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Ruminal degradation of dry matter and neutral detergent fiber of banana peel treated with limestone

Degradação ruminal da matéria seca e da fibra em detergente neutro da casca de banana tratada com calcário

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Abstract

This study aimed to evaluate the kinetics of dry matter degradation and neutral detergent fiber of banana peel treated with limestone. The banana peel has been acquired from a candy manufacturer that after washing with chlorinated water to 1% and pulp removal was discarded. The banana peel *in nature* was treated with 1, 2, 3 and 4% of limestone in the natural matter, homogenized and pre-dried in the sun for 120 hours. The experiment was conducted in a completely randomized experimental design, with five treatments (0 (control), 1, 2, 3 and 4% inclusion of limestone) with 3 repetitions. The dry matter potential degradability, showed no difference (P>0.05) in the levels compared to the control with an average of 67.58%. The insoluble degradation fraction rate of dry matter and the fiber fraction did not differ (P> 0.05) between levels and control. In relation to effective degradability of neutral detergent fiber, there was an increase of 3.47% for each percentage unit increased limestone. In relation to the ruminal degradation parameters of dry matter and neutral detergent fiber is not recommended the utilization of limestone as an additive in the treatment of banana peel.
Key words: Feed evaluation, coproducts, degradability, calcium oxide, ruminants

Resumo

Objetivou-se avaliar a cinética de degradação da matéria seca e fibra em detergente neutro da casca de banana tratada com calcário. A casca de banana foi adquirida de uma indústria de doces que após lavagem com água clorada a 1% e retirada da polpa, era descartada. A casca de banana *in natura* foi tratada com 1, 2, 3 e 4% de calcário na matéria natural, homogeneizadas e pré-seca ao sol durante 120 horas. O experimento foi realizado em um delineamento experimental inteiramente ao acaso, sendo

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cinco tratamentos (0 (controle), 1, 2, 3 e 4% de inclusão de calcário) com 3 repetições. A degradabilidade potencial da matéria seca, não apresentou diferença (P>0,05) dos níveis em relação ao controle com média de 67,58%. A taxa de degradação da fração insolúvel da matéria seca e da fração fibrosa não diferiu (P>0,05) entre níveis e o controle. Em relação á degradabilidade efetiva da fibra em detergente neutro, houve incremento de 3,47% para cada unidade percentual de calcário aumentada. Em relação aos parâmetros da degradação ruminal da matéria seca e da fibra em detergente neutro, recomenda-se a utilização 4% de inclusão de calcário na matéria natural da casca de banana.

Palavras-chave: Avaliação de alimentos, coprodutos, degradabilidade, óxido de cálcio, ruminantes

Introduction

Animal feed is one factor that affects the cost of ruminant production by reducing the profit margin for producers (BOSA et al., 2012). The coproducts of agro industries have received special attention because they may lower costs for animal feed (GOES et al., 2008). The food processing industry produces large amounts of coproducts with potential utility in animal feed. These include banana peels, which are wasted and are quickly becoming a source of environmental pollution (OMER, 2009).

However, the primary difficulty of using banana peels in animal feed during strategic times is related to the management of this coproduct and lack of information about its nutritional value. Banana peels contain approximately 85% water. This water inhibits storage and reduces the storage time. Balancing the diets of animals also presents an issue.

In this sense, dolomitic limestone has been used as an alkalizing agent and desiccant with the intent of promoting the degradability and digestibility of forager feeds with low nutritional value (MACEDO et al., 2011), as well as improving the fermentation profile and allowing greater nutritional benefit (CALDERÓN; SHIMADA, 1980).

Current systems of ruminant diet adequacy require information on the proportions of feed fractions, as well as their degradation rates. This is relevant to synchronize the energy availability and nitrogen in the rumen, maximize the efficiency and microbial degradation of feed, and reduce losses from ruminal fermentation (GOES et al*.,* 2010).

The fit between fermentation protein and carbohydrates (for the same rate of degradation) promotes maximum microbial synthesis and enhances the metabolizable protein (VIANA et al., 2012). However, robust technical and nutritional foundations should be applied, and a truly viable economic alternative should be ensured, as the ingredients that are usually used undergo price variations. It is thus necessary to obtain more knowledge regarding the potential benefit from the nutrients in banana peels (CABRAL et al., 2005).

