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Forage crops

Wilting whole crop black oat with glyphosate for ensiling: effects on nutritive, fermentative, and aerobic stability characteristics

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ABSTRACT - We aimed to evaluate the effects of glyphosate as a chemical desiccant on the nutritional quality, fermentation pattern, and aerobic stability of wilted black oat (*Avena strigosa* Schreb) silage. Black oat sowing occurred in the first fortnight of May 2013. Desiccant application took place when oat reached milky/dough grain stage (96 days after planting). Glyphosate dosages evaluated were 0, 500, 750, 1000, and 1250 mL ha^{-1}. Three days after desiccation, all treatments were ensiled, and the silos were kept stored for 150 days. A completely randomized design was used, and all statistical procedures were performed by means of Bayesian Inference. Treating herbage prior to ensiling from 500 mL ha^{-1} glyphosate increased dry matter and organic matter contents compared with control. On the other hand, fiber content decreased linearly for desiccated silages, as shown by the negative slopes for neutral detergent fiber, acid detergent fiber, and cellulose. The highest concentrations of hemicellulose and neutral detergent insoluble nitrogen occurred for the dosages of 729.96 mL ha^{-1} and 759.52 mL ha^{-1} glyphosate, respectively. Wilted silage had less concentration of acetic acid and isovaleric acid and presented a higher amount of 2,3-butanediol. Due to the lack of beneficial short-chain fatty acids, treated silages had a higher organic matter loss (0.1 g mL^{-1}) and reached a maximum pH (0.009 h mL^{-1}) more quickly than control silage, after aerobic exposure. In this way, for wilted black oat silage production, harvested at milky/dough grain stage, the application of 500 mL ha^{-1} glyphosate is recommended.

Key Words: aerobic deterioration, dry matter, pre-drying, volatile organic compounds

Introduction

Black oat (*Avena strigosa* Schreb) is one of the most important small grain cereals for animal feeding in southern Brazil. Despite grain yield, black oat is also cultivated for ensiling; however, it is not an easy forage to be stored in anaerobic conditions, compared with maize, for example. According to Haigh (1990), a content of water-soluble carbohydrates (WSC) over 37 g kg^{-1} DM in herbage is necessary for an adequate fermentation. Whole crop black oat WSC content ranges from 139 g to 79 g kg^{-1} DM, for milky and dough grain stages, respectively.

However, black oat herbage has a high buffer capacity, demanding a larger quantity of water-soluble carbohydrates for organic acid synthesis and pH drop (Bergen et al., 1991, Meinerz et al., 2011). A slower pH decline leads to undesirable microorganism development and exacerbated plant enzymatic activity, reducing the nutritive value and sanitary quality of silage (Cazzato et al., 2011). Wilting controls the extent of fermentation, once the buffer capacity and water activity of the crop are reduced, being necessary less organic acid synthesis to inhibit spoilage fermentation, dropping the soluble sugar consumption (Weißbach and Honig, 1996; McEniry et al., 2007).

Wilting raises forage dry matter content regardless of the plant development stage at harvesting. However, this technique demands skilled work force and adequate machinery. In this context, chemical desiccation is a promising technology, raising forage dry matter content quickly and efficiently without the need of heavy machinery.

The objective of this study was to evaluate the effect of chemical desiccation on the ensiling process of whole crop black oat and changes in silage nutritional quality, fermentation pattern, and aerobic stability.

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Material and Methods

The experiment was carried out in a field in Maringá, PR, Brazil, during the 2013 harvest. The field is located at an altitude of 550 m, 23°21' S latitude, and 52°04' W longitude. The local climate is classified as Cfa (warm climate, fully humid with a hot summer), according to Peel et al. (2007), with mean annual rainfall of 1276 mm, annual potential evapotranspiration of 1070 mm, mean annual temperature of 17.5 °C, with minimum mean of 13.6 °C in July, maximum mean of 20.6 °C in January, and relative humidity of 66% (Embrapa, 2012). Soil is classified as Red Latosol of sandy texture (Embrapa, 2006). The climate conditions during field experiment (Figure 1) were adequate for the oat development, according to Carvalho et al. (2010).

