TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 1243-1249

doi:10.3906/vet-2006-94

Chemical compounds and gas production kinetics of annual ryegrass hay in distinct nitrogen levels and cutting heights

Juliana Reolon PEREIRA[1], Marcela Abbado NERES[1], Itacir Eloi SANDINI[2], Ana Carolina FLUCK[3], Olmar Antônio Denardin COSTA[3,*], Laércio Ricardo SARTOR[3]

[1]Department of Animal Science, State University of the West of Paraná, Marechal Cândido Rondom, PR, Brazil
[2]Department of Agronomy, State University of Midwest Paraná, Guarapuava, PR, Brazil
[3]Animal Science Research Program, Federal Technological University of Paraná, Dois Vizinhos, PR, Brazil

Received: 19.06.2020 • **Accepted/Published Online:** 12.11.2020 • **Final Version:** 18.12.2020

Abstract: The aim of our work was to evaluate the chemical composition and cumulative gas production profiles of annual ryegrass hay through in vitro incubations for two cutting heights and different nitrogen fertilization doses. The experimental design was randomized blocks with split-plots. The nitrogen doses (0, 75, 150, and 225 kg N ha^{-1}) were distributed in the main plot and the subplots. To make the hay, the annual ryegrass was cut at heights of 5 and 10 cm from the ground, with three replications each. In the experimental area, corn was cropped in the summer and annual ryegrass pasture in the winter. We evaluated the nutritional compounds, with fractionation of protein and carbohydrate content as the kinetics of ruminal degradation in the gas production technique in vitro. The carbohydrates decreased linearly with the increasing levels in N, as well as the kinetic parameters. Protein fractions increased with the increase in N. A higher nonfibrous content was obtained at a cutting height of 10 cm. Regardless of the cutting height, nitrogen fertilization increased the crude protein content and decreased the total carbohydrates content, especially the nonstructural carbohydrates. The increase of N in the plant decreased the specific rate of gas production due to the degradation of the soluble fraction of rapid digestion and caused a decrease in gas production of the slow digestion fraction.

Key words: Haymaking, kinetics parameters, *Lolium multiflorum* Lam., winter grasses

1. Introduction

In temperate regions, the use of winter grasses is a strategy to minimize the climatic effects on the production systems of animals on pasture due to their characteristics of adaptation to subtropical climates, as they show peak mass production in cold weather. [1]. Thus, annual ryegrass (*Lolium multiflorum* Lam.) is a winter grass used primarily as pasture, silage, and hay, but also as a good source of protein, and for its several benefits due to its versatility of use. With climate changes, it is not always possible to provide a feed that meets the animals' requirements for long periods, mostly during winter. Thus, an alternative to this problem is the production of hay, a technique that requires proper handling and fertilization to generate a good-quality product. The cutting height of the plant can influence the nutritional values of the hay, as it affects the production of dry matter, lignin, and fiber content [2,3].

Another aspect to be observed is the nitrogen content. Being part of the chemical composition of biomolecules such as ATP (adenosine triphosphate), electron transporters (NADH, NADPH), chlorophyll, proteins, and also some enzymes, nitrogen is an essential element for plants. [4]. Nitrogen fertilization provides gain in forage mass and increased availability of dry matter for animal intake. The optimization of the use of nitrogen through the efficiency of the plant in absorbing what is being supplied results in higher leaf production and lower stem production, which favors the increase of forage protein content.

Within animal production systems, the knowledge about protein degradation is indeed important for ruminants, because they can produce microbial protein which is directly linked to the dietary N source. Measuring the protein content in diets and its fractions is necessary to understand how the nitrogen uptake from forage behaves inside the rumen. However, little is known about the nutritional details of ryegrass hay of different doses of nitrogen fertilization and harvest at different heights. Considering the influence of nitrogen on plants and also on ruminal fermentation, the aim of our work was to evaluate the chemical composition and in vitro ruminal kinetics of annual ryegrass hay from two cutting heights and grown with different doses of nitrogen fertilization.

