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Nutritional value of Tifton 85 grass haylage under different layers of polyethylene film and storage times

Valor nutricional do pré-secado de capim Tifton 85 com diferentes camadas de filme de polietileno e tempos de armazenamento

Kácia Carine Scheidt[1]*; Eduardo Eustáquio Mesquita[2]; Caroline Daiane Nath[3]; Marcela Abbado Neres[2]; Marilda Schmoeller[4]; Deise Dalazen Castagnara[5];Ériton Egidio Lisboa Valente[2]

Highlights

4 or 6 film layers does not change the nutritional value of Tifton 85 grass haylage.
Storage for 90 days changes the chemical composition of haylage.
Storage for 90 days reduces *in vitro* dry matter digestibility of haylage by 10%.

Abstract

Forage conservation processes are fundamentally aimed at maintaining the nutritional quality of forages so that they can overcome the low availability and quality of roughage feed in unfavorable times. The objective of this study was to examine the effect of the number of layers of polyethylene film and storage time on the nutritional value of haylage of Tifton 85 grass (*Cynodon* spp cv. Tifton 85). Four and six layers of milky white polyethylene film were used in the wrapping of silage bales with a capacity of 400 kg (100 and 150 µm in total thickness, respectively), for four different storage times (at baling and 30, 60, and 90 days). The experiment was laid out in a randomized-block design with four replicates, in a split-plot arrangement in time, in which the number of layers of polyethylene film that wrapped the haylage bales constituted the plots and the storage times the sub-plots. Neutral detergent insoluble protein (NDIP) levels were higher in the haylage wrapped with four layers of polyethylene film (35.8 g kg^{-1} CP). Storage time affected the neutral detergent fiber (NDF), acid detergent fiber (ADF), mineral matter (MM), NDIP, and lignin contents and *in vitro* dry matter digestibility (IVDMD). The ADF and lignin contents increased linearly, whereas IVDMD decreased linearly.

[1] Dra in Animal Science, Universidade Estadual de Maringá, UEM, Maringá, PR. Brazil. E-mail: kaciacarine_19@hotmail.com
[2] Profs., Center for Agrarian Sciences, Universidade Estadual do Oeste do Paraná, UNIOESTE, Marechal Cândido Rondon, PR, Brazil. E-mail: pastureresearch@gmail.com; mabbadoneres@yahoo.com.br; eritonvalente@yahoo.com.br
[3] Dra in Animal Science, UNIOESTE, Marechal Cândido Rondon, PR, Brazil. E-mail: karolynedayane@hotmail.com
[4] M.e in Animal Science, UNIOESTE, Marechal Cândido Rondon, PR, Brazil. E-mail: marildasch@hotmail.com
[5] Profa, Department of Veterinary Medicine, Universidade Federal do Pampa, UNIPAMPA, Uruguaiana, RS, Brazil. E-mail: deisecastagnara@yahoo.com.br
* Author for correspondence

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Mineral matter showed a negative quadratic response. The NDIP and NDF contents exhibited a positive quadratic response. Storage time did not influence the dry matter (550.3 g kg^{-1}), crude protein (102.3 g kg^{-1} DM), or acid detergent insoluble protein (28.9 g kg^{-1} CP) contents or *in vitro* NDF digestibility (582.1 g kg^{-1} DM). In conclusion, the number of layers of polyethylene film does not influence the nutritional value of Tifton 85 grass haylage. Storage for 90 days reduces the nutritional value of Tifton 85 grass haylage.
Key words: Chemical composition. Silage. Plastic film. Tropical grass.

