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Evaluation of Winter Cereal Silages Subjected to Pre-Drying at Different Phenological Stages with and without the Use of Additives

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ABSTRACT

This study aimed to evaluate the productivity, nutritional, and fermentative characteristics and aerobic stability of white oat, barley, and wheat silages that had been subjected to pre-drying at different phenological stages, with or without the application of additives. The experimental design used was completely randomized, in a 3×3×2 factorial design (three forage species, three additives, and two phenological stages), with three replications. After harvest, the cereals were exposed to the sun, and prior to ensiling, were treated (or not) with an inoculant containing a mixture of fermentative bacteria and enzymes and/or propionic acid. Comparing the cereals harvested at different vegetative and reproductive stages, revealed higher percentages of crude protein when the cut was made in the vegetative stage, lower contents of neutral detergent fiber, acid detergent fiber, and lignin; and higher levels of neutral detergent fiber digestibility after 30 hours of incubation. Addition of the inoculant containing homofermentative and heterofermentative bacteria promoted the production of silages with higher lactic acid levels, lower pH values, and losses of ammonia-N. Compared with the control group, addition of propionic acid did not improve fermentative characteristics. Cereals harvested at the vegetative stage produced silage with a best bromatological composition. Although the use of biological additives did not alter the bromatological composition of the pre-dried silages, treatment with the bacterial inoculant improved most fermentative parameters; however, it was ineffective in enhancing the aerobic stability of silage after exposure to air.

Keywords: Aerobic deterioration, Bacterial inoculant enzyme, Fermentative parameters Propionic acid, Silage quality.

INTRODUCTION

Pre-dried silage is produced by the dehydration of forage after cutting, with partial removal of water from the plant through wilting. After reaching the ideal dry matter content. The forage is ensiled in an anaerobic environment, in order to create adequate conditions for lactic fermentation, and thus reducing the incidence of undesirable secondary fermentations (Bragachini *et al.*, 2008).

In the southern region of Brazil, the prevailing subtropical climate makes it possible to use different winter cereal cultivation practices for production of forage, as these materials provide good dry matter (DM) combined with a high productive potential and nutritional quality (Rosário *et al.*, 2012). However, several factors are known to influence the quality of silage derived from

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winter cereals, including the variability between species, differences between genotypes of the same species and their adaptability to different edaphoclimatic conditions (Meinerz *et al.*, 2011b), and the time of harvest.

In this context, additives are often used to improve the fermentation process and to reduce nutritional losses and aerobic deterioration (Yitbarek and Tamir, 2014; Oladosu *et al.*, 2016). Chemical and microbial additives can be used to prevent the development of degradative microorganisms, thereby reducing quality losses (Pedroso *et al.*, 2007), and the utility of bacterial and enzymatic inoculants has been assessed as an economically viable alternative to improve aerobic stability (Gimenes *et al.*, 2005).

However, there is currently a lack of information regarding the use of additives in the ensiling process of winter cereals and, therefore, the objective of this study was to evaluate the productivity, nutritional characteristics, fermentative characteristics, and aerobic stability of white oats, barley, and wheat preserved in the form of pre-dried silage, with or without the use of additives.

MATERIALS AND METHODS

Location and Preparation of the Experimental Area

The experiment was carried out from April to December 2017, at the Frederico Westphalen Campus of the Farroupilha Federal Institute of Education, Science and Technology, which is in the physiographic of Alto Uruguai (latitude 27° 23′ South and longitude 53° 25′ West; 480 m above sea level). According to the Köppen climate classification, the region's climate is Cfa, of a humid subtropical type. The average values of temperature, precipitation, and humidity during the study period are shown in Table 1.

Preparation of the experimental area was performed 20 days prior to the beginning of the experiment through drying and harrowing, during which time, soil samples were collected. Potassium and phosphorus fertilization was carried out according to published recommendations (CQFS-RS/SC, 2016), with the application of 90 kg ha^{-1} of phosphorus and 20 kg ha^{-1} of potassium during sowing. Nitrogen fertilizer in the form of urea was applied at three times at the rate of 80 kg ha^{-1}. The initial application was carried out 30 days after sowing and the second and third applications were made at 25-day intervals thereafter. Seeds of the three cereals white oats, barley, and wheat were sown in rows at a depth of 3 cm and spacing of 17 cm. The sowing density of the cereals were as follows: white oats, 450 plants per m², barley, 307 plants per m²; and wheat, 400 plants per m². Before the cuts, the productivity of the different cereals was estimated using a metallic square (50×50 cm) which was randomly throw five times per plot and each time a sample was cut off at the ground level.