* Correspondence: odenardin@gmail.com

2. Materials and methods

An experiment was carried out in the county of Guarapuava, Paraná State, Brazil. The experimental area is located in the physiographic region named Terceiro Planalto Paranaense (25° 33′ S and 51° 29′ W, elev. 1098 m a.s.l.). The climate is humid subtropical Cfa according to the Köppen classification, with an average temperature of 23.5 °C in the hottest months and below 12.7 °C in the coldest [5], and presents an average rainfall ranging from 1400 to 2000 mm. The soil is classified as a typical dystrophic latosol bruno [6].

We applied a topdressing when the ryegrass was sown using 250 kg ha^{-1} of formulated 0-20-20 (N, P_2O_5, K_2O) according to the criteria of soil analysis (0–15 cm). The soil chemical characteristics were: pH ($CaCl_2$) of 4,65; organic matter: 39.04 g dm^{-3}; P-Mehlich: 2.23 mg dm^{-3}; K^+: 0.45 cmolc dm^{-3}; Ca^{+2}: 2.95 cmolc dm^{-3}; Mg^{+2}: 1.69 cmolc dm^{-3}; Al + 3: 0.17 cmolc dm^{-3}; H + Al: 5,47 cmolc dm^{-3}.

The experimental design was randomized blocks with split-plots. The Nitrogen doses (0, 75, 150, and 225 kg N ha^{-1}) were distributed in the main plot and in the subplots where the hay was harvested (5 and 10 cm cutting heights). The experimental area was divided into 12 uniform plots of 19.2 m^2 each. In the summer, there was a corn crop in the experimental area and ryegrass pasture in the winter as established in a 10-year protocol of crop rotation and integrated crop systems.

The sowing of the annual ryegrass (*Lolium multiflorum* Lam. 'BAR HQ') was carried out in May 2016 with a 30 kg ha^{-1} density of viable seeds. It was done by direct sowing with a line spacing of 17 cm. The fertilization with N was applied once, right after the plants emerged. The N source was urea (45% N). The plants were harvested at the preflowering stage, when it presented 50% exposed spikelets, by cutting at 5 and 10 cm from the ground. The ryegrass was harvested for haymaking in September 2016. After the harvest, the plants were exposed to dehydration in the field, turned twice a day, and baled after 120 h.

After baling, the material remained stored in a covered shed for 30 days. A 100-g sample was taken from each hay bale and the samples were dried at 55 °C for 72 h in a forced-air oven and ground in Willye-type mill™ fitted with a 1-mm-sieve to yield the partially dried samples (Thomas Scientific).

The chemical analyses were performed for dry matter (DM) and ash (with AOAC 967.03 and 942.05 methods [7]). The organic matter (OM) was calculated as 1000 - MM (Method 942.05) [7]. The insoluble fiber in acid detergent (ADF) and the insoluble lignin in acid detergent (ADL) were determined with the AOAC 973.18 method [8]. The insoluble fiber in neutral detergent (aNDF) was determined using the method described by Mertens et al. [9] without sodium sulfite. The analysis of crude fat (CF) was performed using semiautomatic equipment (ANKOM XT^{15} Extraction System, ANKOM Technology Corporation, Fairport, NY, USA) with filter bags. Crude protein (CP) was estimated from the total nitrogen value (N), using the Kjeldahl method (Method 2001.11; AOAC [10]. The nitrogen fractions (A, B_1+B_2, B_3, and C) were calculated following the method described by Licitra et al. [11]. Total carbohydrates and their fractions were also calculated [12]. The soluble sugars were assessed by colorimetric phenol-sulfuric acid trial according to Dubois et al. [13].

