Vol. 14(6), pp. 369-378, 7 February, 2019
DOI: 10.5897/AJAR2018.13675
Article Number: C0B84DA60208
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Different dry matters content used for the conservation of annual ryegrass (*Lolium multiflorum* Lam.) in anaerobic environment

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Received 26 October, 2018; Accepted 31 January, 2019

Annual ryegrass (*Lolium multiflorum* Lam.) is widely used for feeding ruminants, and the conservation of this material as haylage can be an alternative to farms. The aim of this work is to study the nutritional and microbiological value of annual ryegrass (*L. multiflorum* Lam.) pre-dried and stored with different dry matter contents (250, 350, 450, 550, 650 and 750 g kg^{-1}) in anaerobic environment. The experimental design was completely randomized with six treatments and four replications. The pH presented at the time of opening of the silos showed a linear increase of 0.003 pH units for each 1 g kg^{-1} of dry matter increase in ryegrass, as the materials with contents above 550 g kg^{-1} of dry matter showed a pH above the desired pH. Materials containing 650 and 750 g kg^{-1} dry matter showed the lowest concentrations of NH_3-N. This fact indicates that haylages with higher levels of dry matter present lower protein losses after aerobic exposure. The increase in dry matter contents of the treatments provided higher crude protein contents to the materials (being 112.91 g kg^{-1} of dry matter) in the treatment with 750 g kg^{-1} of dry matter. The best results for the proliferation of LABs and efficiency in pH decrease were in the treatment with 450 g kg^{-1} of dry matter.

Key words: Haylage, chemical composition, *Lolium multiflorum,* silage, microorganism.

INTRODUCTION

Annual ryegrass (*Lolium multiflorum* Lam.) is widely cultivated in the world for various purposes (Choi et al., 2017), especially in animal feed. Dairy farms depend heavily on ryegrass for high-quality winter food to cows (Han et al., 2014). In this food, the conserved forms used with silage, hay and haylage are keys to herd feeding in shortage periods or during the year (Meinerz et al., 2015). Using this temperate cereal for conservation can be a viable option given their shorter cycle than maize; they can adapt to decreased rainy seasons and frost

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resistant as well as make better use of water by providing early forage or a silage cut for dry season (Celis-Alvarez et al., 2016).

Corn silage is of great importance for dairy cattle feeding systems, demonstrating high rates of mass and energy production at a relatively low cost (Hernandez-Ortega et al., 2011). However, maize silage has low protein content compared to ryegrass. Thus, ryegrass conservation can be a promising option for dairy farms, especially in regions favorable to the production of this forage.

In the conservation of fodder, conservation is sought by the reduction of available water in the hays, anaerobic fermentation in the silages, and both preservation tools in the haylage. The principle of forage conservation through haylage and silage is based on anaerobic fermentation, aiming to provide sufficient amounts of lactic acid to promote a drop in pH (Soundharrajan et al., 2017) and inhibit undesirable microorganisms (Nath et al., 2018). After harvester, silos are used in silage storage, while in the haylage, the bales are individual with plastic wrapped (Han et al., 2014).

In haylage of ryegrass, the pre-drying after the cut is an excellent technological alternative for improving the fermentation pattern of haylages (Nath et al., 2018) and to preserve the nutritional characteristics of original forage. However, the adequate DM content for pre-drying is not known to retain the original nutritional characteristics of ryegrass and to prevent the proliferation of undesirable microorganisms.

Thus, the objective of this work is to evaluate the nutritional and microbiological value of pre-dried annual ryegrass (*L. multiflorum* Lam.) stored with different dry matter contents in anaerobic environment.

MATERIALS AND METHODS

The experiment was conducted at the Animal Nutrition Laboratory of Unipampa - Uruguaiana Campus, located at Latitude 29° 45 '17 "S and Longitude: 57° 05' 18" W, at an altitude of 66 m, Rio Grande do Sul, Brazil. The design was completely randomized with six treatments and four replications. The treatments studied were different dry matter (DM) contents (250, 350, 450, 550, 650 and 750 g kg^{-1}) of annual ryegrass stored in anaerobic environment.

The ryegrass implantation was done in the month of May and its harvest was done with a tractor harvester placed 5 cm in the soil, during the phenological stage of pre-flowering. The cut material was ground in a hammer mill for conditioning and was exposed to the sun for natural dehydration. In dehydration, the material was placed on a plastic canvas with a layer of 10 cm where it remained for a period of time sufficient to reach the desired DM content for ensiling according to the treatments. In the monitoring of DM contents, 100 g samples at 15 points were collected every 2 h for instantaneous measurement (Lacerda et al., 2009).

