RESEARCH PAPER

Kinetic parameters of ruminal degradation of Marandu grass silage supplemented with brewer's grain

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Abstract

D.J. Ferreira, A.M. Zanine, R.P. Lana, A.L. Souza, F.M. Negrão, L.J.V. Geron, H.N. Parente, and C.C.O. Dantas. 2016. Kinetic parameters of ruminal degradation of Marandu grass silage supplemented with brewer's grain. Cien. Inv. Agr. 43(1):135-142. This study measured the kinetic parameters of the ruminal degradation of the silage of *Brachiaria brizantha* cv. Marandu. The experiment had a completely randomized design with five treatments and five repetitions; the treatments featured the inclusion of natural brewer's grain to Marandu grass silage at levels of 0, 10, 20, 30 and 40%. The regression equation for the degradability of the soluble Fraction A of dry matter showed a linearly increasing pattern (P≤0.05) with an addition of 0.19% for each 1% of brewer's grain included. The percentage of insoluble potentially degradable fraction (B) of dry matter was reduced linearly (P≤0.05). The ruminal degradation rate in the dry matter (c), expressed as % h^{-1}, increased linearly (P≤0.05). The crude protein percentage in the Fraction B demonstrated a linear regression pattern as brewer's grains were added (P<0.05), and there was a 0.06% reduction per unit of additive to Marandu grass silage. There was a linear increase in degradation rate c in the crude protein (P≤0.05) as the additive was included in the silage. A linear increase (P≤0.05) was observed for the regression of the potentially degradable fraction of NDF, represented by Fraction D. The inclusion of 20% of brewer's grain to Marandu grass silage resulted in an improvement in the kinetic parameters of ruminal degradation.

Key words: Byproduct, forage conservation, nutritive value

Introduction

Low beef production in the tropics can be primarily attributed to inappropriate nutrition resulting from the characteristic seasonality of forage production under these conditions. In continuous meat production, it is essential to eliminate negative phases of development and promote conditions for normal animal development throughout the year and to reach conditions of slaughter, weight and/or termination at an earlier age. Forage supplements,

such as preserved hay and silage, are necessary to balance animal food requirements during this process (Ferreira *et al.,* 2013).

The conservation process results in a pronounced reduction in energy and dry matter. Losses from secondary fermentation of the effluent produced and aerobic deterioration can range from 7 to 40% (McDonald *et al.,* 1991; Zanine *et al.,* 2010); however, the use of additives to the ensilage of tropical grass can circumvent these limitations, which result from high humidity content, low content of soluble carbohydrates and high buffering capacity. These factors inhibit the occurrence of an adequate fermentative process and hamper the production of high quality silage. To reduce these losses, numerous experiments have been carried out and need to be validated for effectiveness (Zanine *et al.,* 2006a; Zanine *et al.,* 2006b; Negrão *et al.,* 2014).

Brewer's grain is a byproduct with good nutritive value for this application. When this byproduct is dehydrated, it presents hygroscopic characteristics that function as a moisture absorber, enabling the reduction of effluent in grass silage and possibly promoting improvements to fermentative patterns.

According to Van Soest (1994), chemical constituents and degradation rates can greatly differ according to various residues. Therefore, it is essential to evaluate residues with potential for inclusion in animal feeding.

The ruminal in situ incubation technique is considered to be a reference method to estimate degradation parameters, such as soluble, insoluble but degradable and undegradable fractions, in addition to determining potential and effective degradability when adjusted to suitable nonlinear models (Ørskov and McDonald, 1979). The characterization of the fractions that constitute carbohydrates of food obtained in tropical conditions and the evaluation of its degradation kinetics pattern are valuable tools in diet formulation because they aim to maximize ruminal microbial growth and consequently result in a better predic-

tion of animal performance (Dantas *et al*., 2014; Negrão *et al*., 2014).

To our knowledge, there is no information regarding the degradation kinetics of silage with different brewer's grains to Marandu grass silage. In this context, this experiment was conducted to evaluate in situ ruminal degradation of dry matter, crude protein, and fiber in neutral detergent of Marandu grass silage supplemented with brewer's grain.

Materials and methods

The experiment was carried out in the experimental area of the Animal Science Course of Technology and Agriculture Science Institute of Federal University of Mato Grosso, Rondópolis Campus (geographical coordinates 16º28' S and 50º34' W, average altitude of 270 m) in the South-Central region of Mato Grosso, a micro-region of Rondonópolis (Brazil).