Pre-sowing fertilization was equivalent to 180 kg ha^{-1} using the NPK formula 12-17-17 (N, P_2O_5, K_2O) as recommended by CQFS RS/SC (2004). Black oat (*Avena strigosa* Schreb) planting took place (May 15th, 2013) in an area of 0.2 ha (73 m long and 28 m wide). Seed density was equivalent to 100 kg of seeds ha^{-1}. Nitrogen fertilization was performed (June 6th, 2013) in a single application of 112 kg ha^{-1} as urea.

Herbage reached the milky/dough grain stage on August 19th, 2013 (96 days after planting), and the cultivated area was subdivided into five plots of 360 m^2 (70 m long and 5 m wide) for Roundup Transorb® application. Dosages of glyphosate evaluated in this study were 0 (control), 500, 750, 1000, and 1250 mL ha^{-1}.

Monitoring of oat dry matter (DM) content occurred according to Lacerda et al. (2009) by using a microwave oven, aiming forage harvesting at 300 to 350 g kg^{-1} as fed. Three days after desiccant application (August 22th, 2013), treated herbage reached the expected DM content; however, for logistic reasons, all treatments were harvested and ensiled on the same day.

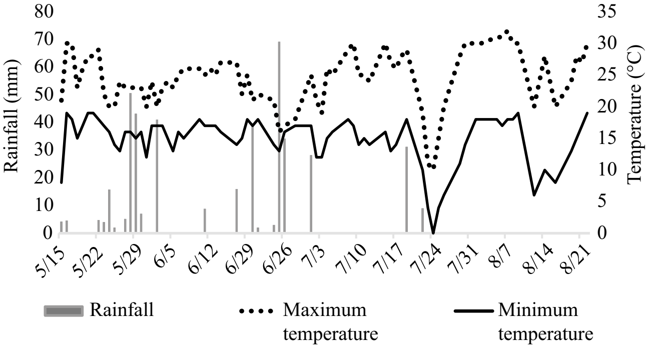

Figure 1 - Rainfall (mm), maximum temperature (ºC), and minimum temperature (ºC) during the experiment in Maringá, PR, in 2013.

Crop was harvested with an ensiling machine Menta brand – Premium Flex model. After cutting, forage was inoculated with a bacterial additive (Tropical Master – Katec Lallemand) containing *Lactobacillus plantarum* MA 18/5U and *Pediococcus acidilactici* MA 18/5M to reach a theoretical rate of 1×10^5 cfu/g of forage. Fresh forage (10 kg) was packed manually into experimental PVC tube silos (0.015 m^3). On day 0, as well as at the opening time, silos were weighed for subsequent determination of dry matter recovery index (DMRI) according to Jobim et al. (2007). Silos remained stored for 150 days.

At the opening of silos, samples were collected for pH measurement according to Silva and Queiroz (2002). Ammonia nitrogen was determined according to Detmann et al. (2012) from the silage juice extracted with the aid of an 8-t hydraulic press. Approximately 500 g of silage were sampled from each silo for DM content determination using a forced-air circulation oven at 55 ºC during 48 h.

Dried samples were grounded to 1-mm particle size and subjected to the following analysis: dry matter at 105 °C (method 967.03; AOAC, 1990); ash (method 942.05; AOAC, 1990); organic matter (OM) was calculated by the formula OM = 100 − ASH; neutral detergent fiber (NDF) and acid detergent fiber (ADF), according to Van Soest et al. (1991); lignin (LIG) was determined by the method LAD (lignin in acid detergent), according to Detmann et al. (2012); hemicellulose (HEM) was calculated by the difference between NDF and ADF fractions (HEM = NDF − ADF); cellulose was determined by the subtraction of lignin from the ADF fraction (CEL = ADF − LIG); crude protein (CP; method 990.03; AOAC, 1990); and neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were obtained by analyzing the nitrogen from the NDF and ADF residues.

The aerobic stability trial was performed as described by Jobim et al. (2007). In each silo, approximately 3 kg of fresh silage was decompressed to facilitate exposure of the ensiled material to air. Buckets were stored in a controlled temperature chamber during 74 h at 25 °C. The pH measurement was performed daily at 15:00 h according to Silva and Queiroz (2002). In addition, a sample of 25 g was taken daily from silos for further determination of DM and ash content.

