DOI: 10.5433/1679-0359.2020v41n3p971

Chemical composition, aerobic stability, and fermentation pattern of white oat silage wilted with glyphosate

Composição química, estabilidade aeróbia e padrão fermentativo de silagens de aveia branca dessecadas com glifosato

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Highlights:
Glyphosate application at the doses 500 mL ha^{-1}, 750 mL ha^{-1} and 1000 mL ha^{-1} increased dry matter content compared to control.
Glyphosate application did not change lactic acid concentration in silages; however, pH linearly decreased due to desiccation.
Wilting did not affect acetic acid concentration in silages, as well as aerobic stability.
The greatest dry matter recovery index (934 g kg^{-1}) was observed at a dose of 864.20 mL ha^{-1} glyphosate.
Glyphosate application prior to ensiling up to 1000 mL ha^{-1} did not cause any impairment to silage conservation and aerobic stability, as well as increased dry matter recovery.

Abstract

White oat has good nutritional quality but is not an easy forage to ensile due to its high buffer capacity and moisture content at ensiling moment. Therefore, wilting is necessary to offset such negative aspects. However, this process demands skilled workforce and adequate machinery. In this way, chemical desiccation is a promising technology to reduce the steps needed for wilting. Thus, we aimed to evaluate the effects of glyphosate as a chemical desiccant on the nutritional quality, fermentation pattern, losses, and aerobic stability of wilted white oat (*Avena sativa*) silages. White 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 doses evaluated were 0, 500, 750, 1000, and 1250 mL ha^{-1}. Three days after desiccation, all treatments were ensiled, and the silos were stored for 150 days. A completely randomized design was used, and all statistical procedures were performed by means of Bayesian Inference. No differences were found for lactic acid, but treated-silage pH linearly decreased. The lowest concentration of butyric acid (3.40 mg kg^{-1}) was observed at 900.80 mL ha^{-1}. For ammonia, the highest point (50 g kg^{-1}) occurred at 916.51 mL ha^{-1}. Aerobic stability was not influenced by treatments. Maximum dry matter recovery index (934 g kg^{-1}) was observed at 864.20 mL ha^{-1} glyphosate. Wilted forage from treatments 500 mL ha^{-1}, 750 mL ha^{-1}, and 1000 mL ha^{-1} had greater dry matter content compared to control (320.1, 326, 301.3, and 270.7 g kg^{-1} respectively). Hemicellulose linearly decreased and crude protein linearly increased. The lowest concentrations of neutral detergent fiber (642.8 g kg^{-1})

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Received: May 21, 2019 - Approved: Nov. 12, 2019

and neutral-detergent insoluble nitrogen (2.30 g kg^{-1}) occurred at doses of 1141.32 mL ha^{-1} and 829.14 mL ha^{-1}, respectively. In brief, for wilted white oat silage production, harvested at milky-dough grain stage, glyphosate application prior to ensiling up to 1000 mL ha^{-1} led to better conservation compared to non-treated silage.
Key words: Wilting. Dry Matter Losses. Neutral Detergent Fiber. Ammonia Nitrogen.