The experiment was a completely randomized design with five treatments and five repetitions, with the treatments including natural brewer's grain to *Brachiaria brizantha* cv. Marandu grass silage at levels of 0, 10, 20, 30 and 40%. The Marandu grass was obtained at 60 days of regrowth after being harvested 10 cm from the ground; the grass was ensiled in an experimental silo of 10 L. The silo was opened after 45 days.

After collection, the samples of the silage were immediately dried in a forced air oven at 65 °C for 48 h and then ground in a Wiley mill equipped with sieve mesh of 1 mm, according to the recommendations of the NRC (2001).

The ground samples were stored in polyethylene containers for further analysis of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEM), lignin (LG), mineral matter (MM), and organic matter (OM) according to the methodology described by AOAC (2005) (Table 1).

Table 1. Chemical composition, pH and N-NH$_3$ of brewer's grain and Marandu grass silage plus various levels of brewer's grain.

Variable	Levels of brewer's grain (%)					Brewer's grain
	0	10	20	30	40	
pH	4.28	4.19	4.13	4.12	4.09	---
N-NH$_3$ (%TN)	7.61	7.03	6.45	5.89	5.29	---
Dry matter[1]	24.13	27.91	31.02	39.5	43.9	89.96
Nitrogen[2]	1.15	1.80	2.02	2.16	2.35	3.90
Carbohydrate solubles[2]	4.06	4.09	4.12	4.15	4.19	4.18
Ether extract	3.52	5.08	5.19	5.29	5.42	6.09
Total digestible nutrient[2]	33.27	39.06	44.85	50.60	56.40	60.18
Neutral detergent fiber[2]	73.63	64.06	61.25	61.60	60.50	60.75
Acid detergent fiber[2]	29.45	30.28	30.96	32.30	32.80	30.09
Hemicellulose	44.18	33.78	30.29	29.30	26.00	30.66
Lignin[2]	6.89	6.48	6.40	6.32	6.18	5.23
Minerals[2]	6.31	7.13	7.02	6.52	6.55	7.36
In vitro dry matter digestibility[2]	60.93	64.37	65.10	65.60	66.10	64.35

[1]Percentage.
[2]Percentage of dry matter.

The total digestible nutrient (TDN) values were estimated according to Van Soest (1994) using the equation: TDN (%) = Deg + (1.25 * EE) – MM, where Deg = degradability, 1.25 = correction factor, EE = ether extract and MM = mineral matter. The content of NDF was determined according to AOAC (2005).

In situ incubation was carried out using four fistulated crossbred cows (Holstein × Zebu) with a mean body weight of 400 kg and kept in Marandu pastures.

Grounded food was weighed and placed in non-woven (TNT) bags to provide a useful area of 10 to 20 mg cm^{-2} of sample in the bags; a vacuum-sealed bag was used as the correction factor according to Nocek's (1988) methodology. The experiment was carried out in duplicate for each food, and each animal represented five repetitions.

The bags were placed into a rumen and then removed from the rumen at 0, 2, 4, 8, 16, 24, 48, 72, 96 and 144 h of incubation. After removal, they were uniformly washed in running water until clean, then dried in a forced air oven for 48 h, according to NRC (2001). DM determination was carried out at 105 °C for 72 h, and the residue obtained from this stage was used for analysis of CP, NDF and NDFi according to methodologies described by AOAC (2005).

The data from the *in situ* degradability of DM were obtained using the difference in weight for each component before and after ruminal incubation, expressed as a percentage. To interpret the degradation profiles of DM and CP, a first class asymptotic exponential model according to Orskov and McDonald (1979) was used; Mertens' (1979) model was used for the degradation profile of NDF.

Throughout these models, the DM and CP contents were estimated for Fraction A, which corresponds to the soluble fraction in water (of DM) and disappears from the bag at time zero; Fraction B, which corresponds to the insoluble

fraction in water but with potential degradation in the rumen according to time; Fraction C, which is the degradation rate of Fraction B, expressed in the percentage per hour; and Fraction I, which corresponds to the indigestible fraction of DM and CP in the rumen, which means that it represents food remaining in the bag after 144 h of incubation.

