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In situ degradation kinetic of *Andropogon gayanus* grass silages harvested at three stages of maturity

[*Cinética de degradação* in situ *das silagens de capim* Andropogon gayanus *produzidas em três idades de corte*]

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ABSTRACT

Maturity is considered the primary factor affecting the nutritive value of forages. The aim of this study was to evaluate and compare dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF) ruminal degradation kinetics of *Andropogon gayanus* grass silages harvested at three stages of maturity (56, 84 and 112 d). Dried and ground silage samples (5 mm) were incubated in nylon bags inside the rumen for 0, 6, 12, 24, 48, 72 and 96 h to estimate the kinetics of ruminal DM, CP and NDF degradation. The ruminal kinetic parameters of the silages from each treatment (56, 84 and 112 d of regrowth) in each animal (5 cows) were determined by the model: $y = a + b(1 - e^{-c(t-L)})$. The parameters generated by the model were analyzed as randomized block design, with grass regrowth age as the fixed effect and animal the as random effect (blocks). The grass silage ensiled with 56 days of regrowth had the highest (P<0.05) effective degradability of DM and NDF calculated for fractional rate of particulate passage of 2%/h. All silages had low CP soluble fraction (<25.4%) and effective degradability calculated for fractional rate of particulate passage of 2%/h (<40.1%). A greater (P<0.05) NDF lag time was observed for the silages ensiled with 84 and 112d compared to the silage produced with 56d of regrowth. The ruminal degradation kinetics of *Andropogon gayanus* grass silages showed that the grass ensiled with 56 days of regrowth had higher nutritional value.

Keywords: gamba grass, maturity, preserved forage, ruminal degradability, tropical grass

RESUMO

A idade ao corte é considerada o principal fator a afetar o valor nutritivo das forragens. Objetivou-se avaliar e comparar a cinética de degradação ruminal da matéria seca (MS), proteína bruta (PB) e fibra insolúvel em detergente neutro (FDN) das silagens de capim Andropogon gayanus obtidas em três diferentes idades de corte (56, 84 e 112 dias). As amostras das silagens secas e trituradas (5 mm) foram incubadas em sacos de nylon no rúmen por 0, 6, 12, 24, 48 e 72 h. Os parâmetros da cinética de degradação das silagens de cada tratamento (3 silagens) em cada animal (5 vacas) foram determinados pelo modelo: $y = a + b(1 - e^{-c(t-L)})$*. Os parâmetros gerados pela equação foram analisados como blocos inteiramente ao acaso, sendo que as idades de corte foram inseridas como efeito fixo e os animais como efeito aleatório (blocos). A silagem da planta aos 56 dias de rebrote apresentou maiores (P<0,05) valores de degradabilidade efetiva da MS e da FDN calculada para a taxa de passagem de 2,0%/h. Todas as silagens apresentaram baixa fração solúvel (<25,4%) e degradabilidade efetiva (<40,1%) da proteína bruta com taxa de passagem de 2%. Foi observado maior (P<0,05) tempo de colonização da FDN para as silagens realizadas com as plantas aos 84 e 112 dias de crescimento em comparação com a silagem produzida aos 56 dias. Os resultados da cinética de degradação ruminal das silagens de capim* Andropogon gayanus *indicam que a planta ensilada aos 56 dias de rebrote apresentou melhor valor nutricional.*

Palavras-chave: degradação ruminal, estádio de maturação, forrageira tropical, forragem conservada

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INTRODUCTION

Forages are the basis for ruminant nutrition and cell-wall digestibility is a major limiting factor of forages' nutritive value (Wattiaux *et al*., 1991). According to Wilson (1993) and Jung and Allen (1995) the quality of roughages is closely related to cell wall degradation, as this is the main component of the forage tissue composing up to 80% of the organic matter and its fermentation by rumen microorganisms is the major source of energy for animal production. Thus, high intake of forage and high digestibility of cell walls are essential for high live weight gain or milk production in ruminant systems based on forages as the main source of metabolizable protein and energy. However, both intake and digestibility of forages are influenced by the proportion of cell wall and by the resistance of forage and cell walls to degradation into small particles during mastication and digestion (Wilson and Mertens, 1995).