Resumo

Os processos de conservação de volumosos têm como objetivo fundamental manter a qualidade nutritiva das forrageiras, para que essas possam suprir a baixa disponibilidade e qualidade de alimento volumoso em épocas desfavoráveis. O objetivo do presente trabalho foi avaliar o efeito de número de camadas de filme de polietileno e tempos de armazenamento sobre o valor nutricional do pré-secado de capim Tifton 85 (*Cynodon* spp cv. Tifton 85). Foram utilizadas quatro e seis camadas de filme de polietileno branco leitoso no revestimento de silos-fardos com capacidade de 400 kg (100 e 150 µm de espessura no total, respectivamente) e quatro diferentes tempos de armazenamento (enfardamento, 30, 60 e 90 dias). Utilizou-se o delineamento experimental em blocos casualizados, com quatro repetições, em esquema de parcelas subdivididas no tempo, sendo que o número de camadas de filme de polietileno que revestiram os fardos do pré-secado constituiu as parcelas e os tempos de armazenamento as subparcelas. Os teores de proteína insolúvel em detergente neutro (PIDN) foram superiores no pré-secado revestido com quatro camadas de filme de polietileno (35,8 g kg^{-1} PB). Houve efeito de tempo de armazenamento sobre os teores de fibra em detergente neutro (FDN), fibra em detergente ácido (FDA), matéria mineral (MM), PIDN, lignina e digestibilidade *in vitro* da matéria seca (DIVMS). Constatou-se acréscimo linear para os teores de FDA e lignina, e decréscimo linear para o teor de DIVMS. A MM apresentou comportamento quadrático negativo. Observou-se comportamento quadrático positivo sobre os teores de PIDN e FDN. Para os teores de matéria seca (550,3 g kg^{-1}), proteína bruta (102,3 g kg^{-1} MS), proteína insolúvel em detergente ácido (28,9 g kg^{-1} PB) e digestibilidade *in vitro* da fibra em detergente neutro (582,1 g kg^{-1} MS) não houve influência de tempo de armazenamento. Conclui-se que o número de camadas de filme de polietileno não influenciou o valor nutricional do pré-secado de capim Tifton 85. O armazenamento durante 90 dias reduz o valor nutricional do pré-secado de capim Tifton 85.
Palavras-chave: Composição química. Ensilagem. Filme plástico. Gramínea tropical.

In an attempt to alleviate the negative effects of seasonality of forage production on animal performance, there has been a growing interest in the production of haylage stored as silage bales wrapped with plastic film. This forage conservation technique is attractive to producers because it reduces the risk of damage caused by rainfall, given the earlier process of baling compared with haymaking (Coblentz & Akins, 2018).

In silage bales wrapped in plastic film, it is the presence of plastic that creates anaerobic conditions for the bale, which leads to lactic fermentation, as occurs in other

types of silage (Bernardes & Weinberg, 2013). Polyethylene film has been the material most commonly used to seal silage bales, mainly due to its low cost. However, research shows that the polyethylene polymer has oxygen permeability, which tends to increase with increasing temperature (Amaral et al., 2014). Therefore, the protection provided by this material is highly variable and may change during the storage period, especially during the summer, leaving the silages more prone to aerobic deterioration due to the increased permeability of the film. Accordingly, the number of layers of plastic film used to seal the silo is critical to prevent the penetration of oxygen into the ensiled material and thus control the proliferation of undesirable microorganisms (Nath et al., 2018), which cause losses of important components and aerobic deterioration (Coblentz et al., 2016), besides reducing the hygienic quality of the silage and increasing production costs.

Moreover, in the silage-making process, extending the storage time may induce changes in the nutritional value (Nath, 2019) and cause the development of undesirable microorganisms that influence the chemical composition, intake, and digestibility of the forage. Nath et al. (2018) highlighted that under tropical conditions, haylage stored as silage bales has a shorter shelf life. According to the authors, loss of stability in conserved forage occurs around 60 days of storage.

In view of the foregoing, this study was developed to evaluate the nutritional value of Tifton 85 grass haylage (*Cynodon* spp cv. Tifton 85) stored in silage bales wrapped with different numbers of layers of polyethylene film and stored for different periods.

