).

## Results and discussion

There was a difference when comparing the control and the combination of factorial treatments between urea doses for DM and EE, treatment period effect for EE and linear decreasing dose effect for DM (Table 1).

**Table 1.** Dry matter (DM), Ether extract (EE), Crude Protein (CP) and acid detergent insoluble nitrogen (ADIN), of Tifton 85 hay ammoniated with urea in two treatment periods (30 and 45 days).

| Urea, % DM | Variables | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DM[a] | | Ash[b] | | EE[b] | | CP[b] | | ADIN[b] | |
| | 30 | 45 | 30 | 45 | 30 | 45 | 30 | 45 | 30 | 45 |
| Control | 834.2A | | 34.7 | | 5.3B | | 90.1B | | 1.6 | |
| Mean (Factorial) | 789.3B | | 35.0 | | 6.1A | | 139.7A | | 1.8 | |
| 1 | 813.3 | 792.2 | 31.6 | 33.9 | 5.7 | 6.5 | 83.2 | 91.3 | 2.0a | 1.6b |
| 2 | 793.5 | 782.1 | 34.0 | 34.8 | 5.8 | 6.2 | 127.3 | 122.7 | 1.7a | 1.7a |
| 3 | 808.3 | 762.8 | 36.9 | 39.2 | 5.4 | 6.3 | 122.7 | 121.8 | 1.8b | 2.2a |
| 4 | 791.2 | 771.0 | 36.3 | 33.5 | 6.2 | 6.4 | 243.4 | 205.4 | 1.8a | 1.7a |
| Mean | 801.6 | 777.0 | 34.7 | 35.4 | 5.8b | 6.4a | 144.2 | 135.3 | 1.8 | 1.8 |
| CV, % | 1.7 | | 11.8 | | 10.4 | | 18.3 | | 14.8 | |
| | p value | | | | | | | | | |
| Regression | L* | | NS | | NS | | L* | | NS | Q** |
| $R^2$ | 0.79 | | - | | - | | 0.89 | | - | 0,56 |
| Equation | $\hat{Y} = 777 - 10.5x$ | | | | | | $\hat{Y} = 38 + 40.7x$ | | $\hat{Y} = 0.7 + 0.9x - 0.16x^2$ | |
| Control *vs* Factorial | * | | NS | | * | | * | | NS | |
| Period *vs* Urea | NS | | NS | | NS | | NS | | ** | |
| Period | NS | | NS | | * | | NS | | NS | |

Means followed by different uppercase letters in the same column and different lowercase letters in the same row are different by Tukey's test at 5% probability. NS: Non-significant. *p < 0.01, **p < 0.05; L: linear effect; Q: quadratic effect. Expressed in: [a]g $kg^{-1}$ hay; [b]g $kg^{-1}$ DM.

There was increasing linear effect of urea dose for CP and quadratic effect for ADIN, with the dose of urea that promoted the absolute maximum points obtained by derivation of the regression equation, 2.81% according to the quadratic regression. Moreira Filho, Alves, Vale, Moreira, and Rogério (2013) ammoniated corn straw with 3% urea and observed a reduction in DM content; this reduction was attributed to the water added during ammoniation, necessary to reduce the dry matter content to 70%. The linear reduction in DM with increasing doses of urea is explained by the formation of ammonium hydroxide, promoting the hydrolysis of the cell wall.

There was difference between the control and the combination of urea treatments for NDF, ADF, lignin, cellulose, and linear decreasing effect of urea dose for NDF and ADF (Table 2).

There was interaction effect between dose and treatment period and linear decreasing dose effect for lignin. Treatment period effect was observed for cellulose. Interaction between treatment period and urea dose for hemicellulose was detected, as well as increasing quadratic effect with absolute maximum point at 2.74% urea dose, obtained by derivation of the regression equation.

**Table 2**. Neutral detergent fiber (NDF), Acid detergent fiber (ADF), Hemicellulose (Hem) expressed in g $kg^{-1}$ DM, of Tifton 85 hay ammoniated with urea in two treatment periods (30 and 45 days).