After reaching the desired MS content, the forage was stored in experimental silos made with polyvinyl chloride (PVC) pipes of 50 cm high and 10 cm diameter. The silos were sealed with caps equipped with Bunsen type valves for the free escape of the gases fixed with the aid of adhesive tape. For the drainage of the effluent produced, 0.5 kg of dried and autoclaved sand, insulated by a cotton cloth, was conditioned at the bottom of each silo. Quantities of 2.210, 2.100, 2.090, 1.240 and 1.000 kg for the DM content of 250, 350, 450, 550, 650 and 750 g kg^{-1} were conditioned. Thus, the stocking densities of forage were obtained: 563, 535, 532, 420, 316 and 255 kg m^{-3} and forage DM of 141, 187, 240, 231, 205 and 191 kg m^{-3}.

In the silos unloaded after 60 days, the upper and lower portions of each silo were discarded (5 cm) with posterior homogenization and sampling of the remaining silage to study the microbiological and bromatological profile of the silages. The fermentative characteristics were studied by determination of pH and ammoniacal nitrogen (AN), being the previous item determined in relation to the total nitrogen (NH_3-N/TN) in the 60 days of fermentation. The pre-drying was determined in samples of 350 g, by drying in oven with forced circulation of air under a temperature of 55°C for 72 h. The pH and NH_3-N were determined in the independent samples according to Silva and Queiroz (2009) and Bolsen et al. (1992), respectively.

The bromatological profile was determined in the samples after obtaining the DM and milling in mill of Willy type knives with stainless steel chamber and sieve, using 1 mm mesh. The dry matter correction at 105°C and contents of organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose and hemicellulose were determined (Van Soest et al., 1991). Nitrogen bound to neutral detergent fiber (NIDN) and nitrogen bound to acid detergent fiber (NIDA) were estimated in relation to total nitrogen (TN). Fractions of carbohydrates were estimated according to Sniffen et al. (1992). Total digestible nutrients (TDN) were estimated according to Bolsen et al. (1996) and the relative value of forage.

The microbiological characteristics were studied through the determination of the microbial populations according to Silva et al. (2007). After collection of samples these were homogenized and diluted in the proportion of 10 g to 90 mL of peptone water, obtaining a dilution of 10^1 until 10^8. Afterwards, the samples were inoculated in selective culture media. For the growth and counting of filamentous fungi and yeasts, the Potato Dextrose Agar media was used, maintaining the plates at room temperature for 5 to 7 days. For developing the Acid lactic bacteria (LAB), Lactobacillus MRS Broth media were used in the oven at 35°C for 72 h; for developing Enterobacteria, the Violet Red Bile Agar (Oxford) media were used and maintained at 35°C for 48 h; for developing Clostridia, the Reinforced Clostridial Agar media were used and maintained at 35°C for 48 h in anaerobic chamber. After the incubation period, colony forming units (CFU) between 30 and 300 CFU per Petri dish were counted, and the results expressed in $\log_{10}$ CFU g^{-1} of DM (McDonald et al., 1991).

For statistical analysis of the data, they were submitted to analysis of variance and the means were analyzed by the regression analysis (linear and quadratic models tested). The chosen models were based on determination coefficients (R^2), and significance level (to a 5% level) of the regression coefficients. All the analyses were carried out on Sisvar Statistic Program (Ferreira, 2011).

RESULTS AND DISCUSSION

NH_3-N showed a decrease with increasing DM content of forage stored in 0.02 g kg^{-1} of total N for each gram plus DM (Table 1). The NH_3-N quantification in silages is relevant, since it points out the protein that was degraded during the fermentation phase, being one of the main indicators of the quality of the fermentation process (Santos et al., 2010). Its reduction implies better nutritional quality silage, due to the lower loss of nitrogenous compounds. This is used for the development of undesirable microorganisms and is explained by the decrease in CP and TDN (Table 1), with increase of NH_3-N in the forage (250 g kg^{-1} DM), where these microorganisms are greater among the treatments studied (Table 1).

According to McDonald et al. (1991), the values of NH_3-

N above 100 g kg^{-1} in silages indicate poor preservation of ensiled mass; this ammonia arises from the catabolism of amino acids during the fermentation process. At the lower DM content studied, the percentage of NH_3-N was higher in relation to the others and exceeded the limit suggested by McDonald et al. (1991) for good quality silages. High amounts of NH_3-N decrease the amount of N available for ruminal metabolism due to its high volatilization rate after silo opening.

The dehydration process in the field provided an increase in DM content of ryegrass; however, this was accompanied by an increase in pH values of 0.0107 pH units for each 1 g kg^{-1} increase in DM content (Table 1). This increase makes it difficult to lower the pH after ensilage for adequate food preservation, favoring the proliferation of undesirable microorganisms inside the silo. The pH presented at the time of opening of the silos also showed a linear increase of 0.003 pH units for each 1 g kg^{-1} of DM increase in ryegrass, being that the materials with contents above 550 g kg^{-1} of DM showed a pH above the desired pH (Table 1).