The in vitro analysis of ruminal kinetics was performed in a water bath at 39 °C. We used 100-mL serum amber bottles sealed with butyl rubber stoppers and aluminum crimp seals. One by one, the ryegrass ground samples of approximately 0.5 g were transferred into the bottles and incubated with 40 mL of reduced solution, and culture medium with 10 mL of rumen inoculum, as previously described by Goering and Van Soest [14], and the inoculum according to the methodology of Hall and Mertens [15]. The inoculum was collected from two Holstein steers, with permanent rumen cannulas. The steers were three years old and had ±550 kg of body weight (Protocol 2014 - 008 by the Institutional Committee on Animal Research and Experimentation). Before the in vitro trials, the steers were kept in a paddock with black oat pasture, and we provided supplementation for 8 days with corn silage and ground corn (1 kg day^{-1}) [16]. Briefly, the system used a gauge gas pressure and volume similar to the one described by Abreu et al. [16]. The gas pressures generated from the onset of fermentation were recorded by manometric readings (0–7psi; 0.05 psi increments), and the volume was measured by using a graduated pipette (0–25 mL; 0.1 mL increments) [17]. Pressure and volume readings were taken at 1, 2, 3, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 72, 96, 120, and 144 h after the rumen inoculum addition and expressed as mL 0.1 g DM^{-1} of the incubated sample. The parameters of ruminal kinetics in vitro were estimated using the two-compartmental model [18], as suggested by Oliveira et al. [17] to evaluate the degradation kinetics of temperate grasses.

$$Vt = Vf_1 [1 - \exp(-k_1 * t)] + Vf_2 \exp\{- \exp[1 + k_2 * e(L- t)]\} + \varepsilon;$$

where Vt is the accumulated volume at time t; Vf_1, the final gas volume of the fast degradation fraction; k_1 (h^{-1}), the rate of degradation of the fast fraction; Vf_2, the final volume of gases from the slow degradation fraction; k_2 (h^{-1}), the rate of degradation of the slow fraction; L, lag time; and T, the incubation time (hs), following the Nlin procedure [19].

The data were analyzed following the Glimmix procedure [19], in a split-plot scheme, and followed the general linear mixed models methodology, in which the

data fitted on the exponential family of the distributions ($0 \leq x \leq 1$; beta or gamma distribution), with the choice of the distribution that would best fit the data. This decision was made using the corrected Akaike information criterion value (AICc) [20]. The analysis of variance followed the mathematical model:

$$Y_{ijk} = \mu + N_i + b_j + C_k + (N^*C)_{ik} + \varepsilon_{ijk}$$

where Y_{ijk} is the observation concerning the i^{-th} N level (Ni) in the j^{-th} block (bj) of the k^{-th} height of cut (C_k). We considered the nitrogen level and cutting height as fixed effects and the block as a random effect. The data were submitted to analysis of variance. The variables that showed a significant effect (P = 0.01) to fertilization doses or interaction between the treatments were analyzed through polynomial regression using the corresponding link function. The means that showed a significant effect for cutting height were compared. Pearson correlation by the CORR procedure [19] was tested between the parameters obtained from the in vitro gas production profiles and the chemical composition. For the statistical analysis, we used the SAS University Edition.

3. Results

The nitrogen fertilization and cutting height did not affect (P > 0.01) the levels of dry matter, organic matter, ash, lignin, and the fraction C of carbohydrates (Table 1). An interaction effect between the treatments was observed (P < 0.01) for the variables aNDF, ADF, crude fat, crude protein, and protein fraction $B_1 + B_2$ (Table 1).

For aNDF and ADF, at 5 cm cutting height, there was a linear decrease in the contents of these fractions with an increase in nitrogen fertilization (Table 2). For 10 cm

Table 1. P-values of the effects of the nitrogen doses, cutting height, and their interaction on the chemical compounds and in vitro gas production parameters of annual ryegrass hay. Guarapuava, 2016.