The variables accessed during aerobic stability trial were: pH, time to reach the maximum pH (hours), and average pH during aerobic exposure. Organic matter losses (OML) were estimated as proposed by Paredes et al. (2000).

Fermentation profile was determined using the aqueous extract of silages obtained by homogenizing 25 g of silage with 225 mL of distilled water for 1 min using a blender. The pH of aqueous extracts was determined after 30 min. The supernatant (2 mL) was pipetted and stored in Eppendorf tubes at −20 ºC for further analyses.

Lactic acid concentration was determined by a colorimetric method (Pryce, 1969) in a MARCONI® Janway 6305 spectrophotometer, with λ = 565 nm. Alcohol content, esters, and volatile fatty acids were determined by means of gas chromatography-mass spectrophotometry (GCMS QP 2010 plus, Shimadzu®, Kyoto, Japan) using a capillary column (Stabilwax, Restek®, Bellefonte, USA; M, 0.25 mmø, 0.25 µm Crossbond Carbowax polyethylene glycol).

The experimental design was completely randomized, evaluating control silage and four dosages of glyphosate, with four replicates per treatment, resulting in 20 silos. The mathematical model adopted for statistical procedures was: $Y_{ij} = \mu + D_j + e_{ij}$, in which: Y_{ij} = observation of the j-th treatment in the i-th observation; μ = general average; D_j = dosage effect j; and e_{ij} = random error associated with each observation Y_{ij}. All data analyses were performed according to Rossi (2011) by means of Bayesian Inference.

We considered that the response of the control group (y_{ci}) followed a normal distribution $y_{ci} \sim N(\mu_c, \sigma^2_{ce})$. For the treatment levels, regression models such as linear [1] and quadratic [2] were considered as $y_i \sim N[f(\beta,x_i), \sigma^2_e]$, in which [1] $y_i = f(\beta,x_i) = \beta_0 + \beta_1 x_i + \mathcal{E}_i$, and [2] $y_i = f(\beta,x_i) = \beta_0 + \beta_1 x_i + \beta_2 x^2_i + \mathcal{E}_i$, in which $i = 1, 2, ..., n$, x = treatment level = 500, 750, 1000, and 1250 mL ha^{-1} of chemical desiccant, assuming $\mathcal{E}_i \sim N(0, \sigma^2_e)$. For instance, the vector of regression parameters β_p in models (1 and 2) was considered unrelated. Non-informative *a priori* distribution was considered for all parameters of the models, being for control/treatment, respectively $\mu_c \mid \sigma^2_{ce} \sim N(0,10^{-6})$ and $\tau_{ce} \sim Gama(10^{-3},10^{-3})$; $\beta_p \mid \sigma^2_e \sim N(0,10^{-6})$; and $\tau_e \sim Gama(10^{-3},10^{-3})$, with $\sigma^2_e = \tau_e^{-1}$ (OpenBUGS parameterization). Control was considered as the mean from each group data. For the regression coefficients of the treatments, estimates of maximum likelihood frequency and the value 1 for τ, considering both groups, were used as the initial value.

The critical point coordinates of $f(\beta,x)$, respectively, x_{cr} and y_{cr}, were obtained by $\left(-\frac{\beta_1}{2\beta_2}, -\frac{\beta_1^2 - 4\beta_2\beta_0}{2\beta_2}\right)$, being possible to compare averages with the *a posteriori* mean of the control level $\Delta = y_{cr} - \mu_c$ (Souza, 2014). Results were considered different at a 0.05 significance level when the credibility interval of Δ excluded zeroes.