Considering the above, this study evaluated the ruminal degradation kinetics of dry matter (DM) and neutral detergent fiber (NDF) of banana peels treated with dolomitic limestone.

Material and Methods

The experiment was performed at the experimental farm at the State University of Montes Claros, campus Janaúba, from October 2012 to March 2013. The geographic coordinates are 15º47’50” south latitude and 43º18’31” west longitude. The altitude was 516 m.

The samples of fresh ripe banana peels (5 kg) were conditioned in polyethylene containers with a 25-liter capacity; calculations were then performed to determine the amount of limestone to be added at concentrations of 0 (control), 1.0, 2.0, 3.0 and 4.0% of the fresh matter (FM). Table 1 contains the chemical composition of dolomitic limestone.

After liming and homogenization, the banana peels were pre-dried in the sun for five days. After losing 85% of their water content, the peels were stored for analysis.

Table 1. Chemical composition of dolomitic limestone micropulverized.

Components	Concentration
Oxides Sum (%)	71.70
Calcium Oxide (%)	53.14
Magnesium Oxide (%)	18,56
Power Neutralization(%)	128.48
TRNP (%)	119.91
Humidity (%)	8.00

TRNP - Total Relative Neutralizing Power.

Subsequently, part of the samples were milled (Wiley mill type) in 5-mm diameter sifter sieves and applied to promoting the kinetic study of ruminal degradation. The rest of the material was milled (Wiley mill type) using a 1-mm sifter sieve to evaluate the chemical composition (Table 2). Detailed data and a discussion on the chemical composition of banana peels treated with limestone can be found in Monção et al. (2014a). The definitive dry matter content was determined using the pre-dried samples, which were stored at 105ºC in the hothouse for 8 h according to the methodology described by Detman et al. (2012) (INCT-CA G-003/1). The residue was incinerated in a muffle furnace, and the ash content was evaluated. The crude protein contents (CP) were analyzed in accordance with the procedures described by the Association of Official Analytical Chemists (AOAC, 1990). The fiber content in the neutral detergent corrected for ashes and protein (NDFap), acid detergent fiber (ADF) and lignin (LIG) were estimated using the method described by Van Soest et al. (1991). The total carbohydrate content (TC) was estimated using the equation TC (%) = 100 - [CP (%) + EE (%) + ashes (%)]. The non-fiber carbohydrates (NFC) were estimated using the equation (CNF = TC - NDFap) according to Sniffen et al. (1992).

Table 2. Chemical composition of banana peel treated with limestone.

	Inclusion level (% FM)					
Variables	0	1	2	3	4	CV (%)
DM (%)	78.75	78.71	79.05	79.23	78.19	0.27
PB[1]	9.13	8.22	7.96	7.63	7.27	7.15
NDFap[1]	50.15	51.43	50.97	50.26	49.83	8.71
Ahess[1]	13.38	29.53	20.92	20.21	10.18	3.45
ADF[1]	28.67	29.53	20.92	20.21	10.18	11.03
LIG[1]	10.77	13.33	10.59	11.03	8.50	5.33
TC[1]	72.93	67.57	60.90	57.32	56.43	1.42
NFC[1]	15.66	15.47	11.84	8.87	9.13	7.86

DM - Dry matter; CP - Crude Protein; NDFap - Neutral detergent fiber corrected for ash and protein; ADF - Acid detergent fiber; LIG - Lignin; TC - Total Carbohydrates; NFC - Non-fiber carbohydrates; TEST - Control; [1] in % of dry matter; CV - Coefficient of variation.

For the degradability assay, we used three Holstein x Nellore crossbred steers with 400 kg of body weight. The steers were castrated, properly identified and cannulated in the rumen. They were part of an intensive breeding system (confinement in collective pens) and were provided with troughs and watering holes.

The animals were adapted to the diet for 14 days, whereby they received as roughage (R) sugar cane and banana peel that was pre-dried (50% of the diet) and concentrated (C) based on corn and soybean meal (an 80:20, R:C ratio), which provided necessary conditions for the normal functioning of the rumen.