Height	5 cm				10 cm				P-value		
N Doses	0	75	150	225	0	75	150	225	N Doses	Height	N * H
Dry matter	856.63	852.66	852.66	850.03	853.33	851.86	852.60	852.47	0.1547	0.9421	0.3226
Organic matter	934.44	915.90	908.58	912.87	934.19	916.03	934.09	909.96	0.0544	0.1357	0.0561
Ash	65.56	83.73	91.24	87.14	64.63	83.91	65.34	90.02	0.0721	0.1553	0.0987
Crude fat	50.52	52.11	33.59	54.60	58.04	58.40	47.50	49.59	<0.0001	0.0003	0.0004
Insoluble fiber in neutral detergent	560.80	540.38	536.46	485.10	480.69	495.11	498.16	497.31	0.0430	0.0002	0.0044
Insoluble fiber in acid detergent	328.74	336.05	300.84	264.89	269.76	295.20	339.01	287.77	0.0069	0.0989	0.0003
Lignin	38.63	38.57	35.90	35.87	34.33	34.97	35.47	37.17	0.9615	0.2521	0.5139
Total carbohydrates	793.24	737.64	690.92	633.75	759.43	729.14	685.37	650.84	<0.0001	0.1792	0.0261
Solubles carbohydrates	69.50	39.90	29.07	22.83	76.07	47.83	36.80	23.87	<0.0001	0.0101	0.4917
Carbohydrates B_1 fraction	162.90	157.35	125.37	125.45	202.59	186.21	129.30	129.72	0.0058	0.0916	0.6018
Carbohydrates B_2 fraction	468.00	447.80	450.22	398.62	398.36	411.14	433.42	407.97	0.0981	0.0034	0.0193
Carbohydrates C fraction	92.73	92.53	86.20	86.07	82.33	83.87	85.07	89.23	0.9624	0.2479	0.5096
Crude protein	90.63	126.10	183.97	224.53	116.77	128.47	201.27	209.50	<0.0001	0.0033	0.0005
Non-protein Nitrogen	38.10	27.07	57.53	85.47	34.33	27.83	87.43	83.53	<0.0001	0.1897	0.0273
$B_1 + B_2$ Protein fraction	19.07	52.42	56.53	58.04	44.43	42.34	41.94	44.36	0.0125	0.8893	0.0080
B_3 protein fraction	17.99	27.62	40.28	55.68	21.93	38.34	44.79	53.46	0.0013	0.0105	0.0927
Indigestible protein (C fraction)	15.12	18.73	27.89	25.47	15.97	19.40	26.36	27.43	0.0002	0.4601	0.5805
Vf_1	19.41	21.37	24.42	23.93	21.97	20.13	24.51	23.58	0.0156	0.7489	0.4566
Vf_2	17.78	13.66	9.51	9.99	14.39	13.59	9.53	9.60	0.0040	0.5264	0.8550
k_1	0.1288	0.1063	0.0911	0.0919	0.1121	0.1147	0.0973	0.0920	0.0100	0.9849	0.5223
k_2	0.0205	0.0200	0.0161	0.0172	0.0208	0.0199	0.0156	0.0166	0.0157	0.8248	0.9917

Values in g kg^{-1} of Dry Matter; $B_1 + B_2$ Fraction= True soluble protein + soluble protein in neutral detergent; PB_3 = Insoluble protein in neutral detergent - insoluble protein in acid detergent; P-value of the effects of the nitrogen doses, cutting height and their interaction; Vf_1 and Vf_2 = The maximum volume of gas produced by degradation of the rapid and slow digestion fractions, respectively (mL 0.1 g^{-1} dry matter). k_1 and k_2 = The specific rate of gas production by degradation of the rapid and slow digestion fractions, respectively.

Table 2. Regression equations for the variables of annual ryegrass hay that presented interaction between N doses and cut height. Guarapuava, 2016.