The pH increased with increasing DM content of the ensiled fodder. This increase is due to the higher percentage of oxygen contained in the stored fodder, since the increase in DM also entails greater compaction difficulties, and the presence of residual oxygen delays the establishment of lactic acid bacteria. To limit the growth and proliferation of undesirable microorganisms and to guarantee an adequate fermentation inside the silo, it is necessary that the pH of the silage is less than 4.0 to promote the conservation of nutrients for long periods (Arriola et al., 2011). However, the verification of the pH of the ensiled material alone is not a definitive parameter for silages with high MS content, as opposed to materials with lower concentrations, which is still considered a good standard of evaluation of the fermentative quality of silages (Cherney and Cherney, 2003).

A linear increase of 0.333 g kg^{-1} in DM losses for each 1 g kg^{-1} of increase in DM content of ryegrass was observed (Table 1). These losses are due to the continuity of the respiratory processes inside the silos. This is due to compaction of forage, which is difficult during the ensiling process of materials with high DM contents. The increasing losses of DM in silages occur mainly in grasses that contain reduced levels of fermentable carbohydrates. With the increase of the DM content of the forage to be ensiled, a reduction in the density of the silage fodder also occurred, allowing the maintenance of a greater amount of air inside the silos, favoring the prolongation of the aerobic phase of the ensilage and the continuity of the respiratory processes. During the cellular respiration inside the silos, part of the fermentable substrates is used by the aerobic microorganisms for energy production, causing the consumption of DM and the production of CO_2 and water.

Around 75-90% of the total nitrogen is in the form of protein. During ensiling, proteolysis occurs, causing 40-60% of the nitrogen to be solubilized in non-protein nitrogen compounds (McDonald et al., 2010). CP increased with increasing DM contents (Table 1). This result is due to the lower proteolytic activity and lower loss of nitrogen compounds during the fermentation, since the extent of proteolysis decreases with increasing MS contents. According to Özelçam et al. (2015), determining CP of ryegrass silages and hay presented lower results than those found in this study, being 8.91 g kg^{-1} of DM for silage and 6.35 g $kg^{-1}$of DM for hay, which indicates that the pre-drying of the materials contributed to the increase of CP.

The levels of nitrogen bound to neutral detergent fiber (NIDN) and nitrogen bound to acid detergent fiber (NIDA) decreased with increasing DM content of the ensiled material, indicating a reduction of the proteolysis inside the silos. This results in a lower loss of nitrogen compounds. Elevation of DM content inhibits the development of microorganisms' population of the *genus* Clostridia and proteolytic potentials in silages.

Large swings were observed in the materials (Figure 1). In most of the samples, the silos temperature was lower than the ambient temperature, evidencing that there was no excessive heating of the ensiled material regardless of the DM content adopted. Materials stored immediately with adequate compaction tend not to obtain temperatures above 5 to 8°C at room temperature (Kung et al., 2018), which can be observed at the materials with lower DM contents, and remained less exposed during pre-drying to the field, being ensiladed of faster form.

According to McDonald et al. (1991), in the first days after ensiling, until the end of the aerobic phase, it is common to observe the heating of the ensilage material which can last from 48 to 144 h. After this period, when there is anaerobic fermentation inside the silo accompanied by the drop in pH, the temperature decreases and tends to equal the temperature of the environment. This fact can be observed after the 144 h of fermentation (Figure 1), where the temperature inside the silo was lower than the ambient temperature, extending up to the fifteen days of anaerobic fermentation. In materials with DM contents above 400 g kg^{-1} by the slow fermentation, it is common to observe high temperatures, when compared to silage forages with lower DM content; however the results obtained in the treatments with higher DM values were not affected by high temperatures.

Excessive heating of the ensiled material is undesirable. This condition favors the occurrence of Maillard Reaction which is characterized by the non-enzymatic chemical polymerization of soluble sugars and hemicellulose with amino acids of food when the silage temperature rises above 55°C (McDonald et al., 2010). Silages that go through this process will have reduced protein digestibility. If there is high temperature prolongation above 45-50°C, it will result in protein denaturation with