Resumo

A aveia branca (*Avena sativa*) apresenta boa qualidade nutricional, contudo, devido à sua alta capacidade tampão e elevado teor de umidade no momento do corte, acaba por dificultar o processo de conservação como silagem. Dessa forma, a pré-secagem é necessária para compensar os aspectos negativos relacionados à ensilagem da aveia. No entanto, a pré-secagem exige mão-de-obra qualificada bem como maquinário específico. Desta forma, a dessecação química é uma tecnologia promissora, reduzindo as etapas necessárias para a pré-secagem, facilitando o processo. Sendo assim, objetivou-se foi avaliar os efeitos do uso do glifosato na pré-secagem da avia branca sobre a qualidade nutricional, padrão de fermentativo e perdas bem como na estabilidade aeróbia das silagens resultantes. A semeadura de aveia branca ocorreu na primeira quinzena de maio de 2013. A aplicação do dessecante ocorreu quando a aveia atingiu o estágio de grãos leitoso/pastoso (96 dias após o plantio). As doses de glifosato avaliadas foram 0, 500, 750, 1000 e 1250 mL ha^{-1}. Três dias após a dessecação, todos os tratamentos foram ensilados e os silos foram armazenados por 150 dias. O delineamento experimental foi o inteiramente casualizado e todos os procedimentos estatísticos foram realizados por meio da inferência bayesiana. Não foram encontradas diferenças para as concentrações de ácido láctico, no entanto, o pH diminuiu linearmente nas silagens tratadas. A menor concentração de ácido butírico (3,40 mg kg^{-1}) foi observada para a dosem de 900,80 mL ha^{-1}. A maior concentração de amônia (50 g kg^{-1}) foi encontrada na dosem de 916,51 mL ha^{-1}. A estabilidade aeróbica não foi influenciada pelos tratamentos neste estudo. O maior índice de recuperação de matéria seca (934 g kg^{-1}) foi observado para a dosem de 864,20 mL ha^{-1} de glifosato. As silagens provenientes dos tratamentos 500 mL ha^{-1}, 750 mL ha^{-1} e 1000 mL ha^{-1} apresentaram teores de matéria seca mais elevados em relação ao controle (320,1, 326, 301,3 e 270,7 g kg^{-1}, respectivamente). A hemicelulose diminuiu linearmente e a proteína bruta aumentou linearmente devido a dessecagem. As menores concentrações de fibra em detergente neutro (642,8 g kg^{-1}) e nitrogênio insolúvel em detergente neutro (2,30 g kg^{-1}) ocorreram nas doses de 1,141,32 mL ha^{-1} e 829,14 mL ha^{-1}, respectivamente. Sendo assim, para a produção de silagem de aveia branca pré-seca, colhida no estágio de grão leitoso/pastoso, a aplicação de glifosato antes da ensilagem em até 1000 mL ha^{-1} possibilitou uma melhor conservação da silagem em comparação à silagem não tratada.
Palavras-chave: Pré-Secagem. Perdas de Matéria Seca. Fibra em Detergente Neutro. Nitrogênio Amoniacal.

Introduction

Small grain crops are largely cultivated in southern Brazil for grain production, foraging and even aiming soil covering during the winter. Regarding forage production, special attention is given to oat species once the cultivation technologies for these crops are widely diffused among farmers. White oat (*Avena sativa*) has high dry matter (DM) and grain yield per hectare compared to other small grain crops, raising the interest for ensiling of this crop (Fontaneli et al., 2009; Meinerz et al., 2011). However, this cereal normally has low DM content (from 26 to 31% DM at flowering and dough stages, for example) and high buffer capacity (over 70 meq NaOH 100g^{-1} DM), hampering an adequate conservation of its silage (David et al., 2010; Zamarchi, Pavinato, Menezes, & Martin, 2014). Wilting reduces moisture content and buffering capacity, hence preventing spoilage during fermentation and reducing effluent production, DM losses, and gas synthesis (Cazzato, Laudadio, Corleto, & Tufarelli, 2011; Paris, Zamarchi, Pavinato, & Martin, 2015; Gomes et al., 2019).

Besides that, if compared to non-wilted forage, the wilting process improves silage nutritional quality by increasing total digestible nutrients and DM digestibility, without hampering aerobic stability (Jacobs, Hill, & Jenskin, 2009; Paris et al., 2015; Gomes et al., 2019). On the other hand, the wilting technique demands skilled workforce and adequate machinery. In this context, chemical desiccation is a promising technology, reducing the steps needed for wilting, independent of the forage maturity stage. We hypothesize that white oat wilting with glyphosate would support lower dry matter losses, improved fermentation pattern, and better chemical composition, without detrimental effect on aerobic stability. Given this background, this study aimed to evaluate the effect of chemical desiccation with different glyphosate doses on the fermentation pattern, fermentative losses, aerobic stability, and chemical composition of respective silages.