The samples were packed in TNT (non-woven) bags with 100 g/m^2 gram mage, as recommended by Casali et al. (2008) [15 x 7.5 cm with meshes of 52 microns, amount of 2,0 g of the DM bag^{-1} to maintain a ratio of 20 mg of DM cm^{-2} for the surface area of the bag] (NOCEK, 1988).

The TNT bags containing the samples were sealed in the sealing machine and put in tulle bags with a small lead weight of 100 g that was tied to a nylon line approximately 80 cm in length that was then tied to the cannula lid by one of the extremities. This allowed the tulle bag with the samples to be stored in the ventral region of the rumen.

The incubation periods corresponded to 0 (incubated for 15 minutes), 3, 6, 12, 24, 36, 48, 72, 96 and 120 hours, and the TNT bags were incubated in decreasing order of time to be removed at once at the end of the incubation period and thereby promote uniform washing of the material at rumen removal. After a total incubation period of 120 hours, the TNT bags were manually washed in cold running water until clean. The bags then underwent forced ventilation oven drying at 55ºC for 72 hours as described in the methodology by Detmann et al. (2012).

After obtaining the DM of the samples, they were used to estimate the NDF according to the methodology of Van Soest et al. (1991).

The data from the *in situ* degradability of DM and NDF were obtained using the ratio of the weight difference for each component between the weighings performed prior to and after the ruminal incubation (expressed as a percentage).

The experimental design was completely randomized with five treatments (0 (control), 1, 2, 3 and 4% of limestone). The control had three replicates.

The data obtained regarding incubation time for DM and NDF were adjusted for non-linear regression using the Gauss-Newton method according to the equation proposed by Ørskov and McDonald (1979): $Y=a+b\ (1-e^{-ct})$, wherein: Y = cumulative degradation of the nutritional component analyzed after time t; a = the intercept degradation curve when t = 0, which corresponds with the soluble fraction in the water of the nutritional component analyzed; b = potential degradation of the insoluble fraction in the water of the nutritional component analyzed; a + b = potential degradation of the nutritional component analyzed because time is not a limiting factor; c = degradation rate by the fermentative action of b; and t = incubation time.

Once calculated, the coefficients a, b and c were applied to the equation proposed by Ørskov and McDonald (1979): $RD=a + (b*c/c+k)$, where RD = effective ruminal degradation of the nutritional component analyzed and k = passage rate of food. We assumed a particle passage rate in the rumen of 5% h^{-1}, as suggested by the AFRC (1993).

The NDF degradability was estimated using the model of Mertens and Loften (1980): $Rt = B \times e^{-ct} + I$, where Rt= fraction degraded at time t, B= insoluble fraction potentially degradable and I = undegradable fraction. After the adjustments of the FDN degradation equation, we proceeded to the standardization of fractions, as proposed by Waldo and Smith (1972), using the equations $Bp= B/(B+I) \times 100$ and $Ip= I/(B+I) \times 100$, wherein Bp = potentially degradable standardized insoluble fraction (%), IP= standardized undegradable fraction (%) B= potentially degradable insoluble fraction and I = indigestible fraction. When calculating effective NDF degradation, the model $DE= Bp \times c/(c+k)$ was used, in which Bp is the standardized potentially degradable fraction (%).

When significant, the means were subjected to Dunnett's test compared with the control using the GLM procedure of SAS (SAS INSTITUTE, 2000) at 5% probability.

The effect of the inclusion levels of limestone on the degradation parameters of DM and NDF banana peels were analyzed using regression equations for the independent variable (inclusion levels) and the dependent variables obtained (parameter degradation) using the REG procedure by SAS (SAS INSTITUTE, 2000) at 5% probability. The tested regression models were linear, quadratic and cubic. To assess whether the parameters of the equation were the same as or different from zero, we used the "t" test at 5 (*) and 1% (**) probability.

Results and Discussion

There was a significant reduction (P<0. 05) in the soluble fraction "fraction a" as opposed to the insoluble fraction in the treated peels compared with the control. There was also potentially greater degradable "fraction b" and effective degradability (ED) in the banana peel DM in the treated peels compared with the control (Table 3).