For NDF and ADF, Fraction D was determined as the fraction that corresponds to the portion of NDF that is potentially degradable in the rumen; Fraction C was determined as the ruminal degradation rate of fraction D expressed as the percentage per hour; Fraction L corresponds to the latency time; and Fraction I corresponds to the fraction that is indigestible in the rumen, or food residue from 144 h of incubation, according to Orskov and McDonald (1979).

The data were statistically analyzed, and the choice of models was based on the significance of the regression parameters tested using the t-test (P≤0.05) and the coefficients of determination (SAEG program, 1999, version 8.1, Federal University of Viçosa, Brazil).

Degradation curves for the DM, CP and NDF of the food evaluated for each animal were submitted for adjustment using their respective models with the procedure of "Marquardt Regression" of SAEG software (1999), which enabled estimates of the parameters analyzed.

Results and discussion

Table 1 shows the values of the chemical composition of brewer's grain and Marandu grass silage plus various levels of brewer's grain.

In Table 2, the means of degradability parameters of fraction A, B and I are presented with the degradation rate of dry matter and crude protein c of Marandu grass ensiled with different levels of brewer's grain.

The regression equation for the degradability of the soluble Fraction A of dry matter shows a linearly increasing pattern (P≤0.05) with an addition of 0.19% for each 1% of brewer's grain included (Table 2). This pattern was similar to that observed by Dantas (2012), who reported that the degradability of the soluble Fraction A of dry matter increased linearly with the inclusion of soybean hulls in *Brachiaria* grass silage. Rego *et al.* (2009) included dehydrated cashew apple in elephant grass silage and also observed a linear degradability of the soluble Fraction A with 0.64% for each 1% inclusion of the cashew byproduct.

This increase in the degradability of soluble Fraction A can be attributed to a greater solubility of the product when it is isolated compared with the grass, contributing to a higher soluble fraction of silage where the highest concentration of the byproduct was added.

The percentage of insoluble potentially degradable fraction (B) of dry matter was reduced linearly (P≤0.05) (Table 2). The estimated reduction was approximately 0.31% for each 1% of brewer's grain added and estimated using the equation to 50.58, 47.47, 44.35, 41.23 and 38.12%, respectively. Rêgo *et al.* (2009) evaluated levels of annatto grain byproduct (0, 4, 8, 12 and 16%) in grass silage and observed that the insoluble potentially degradable fraction (B) of dry matter presented values of 45.72% for the highest concentration of byproduct. It can be inferred that changes in fiber, hemicellulose and pectin contents caused by the inclusion of byproduct can directly interfere with the degradability of this fraction (Rêgo *et al.,* 2010).

The ruminal degradation rate in dry matter (c), expressed as % h^{-1}, increased linearly (P≤0.05) with estimated means of 0.0003% per unit of additive added (Table 2). The observed means are lower than the optimal range proposed by Rezende *et al.* (2007), who reported that values above 0.15% h^{-1} are sufficient for an adequate passage rate of the

food in the gastrointestinal tract of the animal. These authors observed values superior to that of the present study when they added potato meal to elephant grass silage in a range of 0 to 20% using the same methodology of this study.

The inclusion of brewer's grain in Marandu grass silage affected (P≤0.05) the concentration of indigestible fraction (I) of dry matter. Quadratic behavior (P≤0.05) of this fraction was observed when the brewer's grain was added, with the maximum value estimated as 29.34 of fraction (I) at a level of 10.60% of brewer's grain (Table 2).

Brewer's grain is a byproduct generated by the industry after starch from the cereal grains is removed for alcohol production. In brewing, barley grains germinate to convert starch into dextrin and sugar. This process is interrupted by heating it at the point of maximal conversion. Mertens (1979) suggested that the primary mechanism causing decreases in fiber digestion *in vivo* is the lower activity of cellulolytic bacteria due to acid conditions caused by the rapid fermentation of starch. However, brewer's grain showed reduced starch content and did not harm fiber digestion when extensively digested in the rumen.

The regression equation of the degradability rate of the portion of highly soluble in water, represented by Fraction A of crude protein, showed a linearly increasing pattern (P≤0.05), indicating a mean increase of 0.16% for each 1% of additive added. Compared with the control silage (33.65%), 20% (37.00%) and 40% (40.35%) additions of brewer's grain led to 3.35 and 6% increases for the intermediate and highest levels, respectively.