Material and Methods

White oats (*Avena sativa* L. Corona) were grown at the Experimental Farm of Iguatemi, State University of Maringá, Maringá – PR, Brazil (23°21'13'' S – 52°04'27'' W; 550-m altitude). The local climate is classified as Cfa (warm climate, fully humid, with a hot summer), according to Peel, Finlayson and McMahon (2007). Annual means of climate variables are the following: rainfall of 1276 mm, potential evapotranspiration of 1070 mm, average temperature of 17.5 °C, minimum temperature of 13.6 °C (in July), maximum temperature of 20.6 °C (in January), and relative humidity of 66% (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2012). Soil is classified as a sandy Red Latosol (Santos et al., 2006).

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 the *Comissão de Química e Fertilidade do Solo* [CQFS-RS/SC] (2004). Grass planting took place on May 15th, 2013, in an area of 0.2 ha (73 m long and 28 m wide). Seed density was equivalent to 100 kg seeds ha^{-1}. Nitrogen fertilization was performed on June 6th, 2013, in a single application of 112 kg ha^{-1} as urea.

At the milky-dough phenological stage (August 19, 2013), the area was subdivided into five plots of 360 m^2 (70 m long and 5 m wide) for glyphosate application. Desiccation was performed using a backpack sprayer. Doses evaluated in this study were 0 (control), 500, 750, 1000, and 1250 mL ha^{-1}.

Forage dry matter (DM) content was monitored aiming at 30 to 35% DM. Three days after desiccation (August 22, 2013), all plots reached the expected DM content, and forages were ensiled on the same day.

The crop was harvested using a Premium Flex forage harvester (Menta Mit, Cajuru, SP, Brazil). After cutting, forage was inoculated with a bacterial additive (starter culture) containing *Lactobacillus plantarum* MA 18/5U and *Pediococcus acidilactici* MA 18/5M in order to reach a theoretical rate of 1×10^5 CFU/g of forage.

Fresh forage (10 kg) was packed into experimental PVC tube silos (0.015 m^3). Packing was done manually, and silos were sealed with double-sided tarpaulin sheet and adhesive tape. On day 0 and at the opening time, silos were weighed for subsequent determination of dry matter recovery index (DMRI), according to Jobim, Nussio, Reis and Schmidt (2007). Silos remained stored during 150 d.

At silo opening, samples were collected for pH measurement, according to Silva and Queiroz (2009). Ammonia nitrogen ($N\text{-}NH_3/N$) was determined according to Detmann et al. (2012), using silage juice extracted with the aid of an 8-ton hydraulic press. About 500 g of silage were sampled from each silo for DM content determination, using a forced-air circulation oven at 55 ºC for 48 hours. Dried samples were grounded to 1-mm particle size and submitted to the following determinations: dry matter at 105 °C and ash using Association of Official Analytical Chemists [AOAC] (1990) methods 967.03 and 942.05, respectively; organic matter (OM) by the formula OM = 100-ASH; neutral

detergent fiber (NDF) and acid detergent fiber (ADF) according to Mertens (2002) and Van Soest (1963) methods, respectively; lignin (LIG) by the LAD (lignin in acid detergent) method according to Detmann et al. (2012); hemicellulose (HEM) by the difference between NDF and ADF fractions (HEM = NDF-ADF); cellulose by subtracting lignin fraction from ADF fraction (CEL = ADF-LIG); crude protein AOAC (1990) method 990.03; neutral-detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) by analyzing nitrogen content in NDF and ADF residues, respectively.

Aerobic stability was measured as described by Jobim et al. (2007). About 3 kg of fresh silage was decompressed in each silo to facilitate exposure of ensiled material to air. Buckets were stored in a controlled temperature chamber for 74 h at 25 °C. The pH was measured daily at 15:00 h, according to Silva and Queiroz (2002). Also, a 25 g sample was collected daily from silos for further DM and ash content determinations. 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, Roig, Bernal, Sánchez-Monedero and Cegarra (2000).