Table 1. Temperature, precipitation and air humidity observed during the experimental period. [a]

	Temperature (C°)		Precipitation (mm)		Air humidity (%)	
Months	Average occurred	Average five years	Average occurred	Average five years	Average occurred	Average five years
June	15.1	13.9	232.8	181.4	82.0	82.8
July	15.6	14.3	15.8	170.4	67.0	78.6
August	17.3	17.2	244.8	124.0	70.0	70.6
September	21.3	17.8	95.2	150.2	68.0	71.6
Average	17.3	15.8	147.1	156.5	71.7	75.9

[a] Source: INMET - National Institute of Meteorology, 2017. Location of the weather station: Latitude 27° 23' 44" South, and Longitude 53° 24' 46" West.

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Preparation and Elaboration of Silage

The total experimental area covered 5,500 m², divided into 54 experimental plots of dimension 10×9 m, which were separated by 1-m-wide corridors. The crops of oats (cv. URS Taura), barley (cv. BRS Cauê), and wheat (cv. TBIO Energia I) were harvested on August 25, 2017 (considered the vegetative stage) and September 19, 2017 (considered the reproductive stage), at 73 and 98 days after sowing, respectively.

The harvested forage was subjected to the following treatments: (1) Untreated forage (Control); (2) Treatment with LALSIL® DRY inoculant (*Lactobacillus buchneri* NCIMB 40788 (7.5×10^{10} CFU g^{-1}); *Pediococcus acidilactici* CNCM MA 18/5M (5×10^{10} CFU g^{-1}); beta-glucanase from *Aspergillus niger* MUCL 39199 (5,750 UI g^{-1}); and xylanase from *Trichoderma longibrachiatum* MUCL 39203 (3,000 UI g^{-1}); and (3) Treatment with a product based on propionic acid (MOLD-ZAP®-ALLTECH: 55% propionic acid and 12% ammonia hydroxide). The additives were manually sprayed to forage during collection in the form of aqueous solutions to ensure even distribution over the forage mass.

The cereals were cut 10 cm from the ground, using a mower equipped with a metal roller conditioner, and then fragmented in a crusher set to an average particle size of 2 cm. The different cultivars were ensiled when it accounted for about 35% of the pre-dry matter, obtained after approximately 6 hours of exposure to the sun, and measured with the aid of the Koster moisture meter. Subsequently, the material was ensiled with six layers of plastic film with 50% overlap in rolls of approximately 50 kg. Prior to ensiling, a sample of fresh forage was taken following the methodology of Playne and McDonald (1966) for the determination of Buffering Capacity (BC). All pre-dried silages were sampled after 45 days of ensiling, using a "probe" sampler (50 cm in length×4.8 cm in diameter). The silage samples were homogenized prior to determining pH and chemical composition. The pH of the ensiled materials was measured using a bench pH meter based on the methodology described by Silva and Queiroz (2002).

Chemical Analysis and Estimation of Milk Production

To determine the chemical composition of the silages and the levels of acetic acid, lactic acid, and ammonia-N (mg N 100 g^{-1} silage), the samples were dried in an oven with forced air ventilation at 60°C for 72 hours, then, ground in a mill and passed through a Willey type 1-mm-mesh sieve. Thereafter, samples of the powdered material were analyzed using a Foss NIR 5000 system, following instructions in the user manual. Calibration for estimating nutritional quality variables was based on the system developed by the Dairy One Forage Lab (Ithaca, NY, USA) and validated with batches of independent samples from several locations in Brazil by ESALQLab/USP.

The estimate of the productive potential of milk (kg t^{-1} DM) were estimated according to the MILK2006 spreadsheet, considering the Total Digestible Nutrients (TDN) values and dry mass per hectare of the plants (Shaver and Lauer, 2006). Estimates of TDN content were calculated according to the NRC (2001).