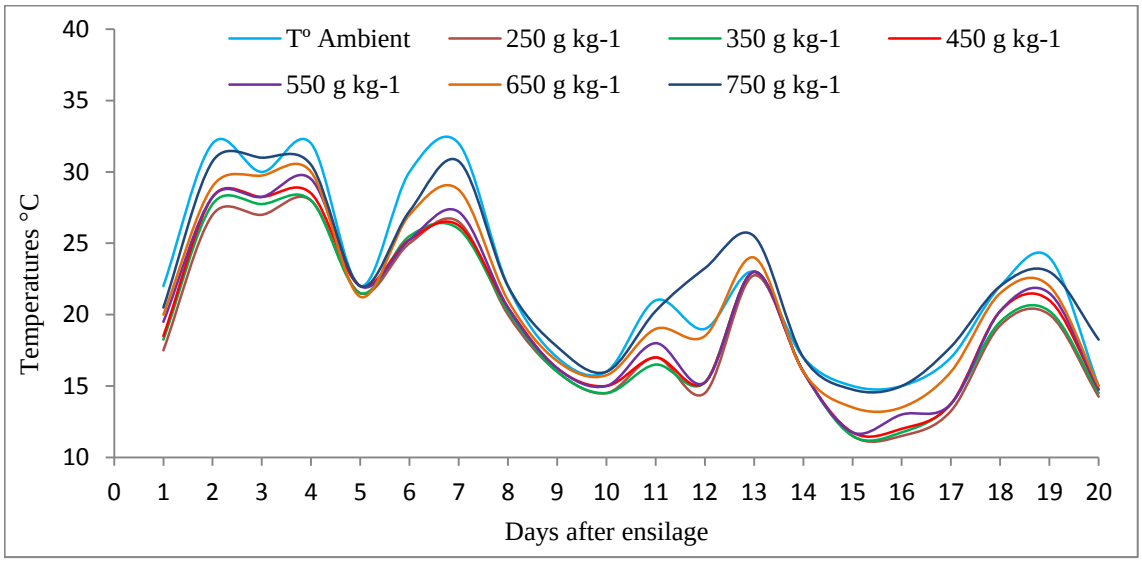

Figure 1. Oscillations at temperatures of stored ryegrass with different levels of dry matter.

consequent increase in NIDA in the ensiled material (Kung et al., 2018). In addition to temperature monitoring, the NIDA contents of the forage before and after ensilage act as indicative of the occurrence or not of this reaction; also, the higher the NIDA content, the greater the losses and the lower the protein available for the ruminal microorganisms.

Materials with high DM contents and prolongation of the aerobic phase due to delay in silo loading and sealing failures are the main factors that predispose Maillard Reaction in silages. In the present study, temperatures above 35°C (Figure 1) were not observed, even in treatments with higher dry matter content, suggesting that ryegrass can be conserved by means of anaerobic fermentation with higher dry matter contents and pre-drying.

No statistical difference was observed between the EE contents of the different treatments (Table 1). Its measurement in animal feed is fundamental because EE provides 2.25 times more energy when compared with carbohydrates and proteins (Garcez Neto et al., 2018). In a study of oat silage, Garcez Neto et al. (2018) obtained a mean result of 29.70 g kg^{-1} of DM of EE after 20 days of storage of the material, lower than the minimum value obtained in this study, which was 31.38 g kg^{-1} of DM (Table 1); also, it is within the range used for conventional ruminant diets. This demonstrates that ryegrass haylage and silages have great potential to provide energy to ruminants when related to their EE.

One of the main sources of energy for the microorganisms present in the rumen are carbohydrates, just as they are needed for food storage in the anaerobic environment. NFCs are the main substrates used for the production of lactic acid to achieve stability inside the silo. The TC contents presented a linear reduction of 0.0590 g for each 1 g kg^{-1} of DM (Table 1). This result is related to the pre-drying of the materials before storage, which during the time that it exposed to the field may have led to volatilization and leaching, consequently causing nutrient losses (Singh et al., 2018). This fact can also be explained by the NFC values, which also obtained a linear reduction, but more marked when compared to the reduction observed in TC; in counterpoint, the contents of FC increased to 0.1398 g in each 1 g kg^{-1} in DM increase, where the pre-drying of the food increased the less volatile fractions, such as FC and as a result decreasing the NFC contents of the forage.

Pre-drying contributed to getting silages with better NDF contents. The data presented a quadratic behavior, with the reduction up to the material with 0.450 g kg^{-1} of DM and subsequent increase (Table 1), but without any result that would impair the nutritional quality of the materials with higher DM content. All treatments generated satisfactory results, being below the 55-60% limit of NDF in the diet of ruminants (Van Soest, 1994).

The ADF contents did not present significant differences among the treatments studied (Table 1). However, it is possible to observe that in the treatment with content of 350 g kg^{-1} DM that obtained high quantification of undesirable microorganisms. The ADF content also increased when compared to the other treatments (Table 2), because these microorganisms consume non-fibrous carbohydrates. When comparing the bromatological composition and microbiological profile of silages of different grasses, Gentu et al. (2018) obtained NDF and ADF higher than those found in this study, but found that the NDF and ADF contents decreased with ensilage of the material and attributed the possibility of bacteria producing enzymes capable of degrading fibers, thus reducing the NDF and ADF content

Table 1. Chemical-bromatology composition of silage and haylage ryegrass with different contents of dry matter.