This result was similar to that of Rego *et al.* (2010), who registered a linear pattern in elephant grass silage with 1, 4, 8, 12 and 16% of annatto grain byproduct added. Santos *et al.* (2012) evaluated the degradability of Fraction A of crude protein on elephant grass silage with algarroba meal (*Prosopis juliflora*) and registered a mean of 36.8%, which was similar to the present study using brewer's grain.

Notably, the different components of brewer's grain used in the silage contributed to an increased Fraction A ruminal degradability of dry matter and crude protein (Table 2). This fraction represents the portion of the plant that is readily available to rumen microorganisms. According to Rego *et al.* (2009), Fraction A disappearance characterizes

Table 2. Means of the water-soluble fractions (A), potentially digestible insoluble (B), degradation rate of fraction (B), potentially degradable (c) and indigestible fraction (I) of dry matter and crude protein and their respective equation of regression (R^2) and coefficient of variation (CV) of Marandu grass ensiled with different levels of brewer's grain.

| Variable | Level of dry brewer residue (%) | | | | | Regression Equation ** | CV (%) | R^2 (%) |
	0	10	20	30	40			
Dry matter								
Fraction A		22.52	25.18	27.48	28.88	30.27	Y=22.56+0.193X	1.23	99.78
Fraction B		50.47	47.3	44.3	41.2	38.1	Y=50.58-0.3117X	1.83	94.9
c (% h^{-1})		0.01	0.02	0.02	0.02	0.03	Y=0.01+0.0003X	7.87	90.64
Fraction I		27	27.5	28.2	29.9	31.6	Y=28.15+0.227X-0.0107X^2	2.79	95.43
Crude protein								
Fraction A		33.7	35.37	37.06	38.76	40.36	Y=33.6+0.1675X	1.97	99.88
Fraction B		33.47	32.8	32.1	31.4	30.8	Y=33.13-0.0667X	3.35	90.8
c (% h^{-1})		0.033	0.03	0.04	0.04	0.04	Y=0.03+0.0003X	3.36	78.38
Fraction I		32.88	31.8	30.8	29.8	28.8	Y=32.909-0.102X	4.1	93.84

** Significant at 5% of probability via t test.

the solubilization of sugar and soluble nitrogen compounds remaining from the fermentation in the silo and is primarily composed of sucrose, fructose and glucose plus low amounts of mannose and galactose.

The degradability rate of the portion insoluble in water but potentially degradable in crude protein as a function of time, which was represented by Fraction B, showed a linear pattern and decreased as brewer's grains were added (P≤0.05). There was a reduction of 0.06% per unit of additive included on Marandu grass silage (Table 2).

The estimated values from the equation of the degradability of Fraction B showed that levels of inclusion of 0, 20, 30 and 40% of brewer's grain promoted reductions of 33.13, 32.47, 31.80 and 30.47%, respectively. These results contrasted with those reported by Dantas (2012), who observed a linear increase in Fraction B degradability at levels of 0, 20, 30 and 40% of inclusion of soybean hulls with values of 28.48, 32.78, 37.09 and 45.71%, respectively.

There was linearly increasing pattern of degradation rate c in the crude protein (P≤0.05) as the additive was included in the silage. A reduction of 0.0003% for each 1% of byproduct added was estimated and, for Fraction I, there was a linear reduction of 0.10% per unit of additive included (Table 2). Kung and Ranjit (2001) suggested that the increase in the carbohydrate proportion of the cell wall and lignin content of forage are the primary causes of decreased degradability.

A linear pattern increase (P≤0.05) was observed for the regression of the potentially degradable fraction of NDF, represented by Fraction D (Table 3). There was an increase of 0.62% in the degradability of Fraction D per unit of brewer's grain added.

Given the findings of the present experiment, it could be stated that the action of the microorganisms present in the digestive tract of ruminants can be facilitated by the difference obtained between the chemical composition of the byproduct, which presents low contents of lignin, cellulose and hemicellulose.

For degradation rate c, there was no effect (P≤0.05) for the inclusion of the additive in the silage with a mean of 0.022% h^{-1} (Table 3). The result was antagonistic to those found by Dantas (2012) who observed a reduction of the rate (c) in approximately 0.0003% h^{-1} for each 1% of byproduct added to silage of *Brachiaria decumbens*.

For latency time (L), there was no significant effect for the inclusion of byproduct on the kinetic parameters (P≤0.05). Latency is defined as the duration over which the component (NDF) is not digested (Table 3). This event normally corresponds to initial events of ruminal microorganisms, such as substrate adherence and enzyme synthesis. According to Pires *et al.* (2010), this can be explained by the presence of microorganisms present in the silage. The present study registered contrary results to those observed by the aforementioned authors, who evaluated the *in situ* degradability of silages made from corn, sorghum and Marandu.