Aerobic stability test

To estimate aerobic stability, 4 kg of material was collected from each silo and transferred to plastic bags for subsequent homogenization. Thereafter, 1.5 kg of the material was placed in trays and transferred to a temperature-controlled room (24±1°C). The temperature of each silage was measured six times a day for 5 days, by means of a permanent thermometer inserted in the center of the ensiled mass at a depth of 10 cm in the center of the mass. Aerobic

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stability was calculated by determining the length of time taken for the temperature of the silage to rise by 2°C in relation to room temperature (Honig, 1990).

Statistical Analysis

For productivity variable, the experimental design used was completely randomized, in a 3×2 factorial design (three forage species and two phenological stages of the crop), with three replications. For the bromatological variables, the experimental design used was completely randomized, in a 3×3×2 factorial design (three forage species, three additives, and two phenological stages of the culture), with three replications. After the normality test, the data were analyzed by analysis of variance.

Statistical analyses were performed using the MIXED procedure of the SAS® statistical program (University Edition version, 2016), with forage species, additives, phenological stages of the crop, and their interactions being considered fixed effects, and the repetitions and residue considered random effects. Averages were compared using the LSMEANS (Statistical package feature to compare means) resource and interactions were considered significant at the 5% level. The data obtained for NH_3-N were found to be non-normally distributed, and, therefore, were initially transformed prior to analysis with the non-parametric Wilcoxon test. For correlation analysis, we used the PROC CORR command of the SAS statistical package.

RESULTS AND DISCUSSION

Production Estimates

A difference in yield was observed between the evaluated cereals and between the phenological stages (Table 2). Oats and barley showed the highest yields (P< 0.0001), in relation to wheat. When comparing the stages, it was observed that lower productivity was obtained when cutting in the vegetative phase, compared with the reproductive stage (P= 0.0009).

Compared with the findings obtained in the present study, similar yields for wheat, but different yields for barley and oats, were observed in a previous study conducted at EMBRAPA Trigo in Passo Fundo, RS (Fontaneli *et al.*, 2009). On the basis of their evaluation of 14 crops of winter forage plants destined for silage production, cut at the grain stage in soft mass (30 to 35% DM), these authors obtained average yields for oats (UPF 18, IPFA, and Agro Zebu), barley (BRS 195, 224, and 225), and wheat (BRS Figueira, Umbu, and 277) of 6.011, 4.099, and 5.096 kg DM ha^{-1}, respectively. In further study conducted at the same location, Lehmen *et al.* (2014) evaluated the silages of winter cereal cut at the pasty grain stage (30 to 35% DM), and obtained productivities of 6.500 kg DM ha^{-1} for the barley crop (BRS Cauê) similar to those measured for the same cultivar in the present study. However, higher productivity of 9.055 kg DM/ha^{-1} was observed for white oats (URS 21 and URS Guapa), whereas wheat (BRS' Umbu and Tarumã) showed approximately

Table 2. Productivity and buffering capacity of different forage species at two phenological stages. [a]

Variables [b]	Species			Stages		SEM	Interactions
	Oat	Barley	Wheat	VEG	REP		ESP×EST
Productivity	5.033[ab]	7.079[a]	3.722[b]	3.479[b]	7.645[a]	35.7	< 0.0001
Buffering Capacity (BC)	21.35[a]	18.12[ab]	14.61[b]	23.97[a]	12.09[b]	0.94	0.59

[a] VEG= Vegetative, REP= Reproductive, SEM= Standard Mean Error, ESP= Forage Specie, EST= Stages Phenological, ADT= Additives. [b] Productivity in kg DM ha^{-1}, BC= In eq mg NaOH100 g^{-1} DM. (a-b) Means that do not share a letter are significantly different.

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three-fold higher productivity at 9.812 kg DM/ha^{-1}.

Differences in productivity between different phenological stages is in line with expectations, given that with the progression of plant development there is a greater production of phytomass, and, consequently, a higher accumulation of DM (Beck *et al.*, 2009). Nevertheless, we expected higher productivity from the three crops examined in the present study, based on average yields of the same crop plants reported in other studies conducted in the southern region of Brazil. The differences in yield could, however, be explained by the fact the experimental period of the present study coincided with a period of relative drought, when compared with the 5-year average (Table 1), with less than 10% of the historical average precipitation falling during the month of July. This deficiency in rainfall suppressed forage development to a certain extent, resulting in lower productivity per hectare.