Table 3. Parameters of dry matter (DM) ruminal degradation of the banana peel treated with limestone.

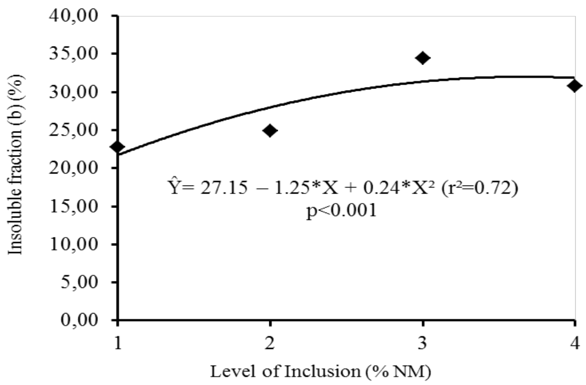
Parameters	Inclusion level (% FM)						
	0	1	2	3	4	CV (%)	EPM
a (%)	43.61	* 42.27	* 41.69	* 35.17	* 38.85	2.37	0.20
b (%)	23.39	22.77	24.88	* 34.47	*30.80	1.66	0.10
c (% h^{-1})	1,47	1.85	2.16	2.46	1.12	14.66	0.00
PD (%)	67.01	65.04	66.58	69.65	69.65	1.56	0.23
ED (%)	48.95	48.42	48.93	* 44.86	* 44.48	2.55	0.27
R^2 (%)	0.93	0.90	0.96	0,97	0.92	-	-

* Averages differ from the control treatment by the Dunnett test (P<0,05). a - soluble fraction; b - insoluble fraction, but potentially degradable; c - degradation rate of fraction "b"; PD - Potential Degradability; ED - Effective Degradability; R^2 - Coefficient of determination.

For "fraction a", there was a reduction of 3.07, 4.40, 19.35 and 10.91% for 1, 2, 3 and 4% limestone levels compared with the control (43.61%), respectively. This reduction can be explained by excessive heat during the alkaline treatment in the first hours after the addition of limestone, which may have caused the combustion of soluble carbohydrates (sugars), thereby promoting the reduction of non-fiber carbohydrate levels (NFC) (Table 2). No differences were observed between the limestone levels, with a 39.49% average soluble fraction. Romão et al. (2013) evaluated the degradation parameters of dry matter and sugarcane cell wall components treated with calcium oxide using an *in situ* technique and found a reduced soluble fraction when working with 4.5% calcium oxide.

For "fraction b", there was an increase only in the 3 to 4% levels of limestone. These levels were 47.37 and 31.68% higher than the control (23.39%), respectively. Between the levels, the averages adjusted to the quadratic regression model, and the 2.60% level maximized the fraction b content of banana peel DM (Figure 1).

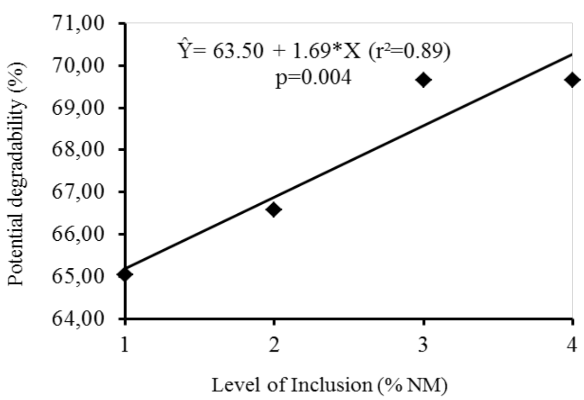
Figure 1. Behavior of the insoluble fraction, but potentially degradable (fraction b) of banana peel dry matter as a function of limestone inclusion in the Fresh Matter (FM).



Omer (2009) evaluated three components of the banana plant. Among these peels *in nature* the "b" fraction content of DM was 38.74%. This value is higher than that observed in this study for the peel control. This variation can be explained by edaphoclimatic variations, genetics and growth stage, which promote changes in the peel chemical composition.