Fermentation profile was determined using the aqueous extract of silages obtained by homogenizing 25 g silage and 225 mL distilled water (1:10) (Pedroso, Pedroso, Barioni, & Souza, 2014). Aqueous-extract pH was determined after 30 minutes. The supernatant (2 mL) was pipetted and stored in Eppendorf tubes at -20º C for further analyses.

Lactic acid concentration was determined by a colorimetric method (Pryce, 1969), using a MARCONI® Janway 6305 spectrophotometer at λ = 565 nm. Alcohols, esters, and volatile fatty acids were measured by 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 polyethyleneglycol).

The experimental design was completely randomized, evaluating control silage and four doses of glyphosate, with four replicates per treatment, resulting in 20 silos. The mathematical model used 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 = dose-effect j; and e_{ij} = random error associated with each observation Y_{ij}. All data analyses were performed according to Rossi (2011) using Bayesian inference.

Control group response (y_{ci}) was deemed to follow a normal distribution $y_{ci} \sim N(\mu_c, \sigma^2_{ce})$. For treatment levels, regression models such as linear [1] and quadratic [2] were considered as $y_i \sim N[f(\beta,x_i),\sigma^2_e]$, being [1] $y_i = f(\beta,x_i) = \beta_0 + \beta_1 x_i + \varepsilon_i$, and [2] $y_i = f(\beta,x_i) = \beta 0 + \beta_1 x_i + \beta_2 x^2_i + \varepsilon_i$, where i = 1 ,2, ..., n, x = treatment level = 500, 750, 1000, and 1250 mL ha^{-1} chemical desiccant, assuming $\varepsilon_i \sim N(0,\sigma^2_e)$. For instance, the vector of regression parameters β_p in models (1 and 2) was regarded as unrelated. A non-informative *a priori* distribution was considered for all model parameters, being for control and treatment, respectively, $\mu_c|\sigma^2_{ce} \sim N(0,10^{-6})$ and $\tau_{ce} \sim Gama(10^{-3}, 10^{-3})$ $\beta_p|\sigma^2_e \sim N(0,10^{-6})$; 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 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, respectively, x_{cr} and y_{cr}, were obtained by, 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.

The deviance information criterion (DIC) was used to select the best model (quadratic or linear) for each variable measured. Spiegelhalter, Best, Carlin, & Van Der Linde (2002) suggested the following for DIC values for models [1] and [2]: $D = |DIC_1 - DIC_2|$. Differences were regarded as non-

significant if D<5; significant if 5≤D≤10; and highly significant if D>10. In the case of non-significance for a quadratic model, the response (y_{ij}) followed a normal distribution, being $y_{ij} \sim N\left(\mu_j, \sigma^2_{je}\right)$, 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 σj^2, non-informative distributions were considered *a priori* $\mu_j|\sigma^2_e \sim N(0,10^{-6})$ and $\tau_j \sim Gama\ (10^{-3},10^{-3})$, respectively.

Multiple comparisons were performed between the *a posteriori* distributions of the means of each treatment versus control. Treatments whose credibility intervals for the mean differences do not include the value zero were considered as 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 R software (R Development Core Team [R], 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 by Heidelberger and Welch (1983) and Geweke (1991) criteria using the CODA package of R software (R, 2014).