Chemical Composition of Silages

No interactions were observed among the assessed variables with respect to DM, EE, NDF, ADF, lignin, or TDN (Table 3). In this regard, there has been considerable discussion concerning the ideal phenological stage at which to harvest winter cereals for silage production. However, it is known that maturity affects production of DM and the relative compositions of forage components and, consequently, differences in the nutritive value of materials (Meinerz *et al.*, 2011b). It was possible to verify that there was a significant difference ($P< 0.0001$) in the DM percentage of crops harvested at the two assessed phenological stages, being lower for forage when harvested in the vegetative stage than for forage harvested in the reproductive stage.

This difference can be explained with respect to two factors. Firstly, it is believed that by exposing cereals to solar radiation for a period of 6 to 8 h for pre-drying, it is possible to reach a DM content considered adequate (Fluck *et al.*, 2018), which for the studied materials should reach at least 300 g kg^{-1} of DM to be effectively ensiled. However, when harvested at the vegetative stage, the solar radiation on the day of ensiling of the cereals is likely to be less intense than later in the season and, consequently, the material may not lose sufficient amounts of water. The second factor relates to an increase in the DM content of plants associated with the inclusion of grains in the forage when the crop is cut during the reproductive stage (Fluck *et al.*, 2018; Weinberg *et al.*, 2010). The findings of the present study tend to be consistent with this latter explanation, since the starch content of forage, which is indicative of grain content, was significantly higher ($P< 0.0001$) in the reproductive stage (Table 3).

With respect to the percentage CP among the different cereals, there was no significant difference between additive treatments ($P= 0.8232$), and the values recorded tended to be consistent with those reported in the literature (Meinerz *et al.*, 2011a; Hastenpflug *et al.*, 2011; Leão *et al.*, 2016). However, the percentage CP was higher in vegetative-stage forage than in that cut at the reproductive stage ($p < 0.0001$), which is consistent with a decrease in the levels of CP that are known to occur concomitant with the increasing physiological maturation of winter cereals (Coblentz and Walgenbach, 2010). This is probably related to the fact that during the period of vegetative growth, plants, and particularly leaves, have high levels of Nitrogen (N), which are mainly components of the compounds associated with photosynthesis. With advancing phenological stage, there is an increase in the structural parts containing a lower proportion of N and, consequently, a decline in the production of biomass and a dilution of the N in plants (Taiz and Zeiger, 2004). The reduction in CP levels can be explained by the fixation of N in the structures of the cell wall and in an increase in the rate of senescence (Van Soest, 1994),

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Table 3. Nutritional value of winter cereal silages cut at different phenological stages with or without addition of inoculant or propionic acid.[a]

Variables	Species			Stages		Additives			SEM	Interactions		
(g kg^{-1} DM)	Oat	Barley	Wheat	VEG	REP	C	I	P		ESP×EST	ADT×ESP	ADT×EST
DM	281^{c}	325^{b}	356^{a}	258^{b}	382^{a}	300^{b}	312^{b}	312^{a}	0.63	0.06	0.50	0.13
Ash	106	101	95.1	120	82.2	106	98.1	98.9	0.51	0,13	0,68	0,89
STARCH	19.9	29.2	16.4	2.7^{b}	40.5^{a}	18.0	22.4	25.4	0.39	0.37	0.20	0,73
CP	189	201	197	245^{a}	148^{b}	211	188	188	0.92	0.58	0.85	0,15
NDF	599	582	576	546^{b}	625^{a}	580^{b}	587^{ab}	591^{a}	0.55	0.35	0.71	0.79
ADF	377^{a}	336^{b}	337^{b}	321^{b}	379^{a}	348	350	351	0.52	0.14	0.19	0.53
Lignin	47.1^{a}	38.5^{b}	36.3^{b}	33.5^{b}	47.8^{a}	41.2	37.6	43.2	0.15	0.27	0.15	0.96
NFC	96.9^{b}	113^{ab}	124^{a}	89.2^{b}	134^{a}	101	118	116	0.54	0.50	0.00	0.71
EE	44.6^{a}	40.4^{a}	38.1^{b}	46.0^{a}	36.1^{b}	42.5	40.4	40.1	0.11	0.10	0.07	0.29
TDN	596^{b}	615^{a}	622^{a}	626^{a}	596^{b}	609	618	606	0.57	0.29	0.32	0.81
NDFD[b]	66.8^{b}	72.2^{a}	73.4^{a}	77.3^{a}	64.3^{b}	70.8	69.5	72.1	0.73	0.02	0.76	0.18
MILK[c]	1.849^{b}	1.921^{a}	1.935^{a}	1.975^{a}	1.829^{b}	1.899	1.891	1.915	15.66	0.63	0.61	0.58