| Urea, % of DM | Variables | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NDF | | ADF | | Lignin | | Celullose | | HEM | |
| | 30 | 45 | 30 | 45 | 30 | 45 | 30 | 45 | 30 | 45 |
| Control | 748.3A | | 394.3B | | 82.2B | | 303.4A | | 337.3 | |
| Mean (Factorial) | 710.9B | | 414.3A | | 93.1A | | 289.3B | | 355.0 | |
| 1 | 728.4 | 730.3 | 430.4 | 433.2 | 104.5a | 96.6b | 290.4 | 299.3 | 337.8a | 354.6a |
| 2 | 714.3 | 724.8 | 418.5 | 425.3 | 96.9a | 92.4a | 289.6 | 288.6 | 345.3a | 346.5a |
| 3 | 715.5 | 726.6 | 419.6 | 411.1 | 97.3a | 87.5b | 284.3 | 297.7 | 377.4a | 358.7a |
| 4 | 682.3 | 665.2 | 395.7 | 380.5 | 98.6a | 70.6b | 273.8 | 290.4 | 343.5b | 376.2a |
| Mean | 710.1 | 711.7 | 416.1 | 412.5 | 99.3a | 86.8b | 284.5[b] | 294.0[a] | 351.0 | 359.0 |
| CV, % | 1.1 | | 15.3 | | 5.4 | | 3.9 | | 4.5 | |
| | P – Value | | | | | | | | | |
| Regression | L* | | L* | | NS | L* | NS | | Q** | NS |
| $R^2$ | 0.71 | | 0.90 | | NS | 0.88 | - | | 0,57 | - |
| Equation | $\hat{Y} = 752 - 16.5x$ | | $\hat{Y} = 448 - 13.9x$ | | $\hat{Y} = 107 - 8.2x$ | | | | $\hat{Y} = 287 + 56.0x - 10.2x^2$ | |
| Control *vs* Factorial | * | | ** | | * | | ** | | NS | |
| Period *vs* Urea | NS | | NS | | * | | NS | | ** | |
| Period | NS | | NS | | * | | ** | | NS | |

Means followed by different uppercase letters in the same column and different lowercase letters in the same row are different by Tukey's test at 5% probability. NS: Non-significant. *p < 0.01, **p < 0.05; L: linear effect; Q: quadratic effect.

There was interaction between treatment period and urea dose for ADIN, with the highest mean with application of 3% urea during 45 days of treatment. Oji, Etim, and Okoye (2007) ammoniated corn straw and observed an increase in CP content between 75% and 95%. This increase in the CP content was attributed to the greater availability of nitrogen from the hydrolysis of urea, which is retained on the forages (Sarwar, Nisa, Hassan, & Shahzad, 2006). Carvalho et al. (2006) ammoniated sugarcane bagasse with 0; 2.5; 5.0 and 7.5% urea and reported an increasing quadratic effect for the ADIN content with the increase in the dose applied. The increase in ADIN content in forages subjected to ammoniation is attributed to the binding between part of the applied nitrogen and the components of the cell wall (Guedes et al., 2006). From the nutritional point of view, this increase is undesirable since this nitrogen source is not available to the ruminant.

The study of (García-Martínez et al., 2009; Ramírez, Aguilera-Gonzalez, Garcia-Diaz, and Núñez-González (2007)) evaluated the ammoniation in buffel and bermudagrass hay, and corn straw residue, respectively, and obtained similar behavior to that observed in the present study, regarding those fractions. One of the benefits of the reduction in cellulose contents is the better access of cellulolytic microorganisms to the cell wall (Oji et al., 2007) improving fiber fermentation.

There was a difference between the control and the combination of factorial treatments for NDFap, fraction A + B1, fraction B2 and fraction C of carbohydrates (Table 3).

A linear decreasing effect of urea and interaction between dose and treatment period for NDFap were verified, and the lowest values of NDFap were found when 3 and 4% were applied for 30 days. There was a linear increasing effect for the A + B1 fraction, and decreasing for C fraction, as well as effect of the treatment period for those two variables. Dean et al. (2008) ammoniated Pensacola Bahiagrass and Coastal bermudagrass hay and obtained an increase in the soluble carbohydrate content attributed to the urea treatment that promoted the hydrolysis of the fiber, increasing the content of soluble compounds. The A + B1 fraction of carbohydrates is represented by soluble sugars with rapid degradation by the ruminal microorganisms (Sniffen et al., 1992), and its increase represents an increase in energy source of fast release for the development of these microorganisms.

There was difference between the control and the combination of factor treatments for $V_{f1}$ and $V_{f2}$ (Table 4). In $V_{f1}$, there was an increasing quadratic effect of urea doses, with the absolute maximum point at the application of 1.7% urea, observed by the derivation of the regression equation. For $V_{f2}$, it was observed a linear effect. However, the highest values for both $V_{f1}$ and $V_{f2}$ were found in 45 days.

The action of ammonia on the fiber components, associated with a higher nitrogen supply to the ruminal microorganisms, resulted in a higher production of gas from the soluble fraction ($V_{f1}$) and from the fiber fraction ($V_{f2}$). From the nutritional point of view, the increase in gas production reflects an improvement in the quality of ammoniated hay, since the production of short chain fatty acids (acetate, butyrate and propionate) will be a source of energy for the ruminant.