Coefficients[§]	β0		β1		β2	
	Insoluble fiber in neutral detergent					
Height	5 cm	10 cm	5 cm	10 cm	5 cm	10 cm
Estimative	6.3394	6.1837	–0.0002	0.00014	-	-
SE	0.017	0.0096	0.00025	0.00007	-	-
P-value	<0.0001	<0.0001	0.4530	0.0703	-	-
	Insoluble fiber in acid detergent					
Estimative	5.8394	5.5808	–0.00101	0.003	-	- 0.00001
SE	0.032	0.035	0.0002	0.0007	-	0.0000032
P-value	<0.0001	<0.0001	0.0014	0.0029	-	0.0058
	Crude fat					
Estimative	3.88	4.076	–0.00016	- 0.0009	-	-
SE	0.099	0.034	0.0007	0.00024	-	-
P-value	0.0001	<0.0001	0.8212	0.0041	-	-
	Crude protein					
Estimative	4.5163	4.7386	0.0041	0.0029	-	-
SE	0.0977	0.050	0.0002	0.0003	-	-
P-value	0.0138	<0.0001	0.0001	<0.0001	-	-
	$B_1 + B_2$ protein fraction					
Estimative	3.2987	4.1608	0.004	0.00002	-	-
SE	0.1835	0.2191	0.0013	0.00078	-	-
P-value	<0.0001	<0.0001	0.0078	0.9781	-	-

[§]$y = \exp(g) = \beta0 + \beta1\ X + \beta2\ X^2$; $B_1 + B_2$ Fraction = True soluble protein + soluble protein in neutral detergent; SE = Standard error.

Table 3. Regression equations for the variables of annual ryegrass hay that showed effect for the N doses. Guarapuava, 2016.

Variables[§]	TC	CHOs	B_1	NPN	PB_3	PC	Vf_2	k_1
	β0							
Estimative	6.658	4.2353	5.22	3.4257	3.042	2.779	2.7591	- 2.1214
SE	0.00711	0.0467	0.0539	0.1024	0.2039	0.04465	0.0737	0.0437
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0218	<0.0001	<0.0001	0.00031
	β1							
Estimative	–0.00084	–0.00494	–0.00183	0.00453	0.00435	0.00261	–0.0024	–0.00129
SE	0.00005	0.00033	0.00039	0.00073	0.00052	0.00032	0.000525	0.000312
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004

[§]$y = \exp(g) = \beta0 + \beta1\ X + \beta2\ X^2$; TC = Total Carbohydrates; CHOs = Solubles Carbohydrates; NPN = Nonprotein nitrogen; PB_3 = Insoluble protein in neutral detergent - insoluble protein in acid detergent; PC = Indigestible protein; Vf_1 and Vf_2 = The maximum volume of gas produced by degradation of the rapid and slow digestion fractions, respectively (mL 0.1 g^{-1} dry matter). k_1 and k_2 = The specific rate of gas production by degradation of the faster and slower digestion fractions, respectively. SE = Standard error.

cutting height, there was an increase in aNDF and ADF, with decreasing quadratic effect, with a maximum point of 150 kg of N for ADF, influenced by the increase in insoluble protein fractions (Table 1).

The crude fat showed an interaction effect between treatments (Table 1). At a 5 cm cutting height, the slope did not present any significant effect (Table 2). For 10 cm cutting height, there was a negative linear effect, a decrease in fat contents with an increase in nitrogen fertilization (Table 2).

The crude protein and fraction $B_1 + B_2$ had an increasing linear effect with N fertilization regardless of height (Table 1). In the two variables, the effect of the cutting height was evidenced by the lower intercept (β0) and the higher slope (β1) at the height of 5 cm (Table 2). The cutting height showed an isolated effect only for the B_2 fraction of the CHO (Table 1), with higher values for the height of 5 cm.

Nitrogen fertilization showed a significant statistical effect for total carbohydrates, soluble carbohydrates, and B1 fraction, for the protein fractions NPN (Nonprotein nitrogen - A fraction protein), B_3, and C, and also for the parameters of the in vitro gas production profiles, Vf_2 and k_1 (Table 1). The carbohydrates showed a decreasing linear effect with the increasing of the N dose, as well as the in vitro ruminal kinetic parameters (Table 3). Conversely, protein fractions showed a linear effect increasing with the increase of N. The model applied to estimate the parameters of in vitro gas production profiles did not present a lag time, regardless of the treatment.