[a] VEG= Vegetative, REP= Reproductive, C= Control, I= Inoculant, P= Propionic acid, SEM= Standard mean error, ESP= Forage specie, EST= Stages phenological, ADT= Additives, DM= Dry Matter, CP= Crude Protein, NDF= Neutral Detergent Fiber, ADF=acid detergent fiber, NFC=non-fibrous carbohydrates, EE= Ether Extract, TDN= Total Digestible Nutrients through the NRC 2001. [b] NDFD= Digestibility NDF at 30 hours of incubation (% of NDF). [c] To estimate milk production, the Milk 2006 model was used Shaver and Lauer (2006) in Kg t^{-1} of DM. Letters (a-c) Means that do not share a letter are significantly different.

with a consequent reduction in photosynthesis.

In terms of NDF contents (Table 3), there was no significant difference (P= 0.36) among the forages of cereal species examined. However, differences were observed in the forage cut at different phenological stages (p = 0.008), with higher values being found in material cut at the reproductive stage compared with that cut at the vegetative stage, consistent with the findings of previous studies (Taiz and Zeiger, 2004). This difference can be attributed to the deposition of cell wall material such as lignin with advancing phenological stage (Van Soest, 1994). In addition, developmental changes, such as the lengthening of internodes and emergence of inflorescences, result in plant material of lower nutritional value (Costa *et al.*, 2018). Nevertheless, we found that the average NDF values of the three cereal species examined were close to the limits previously reported by Van Soest (1965), who considers that contents of cell wall constituents in excess of 55 to 60% are limiting with respect to forage consumption. In addition, treatment with propionic acid had the effect of increasing the NDF, which can be attributed to degradation of soluble fractions in the medium during the fermentation process, resulting in the concentration of other components (Table 3).

With respect to phenological stage, levels of ADF showed a pattern like those of NDF (Table 3), with higher levels being detected at a more advanced stage of plant maturation (P< 0.0001). These observations are consistent with those previously reported in the literature. For example, Fontaneli *et al.* (2009) have reported an average ADF content of 352.20 g kg^{-1} DM from their evaluation of three varieties of oats, two of rye, three of barley, three of triticale, and three of wheat. Similarly, in their evaluation of the nutritional value of winter cereal silages (twelve grass genotypes), Lehmen *et al.* (2014) recorded an average ADF content of 355.50 g kg^{-1}.

In addition to having low digestibility, forages with ADF values of approximately 40% or higher are less readily consumed, as this parameter represents the cellulose and lignin fractions, the latter of which is the main non-digestible fraction of plants (Van Soest, 1994). This is reflected in the higher ADF content of oats (P< 0.0001) when compared with those of barley and wheat, which is attributable to the higher (P= 0.0002) percentage of lignin found in oat forage. Moreover, lower (P< 0.0001) concentrations of lignin was observed at the vegetative stage compared with the reproductive stage, thereby contributing to an increase in ADF values as plants mature, as discussed previously.

The NDF comprises ADF and the hemicellulose fraction, which explains the lower Digestibility NDF at 30 hours of incubation.at more advanced stages of plant maturation, during which ADF levels are higher. Dado and Allen (1996) fed lactating cows with silages containing similar contents of NDF and CP, and with different NDFD, and observed higher DM consumption and milk production for cows that consumed silages with higher digestibility coefficients.