Variable	Dry matter content g kg^{-1}										Mse (EPM)
	250	350	450	550	650	750	L	Q	Equation	R^2	
NH_3-N	12.96	9.43	7.34	5.18	3.35	2.61	0.000	0.000	Y=17.1196-0.0206x	0.96	0.2283
pH E	6.202	6.213	6.217	6.221	6.228	6.248	0.000	0.115	Y=6.1901+0.0107x	0.92	0.0298
pH	3.84	4.00	4.07	4.66	5.25	5.44	0.000	0.000	Y= 2.7819+0.0035x	0.93	0.0335
DML	4.72	6.52	9.77	16.45	23.13	17.18	0.006	0.142	Y=4.0660+0.3330x	0.80	0.6224
CP	98.16	102.70	103.38	104.37	108.10	112.91	0.021	0.735	Y=91.9396+0.0259	0.93	4.2973
NIDN	11.73	10.86	9.78	9.68	9.58	9.55	0.008	0.153	Y=12.3208-0.0042x	0.78	0.5937
NIDA	6.52	6.03	5.85	5.71	5.32	5.08	0.007	0.909	Y=7.1038-0.0027x	0.97	0.3748
EE	31.57	31.38	31.60	31.50	31.41	31.63	0.968	0.920	-	-	0.8550
TC	793.25	788.90	783.25	774.61	768.47	765.93	0.000	0.887	Y=808.5674-0.0590x	0.98	5.4060
NFC	364.60	346.60	333.38	327.64	299.41	296.22	0.000	0.796	Y=397.8605-0.1398x	0.98	5.4061
FC	423.65	427.30	432.37	441.81	451.26	456.80	0.000	0.625	Y=397.8605+0.1398x	0.96	5.8270
NDF	473.77	467.41	454.99	464.43	478.87	494.42	0.000	0.004	Y=536.8684-0.3454x+0.0003x^2	0.94	6.4502
ADF	302.67	307.29	299.99	301.80	303.62	305.02	0.008	0.000	-	-	3.5448
CEL	265.67	263.15	253.05	251.30	249.55	246.43	0.000	0.001	Y=274.6867-0.0396x	0.91	5.5555
LIG	57.00	49.13	46.94	50.50	49.07	46.10	0.001	0.022	Y=67.936-0.0637+0.00005x²	0.68	1.7856
HEM	176.18	152.63	145.00	162.63	180.26	196.89	0.000	0.000	Y=258.8521-0.4809+0.0054x²	0.92	4.6779
TDN	594.87	622.20	632.25	631.34	622.92	609.93	0.508	0.000	Y=499.3604+0.5137-0.0005x²	0.96	4.3852
Frac C	170.80	152.43	147.82	146.80	153.28	157.00	0.028	0.000	Y=225.3774-0.2957X+0.0003x²	0.92	3.3754
Frac B2	430.81	427.00	417.33	428.86	450.39	473.67	0.012	0.038	Y=498.3348-0.3800+0.0005x²	0.96	12.6718
A+B1	378.39	410.57	449.85	396.33	391.33	381.83	0.429	0.075	-	-	13.9057

NH_3-N: ammoniacal nitrogen (% total N); pH E: hydrogen potential in the ensilage; pH: hydrogen potential; DML: dry matter loss (%); CP: crude protein; NIDN: nitrogen bound to neutral detergent fiber; NIDA: nitrogen bound to acid detergent fiber; EE: ether extract; TC: total carbohydrates; NFC: non-fibrous carbohydrates; FC: fibrous carbohydrates; NDF: neutral detergent fiber; ADF: acid detergent fiber; CEL: cellulose; LIG: lignin; HEM: hemicellulose; TDN: total digestible nutrient; Frac C: C fraction of carbohydrates; Frac B: B fraction of carbohydrates; A+B1: A+B1 fraction of carbohydrates.

of silages. This same behavior can also be observed in the present study.

Horst et al. (2017), comparing the bromatological composition of pre-dried silages of different oat cultivars, obtained a mean of 85.70 g kg^{-1} of DM for lignin. It is higher than that found in this study (Table 1) which is a mean of 49.70 g kg^{-1} of DM. The forage that obtained the highest result was the treatment of 250 g kg^{-1} of DM (Table 1); however it remained within the standards for good quality fodder. The lignin represents the completely indigestible portion of the plant, being negatively related with the capacity to take advantage of the ingested fodder. The results of hemicellulose showed a quadratic regression model, with a reduction up to the DM content of 450 g kg^{-1}, and a subsequent increase (Table 1). The increase of this carbohydrate in forage DM can be considered as a reflection of the reduction in the other constituents of the plant. The hemicellulose has constituents like xylan, which occupies a large part of its fraction and requires specialized systems such as xylanolítico to make its degradation (Saratale et al., 2012).

The cellulose fraction was set to linear regression with a reduction of 0.0396 g for each 1 g kg^{-1} of DM. Cellulose is a fraction of the cell wall that is formed basically by long linear chains of high molecular weight and a high degree of polymerization (Giger and Reverdin, 1995). These

Table 2. Estimates of consumption, energy, relative value of forage and microbiological profile of haylage ryegrass after opening.