Results and Discussion

The pH values decreased linearly due to glyphosate application, but regression equations for lactic acid, acetic acid, and propionic acid were not significant (Table 1). However, compared to control, acetic acid concentrations were higher for treatments 500 and 1250 mL ha^{-1}. Glyphosate use may reduce lactic acid synthesis and hence may increase silage pH. However, in this study, glyphosate application had no effect on lactic acid synthesis, allowing a suitable pH drop in the studied silages. Acetic acid synthesis is stimulated by low DM content in silages (Rooke & Hatfield, 2003); however, its production is associated with higher DM losses during storage because of gas production (mainly carbon dioxide) (Pahlow, Muck, Driehuis, Elferink, & Spoelstra, 2003; Gomes et al., 2019). On the other hand, acetic acid has an antifungal action protecting silage from spoilage during aerobic exposure (Danner, Holzer, Mayrhuber, & Braun, 2003). Despite the quadratic effect, no difference was observed between critical point and control of propionic and isobutyric acids. Like acetic acid, propionic acid increases silage aerobic stability, however, this acid is usually found in grass silage with less than 0.1% DM, as observed in this study (Weinberg, Ashbell, Hen, & Azryeli, 1995; Nishino & Shinde, 2007; Li & Nishino, 2011). Isobutyric acid is produced by clostridia and lactic-acid bacteria during fermentation of branched amino acids (Suzzi, Grazia, & Ferri, 1990). A quadratic effect was observed for butyric acid content, therefore, a theoretical glyphosate dose of 900.08 mL ha^{-1} would reduce butyric acid concentration by 24% compared to the control. *Clostridium* species consume soluble nutrients (e.g., soluble sugars, lactic acid, and protein) to produce butyric acid, reducing energy recovery and silage nutritional value (Pahlow et al., 2003; Kung, Shaver, Grant, & Schmidt, 2018). However, ammonia synthesis unexpectedly increased in wilted silages. A quadratic behavior was observed for ammonia, therefore, the highest ammonia level (24% higher compared to control) would be found using 916.51 mL ha^{-1} of glyphosate. Protein degradation increases ammonia in silages, mainly due to amino acids deamination by microorganisms (Pahlow et al., 2003, Kung et al., 2018). Wilting normally reduces ammonia synthesis in silage; however, glyphosate application may increase contents of free amino acids in plants, allowing further deamination and ammonia production (Duke, Hoagland, & Elmore, 1979; Orcaray, Zulet, Zalbaza, & Royuela, 2012). Besides an increase in ammonia due to chemical wilting, metabolites associated with degradation of nitrogen compounds were within acceptable limits (below 1% DM for butyric acid; 800 mg kg^{-1} DM for isobutyric acid; and 12% ammonia) (Kalač, 2011; Lehmen, Fontaneli, Fontaneli, & Santos, 2014; Kung et al., 2018).

Table 1
Bayesian estimates (means, standard deviations, and regression equations) for fermentative pattern of white oat silages

Item	Glyphosate dose (mL ha^{-1})					Regression Equation			DIC$_L$	DIC$_Q$	$^1x_{cr}$	$^1y_{cr}$
0	500	750	1000	1250		*b0*	*b1* X	*b2* X^2				
pH	3.81	3.93*	3.93*	3.87	3.83		4.02	-0.01				
	(0.04)	(0.04)	(0.03)	(0.03)	(0.06)		(0.04)	(0.001)				
					g kg^{-1} of DM							
Lactic acid	94.1	89.0	82.3	87.9	99.9		76.4	0.02				
	(-11.4)	(-9.6)	(-12.7)	(-19.8)	(-17.5)		(-15.6)	(-0.02)				ns
Acetic acid	7.40	10.4^{*L}	7.90	7.90	11.8*		8.00	0.01				
	(-0.6)	(-0.9)	(-0.9)	(-2.3)	(-2.4)		(-2.30)	(-0.01)				ns
Butyric acid	4.50	0.80^L	2.70	3.50	1.20		-10.6	0.03	-0.000017	-9.9	-20.2	900.08	3.40*
	(-3.3)	(-0.3)	(-1.4)	(-1.2)	(-0.5)		(-3.40)	(-0.01)	(0.000005)				(0.5)
2,3-Butanediol	5.70	3.60*	7.50^{*L}	6.20	3.50		-12.8	0.05	-0.000027	1.6	-12.5	861.7	7.30*
	(-0.5)	(-1)	(-0.7)	(-1.5)	(-1.3)		(-4.30)	(-0.01)	(0.000006)				(0.60)
Ethanol	3.60	4.00	4.00	2.40	3.60		4.40	0					
	(-1.3)	(-2.8)	(-0.6)	(-1.2)	(-0.9)		(-1.60)	(0)					ns
Propionic acid	0.80	0.30	0.60	0.60	0.30		-1.10	0.01	-0.000002	-68.9	-73.3	871.12	0.60
	(-0.5)	(-0.2)	(-0.3)	(-0.4)	(-0.4)		(-0.70)	(-0.01)	(0.000001)				(0.04)
Formic acid	1.10	0.70	2.00	1.30	0.60		-4.20	0.02	-0.000008	-29.9	-36.1	874.30	1.8*
	(-0.5)	(-0.3)	(-1)	(-0.4)	(-0.3)		(-2.10)	(-0.01)	(0.000003)				(0.30)
					mg kg^{-1} of DM							
1-Propanol	110	21.7^{*L}	41.5^{*L}	42.3^{*L}	16.8^{*L}		35.4	-0.006					
	(21.85)	(16.59)	(22.31)	(8.03)	(5.73)		(17.9)	(0.020)					ns
Isobutyric acid	266.20	127.20	266.10	287.90	83.68^L		-515.92	1.882	-0.0011	200.7	185.3	845.71	281.3
	(91.52)	(20.23)	(71.26)	(71.49)	(34.03)		(180.7)	(0.450)	(0.0003)				(27.46)
Ethyl lactate	322.80	231.40	160.60^{*L}	127.20*	289.70		603.63	-1.112	0.0007	190.6	180	808.55	146.6*
	(70.05)	(47.32)	(35.12)	(58.19)	(31.35)		(165.2)	(0.408)	(0.0002)				(23.72)
Butanol	59.28	6.99^{*L}	25.51^L	19.52	11.53^{*L}		-60.57	0.191	-0.0001	124.7	117	888.50	24.30*
	(19.44)	(5.94)	(6.24)	(9.22)	(3.71)		(24.33)	(0.060)	(0.0000)				(3.42)