The experiment was carried out on a haylage farm (24º33'18" S, 54º01'19" W and altitude of 412 m), in the municipality of Marechal Cândido Rondon - PR, Brazil. According to the Köppen classification, the local climate is a Cfa type (subtropical), with well-distributed rainfall throughout the year and hot summers. The soil is classified as an eutric Oxisol ("Latosolo Vermelho") (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2018), with 650 g kg^{-1} of clay and the following chemical characteristics in the surface layer (0-20 cm): pH in water = 5.2; P (Mehlich) = 31.6 g dm^{-3}; K (Mehlich) = 0.1 $cmol_c$ dm^{-3}; Ca^{2+} (KCl 1 mol L^{-1}) = 5.9 $cmol_c$ dm^{-3}; Mg^{2+} (KCl 1 mol L^{-1}) = 1.9 $cmol_c$ dm^{-3}; H+Al (calcium acetate 0.5 mol L^{-1}) = 4.6 $cmol_c$ dm^{-3}; CEC = 12.5 $cmol_c$ dm^{-3}; base saturation at pH 7.0 = 63.2%; Cu = 26.87 mg dm^{-3}; Zn = 30.06 mg dm^{-3}; Mn = 87.7 mg dm^{-3}; and Fe = 21.6 mg dm^{-3}.

Tifton 85 grass (*Cynodon* spp cv. Tifton 85) was cut on November 17, 2014, at 17h30, after 35 days of vegetative growth, using a windrower with iron-free fingers (Kuhn®), adjusted for cutting at a height of 5 cm. After harvesting and pre-drying for 17h30 min, the forage with 537.5 g kg^{-1} of dry matter was packed in 24 bales with approximately 100 cm in height and 150 cm in diameter (capacity of 400 kg), using a baler (Kuhn®). Subsequently, the haylage bales were wrapped with a wrapper (Casale®). At this time, the haylage bales were divided into two sealing strategies: 12 were wrapped with four layers of milky white polyethylene film (stretch), 25-µm-thick, and 12 were wrapped with six layers of polyethylene film (stretch) milky white, 25-µm-thick, totaling 100 µm and 150 µm for four and six layers, respectively. The silage bales were stored exposed to weather conditions.

To evaluate the nutritional value of the haylage, samples of approximately 300 g from the central portion of the silage bales were collected at baling and after 30, 60, and 90 days of storage. The samples were pre-dried in a forced-air oven at 55 ºC for 72 h to determine the dry matter (DM) content. Then, they were ground in a Wiley mill with a 1-mm sieve to quantify the mineral matter (MM) and crude protein (CP) contents according to the Association of Official Analytical Chemists [AOAC] (1990); and the neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent insoluble protein (NDIP), acid detergent insoluble protein (ADIP), and lignin contents according to Van Soest et al. (1991).

The *in vitro* digestibility of dry matter (IVDMD) and neutral detergent fiber (IVNDFD) was determined following the technique described by Tilley and Terry (1963), adapted to the artificial rumen TE-150 (TE-150/Tecnal, Scientific equipment, Piracicaba - SP, Brazil), as described by Holden (1999).

The experiment was laid out in a randomized-block design with four replicates in a split-plot arrangement where the number of layers of polyethylene film that wrapped the haylage bales constituted the plots and the storage times the sub-plots.

Data were subjected to analysis of variance (ANOVA), using the following statistical model:

$$Y_{ijk} = \mu + \beta_k + A_i + (A\beta)_{ik} + B_j + (AB)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} = value observed in the experimental plot that received level i of factor A and level j of factor B in block k; μ = overall mean of the experiment; A_i = effect of level i of factor A; $(A\beta)_{ik}$ = effect of level i of factor A in block k (residue a); B_j = effect of level j of factor B; $(AB)_{ij}$ = interaction effect between level i of factor A and level j of factor B; and ε_{ijk} = experimental error (residue b). The means for number of layers of polyethylene film (factor A) were compared by Tukey's test, whereas the effect of storage time (factor B) was studied by regression analysis, choosing the model that showed the highest coefficient of determination (R^2) and a significant P value. A significance level of 0.05 was adopted for all analyses, which were performed using SISVAR statistical software version 5.6.