Ensiling tropical grasses has gained importance due to the high productivity of these forages, which favors the reduction of the cost of feeding ruminants compared to traditional crops like corn and sorghum. In this context *Andropogon gayanus* grass is an important tropical grass due to its ability to tolerate long dry seasons and low fertility acidic soils characteristic of the Cerrado biome (CIAT, 1990), which is currently the main area for the Brazilian livestock expansion. However, very little was found in the literature on its utilization for silage production and the effect of maturity, which is considered the primary factor in reducing forage's nutritive value (Nelson and Moser, 1994), on this grass silage nutritional quality. It is well known that vegetative pastures have better nutritional quality than mature pastures, but they also have lower dry matter (DM) yield and high moisture content which may result in secondary fermentation, production of silage effluents and spoilage of the silage (McDonald *et al.*, 1991). At more advanced maturity, DM yield is higher and DM content (30 to 35% DM) of the pasture can be more favorable for silage production, but the high cell wall and lignin content can limit intake and nutritional value of the forage (Van Soest, 1994). Therefore, it was our aim to compare the *in situ* rumen degradability characteristics of *A. gayanus* grass silage, harvested at three stages of maturity.

MATERIALS AND METHODS

The *Andropogon gayanus* grass silages were produced in the rainy season of 2006-2007 at 19º35''36S, 43º51'56''W; altitude 747m, using an established pasture in Lagoa Santa County, Minas Gerais, Brazil. Soil samples were collected at a depth of 0-20cm and analyzed prior to the beginning of the experiment. To neutralize acidity, lime was applied (2000kg ha^{-1}) at the beginning of the rainy season. After 30 days the grass was cut 20 cm above the soil level and 250kg ha^{-1} of 08-24-12 and 100kg ha^{-1} of 30-00-20 (N:P:K) was applied. *A. gayanus* grass was then harvested at different regrowth ages (56, 84 and 112 days), chopped (10-25mm) and ensiled. The grass was ensiled in plastic bags within 200 L-steel barrels, compacted by trampling, and sealed. The silage sample from each regrowth age used in the *in situ* study was a pool from samples taken from 12 different silos. Extracts of the fresh silages were obtained with a hydraulic press and immediately analyzed for ammonia nitrogen and pH. Silage extracts (10mL) were also taken, acidified with 2mL of metaphosphoric acid (0.25; w/v) and frozen at -20ºC until analyzed for acetic, propionic, butyric and lactic acid. The chemical composition of the silages is shown in Table 1.

Ruminal disappearance of dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) of the silages were determined using a nylon bag technique (Ørskov and McDonald, 1979) and five mature, ruminally fistulated Holstein cows fed 4kg of concentrate (20% CP) and corn silage *ad libitum*. Silage samples were dried at 55ºC in a forced air oven for 72h and ground through a 5mm screen. Ground samples were weighed (5g/bag) into 7.5cm x 15cm monofilament polyester bags (50μm pore size). The bags were attached to a metal chain of approximately 150g, which worked as an anchor in order to keep the bag placed in the ventral sac of the rumen and in constant contact with the rumen fluid. Triplicate polyester bags were incubated in each cow for 6, 12, 24, 48, 72 and 96h. Upon removal, bags were rinsed under cold running tap water until the water running off was clear. Triplicate sets of unincubated bags (0h) containing each treatment were rinsed as described above to estimate the degree of solubility without incubation (soluble fraction). Bags were subsequently dried in a forced air oven at 55ºC for 72h. Bags and contents were weighed, and DM disappearance was calculated. The residues from triplicate bags, from each incubation time, put in the same animal were pooled and ground using a 1mm screen and subsequently analyzed, in duplicate, for DM, CP and NDF. The DM was determined by oven drying at 105°C (Association…, 1990), ash was determined by combustion at 550°C (Association…, 1990) with organic matter (OM) content determined by the difference between DM and ash content. Nitrogen (N) was determined by the Kjeldahl method with CP expressed as N×6.25 (Association…, 1990). The NDF and acid detergent fibre (ADF) contents were determined according to Van Soest *et al*. (1991) using an Ankom[200] fiber analyzer (Ankom Technology Corp., Fairport, NJ). Heat-stable α-amylase and sodium sulfite were not used during the NDF procedure. Concentrations of NDF and ADF are expressed inclusive of residual ash. Lignin analyses were performed on ADF residues, using the direct sulfuric acid method, according to Robertson and Van Soest (1981). Ammonia-N in silage extracts was determined by the Kjeldahl method with the volatile N fraction generated by heating the solution at pH>7 in the presence of MgO. Organic acids in silage extracts were analyzed using a gas chromatograph (GC-17 Shimadzu gas chromatograph) equipped with a flame ionization detector and fitted with a Nukol capillary column according to the methodology described by Playne (1985). The gas chromatograph was operated isothermally with a column temperature of 200ºC and an inlet and detector temperature of 225ºC.