continue

continuation

						g kg^{-1} of N						
Ammonia	38.0	41.6	49.9*^{L}	48.7	43.3	7.00	0.10	-0.000056	32.05	26.1	916.51	50.0*
	(4.2)	(3.4)	(1.6)	(4.1)	(5.9)	(14.4)	(0.04)	(0.000020)				(2.0)

*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}); DIC_L - Deviance Information Criterion for linear regression; DIC_Q - Deviance Information Criterion for quadratic regression; ns - Non-significant effect for regression equation; Values between () - Standard deviation.

Ethanol is the main alcohol in silages. In our study, the contents of this substance in white oat silage were not affected by desiccation with glyphosate. The lowest concentration of butanol would be observed using a dose of 888.50 mL ha^{-1} glyphosate. According to Kalač (2011), butanol presence is indicative of degradation of nitrogen compounds during fermentation. As stated by the mathematical model, a glyphosate dose of 861.70 mL ha^{-1} would lead to an increase in 2,3-butanediol (by 21.92% compared to control). In this experiment, 2,3-butanediol concentrations were higher than those reported by Li and Nishino (2011) for wilted grass silage after 120 days of fermentation. This chemical is a minor electron acceptor and its synthesis increases with the lack of other important electron acceptors (e.g. ethanol and lactic acid) (Daniel et al., 2013). Lactic acid bacteria, yeasts, and enterobacteria produce 2,3-butanodiol under anaerobic conditions (Nishino & Shinde, 2007). Ethyl lactate is an ester formed under low pH conditions by abiotic esterification of lactic acid and ethanol (Weiss, 2017). As lactic acid and ethanol concentrations in the evaluated silages did not change, no differences were expected due to wilting. However, a quadratic effect was observed, therefore, the lowest concentration of ethyl lactate would be observed by applying 808.05 mL ha^{-1} glyphosate.