There was no interaction effect ($P > 0.05$) between the number of layers of polyethylene film and storage time for the studied variables. Thus, mean values between layers of polyethylene film were compared separately and a regression study was carried out for storage times.

The number of layers of polyethylene film did not influence ($P > 0.05$) the evaluated variables, except NDIP, which was 6.0 g kg^{-1} CP higher in the haylage wrapped with four layers of film (Table 1). Considering that the polyethylene film shows permeability to oxygen and that the presence of oxygen favors the development of undesirable microorganisms (Muller, 2018), the haylage wrapped with six layers of polyethylene film was expected to have better nutritional value. However, the magnitude of changes in the thickness of the film layer used to seal the bales of the evaluated haylage was clearly not enough to change its nutritional value.

Storage time did not affect DM, which averaged 550.3 g kg^{-1} (Table 2). The lack of statistical difference for the DM content between storage times indicates that the fermentation process occurred properly, as reported by Nath et al. (2018). Another aspect

to be considered is the high DM content at the time of baling, which contributed to the avoidance of losses due to effluent production during the fermentation period.

The MM content varied quadratically with the increase in storage time, with an estimated maximum value of 62.7 g kg^{-1} DM at 47.44 days of storage (Table 2). Nath (2019) studied Tifton 85 grass haylage prepared with an ideal DM content for silage (above 500.0 g kg^{-1}) and described higher MM values: 86.3 g kg^{-1} DM. The lower MM content is indicative of better forage conservation, since inadequate fermentation causes organic matter to be consumed, making the mineral portion proportionally larger.

Table 1
Nutritional value of Tifton 85 grass haylage wrapped with four and six layers of polyethylene film over 90 days of storage

Variable	Treatment		SEM	P-value[1]
	Four layers	Six layers		
DM (g kg^{-1})	546.2	554.4	0.190	0.096
MM (g kg^{-1} DM)	61.7	60.8	0.032	0.203
CP (g kg^{-1} DM)	102.2	102.4	1.347	0.911
NDF (g kg^{-1} DM)	636.8	634.4	0.139	0.478
ADF (g kg^{-1} DM)	331.8	330.8	0.510	0.963
Lignin (g kg^{-1} DM)	25.3	25.4	0.011	0.972
ADIP (g kg^{-1} CP)	28.9	29.0	0.715	0.951
NDIP (g kg^{-1} CP)	35.8 a	29.8 b	0.360	0.013
IVDMD (g kg^{-1} DM)	602.8	598.3	0.259	0.299
IVNDFD (g kg^{-1} DM)	585.0	579.3	0.666	0.590

[1]F test for the effect of number of layers of polyethylene film
SEM = standard error of the mean; DM = dry matter; MM = mineral matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADIP = acid detergent insoluble protein; NDIP = neutral detergent insoluble protein; IVDMD = *in vitro* dry matter digestibility; IVNDFD = *in vitro* neutral detergent fiber digestibility.
Means followed by different lowercase letters in the same row differ by Tukey's test ($P < 0.05$).

Crude protein was not affected by storage time, averaging 102.3 g kg^{-1} DM (Table 2). Vendramini et al. (2016) evaluated Tifton 85 grass haylage and found an average CP content of 124.0 g kg^{-1} DM. The low CP content may be attributed to the loss of soluble protein due to the leaching of the cellular content during the drying of the plant in the field or the loss of leaf fragments during the collection of wilted forage for baling.

According to Bueno et al. (2018), the cell-wall components in plants change little in response to the silage fermentation period, as they are not the preferred targets

of fermentation bacteria. However, in the present study, the NDF content showed a quadratic response to storage time, with an estimated minimum value of 620.2 g kg^{-1} DM at 24.83 days of storage (Table 2). The ADF contents showed linear increases that reached 9.30% in the evaluated period (Table 2). These results may be attributed to the loss of non-structural components during the silage fermentation process. The ADF levels found in the present study are close to the 321.0 g kg^{-1} DM observed by Arriola et al. (2015) in a study with Tifton 85 grass haylage.