Table 1. Chemical composition of *Andropogon gayanus* grass silages harvested at three stages of maturity

Item	Regrowth age (days)		
	56	84	112
Dry matter (%)	17.7	25.4	26.2
Organic matter (% of DM)	91.0	92.3	93.6
Crude protein (% of DM)	6.9	5.6	5.4
NDF (% of DM)	74.9	74.7	74.4
ADF (% of DM)	45.0	43.3	42.7
Lignin (% of DM)	6.1	5.8	7.1
Ammonia_N (% of total N)	11.2	9.9	5.0
pH	5.3	5.3	4.7
Acetic acid (% of DM)	6.61	0.98	0.82
Propionic Acid (% of DM)	2.41	0.24	0.11
Butyric acid (% of DM)	6.10	2.16	2.03
Latic acid (% of DM)	ND	ND	0.39

NDF = neutral detergent fibre; ADF = acid detergent fibre. ND = not detected.

The kinetics of in situ DM, CP and NDF disappearance were estimated using a non-linear procedure (PROC NLIN) of SAS (Statistical…, 2002). For each cow, the McDonald (1981) model was fitted to the percentage of DM and NDF disappearance as:

$$y = a + b(1 - e^{-c(t-L)}) \text{ for } t > L$$

where a is the soluble fraction (%), b the slowly disappearing fraction (%), c the fractional rate of disappearance (%/h), L the lag time (h), and t is the incubation time (h). Effective ruminal disappearance was estimated using the model:

$$y = a + b\,[c/c+k]\, e^{-kL/100}$$

where k is the fractional rate of particulate passage, assumed to be 0.02 h^{-1}. The undegradable fraction (U) was calculated as: U = 100 – (a + b). The model of McDonald (1981) did not fit to the percentage of CP disappearance. Therefore, the model of Ørskov and McDonald (1979) without lag time was fitted to the percentage of CP disappearance as:

$$y = a + b(1 - e^{-ct})$$

Effective ruminal CP disappearance was estimated using the model:

$$y = a + [(b*c)/(c + k)]$$

The parameters a, b, c, L, U and effective disappearance, calculated from the *in situ* DM, CP and NDF disappearance data, were analyzed as a complete randomized block design for the fixed effect of treatment (regrowth age) with the cow as the random (block) variable using the

PROC MIXED procedure of SAS according to the following model:

$$\gamma ij = \mu + \alpha i + \beta j + \varepsilon ij$$

where γij is the observation (parameter), μ the overall mean, αi the effect of cow (1–5), βj the effect of treatment (silages made with 56, 84 and 112 days of regrowth), and εij is the residual error. Differences between reported means were determined using the PDIFF option of the least square mean linear hypothesis test (LSMEANS) of SAS (Statistical…, 2002). Differences were considered significant when P<0.05.

RESULTS AND DISCUSSION

Andropogon gayanus grass silages ensiled at 56 and 112 d of regrowth had lower (P<0.05) slowly degradable DM fraction (*b*) than the grass ensiled at 84 d (Tab. 2). However, the potentially degradable DM fraction (*a*+*b*) and the undegradable DM fraction (U) did not differ (P>0.05) between the different silages. The lower slowly degradable DM fraction (*b*) in the silages produced at 56 and 112d of regrowth was due to higher soluble DM fraction (*a*). The fractional rate of DM disappearance (*c*) was higher in silage ensiled at 56d of regrowth than in silage ensiled at 84d. The fractional rate of DM disappearance in the silage produced at 112d of regrowth did not differ (P>0.05) from silages produced at 56 or 84d. Lag time was higher (P<0.05) for the silage ensiled at 56 d of regrowth than at 84 or 112 d. The silage made at 56d of regrowth had the highest (P<0.05) and the silage produced at 84d the lowest (P<0.05) DM effective degradability.

Table 2. Ruminal kinetic parameters and effective degradability of DM of *Andropogon gayanus* grass silages ensiled with different regrowth ages

Item	Regrowth age (days)			SEM	*P*-value
	56	84	112		
a (%)	12.3	9.6	11.8	-	-
b (%)	59.6b	64.2a	59.9b	1.33	<0.01
c (%/h)	3.13a	2.41b	2.76ab	0.176	<0.01
a+*b* (%)	71.9	73.8	71.7	1.33	0.19
U (%)	28.1	26.2	28.3	1.33	0.19
Lag (h)	4.3a	3.4b	3.3b	0.18	<0.01
Effective degradability* (%)	45.7a	42.9c	44.7b	0.24	<0.01

Within a row, means followed by different letters differ (P<0.05). SEM = standard error mean; *a* = soluble fraction; *b* = slowly degradable fraction; *a*+*b* = potentially degradable fraction; U = undegradable fraction *c* = fractional rate of disappearance. *Calculated with fractional passage rate (*k*) assumed at 0.02 h^{-1}.