The authors observed differences in latency between silages and additives. The latency high values for forage (9.81) can be an effect of the lack of microorganisms at the moment of the trial. A linear reduction (P≤0.05) of indigestible fraction (I) of NDF of silage was observed as brewer's grain was added with a reduction of 0.55% per unit of residue included in the silage (Table 3). According to Cabral *et al.* (2002), this can be considered a beneficial characteristic because this reduction would result in an increase of energy in the animal.

Fraction I was estimated using the equation at the additive levels of 0, 20 and 40%; 49.42, 38.28 and 27.17% reductions were respectively found, indicating differences of 11.14 and 22.25% at the 20 and 40% brewer's grain levels compared with control

Table 3. Means of the degradability parameters of fractions D and I, degradation rate *c* and latency (L) of neutral detergent fiber (NDF) and their respective equations of regression (R^2) and coefficients of variation (CV) of Marandu grass ensiled with different levels of brewer's grain.

Variable	Level of dry brewer residue (%)					Regression Equation **	CV (%)	R^2 (%)
	0	10	20	30	40			
Fraction D	37	40.35	46.6	52.9	59.24	Y=59.41-0.6297X	14.77	75.39
c (% h^{-1})	0.01	0.022	0.02	0.03	0.028	0.022	-	-
L (h)			14.516			14.516	-	-
Fraction I	48.2	44.34	40.1	32.1	26.54	Y=49.42-0.5567X	4.96	98.85

** Significant at 5% probability via t test.

silage. Thus, these content levels can be considered as acceptable for good quality silage in ruminant diets even at intermediate levels of inclusion.

Notably, the proportion of carbohydrates as cell wall and lignin content are factors that most strongly affect the quality of tropical grass. According to Van Soest (1994), the NDF content of forage negatively correlated with consumption. Therefore, the lower contents of lignin and NDF of brewer's grain compared with their content in Marandu grass represent a possible reason for the reduction in Fraction I.

The inclusion of an intermediate level (20%) of brewer's grain to Marandu grass silage promoted the improvement of protein and carbohydrate fractions and the kinetic parameters of ruminal degradation of dry matter, crude protein and neutral detergent fiber.

Acknowledgments

Resumen

D.D.J. Ferreira, A.M. Zanine, R.P. Lana, A.L. Souza, F.M. Negrão, L.J.V. Geron, H.N. Parente y C.C.O. Dantas. 2016. Parámetros cinéticos de degradación ruminal en ensilajes Marandu con granos de cerveza. Cien. Inv. Agr. 43(1):135-142. Este estudio tuvo como objetivo medir los parámetros cinéticos de la degradación ruminal de ensilaje de *Brachiaria brizantha* cv. Marandu. El experimento se realizó en un diseño completamente al azar con cinco tratamientos y cinco repeticiones, los tratamientos se incluyen granos de cervecería a Marandu ensilado de hierba a niveles de 0, 10, 20, 30 y 40%. Observando la ecuación de regresión de la degradabilidad la Fracción soluble A de la materia seca, había una creciente comportamiento lineal (P≤0,05), un aumento de 0,19 unidades porcentuales con la adición de 1% de la fábrica de cerveza en grano ensilado Marandu. El porcentaje de la fracción insoluble potencialmente degradable (B) de la materia seca reducida linealmente (P≤0,05). La tasa de degradación ruminal (c), expresada en % h^{-1} el aumento linealmente (P≤0,05). El porcentaje de proteína cruda en la fracción B demuestra un patrón de regresión lineal con la adición de granos de cerveza (P≤0,05), hubo una reducción de 0,06% por unidad de aditivo incluido en Marandu ensilado de hierba. Para C velocidad de degradación de proteína cruda, una respuesta lineal (P≤0,05), debido a la inclusión de niveles de aditivos en el ensilaje. Hubo aumento lineal (P≤0,05) de la ecuación de regresión de potencialmente degradable NDF, representado por la fracción D. La inclusión de 20% de grano de cerveza a Marandu ensilado de hierba resultó en una mejora de los parámetros cinéticos de la degradación ruminal.

Palabras clave: Conservación de forraje, subproducto, valor nutritivo.

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