**Table 3**. Neutral detergent fiber corrected for ash and protein (NDFap), total Carbohydrates (TC), fraction A + B1 (A + B1), fraction B2 (B2) and fraction C (C) of Tifton 85 hay ammoniated with urea in two treatment periods (30 and 45 days).

| Urea, % DM | Variables | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NDFap$^a$ | | TC$^a$ | | A + B1$^b$ | | B2$^b$ | | C$^b$ | |
| | 30 | 45 | 30 | 45 | 30 | 45 | 30 | 45 | 30 | 45 |
| Control | 733.4A | | 868.3 | | 170.2B | | 562.5 | | 198.3B | |
| Mean, Factorial | 706.8B | | 868.6 | | 197.3A | | 509.4 | | 222.8$^a$ | |
| 1 | 724.3a | 717.5a | 878.5 | 869.4 | 186.5 | 186.5 | 538.5a | 530.6a | 250.2a | 230.4b |
| 2 | 702.4a | 708.3a | 869.4 | 866.6 | 201.2 | 194.3 | 500.3a | 514.5a | 230.5a | 222.6$^a$ |
| 3 | 698.4b | 725.4a | 865.5 | 862.3 | 205.3 | 177.3 | 492.3b | 548.3a | 233.4a | 209.3b |
| 4 | 672.3b | 705.8a | 867.3 | 869.7 | 230.6 | 196.4 | 441.4b | 509.4a | 236.7a | 169.5b |
| Mean | 699.4b | 714.3a | 870.2 | 867.0 | 205.9 | 188.6 | 493.1b | 525.7a | 237.7a | 208.0b |
| CV (%) | 1.7 | | 2.7 | | 8.4 | | 5.4 | | 2.6 | |
| | P < value | | | | | | | | | |
| Regression | L* | NS | NS | | L* | | L* | NS | NS | L* |
| $R^2$ | 0.93 | - | NS | | 0.67 | | 0.93 | - | - | 0,88 |
| Equation | $\hat{Y} = 739 - 16.1x$ | | | | $\hat{Y} = 178 + 7.4x$ | | $\hat{Y} = 556 - 29.8x$ | | $\hat{Y} = 225 - 19.7x$ | |
| Control *vs* Factorial | * | | NS | | * | | * | | ** | |
| Period *vs* Urea | ** | | NS | | NS | | * | | * | |
| Period | * | | NS | | * | | * | | * | |

Means followed by different uppercase letters in the same column and different lowercase letters in the same row are significantly different by Tukey's test at 5% probability. NS: Non-significant. *p < 0.01, **p < 0.05; L: linear effect; Q: quadratic effect; Expressed in: $^a$g kg$^{-1}$ dry matter; $^b$g kg$^{-1}$ carbohydrates.

**Table 4**. Gas production parameters[a] of Tifton 85 hay ammoniated with urea in two treatment periods.

| Urea, % DM | Parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $V_{f1}$ | | $k_1$ | | L | | $V_{f2}$ | | $k_2$ | |
| | 30 | 45 | 30 | 45 | 30 | 45 | 30 | 45 | 30 | 45 |
| Control | 8.8B | | 0.166 | | 3.03 | | 16.40B | | 0.013 | |
| Mean (Factorial) | 9.2A | | 0.176 | | 2.9 | | 18.7A | | 0.013 | |
| 1 | 8.9 | 10.1 | 0.166 | 0.178 | 2.95 | 2.78 | 15.32 | 17.87 | 0.013 | 0.015 |
| 2 | 8.8 | 10.4 | 0.166 | 0.180 | 2.83 | 2.65 | 16.34 | 18.86 | 0.015 | 0.014 |
| 3 | 9.1 | 9.5 | 0.164 | 0.188 | 2.90 | 3.51 | 16.70 | 20.85 | 0.013 | 0.014 |
| 4 | 7.6 | 9.5 | 0.192 | 0.176 | 3.10 | 2.70 | 21.31 | 22.50 | 0.011 | 0.010 |
| Mean | 8.6b | 9.9a | 0.172 | 0.181 | 2.9 | 2.9 | 17.4b | 20.0a | 0.013 | 0.013 |
| CV (%) | 6.7 | | 9.5 | | 7.8 | | 5.4 | | 3.0 | |
| | P < Values | | | | | | | | | |
| Regression | Q* | | NS | | NS | | L* | | NS | |
| $R^2$ | 0.99 | | - | | - | | 0.91 | | - | |
| Equation | $\hat{Y} = 9.1 + 0.68x - 0.2x^2$ | | | | | | $\hat{Y} = 14.4 + 1.7x$ | | | |
| Control *vs* Factorial | ** | | NS | | NS | | ** | | NS | |
| Period *vs* Urea | NS | | NS | | NS | | NS | | NS | |
| Period | * | | NS | | NS | | ** | | NS | |

Means followed by different uppercase letters in the same column and different lowercase letters in the same row are significantly different by Tukey's test at 5% probability. NS: Non-significant. *p < 0.01, **p < 0.05; L: linear effect; Q: quadratic effect; [a]$V_{f1}$ and $V_{f2}$: Gas production of fast and slow digestion substrates, respectively (mL 0.1 $g^{-1}$ DM); $k_1$ and $k_2$: Mean digestion time of fast and slow digestion substrates, respectively (% $h^{-1}$); L: Lag time (h).

## Conclusion

The ammoniation with at least 2.7% urea during 45 days of treatment was sufficient to improve the nutritional value of Tifton 85 hay, through the increase in protein content and fiber quality, which resulted in improving the kinetic parameters of fiber fermentation.

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