There was no significant difference (P>0.05) for the degradation rate of fraction "b", "c" or potential degradability (PD) of banana peel DM compared with the control, with an average of 1.81% h^{-1} and 67.58%, respectively. Between the limestone levels (except 3 and 4%), an increase was observed in PD of 1.69% for each percentage of limestone (Figure 2).

Figure 2. Potential Degradability behavior (PD) (%) of banana peel dry matter as a function of limestone inclusion in the Fresh Matter (FM).

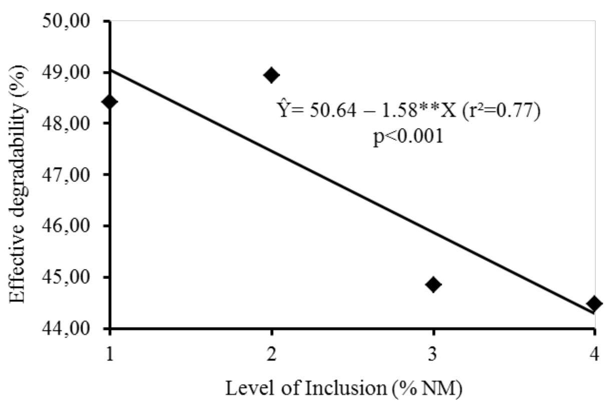


The banana peel presents a high "a" fraction (soluble sugars), which promotes rapid microbial growth in the rumen. However, the low ruminal degradation rate of the potentially degradable fiber fraction "b" can reduce DM intake and energy availability, which limits the animals' productive performance (MACEDO et al., 2011).

Omer (2009) found a degradation rate of the insoluble fraction and potential degradation for the banana peel of 5.1% h^{-1} and 87.45%, respectively. Usually, when limestone is used in low nutritional value roughages, such as sugarcane, an increase in degradation rate as well as the PD of DM was observed (RIBEIRO et al., 2009; MACEDO et al., 2011).

There was a significant reduction (P<0.05) in the ED of banana peel DM in the 3 and 4% levels compared with the control, and the associated averages were 8.35 and 9.13% less than the control. There was a linear reduction (P <0.05) in the ED of MS with a reduction in limestone supply. A reduction of 1.58% was observed in the ED for each 1% increment in fresh limestone matter (FM) (Figure 3). This likely occurred due to the increase in the degradation rate of the fiber fraction (represented by fraction "b"), thereby enabling shorter permanence of food in the rumen.

Figure 3. Effective Degradability behavior (ED) (%) of banana peel dry matter as a function of limestone inclusion in the Fresh Matter (FM).



Another relevant factor that may have affected the ED values of DM is the tannin content, which was not quantified in this study; lignin represents another potential factor (Table 2). The initial and final parts of the banana peel as well as the stem (fruit support structure) may contain high levels of tannin and lignin, a phenolic compound responsible for causing the intoxication of microorganisms in the rumen (MONTEIRO et al., 2005). Also, according to the authors, tannins may form hydrogen bonds with cell components, such as proteins, to confer resistance to rumen degradation. This may be advantageous when the food has a high-value biological amino acid profile and particularly when essential amino acids are included.

There was a significant difference ($P<0.05$) between the treated peels and the control peels regarding the insoluble fraction. There was also a significant difference regarding the degradable standardized (Bp), effective degradability (ED) and standardized undegradable fraction (Ip) of NDF bananas in the treated peels compared with the control (Table 4).

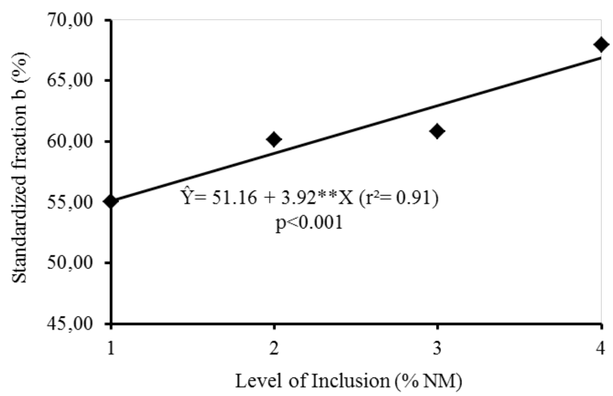
Table 4. Degradation kinetics (Bp, Ip and c), coefficient of determination (R^2) and effective degradability of the fiber in neutral detergent (ED) of banana peel treated with limestone.