Deviance information criterion (DIC) was used to select the best model (quadratic or linear) for each variable measured. Spiegelhalter et al. (2002) suggested the following criterion for DIC values for models [1] and [2]: $D = |DIC_1 - DIC_2|$. Difference was not considered significant when $D<5$; if $5 \leq D \leq 10$, the difference was considered significant; and if $D>10$, the difference was considered highly significant. In case of non-significance for quadratic model, the response (y_{ij}) followed a normal distribution, being $y_{ij} \sim N(\mu_j, \sigma^2_{je})$, $i = 1, 2, ..., n_j$ for the j-th treatment levels. Logarithmic transformation was applied to data responses with high variability. For each μ_j and σ^2_j, non-informative distributions were considered *a priori*, respectively, $\mu_j | \sigma^2_e \sim N(0,10^{-6})$ and $\tau_j \sim Gama(10^{-3},10^{-3})$.

Multiple comparisons were performed between the *a posteriori* distributions of the means from the different treatments versus control. Treatment levels whose credibility intervals for the mean differences excluded zero were considered different at a 0.05 significance level. The initial values for each μ_j were the sample mean of the j-th treatment.

Marginal distributions *a posteriori*, for all the parameters involved in the described procedures, were obtained by the BRugs package of the R program (R Development Core Team, 2014). A total of 5,500,000 values were generated in an MCMC (Monte Carlo Markov Chain) process. Considering a sampling discard period of 500,000 initial values, the final sample taken at intervals of 50 values contained 100,000 generated values. Chain convergence was verified through Heidelberger and Welch (1983) and Geweke (1992) criteria in the coda package of R program (R Development Core Team, 2014).

Results

No significant results for DMRI, OM, LIG, ADIN, and pH were found (Table 1). However, glyphosate application prior to ensiling increased OM concentration in all treated silages compared with the control.

Despite the negative slope of DM equation, average DM contents were higher for all desiccated silages. Equations for NDF, ADF, and CEL ($P<0.05$) showed a negative slope. However, wilting increased ($P<0.05$) NDF in treatments of 500 and 750 mL ha^{-1} compared with control. Silage from treatment 500 mL ha^{-1} glyphosate had higher ADF and CEL values than non-treated silage.

According to the model ($P<0.05$), the highest HEM content (312.8 g kg^{-1} DM) was observed for silage treated with 729.96 mL ha^{-1} glyphosate. Glyphosate applied at 1000 mL ha^{-1} prior to ensiling reduced ($P<0.05$) NDIN compared with control. The 759.52 mL ha^{-1} dosage resulted in the lowest concentration (1.6 g kg^{-1} DM) of NDIN.

Wilted silages had a lower crude protein content compared with control. However, the linear regression equation for this variable showed a positive slope ($P>0.05$).

Desiccation did not affect the concentrations of lactic acid, butyric acid, ethanol, and ammonia nitrogen (Table 2). Glyphosate treatment reduced isovaleric acid concentration in all treated silages. Silages of treatments 500 mL ha^{-1} and 1250 mL ha^{-1} had a lower content of propionic acid than the control. Desiccation reduced butanol in treated silages compared with the control, as shown by the regression equation for this variable.

Equations for acetic acid and 2,3-butanediol showed a quadratic behavior. Wilting reduced acetic acid compared with the control. Desiccant application in a dose of 947.6 mL ha^{-1} resulted in the lowest concentration of acetic acid (5.6 g kg^{-1} DM). On the other hand, silage treated with 869.3 mL ha^{-1} glyphosate presented the highest concentration (3.5 g kg^{-1} DM) of 2,3-butanediol.

In relation to the aerobic stability trial (Table 3), glyphosate application did not influence maximum pH and average pH. However, desiccation reduced the time in hours to reach maximum pH ($P<0.05$).

Wilted silages showed higher OML during aerobic exposure ($P<0.05$). Glyphosate concentrations greater than those evaluated in this study may have increased OML during aerobic exposure due to the probable higher availability of OM and less beneficial fermentation products.

Discussion

Glyphosate affects stomatal conductance, cellular membrane selective permeability, and aquaporin functionality, reducing water absorption by plant and, consequently, increasing DM content (Zobiole et al., 2010). Ensiling herbage with high moisture leads to an increase of spoilage microorganism development, reducing silage nutritional and sanitary quality (Liu et al., 2011; Xie et al., 2012). In this way, desiccation controlled the extent of fermentation, resulting in a greater conservation of nutrients, once treated silage had a higher OM content compared with the control.