Variable	Dry matter content g kg^{-1}										
	250	350	450	550	650	750	L	Q	Equation	R²	Mse (EPM)
CDMRLW	2.54	2.57	2.64	2.58	2.51	2.43	0.000	0.004	Y=2.2032+0.0018x-0.000002x^2	0.94	0.0343
DMD	653.22	649.62	655.31	653.90	652.48	651.39	0.000	0.932	-	-	2.7616
GE	4801.75	4840.45	4853.56	4955.61	5064.41	5109.68	0.000	0.652	Y=4607.0330+0.6610x	0.97	10.6925
DE	2855.57	2833.63	2912.61	2955.72	3002.12	3014.49	0.000	0.000	Y=2737.1398+0.3837x	0.92	36.0055
RVF	128.37	129.49	134.00	130.98	126.75	122.62	0.000	0.004	Y=110.2749+0.0978x-0.0001x^2	0.90	1.8016
FF	5.45	5.31	4.99	4.95	4.86	4.18	0.000	0.178	Y=6.0596-0.0022x	0.86	0.1394
LACT	5.27	5.30	4.93	4.87	4.81	4.60	0.000	0.980	Y=5.6568-0.0013x	0.91	0.1181
ENT	4.62	4.53	4.44	4.44	4.47	4.59	0.654	0.058	-	-	0.0835
CLOST	5.17	5.10	4.69	4.54	4.39	4.08	0.000	0.868	Y=5.7707-0.0022x	0.97	0.1058

CDMRLW: consumption of dry matter in relation to live weight (%); DMD: dry matter digestibility (g kg^{-1}); GE: gross energy (kcal kg^{-1} of DM); DE: digestible energy (kcal kg^{-1} of DM); RVF: relative value of forage; FF: filamentous fungi; LACT: Acid lactic bacteria; ENT: Enterobacteria; CLOST: Clostridia.

characteristics make it difficult to break during fermentative processes inside silos and in the rumen. High levels of this carbohydrate are negatively correlated with forage quality (Van Soest, 1994). The results obtained in this study for constituents of the plant cell wall were similar to those found by Horst et al. (2017), reaffirming that the pre-drying of forages is able to reduce the content of lignin and cellulose proportionally; consequently increasing the content of hemicellulose present in the forage and thus contributing to the nutritional quality of silages. The losses of energy in the silages are generally more associated to losses of DM due to aerobic deterioration than to losses due to fermentation processes, being one of the facts that the treatment with 250 g kg^{-1} of DM presented the lowest levels of TDN. According to Sniffen et al. (1992), the carbohydrates present in the plants can be classified according to the rate of degradation.

The fraction A that is rapidly degradable is composed of soluble sugars; the intermediate degradation fraction B_1 is composed of starch and pectin; B_2 presents slow degradation, containing the available cell wall; and the fraction C that does not present degradation is composed of the cellular wall that is unavailable mainly by the lignin. Frac C presented quadratic regression, with 492 g kg^{-1} reduction of DM, and a subsequent increase (Table 1). This result is related to the LIG content present in fodder, which also set up the quadratic regression model. This fraction is of low nutritional quality and has a very low degradation in the digestive system of ruminants. It needs specified systems of fungi so that other microorganisms may have some access to this phenolic compound, being of low or no rumen degradability and detrimental when present in high concentrations. The highest value was observed in the treatment with 250 g kg^{-1} of DM. The quadratic regression also explains the data of Frac B_2, with reduction up to 380 g kg^{-1} of DM and subsequent increase. The Frac B_2 fate will depend on digestion and passage rates as its fermentation occurs mainly in the rumen and part of the large intestine (Sniffen et al., 1992). The behavior observed in this fraction is related to the HEM and CEL, which are potentially digestible structural carbohydrates of the plant cells and are available for the nutritional exploitation of the ruminants at the digestive level. The fraction A + B_1 is composed of soluble sugars and starch, respectively. The junction of these fractions is accomplished by the difficulty of quantifying these fractions separately. The A + B_1 fraction did not fit the regression models tested (Table 2).

The results of CDMRLW presented quadratic behavior (Table 2) and are directly related to NDF results, where forages that present higher NDF contents, consequently will have a limitation and decrease of CDMRLW. The contents of this variable increased until the use of the 450 g kg^{-1} of DM, with subsequent reduction (Table 2). The treatments of 650 and 750 g kg^{-1} of DM presented the lower results for CDMRLW (Table 2).

When the chemical composition is very variable between crops, both in fresh and preserved fodder, this is reflected in the digestibility of the

Table 3. Main components and indicatives of material quality after aerobic stability (9 days of aerobic exposure).