Our evaluation of TDN levels (Table 3) revealed significant differences among the three cereals examined (P= 0.005), with higher values being recorded for barley and wheat than for oats. These findings can be explained by the lower (P< 0.0002) amounts of indigestible materials (lignin and ADF), and, consequently, higher (P= 0.005) amounts of non-fibrous carbohydrates (NFC) in both barley and wheat compared with oats. These results corroborate those obtained in a study by Meinerz *et al.* (2015), in which higher values of TDN were detected in the silage and hay from cold season pastures, due to the higher levels of NFC found in these materials.

Comparison of the NFC values of forage harvested at the two different phenological stages revealed higher values (P< 0.0001) during the reproductive stage in relation to the vegetative stage, which contrasts with the

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pattern of TDN levels, which were lower (P< 0.0001) in the reproductive stage than in the vegetative stage. The higher NFC values in the reproductive stage can be explained by the larger (P< 0.0001) amounts of starch than in cereals ensiled at the vegetative stage. The higher TDN values recorded at the reproductive stage can be attributed to the higher proportion of lignin at the time of cutting and ensiling.

High levels of NFC in the preserved silage are associated with good quality, as these compounds are favorable fermentation substrates, and are also rapidly and completely digested by ruminants (Mertens, 1987). However, if only NFC is evaluated for determining the optimal cutoff point for harvesting forage plants, the best phenological stage under the conditions of the present study would be the reproductive stage. This, nevertheless, would have failed to take into account the lower NDFD (P< 0.0001) at this stage compared with the vegetative stage. In this regard, Filya (2003), who evaluated different stages of winter cereal crops, observed lower levels of NDFD at the pre-flowering stage (422.20 g kg^{-1} NDF) compared with the meal grain (349.90 g kg^{-1} NDF).

When comparing NDFD to 30 hours in silages with and without additive treatment, no significant difference (P= 0.08) was observed between control group cereals and cereals treated with either propionic acid or an inoculant containing bacteria and enzymes. Given that enzymatic additives are typically applied in combination with bacterial inoculants, it is often difficult to differentiate between responses mediated by bacteria and those attributable to enzymes (Muck *et al.*, 2018). However, Dehghani *et al.* (2012) demonstrated that the addition of fibrolytic enzymes can have a positive effect on the quality of the silage, increasing the NDFD and improving the performance of animals.

In terms of milk production, the efficiency estimates of milk production, that is, the amount of milk produced per ton of DM, were significantly higher for wheat and barley compared with oats (P= 0.005, Table 3). It was also observed that ensiling cereals at the vegetative stage resulted in a higher milk production potential per ton of DM (1.975 kg) compared with cereals ensiled in the reproductive stage (1.829 kg) (P< 0.0001). Thus, from the perspective of milk production, it can be inferred that it would be preferable to cut and ensile wheat and barley during vegetative stage.

Fermentation Characteristics of Silage

Evaluation of the effects of adding an inoculant containing homofermentative and heterofermentative bacteria (*Pediococcus acidilactici* and *Lactobacillus buchneri*) revealed that the treated material was characterized by higher values of lactic acid (P< 0.0008), lower pH values (P< 0.0001), and lower losses via NH_3-N (P= 0.0048) (Table 4). These data are consistent with findings of a meta-analysis study (130 studies) conducted by Oliveira *et al.* (2017), who found that by inoculating a combination of homofermentative and facultative heterofermentative bacteria (*Lactobacillus plantarum, Pediococcus pentosaceus, Enterococcus faecium, and Lactobacillus rhamnosus*) in the production of silages, it was possible to reduce the pH (P< 0.01), increase the lactate concentration (P< 0.01), and reduce losses of NH_3-N (P< 0.01). Similarly, Sucu and Çifci (2016), who evaluated the use of inoculants in triticale silage, found that treated silage had higher levels of lactic acid and, consequently, lower pH values and NH_3-N levels.