Reduction of DM degradability with increasing regrowth age has been reported for several tropical forages (Coblentz *et al*., 1998; Aguiar *et al*., 1999; Rodrigues *et al*., 2004; Silva *et al*., 2008). This reduction of DM degradability with grass maturity is explained by the increase in stem/leaf ratio and reduction of stem nutritional quality (Nelson and Moser, 1994, Wilson and Hatfield, 1997). Stems have in their composition tissues with lower ruminal degradation rate (sclerenchyma and xylem) (Wilson and Hatfield, 1997). These are support and vascular tissues, which have densely packed cells with thick and lignified walls, hard to break down by rumen microorganisms (Wilson and Mertens, 1995).

The grass silage made at 112 d of regrowth had the highest (P<0.05) soluble CP fraction (25.4%) and the grass ensiled at 84 d the lowest (0.8%) (Tab. 3). But the slowly CP degradable fraction was higher (P<0.05) for the grass ensiled at 56 and 84 d of regrowth compared to the silage produced at 112 d. However, there were no differences (P>0.05) between silages for potentially degradable CP fraction (*a*+*b*) and for undegradable CP fraction (U). The silages fractional rate of CP disappearance (*c*) decreased

(P=0.06) with increasing regrowth age. The CP effective degradability was higher (P<0.05) for the silage ensiled at 56 d of regrowth compared to 84 d, but the silage produced at 112 d regrowth did not differ (P>0.05) from the silages produced at 56 or 84 d.

Table 3. Ruminal kinetic parameters and effective degradability of crude protein (CP) of *Andropogon gayanus* grass silages ensiled with different regrowth ages

Item	Regrowth age (days)			SEM	*P*-value
	56	84	112		
a (%)	14.8b	0.8c	25.4a	0.78	<0.01
b (%)	49.1a	52.7a	31.5b	6.32	0.02
c (%/h)	2.32	1.86	1.04	0.319	0.06
a+*b* (%)	63.9	53.5	56.8	6.29	0.35
U (%)	36.1	46.5	43.2	6.29	0.35
Effective degradability* (%)	40.1a	28.7b	34.0ab	2.64	0.03

Within a row, means followed by different letters differ (P<0.05). SEM = standard error mean; *a* = soluble fraction; *b* = slowly degradable fraction; *a*+*b* = potentially degradable fraction; U = undegradable fraction; *c* = fractional rate of disappearance. *Calculated with fractional passage rate (*k*) assumed at 0.02 h^{-1}.

The lower soluble CP fraction observed for *A. gayanus* ensiled at 84 d regrowth compared to 112 d might have been caused by the Maillard reaction. According to Van Soest (1994), the increase in temperature in silages with elevated moisture content may increase the formation of products of the Maillard reaction. During this process part of the silage carbohydrates are linked with part of the silage protein forming a brown undigestible compound similar to lignin. The soluble CP fraction from *A. gayanus* grass silages (from 0.8 to 25.4%) were very low compared to the results found by Rêgo *et al.* (2011) for elephant-grass silages (48.7 to 44.7%) harvested with different regrowth ages (70, 90 and 110 d). This lower soluble CP fraction of *A. gayanus* grass silages is probably related to a higher nitrogen fraction associated to the cell wall and linked to the lignin fractions and to the formation of Maillard reaction products compared to elephant-grass silages.

Rêgo *et al*. (2011) observed values of potentially degradable CP fraction ranging from 59.0% to 64.7% for elephant-grass silages harvested at three different maturities (70, 90 and 110 d of regrowth). Working with corn silages made with bacterial and/or enzymatic inoculants Gimenes *et al*. (2006) found potentially degradable CP fraction ranging from 74.15% to 78.81% and fractional rate of CP disappearance between 1.73% to 3.01%. Evaluating four *Brachiaria* species harvested at 56 days of regrowth, Lopes *et al*. (2010) reported values of soluble CP fraction between 30.7 and 53.4% and of CP effective degradability from 55.4 to 71.6% calculated for fractional rate of particulate passage of 2%/h. These results demonstrate that *A. gayanus* grass silages, besides having low CP content, also have low and slow CP availability. Therefore, a rumen degradable protein supplementation to ensure an ammonia-N concentration of rumen fluid above 5 mg/100 mL (Satter and Slyter, 1974) is essential to prevent limitation of DM degradation in the rumen of animals fed these silages.