4. Discussion

The dry matter contents were not affected by treatments, mainly because they are associated with hay conservation (Table 1). The levels of organic matter and ash went through numerical fluctuations, but it did not influence statistically the response to cutting height and nitrogen level. At a low cutting height, like 5 cm, there is a greater contribution of stems and dead material to the biomass yield, which causes the variation in the content of fibrous compounds. At a 10 cm cutting height, the increase in the levels of aNDF and ADF with the increase in the fertilization may be associated with the larger tiller size in response to nitrogen supply (Table 2). It makes the tiller wider and the cell wall thicker to support the plant structure. However, the protein content linked to these fibrous fractions has also increased, which can directly influence the values of aNDF and ADF (Table 1).

Overall, the nitrogen fertilization might add to the increase of dissolved N in the plant. The greater availability of N affects photosynthesis due to a component of the chlorophyll molecules and part of the molecular structures of the entire protein synthesis apparatus involved in this photochemical process [21,22]. The low availability of N to the plant accelerates the accumulation of carbohydrates, leading to an additional decrease in the nitrogen content of leaves as photosynthetic capacity [23], yet, with the increase in the concentration of these in the leaves, a decrease in the amount of photosynthetic protein is observed.

As for total carbohydrates, its fractions decreased with the increase in supplying N to the plant on account of the increased use of these carbohydrates to transform the available nitrogen into protein, as a plant natural response [24,25]. As observed, there was a decrease in the amount of soluble sugar in the vegetable. Corsi [26] reported a decrease in the content of soluble carbohydrates as nitrogen fertilization increased in the pasture. Also, a linear decrease was observed in B_1 fraction (starch and pectin). Nitrogen fertilization contributes to the increase in NPN and protein fractions linked to fiber in forage. According to Bredemeier and Mundstock [27], plants take up nitrate (NO_3^-) and ammonium (NH_4^+) by the root. The NPN in the vegetable represents compounds with a low molecular weight such as free amino acids, nitrates, amides, amines, and ammonia [28]. Additionally, in preserved forages, a large part of the crude protein is present as NPN [29]. Thus, the plant can prioritize the deposition of nitrogen compounds, which explains the significant increase in protein fractions of slow degradability ($B_1 + B_2$ and B_3).

The increase in nitrogen fertilizer doses caused an increase of the crude protein content and a decrease in the total carbohydrates content (Table 3) regardless of cutting height. At 10 cm cutting height, the average crude protein content was higher, mainly associated with the increase of the fractions $B_1 + B_2$ and B_3, which are directly linked to the aNDF variation (Table 1). Possibly, animals fed ryegrass hay with high protein content need to get a carbohydrate source supplementation to fit the fermentation process with the combination of these two sources [30]. Detmann et al. [31] comment that in medium- to high-quality pastures, animals should receive an energy source of rapid fermentation in the rumen to accomplish the nitrogen and energy balance. Microbial protein synthesis can be maximized if the availability of fermentable energy and undegraded N in the rumen is synchronized [32]. When the rate of protein degradation exceeds that of the carbohydrates, large amounts of N are lost as ammonia [33].

The in vitro ruminal degradation kinetics parameters revealed that Vf_2 and k_1 showed effect only for the doses in a linear decrease (Tables 1 and 3). The presence of fibrous and nonfibrous carbohydrates is directly linked to the volume of gas produced through the degradation of the elements of rapid and slow digestion, and related to the soluble and insoluble fractions [34].

The rate of degradation k_1 had a decreasing effect (–0.52950; P = 0.0078), associated with the fractions of

rapid degradation, with a negative correlation with the protein fraction "A", and positive with the concentration of soluble carbohydrates (0.66332; P = 0.0004). This is due to the possible use of the carbon chains of these compounds for protein synthesis (microbial protein) combined with obtaining energy with the release of ammonia in the ruminal environment, which indicates, in part, the imbalance in the ratio of energy:protein of rapid degradation.

During the metabolism of nitrogenous compounds, it is necessary to have carbon skeletons derived from carbohydrates [35], which are a source of energy for bacteria to metabolize proteins in the rumen. When it fails, the degradation of other compounds, including nitrogenous ones, will be made as a way to obtain energy. In this sense, the balance between carbohydrates and nitrogen compounds is essential [2,12]. Thus, the deamination of amino acids in the rumen, which leads to the loss of NH_3 by the rumen wall, is one of the main causes of inefficient N retention by ruminants [36].