The combined use of enzymes and bacterial inoculants for ensilage of cultures with a low concentration of soluble sugars tends to increase the release of sugars via the hydrolysis of cell walls, thereby providing larger amounts of substrate for the production of organic acids by bacteria (Sucu and Çifci, 2016). In the present study, in addition to containing strains of the bacteria *Lactobacillus buchneri* and *Pediococcus acidilactici*., the inoculant used contained the enzymes beta-glucanase and

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Table 4. Fermentation characteristics of different forage species in two phenological stages and treated or not with propionic acid or biological inoculant.[a]

Variables[b]	Species		Stages		Additives			SEM	Interactions		
	Oat	Barley	Wheat	VEG	REP	C	I	P		ESP×EST	ADT×ESP	ADT×EST
pH	5.61[a]	5.72[a]	5.40[b]	5.53	5.62	5.92[a]	4.97[b]	5.84[a]	0.06	<0.0001	<0.0001	<0.0001
NH_3-N	352[a]	268[b]	244[b]	387[a]	195[b]	323[a]	251[b]	291[b]	0.12	0.003	0.046	0.179
Acetic acid[2]	22.1[a]	21.8[a]	16.0[b]	22.5[a]	1.22[b]	21.6	20.3	18.1	0.13	0.378	0.027	0.491
Lactic acid	37.5[b]	44.9[ab]	47.1[a]	41.4	45,0	41.4[b]	51,7[a]	36,4[b]	0.26	0.376	0.221	0.952
AS	49.1[a]	49.7[a]	44.2[b]	46.6	48.8	47.1	48.2	47.9	1.18	0.415	0.397	0.798

[a] VEG= vegetative, REP= Reproductive, C= Control, I= Inoculant, P= Propionic acid, SEM= Standard Mean Error, ESP= Forage Specie, EST= Stages Phenological, ADT= Additives. [b] NH_3-N= Ammonia-N (mg N 100 g^{-1} silage), [2]g Kg^{-1} DM, AS= Aerobic Stability in hours. Letters (a-b) means that do not share a letter are significantly different.

xylanase, which are believed to have contributed to promoting a reduction in the pH (< 0.0001) of ensiled cereals (Table 4).

In relation to the effects of adding propionic acid on the aforementioned fermentative characteristics, it is evident that this additive was ineffective (P> 0.05) in promoting an increase in lactic acid levels and, consequently, did not reduce the pH (P> 0.05) when compared with the control group. These results are in agreement with the findings of Castro *et al.* (2006), who studied the inclusion of propionic acid or an enzymatic bacterial inoculant in Tifton 85 silages, and concluded that the use of propionic acid did not improve the qualitative characteristics of fermentation, such as pH, BC, and NH_3-N.

Evaluation of the concentration of acetic acid in ensiled materials revealed an inverse relationship (P= 0.0045; r= -0.64) between a higher concentration of this acid and lactic acid/pH. The data obtained in the present study are consistent with those presented in the review by Muck *et al.* (2018), who stated that higher final pH values found in silages are related to lower concentrations of acetic acid, which can be attributed to the prolonged action of heterofermentative lactic acid bacteria and enterobacteria, and, to a lesser extent to that of clostridia. In addition to negatively affecting fermentative parameters, the presence of acetic acid also contributes to greater losses of DM and energy from the ensiled material. The percentages of acetic acid recorded in the materials obtained in the present study are consistent with the silage of well-fermented grasses, as defined by Kung Jr *et al.*, (2018), which are characterized by an acetic acid content of between 10 and 30 g kg^{-1} DM.

The content of NH_3-N in silage is influenced by the amount of water present in the material. In the present study, it was found that the silage obtained from oats had the highest concentration of NH_3-N, which could be attributable to its high buffering capacity and, indeed, we detected a correlation between these two variables (r= 0.68, p < 0.0001). A further observation that

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supports this correlation is the higher value (P< 0.0001) of BC found at the vegetative stage compared with the reproductive stage, which is associated with higher losses (P< 0.0001) of NH_3-N in species ensiled in the vegetative stage, thereby negatively affecting silage preservation. These results are consistent with those obtained by Meinerz *et al.* (2011b), who detected a close correlation between DM levels (r= 0.91; P< 0.0001) and BC of winter cereals, with higher values of BC and lower DM values being observed for common black oats and BRS Marciana barley, indicating that these materials are more resistant to an elevation in pH.

According to McDonald *et al.* (1991), NH_3-N is related to the fermentative quality of the silage, because when the pH reduction occurs more slowly, it causes protein degradation, thus reducing the levels of CP and increasing the concentrations of NH_3-N. The BC of the forage to be ensiled is a key factor in the fermentation process (McDonald *et al.*, 1991; Kung Jr *et al.*, 2018). When the plant has a high BC, the pH slowly decreases, causing greater losses in the ensiling process, thus reducing the nutritional value of the silage.