As expected there was no difference among silages for soluble NDF fraction (Tab. 4), since this fraction is not soluble in water. The slowly degradable NDF fraction (*b*) and the potentially degradable NDF fraction (*a*+*b*) have similar values since the "*a*" value is zero. Higher (P <0.05) slowly degradable (*b*) and potentially degradable (*a*+*b*) NDF fractions were observed for the silage produced at 84 d of regrowth compared to the silage ensiled at 112 d. The slowly degradable (*b*) and potentially degradable (*a*+*b*) NDF fractions of the silage produced at 56 d of regrowth did not differ (P>0.05) from silages harvested at 84 or 112 d. The NDF undegradable fraction (U) was higher (P<0.05) for the silage ensiled at 112 d of regrowth compared to 84 d, but the silage produced at 56 d of regrowth did not differ (P>0.05) from the silages produced at 84 or 112 d. The fractional rate of NDF disappearance (*c*) of the silage produced at 112 d was higher (P<0.05) compared with silage ensiled at 84 d of regrowth. However, the fractional rate of NDF disappearance (*c*) of the silage produced at 56 d of regrowth did not differ (P>0.05) from the silages harvested at 84 or 112 d. A greater (P <0.05) NDF lag time was observed for the silages ensiled at 84 and 112 d compared to the silage produced at 56 d of regrowth. The silage made at 56 d of regrowth had the highest (P<0.05) NDF effective degradability compared to the silages ensiled at 84 or 112 d which did not differ (P>0.05) between themselves.

Reduction of NDF effective degradability with increasing grass regrowth age has been related to structural changes that lead to increased proportion of support and vascular tissues (sclerenchyma and xylem), which have densely packed cells with thick and lignified walls, hard to break down by rumen microorganisms (Wilson and Mertens, 1995, Wilson and Hatfield, 1997). In addition, higher degree cellulose crystallinity with increasing grass maturity, due to the substitution of water molecules by sugars between the fibrils, may increase hydrophobicity and hinder the access of ruminal microbes to the substrate, thereby increasing the NDF lag time (Chesson and Forsberg, 1997).

Table 4. Ruminal kinetic parameters and effective degradability of neutral detergent fibre (NDF) of *Andropogon gayanus* grass silages ensiled with different regrowth ages

Item	Regrowth age (days)			SEM	*P*-value
	56	84	112		
a (%)	0.0	0.0	0.0	-	-
b (%)	73.3ab	74.9a	70.3b	1.61	0.05
c (%/h)	2.79ab	2.47b	3.12a	0.172	0.02
a+*b* (%)	73.3ab	74.9a	70.3b	1.61	0.05
U (%)	26.7ab	25.1b	29.7a	1.61	0.05
Lag (h)	5.0b	7.0a	6.4a	0.33	<0.01
Effective degradability* (%)	39.4a	36.7b	37.5b	0.38	<0.01

Within a row, means followed by different letters differ (P<0.05). SEM = standard error mean; *a* = soluble fraction; *b* = slowly degradable fraction; *a*+*b* = potentially degradable fraction; U = undegradable fraction; *c* = fractional rate of disappearance.*Calculated with fractional passage rate (*k*) assumed at 0.02 h^{-1}.

Campos *et al*. (2006) reported values of slowly degradable NDF fraction of 63.4% for corn silage, 61.7%, for elephant-grass silage and 51.4% for sorghum silage. These authors also reported values of fractional rate of NDF disappearance of 3.08%/h for corn silage, 3.10%/h for elephant-grass silage and 2.53%/h for sorghum silage. According to Campos *et al*. (2003), NDF effective degradability calculated for fractional rate of particulate passage of 2%/h ranged from 30.3 to 39.1% for the silages of different sorghum hybrids. Working with elephant-grass silages ensiled with different regrowth ages, Rêgo *et al*. (2011) observed potentially degradable NDF fraction ranging from 46.0 to 58.5%. In the present work, the potentially degradable NDF fraction ranged from 70.3 to 74.9%, with fractional rate of NDF disappearance ranging between 2.5 and 3.1%/h. The NDF effective degradability values were 39.4, 36.7 and 37.5% for the *A. gayanus* grass silages ensiled at 56, 84 and 112 d of regrowth respectively. These results show a good potential degradation of the fibre fraction from *A. gayanus* grass silages.

CONCLUSIONS

The ruminal degradation kinetics of *Andropogon gayanus* grass silages showed that the grass ensiled at 56 days of regrowth had higher nutritional value.

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