When the rate of protein degradation exceeds the rate of CHO fermentation, large quantities of N can be lost as ammonia [37]. The decrease in the degradation rate of the soluble fraction (k_1) showed a decline with the increase in nitrogen fertilization and also influenced the volume of gas produced (Vf_2), showing a positive correlation between these variables (0.94002; P < 0.0001).

The gas production of the slow degradation fraction (Vf_2) is positively correlated with the total carbohydrates (0.769; P < 0.0001) and negatively correlated with the protein fractions (A: –0.62730; P = 0.0010. B3: –0.70195; P = 0.0001). Thus, the decrease in gas production for the fraction of slow degradability is linked to the high supply of nitrogen compounds. This increase, regarding the carbohydrates, can compromise gas production due to the high release of ammonia (NH_3), which interferes with the pH of the rumen environment. However, the synthesis of microbial mass is negatively correlated with pH [35].

5. Conclusion

A higher nonfibrous content is observed at a cutting height of 10 cm. Regardless of the cutting height, nitrogen fertilization increases the crude protein content and decreases the total carbohydrates content, especially the nonstructural carbohydrates.

The increase of N in the plant decreases the specific rate of gas production due to the degradation of the soluble fraction of rapid digestion and causes a decrease in gas production of the slow digestion fraction.

Acknowledgments

Authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), financial code 001.

References

1. Skonieski FR, Viégas J, Bermudes RF, Nörnberg JL, Ziech MF et al. Botanic and structural composition and nutritional value on intercropped ryegrass pastures. Revista Brasileira de Zootecnia. 2011; 40 (3): 550-556.
2. Van Soest PJ. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press; 1994.
3. Costa OAD, Ferreira OGL, Silva JLS, Fluck AC, Kröning AB et al. Yield, structural composition and nutritive characteristics of ryegrass cultivars used to haymaking in lowland soils. Bioscience Journal 2018; 34 (5): 1232-1238.
4. Miflin BJ, Lea PJ. The pathway of nitrogen assimilation in plants. Phytochemistry 1976; 15 (6): 873-885.
5. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 2013; 22 (6): 711-728.
6. Bhering SB, Santos HG, Bognola IA, Cúrcio GR, Manzatto, C V et al. Mapa de Solos do Estado do Paraná: Legenda Atualizada. In: XXXII Congresso Brasileiro de Ciência Do Solo. Embrapa Solos; 2009 (in Portuguese).
7. AOAC. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemistry, 1998.
8. Möller J. Gravimetric determination of acid detergent fiber and lignin in feed: interlaboratory study. Journal of AOAC International 2009; 92 (1): 74-90.
9. Mertens DR. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International 2002; 85 (6): 1217-1240.
10. Latimer GW (editor). Official Methods of Analysis of AOAC International. 19th ed. Association of Official Analytical Chemistry, 2012.
11. Licitra G, Hernandez TM, Van Soest PJ. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 1996; 57 (4): 347-358.
12. Sniffen C, O'Connor J, Van Soest P, Fox D, Russell J. A net carbohydrate and protein system for evaluating cattle diets: II carbohydrate and protein availability. Journal of Dairy Science 1992; 70: 3562-3577.
13. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 1956; 28 (3): 350-356.
14. Goering HK, Van Soest PJ. Forage Fiber Analysis. Apparatus, reagents, procedures and some applications. Agricultural Handbook 1970; 379.

15. Hall MB, Mertens DR. In vitro fermentation vessel type and method alter fiber digestibility estimates. Journal of Dairy Science 2008; 91 (1): 301-307.

16. Abreu MLC, Vieira RAM, Rocha NS, Araujo RP, Glória LS et al. Clitoria ternatea L. as a potential high quality forage legume. Asian-Australasian Journal of Animal Sciences 2014; 27 (2): 169-178.