Parameters	Inclusion level (% FM)						
	0	1	2	3	4	CV (%)	EPM
Bp (%)	60.56	*55.05	60.14	60.80	*67.91	2.54	0.42
c (% h^{-1})	3.27	1.83	1.99	3.29	2.74	7.74	0.00
ED (%)	23.67	*14.58	17.03	24.03	23.84	2.73	0.19
Ip (%)	39.44	*44.95	39.86	39.20	*32.09	6.20	0.42
R^2 (%)	0.94	0.95	0.96	0.95	0.97	-	-

* Averages differ from the control treatment by the Dunnett test ($P<0,05$). Bp - insoluble fraction, but potentially degradable standardized; c - fraction degradation rate "Bp"; ED - Effective Degradability, 5%; Ip - standardized insoluble fraction; R^2 - Coefficient of determination.

The Bp of NDF varied between 60.56 and 67.91% for the control and the 4% limestone level. An increment of 12.13% was observed in the Bp of the NDF of banana peels.However, when 1% limestone was utilized, there was a reduction of 9.90% compared with the control.Between the levels, there was a linear increase of 3.93% in the Bp of NDF for each 1% of limestone added in FM (Figure 4).

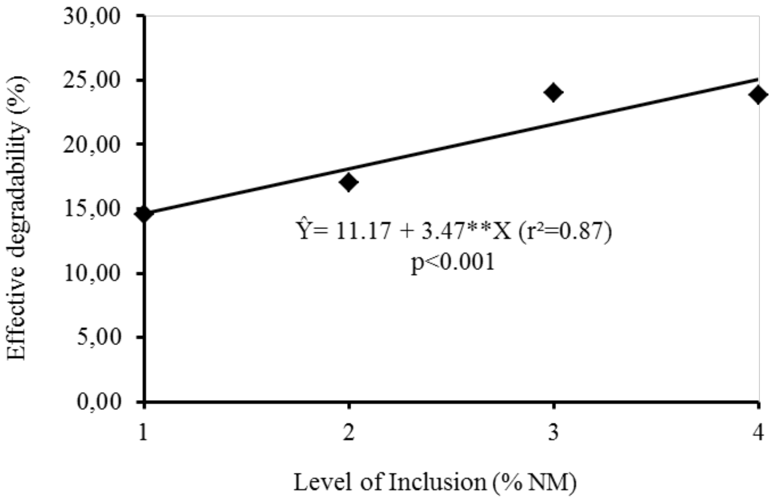
Figure 4. Insoluble fraction behavior but potentially standardized degradable (%) of neutral detergent fiber of banana peel as a function of limestone inclusion in the fresh matter (FM).



The low limestone action observed in this study at the levels of 1 and 2% can be attributed to variations in the calcium oxide content, which acts on cell wall components to reduce the fiber content, favor rumen microbe action and increase the fiber fraction and action of degradation (RIBEIRO et al., 2009).

There was no significant difference (P> 0.05) in the Bp degradation rate between the treatments and the control; the observed average was 2.62% h^{-1}. However, for ED, there was a reduction of 38.40% when using 1% of limestone compared with the control (23.67%).Between the levels, the averages fit the linear regression model, an increase of 3.47% in the ED of NDF was observed per the unit percentage of added limestone (Figure 5).

Figure 5. Behavior of the effective degradability (%) of neutral detergent fiber of banana peel as a function of limestone inclusion in the fresh matter (FM).