Despite the linear positive regression equation for CP, all glyphosate dosages evaluated in this study led to

Table 1 - Bayesian estimates (means, standard deviations, and regression equations) for dry matter, dry matter recovery index, pH, and nutritional composition (g kg^{-1} DM) of black oat silages

Item	Glyphosate dosage (mL ha^{-1})					Regression equation			DIC$_L$	DIC$_Q$	x$_{cr}$^{1}	y$_{cr}$^{1}
	0	500	750	1000	1250	*b0*	*b1* X	*b2* X^2				
DM (g kg^{-1} as fed)	228.1	328.9*	328.4*	308.9*	308.2*	347.2	−0.03		51.6	53.8		
	(1.21)	(0.96)	(1.08)	(0.78)	(0.42)	(0.95)	(0.001)			ns		
DMRI (%)	76.81	80.63	86.03	82.09	85.6	79.65			101	103.2		
	(7.04)	(5.17)	(3.15)	(3.87)	(3.61)	(4.41)			ns	ns		
pH	4.01	3.80	3.86	3.82	3.84	3.80			−53.8	−52.5		
	(0.26)	(0.04)	(0.05)	(0.03)	(0.03)	(0.04)			ns	ns		
Organic matter	933	948.7*	951.4*	950.8*	947.1*	951.4			16.4	14.4		
	(0.06)	(0.13)	(0.29)	(0.35)	(0.30)	(0.31)			ns	ns		
NDF	663.6	737.2*	721.5*	710.2	641.2	807.4	−0.12		70.5	59.6		
	(2.46)	(1.41)	(0.34)	(0.90)	(0.62)	(1.71)	(0.002)			ns		
Hemicellulose	266.5	301.6	298.5	307.3	234.6	158.4	0.42	−0.001	77.5	64.5	729.96	312.8
	(3.71)	(1.24)	(0.53)	(0.89)	(0.58)	(4.61)	(0.012)	(0.0001)				(0.61)
ADF	397.1	435.6*	422.9	402.8	406.5	454.6	−0.04		48.2	46.5		
	(1.49)	(1.03)	(0.61)	(0.45)	(0.42)	(0.85)	(0.001)			ns		
Cellulose	351.6	386.3*	373.3	353.4	358.2	404.3	−0.04		48.9	46.7		
	(1.23)	(0.95)	(0.59)	(0.48)	(0.58)	(0.87)	(0.001)			ns		
Lignin	45.6	49.3	49.7	49.4	48.2	50.3			25.2	27.5		
	(0.32)	(0.57)	(0.26)	(0.20)	(0.52)	(0.41)			ns	ns		
Crude protein	107.2	75.9*	44.1*	82*	90.4*	62.5	0.02		32.6	30.7		
	(0.33)	(0.42)	(0.32)	(0.35)	(0.67)	(0.52)	(0.001)			ns	ns	
NDIN	2.6	1.9	1.9	1.5*	2.8	4.6	−0.01	0.001	−48.5	−58.6	759.52	1.6*
	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.10)	(0.001)	(0.0001)				(0.01)
ADIN	1.6	1.8	1	1.3	1.4	1.7			−50.7	−54.2		
	(0.04)	(0.06)	(0.03)	(0.02)	(0.03)	(0.04)			ns	ns		

DM - dry matter; DMRI - dry matter recovery index; NDF - neutral detergent fiber; ADF - acid detergent fiber; NDIN - neutral detergent insoluble nitrogen; ADIN - acid detergent insoluble nitrogen; DIC$_L$ - deviance information criterion for linear regression; DIC$_Q$ - deviance information criterion for quadratic regression; ns - no significant effect for regression equation.

* Significant difference ($P<0.05$) between treatment and control means by Bayesian contrast.

[1] Critical point coordinates of the quadratic regression (X$_{cr}$ in mL ha^{-1}).

Values between ( ) - standard deviation.

a decrease in CP content. Glyphosate acts on the shikimic acid pathway, inhibiting the production of essential aromatic amino acids and protein synthesis (Zobiole et al., 2010; Orcaray et al., 2012). A reduction of forage CP is not interesting, since it leads to an increase of dietary nitrogen supplementation to reach animal requirement.