17. Oliveira JG, Henrique DS, Abreu MLC, Fluck AC, Mayer LRR et al. Evaluation of mathematical models to describe gas production kinetics of some tropical and temperate forages. Revista Brasileira de Zootecnia 49: e20190197.

18. Schofield P, Pitt RE, Pell AN. Kinetics of fiber digestion from in vitro gas production. Journal of Animal Science 1994; 72 (11): 2980-2991.

19. SAS Institute Inc. SAS/STAT® 13.1 User's Guide. SAS Institute Inc., 2013.

20. Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. SAS® for Mixed Models. 2nd ed. SAS Institute Inc., 2006.

21. Andrade AC, Fonseca DM, Lopes RS. Nascimento Júnior D, Cecon PR et al. Análise de crescimento do capim-elefante "Napier" adubado e irrigado. Ciência e Agrotecnologia. 2005; 29 (2): 415-423 (in Portuguese).

22. Benett CGS, Buzetti S, Silva KS, Bergamaschine AF, Fabricio JA. Produtividade e composição bromatológica do capim-marandu a fontes e doses de nitrogênio. Ciencia e Agrotecnologia 2008; 32 (5): 1629-1636 (in Portuguese).

23. Araya T, Noguchi K, Terashima I. Effect of nitrogen nutrition on the carbohydrate repression of photosynthesis in leaves of Phaseolus vulgaris L. Journal of Plant Research 2010; 123 (3): 371-379.

24. Neumann M, Nörnberg JL, Leão GFM, Horst EH, Figueira DN. Composição química fracional de carboidratos e de proteínas de silagens de milho fertilizadas com doses crescentes de nitrogênio. Ciencia Rural 2017; 47 (5): e20160270 (in Portuguese).

25. Taiz L, Zeiger E. Plant Phisiology. 3rd ed. Sinauer Associates, 2002.

26. Corsi M. Adubação nitrogenada das pastagens. In: Peixoto AM, Moura JC, Faria VP (editors). Pastagens: Fundamentos Da Exploração Racional. FEALQ; 1994: pp. 121-155 (in Portuguese).

27. Bredemeier C, Mundstock CM. Regulação da absorção e assimilação do nitrogênio nas plantas. Ciência Rural 2000; 30 (2): 365-372 (in Portuguese).

28. Fijałkowska M, Pysera B, Lipiński K, Strusińska D. changes of nitrogen compounds during ensiling of high protein herbages – a review. Annals of Animal Science 2015; 15 (2): 289-305.

29. Pacheco D, Waghorn GC. Dietary nitrogen – definitions, digestion, excretion and consequences of excess for grazing ruminants. Journal of New Zealand Grasslands - Proceedings of the New Zealand Grassland Association 2008; 70: 107-116.

30. Russell JB, O'Connor JD, Fox DG, Van Soest PJ, Sniffen CJ. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. Journal of Animal Science 1992; 70 (11): 3551-3561.

31. Detmann E, Valente ÉEL, Batista ED, Huhtanen P. An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. Livestock Science 2014; 162 (1): 141-153.

32. Ørskov ER. Protein Nutrition in Ruminants. 2nd ed. Academic Press, 1992.

33. Rodríguez R, Sosa A, Rodríguez Y. Microbial protein synthesis in rumen and its importance to ruminants. Cuban Journal of Agricultural Science 2007; 41 (4): 287-294.

34. Kozloski GV. Bioquímica Dos Ruminantes. 3rd ed. Editora UFSM, 2011.

35. Bach A, Calsamiglia S, Stern MD. Nitrogen metabolism in the rumen. Journal of Dairy Science 2005;88 (S): E9-E21.

36. Leng RA, Nolan JV. Nitrogen metabolism in the rumen. Journal of Dairy Science 1984; 67 (5): 1072-1089.

37. Nocek JE, Russell JB. Protein and energy as an integrated system. Relationship of Ruminal Protein and Carbohydrate Availability to Microbial Synthesis and Milk Production. Journal of Dairy Science 1988; 71 (8): 2070-2107.