No differences were observed in relation to aerobic stability (Table 2). The highest DMRI (934.4 g kg^{-1}) was observed for silage treated with 864.2 mL ha^{-1} (81.1% higher than control). Silage deterioration after air exposure depends on microorganism contamination (e.g. yeasts) and presence of decay inhibiting compounds (e.g., volatile fatty acids, ammonia, and nitrite) (Wilkinson & Davies, 2013). Yeasts are mainly responsible for spoilage during feed-out phase, consuming lactic acid, raising the pH, thus creating an enabling environment for other spoilage microorganisms (Kung et al., 1998; Wilkinson & Davies, 2013). Acetic acid restricts yeast development controlling spoilage after silo opening (Danner et al., 2003). However, according to the mathematic model, acetic acid concentration did not change among the treated silages in this study, as well as their aerobic stability. The DMRI measures the availability of nutritive compounds for animals after silo opening (Jobim et al., 2007). As observed in this study, glyphosate application (864.20 mL ha^{-1}) increased DMRI by 13.2%.

Table 2
Bayesian estimates (means, standard deviations, and regression equations) for dry matter recovery index and aerobic stability of white oat silages

Item	Glyphosate dose (mL ha^{-1})					Regression Equation			DIC_L	DIC_Q	$^1x_{cr}$	$^1y_{cr}$
	0	500	750	1000	1250	*b0*	*b1* X	*b2* X^2				
DMRI	811.1	824	925.8*	914.8	812.7	314.1	1.44	-0.001	112.9	101.5	864.20	934.4*
	(31.7)	(34.9)	(38.0)	(52.7)	(40.3)	(146.5)	(0.36)	(0.001)				(21.2)
OML[a] (%)	77.4	100	97.9	104.8	35.9	149.7	-0.07					
	4.23	3.99	4.08	3.23	2.34	3.96	0.004				ns	
Maximum pH	8.22	8.15	5.59	6.61	6.52	8.03	-0.002					
	0.96	0.66	1.73	2.17	2.02	1.88	0.002				ns	
Maximum pH (h)	71.5	74.0	74.0	74.0	74.0	81.34	-0.013					
	4.12	0.02	0.02	13.29	14.54	9.75	0.011				ns	
Average pH	5.93	5.24	4.53	5.18	4.81	5.17	-0.001					
	0.59	0.39	0.68	1.17	0.73	253.15	-0.010				ns	

DMRI = dry matter recovery index; [a]OML = organic matter losses during aerobic exposure by Paredes et al. (2000).
*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}); DIC_L - Deviance Information Criterion for linear regression; DIC_Q - Deviance Information Criterion for quadratic regression; ns – Non-significant effect for regression equation; Values between () - Standard deviation.

Unexpectedly, regression equations for DM had a negative linear slope (Table 3). However, when glyphosate was applied at 500 mL ha^{-1}, 750 mL ha^{-1}, and 1000 mL ha^{-1}, DM contents were higher than control. Chemical desiccation prior to ensiling normally increases DM content because glyphosate affects stomatal conductance, cellular membrane selective permeability, and aquaporin functionality, thus reducing water absorption by plants (Zobiole et al., 2010; Carneiro et al., 2017; Bueno et al., 2018). Nevertheless, glyphosate doses above 1000 mL ha^{-1} may induce stomatal closure, which could reduce transpiration and carbon dioxide assimilation, decreasing the DM accumulation by plants (Brecke & Duke, 1980; Yannicari, Tambussi, Istilart, & Castro, 2012). Low DM content in ensiling crops extends fermentation time, increasing fermentative losses and reducing the amount of nutrients available to rumen microorganisms, as observed in control and in silage treated with 1250 mL ha^{-1} glyphosate (Pahlow et al., 2003).

Table 3
Bayesian estimates (means, standard deviations, and regression equations) for dry matter, dry matter recovery index, pH and chemical composition (g kg^{-1} DM) of white oat silages