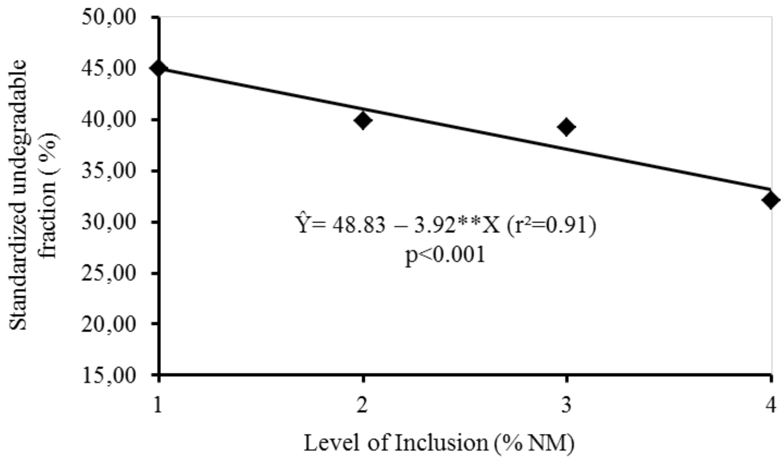


According to Aroeira et al. (1996), the ED of a food can be considered to be the digested energy in the rumen. Therefore, the ingestion of foods with higher DM degradability and the fiber provides more energy for the microorganisms, which can reflect better performance for the animals. Almeida et al. (2015) evaluated the performance of lambs fed with waste from processing agro fruit from these pre-dried banana peels to replace 75% of roughage. The authors observed that the use of banana peel in the diet with 60% roughage and 40% concentrate enabled daily gains of 175.40 g. The positive performance results observed by the authors are largely related to the potential and effective degradability of both DM as the NDF. In this research, the potential degradation values of DM and NDF were above 55%.

In this research, the increase in limestone content on the FM of banana peels occurred due to the changes in the structural integrity of the cell wall components caused by limestone. These changes promoted the rupture of hydrogen bonds and resulted in the expansion of cellulose molecules. This affected the osmotic pressure, as well as the ability to retain water. It also increased intracellular pressure. This caused cell lysis and enabled increased microorganism action. Nutrient degradation into the extracellular medium was thus promoted. In this manner, the ED marginal increased by 38.85% with the inclusion of 1% to 4% limestone. Monção et al. (2014b) evaluated the ruminal degradation of the fiber fraction of banana peels treated with 1, 2, 3 and 4% quicklime in FM. They observed an increase of 7.32% over the ED of NDF incremented with a quicklime level of 1% to 4%. According to the authors, the quicklime with a higher calcium oxide content likely promotes greater action on the cell wall components of the banana peel, thereby enabling higher microbial activity in the rumen and consequently better use of energy.

For the Ip, there was a significant reduction (18.63%, P<0.05) when 4% limestone was used in relation to the control (39.44%). Between the levels, there was a linear reduction of 3. 92% for every 1% of limestone added to the FM of the banana peel. This reduction could be explained by a decrease in the lignin content (Table 2) of the banana peel, resulting in an undegradable lower portion of the fibrous fraction (Figure 6).

Figure 6. Behavior of the standardized undegradable fraction (%) of banana peel neutral detergent fiber as a function of limestone inclusion in the fresh matter (FM).