Neutral detergent fiber, as well as ADF components (CEL, HEM, LIG, heat-damaged protein, and minerals), are not consumed as substrate during silage fermentation due to the lack of enzymes capable of breaking the chemical bonds of fiber components by lactic acid bacteria (Pahlow et al., 2003). Therefore, any observed changes in NDF and ADF are related to a dilution effect; once CP content decreased, a contrary behavior was observed for NDF and ADF.

As shown by the regression equation, the lowest NDIN concentration occurred for 759.52 mL ha^{-1} glyphosate, close to the dosage that resulted in the lowest CP content

Table 2 - Bayesian estimates (means, standard deviations, and regression equations) for fermentative pattern of black oat silages

Item	Glyphosate dosage (mL ha^{-1})					Regression equation			DIC_L	DIC_Q	x_{cr}^1	y_{cr}^1
	0	500	750	1000	1250	*b0*	*b1* X	*b2* X^2				
g kg^{-1} of DM												
Lactic acid	74	70.2	77.9	67.4	71	74.6			58	60.2		
	(2.52)	(1.22)	(0.98)	(1.54)	(0.63)	(1.15)			ns	ns		
Acetic acid	24.2	6.8^{*L}	5.5^{*L}	5.7^{*L}	6.7^{*L}	12.1	−0.02	0.000089	−13.9	−10	947.6	5.6^{*}
	(0.79)	(0.10)	(0.13)	(0.09)	(0.89)	(0.40)	(0.001)	(0.0000018)	ns			(0.05)
Butyric acid	9.4	2.2	2.7	2.9	2.4	2.3			−0.3	1.8		
	(0.39)	(0.15)	(0.14)	(0.30)	(0.14)	(0.19)			ns	ns		
2,3-butanediol	1.6	20	4.1^{*L}	2.6^{L}	2.5	−0.35	0.02	−0.000009	−22.5	−28.4	869.3	3.5^{*}
	(0.03)	(0.04)	(0.04)	(0.05)	(0.09)	(0.30)	(0.001)	(0.0000014)	ns			(0.04)
Ethanol	0.8	0.7	0.9	0.6	1.0	0.06			−58.6	−56.7		
	(0.05)	(0.04)	(0.03)	(0.03)	(0.04)	(0.03)			ns	ns		
Propionic acid	1.9	0.4^{*L}	0.6^{L}	0.5^{L}	0.5^{*L}	0.4			−69	−66.9		
	(0.07)	(0.02)	(0.03)	(0.04)	(0.03)	(0.03)			ns	ns		
mg kg^{-1} of DM												
Isovaleric acid	258	40.2^{*L}	29.75^{*L}	28.76^{*L}	32.55^{*L}	41.23			124.5	124.5		
	(27.94)	(16.21)	(4.20)	(2.83)	(5.98)	9.18			ns	ns		
2-butanol	38.29	1.00^{L}	7.75^{L}	5.76^{L}	11.56	−38.6	0.12		102.2	104.5		
	(20.94)	(0.02)	(1.60)	(3.45)	(7.74)	4.58	(0.005)			ns		
g kg^{-1} of total nitrogen												
Ammonia	36.6	36.9	37.3	50.8	43.3	30.6			47.4	48.9		
	(0.79)	(0.58)	(0.34)	(1.32)	(0.24)	(0.83)			ns	ns		

DIC_L - deviance information criterion for linear regression; DIC_Q - deviance information criterion for quadratic regression; ns - no significant effect for regression equation.
* Significant difference ($P<0.05$) between treatment and control means by Bayesian contrast (L by logarithmic transformation).
[1] Critical point coordinates of the quadratic regression (X_{cr} in mL ha^{-1}).
Values between ( ) - standard deviation.