Item	Glyphosate dose (mL ha^{-1})					Regression Equation			DIC_L	DIC_Q	$^1x_{cr}$	$^1y_{cr}$
	0	500	750	1000	1250	*b0*	*b1* X	*b2* X^2				
DM $_{g\,kg^{-1}\ as\ fed}$	270.7	328.1*	326*	301.3*	279.5	36.83	-0.07					
	(0.71)	(0.33)	(0.48)	(0.58)	(0.93)	(0.81)	(0.001)					
OM	945	948.4	948.6	847.9	941.9	953.7	-0.01					
	(0.4)	(0.22)	(0.1)	(0.28)	(0.23)	(0.26)	(0.001)					ns
NDF	665.3	690.3	663.6	637.6	645.8	809.5	-0.3	0.001	56	43.8	1141.32	642.8*
	(1.46)	(0.19)	(0.65)	(0.95)	(0.13)	(2.41)	(0.006)	(0.0001)				(0.33)
Hemicellulose	309.5	305.9	295.4	259*	274.2	329.5	-0.05					
	(1.21)	(0.94)	(0.95)	(1.05)	(1.81)	(1.6)	(0.002)					
ADF	355.8	384.5*	368.1	378.5*	370.9	385.9	-0.001					
	(0.83)	(1.12)	(0.96)	(0.73)	(1.89)	(1.33)	(0.001)					ns
Cellulose	318.2	342.1*	328.2	337.9	333.9	340.6	-0.01					
	(0.93)	(0.71)	(0.94)	(0.83)	(1.88)	(1.24)	(0.001)					ns
Lignin	35.1	42.3*	39.9	40.6*	36.9	45.3	-0.01					
	(0.16)	(0.45)	(0.31)	(0.12)	(0.26)	(0.32)	(0.001)					ns
Crude Protein	103.8	86.2	106.1	102.3	117.3	71.7	0.04					
	(0.4)	(0.43)	(0.44)	(0.27)	(0.58)	(0.64)	(0.001)					
NDIN	2.40	2.80	2.30	2.60	3.00	5.00	-0.01	0.001	-60.7	-76.0	829.14	2.30
	(0.02)	(0.02)	(0.02)	(0.03)	(0.03)	(0.07)	(0.001)	(0.0001)				(0.01)

DM = dry matter; OM = Organic Matter; NDF - neutral detergent fiber; ADF = acid detergent fiber; NDIN = neutral-detergent insoluble nitrogen.
* 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}); DIC_L - Deviance Information Criterion for linear regression; DIC_Q - Deviance Information Criterion for quadratic regression; ns – Non-significant effect for regression equation; Values between () - Standard deviation.

Glyphosate inhibits the shikimic acid pathway, reducing synthesis of essential aromatic amino acids and proteins (Zobiole et al., 2010; Orcaray et al., 2012); however, in this study, CP contents increased with desiccant application. As nitrogen compounds are less used as fuel during silage fermentation, CP loss during ensiling is not expected; however, consumption of soluble sugars may lead to an increase in CP due to a dilution effect (Pahlow et al., 2003).

No significant results for OM, ADF, CEL, and LIG models were observed. A quadratic effect was observed for NDF and NDIN. Treating silage with 1141.32 mL ha^{-1} glyphosate decreased NDF content by 6%. In addition to the lack of difference between critical point and control, applying desiccant in a theoretical dose of 829.14 mL ha^{-1} resulted in an NDIN content of 2.3 g kg^{-1}. Wilting decreased HEM contents linearly. Cell wall compounds (CEL, HEM, and LIG) are not the main sources of energy for fermentative microorganisms in silages, as they lack enzymes capable of breaking chemical bonds in these molecules (Pahlow et al., 2003). However, HEM is susceptible to hydrolysis under low pH conditions (below 4) (Dewar, McDonald, & Whittenburry, 1963; Rooke & Hatfiled, 2003). In our study pH remained below 3.9, which might have induced HEM hydrolysis. As one of the remaining molecules after neutral detergent extraction, HEM is closely related to NDF; thus, HEM reduction also led to a decrease in NDF content (Van Soest et al., 1991).

Conclusions

Chemical wilting treatment controlled secondary fermentation without any detrimental effect on lactic acid synthesis and aerobic stability. Although dry matter losses were lower than in control, chemical composition was similar among silages. Taken together, results showed that glyphosate doses up to 1000 mL ha^{-1} are suitable alternatives for wilting of white oat harvested at the milky-dough stage, for further ensiling.

Acknowledgments

We would like to thank the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for the financial support.

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