Variable	Dry matter content g kg^{-1}										
	250	350	450	550	650	750	L	Q	Equation	R²	Mse (EPM)
NH_3-N	14.00	16.42	17.89	11.28	4.66	3.01	0.233	0.000	$Y=4.2551+0.6646x-0.0094x^2$	0.88	0.9022
pH	5.82	4.80	4.44	4.98	5.52	5.62	0.000	0.000	$Y=8.5809-0.01595x+0.0016x^2$	0.73	0.2760
CP	91.71	97.94	86.29	95.05	103.81	99.81	0.000	0.135	-	-	50.971
TDN	651.99	642.62	655.33	657.60	659.88	666.44	0.000	0.725	$Y=652.5373-0.3157x+0.0067x^2$	0.76	53.502
CDMRLW	2.13	2.32	2.60	2.57	2.56	2.45	0.000	0.000	$Y=1.0643+0.0540x-0.0004x^2$	0.95	0.0673
DMD	637.03	626.61	640.76	643.29	645.82	653.12	0.000	0.725	$Y=637.64440-0.3511x+0,0075x^2$	0.76	59.532

NH_3-N: ammoniacal nitrogen (% total N); pH: hydrogen potential; CP: crude protein; TDN: total digestible nutrient; CDMRLW: consumption of dry matter in relation to live weight (%); DMD: dry matter digestibility (g kg^{-1}).

silages and haylages, mainly due to the fiber content of the food that affects the digestibility in quality and quantity (McDonald et al., 2010). However, the estimated digestibility levels (Tables 2 and 3) suggest that ryegrass was harvested with a favorable contribution of non-structural carbohydrates; however, it did not fit the regression models tested (Table 2). After the stability period, DMD levels decreased until the treatment with 350 g kg^{-1} of DM and subsequent increase (Table 3).

The maturation stage of the plants influences the conservation of the forage and its nutritive value (Wilkinson and Davies, 2012); thereafter, it was possible to verify the interference of the quality indicators from the energy estimates after the storage of the materials. The estimate of GE is directly related to CP and pH levels of the food. This variable showed a positive linear regression and an increase of 0.6610 kcal kg^{-1} of DM was identified for each gram of DM in treatments (Table 2). The highest result was in the treatment with 750 g kg^{-1} of DM, which coincides directly with the results of CP (Table 1). The DE is not only directly related to the GE but also correlates with the concentrations of ADF present in OM, and lower levels of ADF in OM are indicators of higher levels of ED. For the estimates of ED, a positive linear regression with increase of 0.3837 kcal kg^{-1} of DM for each gram of augmented MS was also observed (Table 2).

Forage RVF is an indicator of quality when referring to concentrations of plants' cell wall constituents. The higher the contents of cellulose, hemicellulose and lignin the lower the RVF of the food, indicating materials of lower or higher quality. The values found in the present study made it possible to generate a quadratic equation that indicates an increase in RVF up to the level of 450 g kg^{-1} of DM with a subsequent decrease in this variable (Table 2). This result corroborates with those presented in Table 1 regarding the NDF contents of the treatments, being the treatment of 750 g kg^{-1} of DM that presented higher levels of NDF and consequently lower levels of RVF.

The presence of anaerobic microorganisms, such as lactic acid bacteria (LAB) during the forage fermentation process is fundamental. The LABs are the principals responsible for using substrate carbohydrates to produce lactic acid, consequently reducing the pH of the ensiled material after the silo closes. However, it is also possible to observe the growth of other agents, such as enterobacteria and Clostridia (McDonald et al., 2010).

In this study, it was observed that, as DM content increased, there was a reduction of 0.0022 $\log_{10}$ CFU g^{-1} of filamentous fungi for each 1 g kg^{-1} DM of ryegrass haylage (Table 2). This result is explained by the low humidity of the materials, inhibiting the growth of these microorganisms, which develop mainly in materials with higher moisture content and the presence of oxygen. These microorganisms are present mainly in silage with less than 300 g kg^{-1} of DM, as can be observed in this study, where the material with DM content of 250 g kg^{-1} obtained the highest result. Fungi are more common in the silo surface layers, either by access to the air due to storage failures, after opening, during anaerobic removal or deterioration. When present in high amounts they may be malefic, many of them being mycotoxin producers, which decrease the quality of silage in terms of their sanity (Kononenko and Burkin, 2014).

To obtain silages of nutritional and sanitary quality, it is necessary to immediately inhibit the cellular respiration of the plant, inhibiting the growth of undesirable aerobic microorganisms,

which consequently will also decrease the proteolysis performed by these agents and thus conditions favorable to the growth of LAB. LAB provides improvement in the fermentation of pre-dried and silage, and when in greater quantity, controls the growth of undesirable micro-organisms (Soundharrajan et al., 2017). This can be noticed in this study, where the number of colonies of LAB prevailed higher compared to enterobacteria and Clostridia (Table 2).

The highest growth of LAB was observed in the ensiled material with a DM content of 350 g kg^{-1}, and a decrease of 0.0013 log_{10} CFU g^{-1} at each 1 g kg^{-1} of DM (Table 2). This was due to the pH of the ensiled mass, which was above the limit of 4.2 in the treatments with higher DM contents. It is common for materials with MS above 400 g kg^{-1} to increase pH (Kung et al., 2018), which can also be noted in this study. This is because as the content of DM increases it limits the availability of metabolic water for the growth of LAB (Whiter and Kung, 2001).