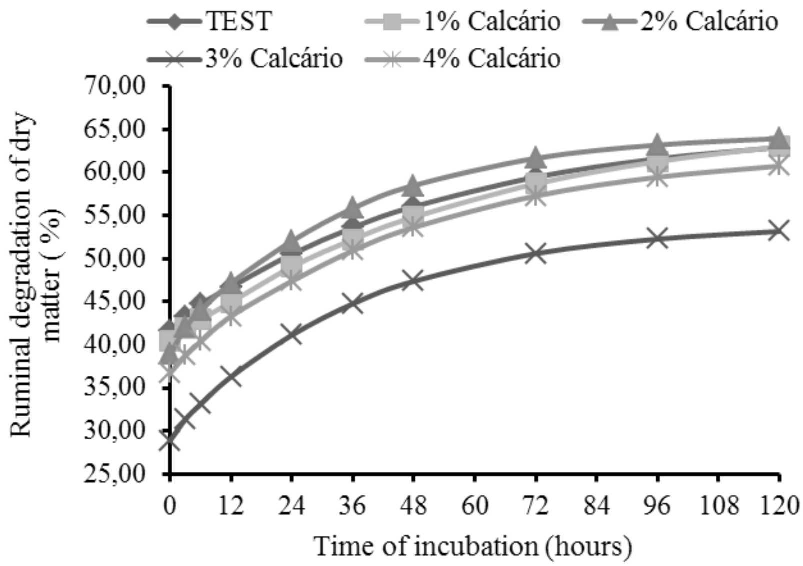


When comparing these results with theories on food intake by animals, a greater NDF intake possibly lowers the food rate of passage and limits the intake of fill rumen. However, other factors may be associated, such as dietary energy and the nutritional quality of the food, the animals' nutrient requirements, the animal species and category (SILVA, 2011). In the same manner, Mertens (1997) reported that feed intake is negatively correlated with the NDF diet content. Therefore, less degradable fiber results in an increased duration during which bulk remains in the rumen. This increased filler limits food intake. Evidently, the associative effect of food can improve a poor fiber passage rate and can cause an increase in dry matter intake of forage with high fiber content. However, this procedure should be used with restrictions and only in economically viable scenarios. This research found that a higher percentage of limestone used in banana peel FM promoted a reduction in cell wall components. This contributed to improving the degradability rate of DM and NDF of treated banana peels. Thus, 4% of limestone in FM should increase DM consumption. However, this variable was not quantified in this study.

The degradability behavior of the treated banana peel DM and the control function of the incubation periods were analyzed. A predominance of the control was degraded following 12 hours of incubation. After this period, the treatment is increasingly degraded with 2% of limestone or higher. The banana peel control was fully degraded at approximately 96 hours (Figure 7).

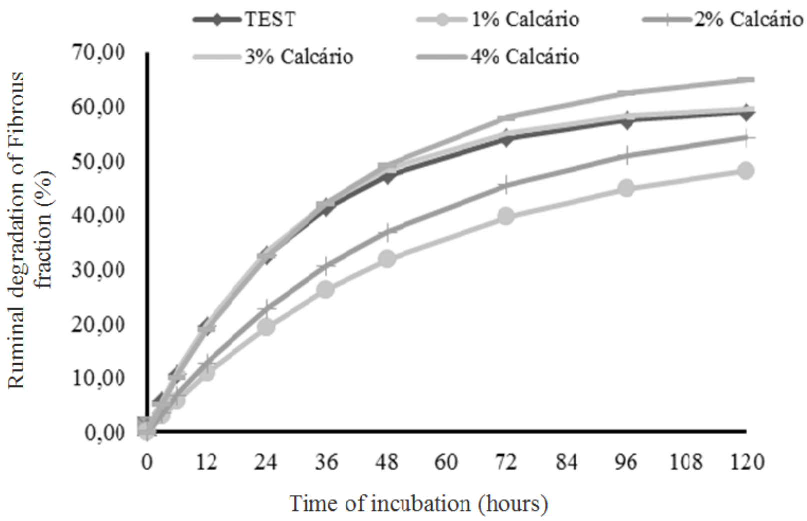
The higher degradation of DM observed for the banana peel control at time zero was likely due to the higher availability of soluble compounds in relation to treatments. Minor soluble carbohydrate availability and ruminal degradation were observed in the treatments with 3 and 4% limestone across all incubation durations. There was no tendency for DM ruminal degradation stabilization in the treatments or the control at any time periods.

Figure 7. Potential degradability of dry matter (DM) (%) of banana peel treated with limestone in function of incubation period in the rumen of crossbred steers.



For NDF, ruminal degradation for the 3 and 4% treatments and the control presented similar behavior for up to 36 hours of incubation. There was a predominance of treatment with 4% of limestone for longer incubation durations. Treatments with 1 and 2% limestone had lower degradation values at all incubation periods, with the exception of time zero, which was equal to the other treatments and the control (Figure 8).

Figure 8. Degradability potential of neutral detergent fiber (NDF) of banana peel treated with limestone in function of incubation period in the rumen of crossbred steers.



Conclusion

Limestone is not recommended as an additive in the treatment of banana peels due to the ruminal degradation parameters of dry matter and neutral detergent fiber.

Further research is recommended for the use of alkaline agents in banana peels, as are analyses of inclusion levels and animal responses to banana peel consumption.

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