Lignin contents increased linearly with storage time (Table 2). This result suggests that there were losses of other components that make up the DM, such as non-structural carbohydrates, while lignin is maintained, causing this fraction to increase proportionally relative to the DM content.

The NDIP levels responded quadratically to the storage times, with a minimum estimated value of 22.7 g kg^{-1} CP at 54.46 days of storage (Table 2). The ADIP levels, on the other hand, did not vary significantly with the storage times, averaging 28.9 g kg^{-1} CP (Table 2). Neutral detergent insoluble protein can be degraded more slowly than the protein present in the cellular content, but ADIP is poorly degraded and, depending on its bonds with lignin, it can make the protein unavailable to the animal. Thus, the higher the concentration of NDIP and ADIP in a feed, the lower or slower the degradation of protein.

In vitro dry matter digestibility (Table 2) declined linearly, reaching a reduction of 10.37% in the evaluated period. This fact is related to the linear increase in ADF contents with storage time. Neres et al. (2014) evaluated Tifton 85 grass haylage and found a mean IVDMD value of 603.6 g kg^{-1}, a result similar to that obtained in the present study. There was no effect of storage time on the IVNDFD of the haylage, which averaged 582.1 g kg^{-1} DM (Table 2).

The use of four or six layers of polyethylene film does not influence the nutritional quality of Tifton 85 grass haylage. However, the nutritional value of Tifton 85 grass haylage decreases up to 90 days of storage; therefore, it should not remain stored for this longer period.

Table 2
Mean values and regression equations for the nutritional components of Tifton 85 grass haylage as a function of storage times

Variable	Storage time				Regression equation	SEM	P-value		R^2	
	0	30	60	90			Linear	Quadratic	Linear	Quadratic
DM (g kg^{-1})	537.5	560.0	561.2	543.7	$\hat{Y} = 550.3$	1.189	0.711	0.110	-	-
MM (g kg^{-1} DM)	59.7	62.6	62.8	60.2	$\hat{Y} = 59.77 + 0.14X - 0.00150X^{2}$*	0.115	<0.001	0.030	0.01	0.65
CP (g kg^{-1} DM)	103.5	95.5	110.3	99.6	$\hat{Y} = 102.3$	1.836	0.703	0.479	-	-
NDF (g kg^{-1} DM)	626.9	620.5	632.9	663.8	$\hat{Y} = 626.90 - 0.52X + 0.01036X^{2}$*	0.384	<0.001	<0.001	0.60	0.88
ADF (g kg^{-1} DM)	315.8	326.5	337.3	348.1	$\hat{Y} = 315.80 + 0.36X$*	0.709	0.003	0.267	0.89	-
Lignin (g kg^{-1} DM)	23.1	24.5	26.0	274.9	$\hat{Y} = 23.11 + 0.05X$*	0.085	0.001	0.099	0.60	-
ADIP (g kg^{-1} CP)	27.2	28.3	29.4	30.4	$\hat{Y} = 28.9$	0.493	0.208	0.493	-	-
NDIP (g kg^{-1} CP)	47.3	27.7	23.0	33.2	$\hat{Y} = 47.362 - 0.902X + 0.00828X^{2}$*	0.708	<0.001	<0.001	0.33	0.99
IVDMD (g kg^{-1} DM)	633.6	611.6	589.5	567.5	$\hat{Y} = 633.7 - 0.73X$*	0.959	<0.001	0.651	0.93	-
IVNDFD (g kg^{-1} DM)	574.0	591.1	601.1	562.0	$\hat{Y} = 582.1$	1.225	0.636	0.051	-	-

*Significant at 5% probability by the F test.
Storage time 0 = baling; SEM = standard error of the mean; R^2 = coefficient of determination; DM = dry matter; MM = mineral matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADIP = acid detergent insoluble protein; NDIP = neutral detergent insoluble protein; IVDMD = *in vitro* dry matter digestibility; IVNDFD = *in vitro* neutral detergent fiber digestibility.

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