Table 3 - Bayesian estimates (means, standard deviations, and regression equations) for aerobic stability of black oat silages

Item	Glyphosate dosage (mL ha^{-1})					Regression equation			DIC_L	DIC_Q	x_{cr}^1	y_{cr}^1
	0	500	750	1000	1250	*b0*	*b1* X	*b2* X^2				
OML (%)	12.29	8.01	10.88	15.57	14.94	3.33	0.010		101.5	103.2		
	(4.84)	(3.61)	(5.17)	(4.88)	(3.57)	(4.48)	(0.005)			ns		
Maximum pH	7.87	7.74	8.21	8.00	6.24	9.15			60.5	59.7		
	(1.06)	(0.54)	(0.80)	(0.25)	(2.17)	(1.24)			ns	ns	ns	
Maximum pH (h)	74.00	74.00	74.00	66.47	68.90	78.72	−0.009		95.4	97.3		
	(0.02)	(0.02)	(0.02)	(4.03)	(5.09)	(3.70)	(0.004)			ns		
Average pH	6.00	5.52	6.69	6.28	5.45	6.19			58.6	58.2		
	(1.07)	(0.61)	(1.36)	(0.62)	(1.51)	(1.18)			ns	ns	ns	

OML - organic matter losses by Paredes et al. (2000); DIC_L - deviance information criterion for linear regression; DIC_Q - deviance information criterion for quadratic regression; ns - no significant effect for regression equation.
[1] Critical point coordinates of the quadratic regression (X_{cr} in mL ha^{-1}).
Values between ( ) - standard deviation.

(750 mL ha^{-1}). On the other hand, HEM increased as the nitrogen content decreased. An increase of HEM content could be beneficial to rumen microorganisms, once this molecule is more soluble than cellulose. However, the lack of nitrogen could be a limiting factor in fermentation processes (Van Soest, 1994).

Lactic acid is the main responsible for the pH drop during silage fermentation (McDonald et al., 1991). A reduction in lactic acid production among treatments may be attributed to a deleterious effect of glyphosate on the lactic acid bacteria. However, treatments used in this study did not affect the lactic acid synthesis.

Desiccation reduced secondary fermentation during the storage phase. The wilting process avoids heterolactic bacteria development due to the reduced water availability. However, the lack of short-chain fatty acids, such as acetic and propionic acids, accelerate silage deterioration after silo opening (Danner et al., 2003; Weinberg et al., 2010; Liu et al., 2011). Isovaleric acid also has a beneficial effect, protecting silo panel against spoilage microflora; however, this acid is synthesized during the leucine catabolism, indicating proteolysis during the storage phase (McDonald et al., 1991; Schönicke et al., 2015).

Alcohol production, in summary, is given during secondary fermentation, mainly by yeasts, as a way to reduce NAD, giving continuity to the fermentation process. However, alcohol synthesis leads to energy losses, due to CO_2 production and the high volatility presented by alcohol molecule (McDonald et al., 1991). Although there was no difference among treatments for ethanol, glyphosate application in a theoretical dose of 869.3 mL ha^{-1} increased 2,3-butanediol in treated silages. Thus, the quadratic behavior presented by the 2,3-butanediol could be related to an increase in yeast development due to the lower concentration of beneficial short-chain fatty acids. Nishino et al. (2007) observed a reduction in alcohol content in whole crop rice silage inoculated with *Lactobacillus buchneri* due to the higher amount of acetic acid presented by treated silage than the control. However, in the present study, wilting reduced 2-butanol concentration, a molecule derived from amino acid degradation (Kalač, 2011).

Silage heating, pH raising, as well as organic matter loss are a consequence of intake of nutrients and fermentation products by spoilage microorganisms during feed-out phase (Basso et al., 2012). These effects may be associated to the reduced concentration of acetic, propionic, and isovaleric acids observed for wilted silages in this trial, since these molecules are capable of inhibiting silage deterioration during feed-out phase (McDonald et al., 1991; Danner et al., 2003; Wilkinson and Davies, 2013).

Conclusions

Glyphosate application from 500 mL ha^{-1} prior to ensiling of black oat at milky/dough grain stage increases dry matter as well as organic matter content of silages. Nevertheless, treated silages have higher fiber content than control silage due to a reduction of crude protein as a side effect of glyphosate application. Wilting decreases the concentration of beneficial fermentation products such as acetic, propionic, and isovaleric acids, increasing deterioration after oxygen exposure. In this way, for wilted black oat silage production, harvested at milky/dough grain stage, the application of 500 mL ha^{-1} glyphosate is recommended.

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