The values obtained in the quantification of enterobacteria varied between the treatments, and the lowest results were in the treatments of 450 and 550 g kg^{-1} DM (Table 2). The growth of these microorganisms is more evident in the initial stages of fermentation, when the pH of the silage is close to neutrality. This is the environment necessary for its development, because the very enterobacteria themselves have buffer capacity and CO_2 production (Collins et al., 2017). Although in less quantity, the enterobacteria also produce lactic acid, but they damage the sanitary profile of conserved foods. These microorganisms compete for water-soluble carbohydrates with LAB, but the product with the highest concentration at the end of this process is acetic acid, which is detrimental to proper fermentation of the material (McDonald et al., 2010). In this experiment, the different contents of DM and pre-drying contributed to the production of silages with less proliferation of enterobacteria in relation to LAB (Table 2), improving the health profile of silage.

As DM levels increased, there was a decrease of 0.0022 log_{10} CFU g^{-1} for each 1 g kg^{-1} DM of the Clostridia populations (Table 2), evidencing that the higher DM content of the haylage fodder helps in the control of these agents. This fact is related to the pre-drying of the materials, which through the wilting of the ryegrass to the field caused a reduction of humidity and consequently a reduction of the presence of these microorganisms. Gentu et al. (2018), ensiling grasses with low moisture content (<54%), observed the reduction of the populations of these microorganisms as well as the results obtained in this study (Table 2), in which the materials with higher MS content presented lower quantification of populations of this bacterium. This is due to the sensitivity that microorganisms of the genus Clostridia present in relation to water scarcity. The treatment with 250 g kg^{-1} of DM obtained a higher concentration of Clostridia (Table 2), because it contains more than 70% humidity, which is considered an environment conducive to its proliferation (Queiroz et al., 2018). Although the pH of this material remained acidic (3.84) (Table 1), in the low DM, even a pH below 4.00 is not enough to inhibit them (McDonald et al., 2010). It can be observed in the haylages with 650 and 750 g kg^{-1} DM (Table 2) that even with a pH above 5.00, a lower presence of Clostridia was obtained, emphasizing that the higher contents of DM contribute to the decrease of these agents independent of their pH. According to Weirich et al. (2018), good quality haylages are those in which LABs are in higher concentration than the other microorganisms, as can be seen in forage with 750 g kg^{-1} DM (Table 2). After the aerobic stability period, the NH_3-N contents presented a quadratic behavior, increasing until the treatment of 450 g kg^{-1} of DM and subsequent reduction in the other treatments (Table 3). These results are related to the higher presence of undesirable microorganisms in the materials with a higher concentration of NH_3-N, especially in filamentous fungi (Table 2), which are mainly responsible for the aerobic deterioration of silages. Materials containing 650 and 750 g kg^{-1} DM showed the lowest concentrations of NH_3-N. This fact indicates that haylages with higher levels of DM present lower protein losses after aerobic exposure. The pH of the studied forages increased with aerobiosis. This suggests the possible development of aerobic microorganisms that cause material deterioration during exposure to air, which degrade the lactic acid produced by the LAB during the anaerobic process, thus raising the pH of the silages (McDonald et al., 1991).

Although all materials analyzed showed increased pH, when compared to the opening of the silos (Table 1) and after opening in aerobiosis (Table 3), it is possible to observe that in materials with 250 and 350 g kg^{-1} of DM the pH increase was accentuated (1.98 and 0.80 respectively). However, in the treatments with 550, 650 and 750 g kg^{-1} of DM, the increase was 0.32, 0.27 and 0.18 (Tables 1 and 3), respectively.

CP values oscillated between treatments. The highest concentration of this nutrient was obtained in the treatments with 650 and 750 g kg^{-1} of DM. This correlates with the result obtained for NH_3-N, with lower CP losses, and a higher concentration (Table 3). The inverse was observed in the treatment of 450 g kg^{-1} of DM, where it presented the highest concentration of NH_3-N and consequently the lowest value for CP (Table 3), because it has greater protein loss during aerobic exposure. TDN data after the aerobic stability period presented a quadratic behavior, with reduction up to the treatment of 350 g kg^{-1} of DM, followed by a subsequent increase (Table 3). These results are possibly related to the higher CP concentrations in treatments with high DM content.

Conclusions

The increase in DM contents of the treatments provided higher CP contents to the materials (being 112.91 g kg^{-1}

of DM) in the treatment with 750 g kg^{-1} of DM. Higher DM levels contributed to the decrease of NH_3-N contents at the time of opening and during the aerobic exposure period. The temperature of the stored forage remained stable, not exceeding 35°C. This shows the reduction of undesirable microorganisms with higher DM levels in the treatments.

The best results for the proliferation of LAB and efficiency in pH decrease was up to treatment with 450 g kg^{-1} of DM. The different contents of MD tested for the conservation of annual ryegrass in an anaerobic environment can be used; however, different nutritional levels were found.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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