Another factor that helps to explain a greater resistance to pH reduction in forages in a vegetative stage is the high percentage of protein, as verified in the data of the present study (245.1 g kg^{-1} of CP). The CP content has a close connection with the forage BC since, as the CP content increases, proteolysis in the silo occurs in greater intensity. It is known that with proteolysis, ammonia is produced, which causes a higher concentration of NH_3N that is an alkalizing substance and, therefore, tends to neutralize the pH of the medium, raising the BC (McDonald *et al.*, 1991). Thus, the high percentage of CP in the vegetative stage contributed to the highest amount of NH_3N (P< 0.0001) and, consequently, higher (P< 0.0001) BC.

In terms of the aerobic stability of silage, there was no significant difference between phenological stages (P= 0.146) or additive treatments (P= 0.842). However, as shown in Table 4, there were notable differences in stability among the silages derived from the different cereals (P= 0.0059). Among the three cereal species evaluated, Of the three cereals evaluated, wheat showed loss of stability in less time having been exposed to air, pre-dried wheat showed the most rapid loss of stability, which can be ascribed to the fact that pre-dried wheat has a higher concentration of lactic acid and a lower concentration of acetic acid compared with the other two cereals. These results are consistent with those reported by Weinberg *et al.* (2011), who found that high residual concentrations of soluble carbohydrates and lactic acid, as well as low levels of acetic acid, in silages were related to aerobic deterioration, as these can serve as substrates for the development molds and yeasts. Therefore, the higher concentration of soluble carbohydrates and lactic acid and the lower level of acetic acid in wheat, associated with aerobiosis, promote the activity of these deteriorating microorganisms, consequently, there is an increase in the temperature of the silo (Table 5), causing the loss of aerobic stability. Related to this, a correlation was

Table 5. Daily temperatures observed for five days after the overture of the silos.[a]

Variables	Species			Stages		Additives		
Day (H)/T (C°)	Oat	Barley	Wheat	VEG	REP	C	I	P
24	21.7	21.7	21.4	22.7	20.5	21.8	21.5	21.5
48	22.1	22.1	22.6	22.2	22.2	22.5	22.2	22.1
72	24.2	24.3	25.3	24.4	24.7	25.1	24.2	24.5
96	21.9	22.2	23.3	22.8	22.0	22.9	21.8	22.6
120	22.2	23	24.2	22.9	23.3	23.4	22.6	23.4

[a] H= Hours, VEG= Vegetative, REP= Reproductive, C= Control, I= Inoculant, P= Propionic acid

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observed between temperature and aerobic stability (r= -0.84).

Compared with stability of 136 h observed for pre-dried wheat in the present study, Horst *et al.* (2018) reported that the silages oats, barley, rye and triticale remained stable for a notably longer period (160 hours).

The aerobic stability of oats (Table 4) corroborates the data of Leão *et al.* (2016), who reported 52 hours for loss of stability in white oats, which they attributed to the higher pH values (5.58) characterizing this cereal. The rapid loss of aerobic stability in the pre-dried silages in the present study can be explained by the high pH values. We found that even the application of an inoculant containing homofermentative and heterofermentative bacteria and/or propionic acid was ineffective in lowering the pH to the desired levels (pH< 4.5). Presumably, fungi, yeasts, and certain species of bacteria may have caused aerobic absorption of the lactic acid in silage, thereby reducing its preservation potential (Pahlow *et al.*, 2003) and causing a rapid loss of aerobic stability.

CONCLUSIONS

When cereals are harvest at the vegetative stage, the resulting silages have a better chemical composition compared with silages derived from cereals harvested at a more advanced stage of development.

The bacterial and enzymatic inoculant improved the fermentative parameters of the pre-dried silages, however, it was not efficient in improving the chemical composition and aerobic stability after the silages had been exposed to air. Similarly, the addition of propionic acid proved to be ineffective in enhancing the fermentative characteristics and aerobic stability of the evaluated silages.

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ارزیابی غلات سیلویی پیشاخشک در مراحل مختلف فنولوژیک با و بدون استفاده ازمواد افزودنی

و. جیاکامازا سروتی، م. جانگبک، س. ناعتزولد پریرا، ا. مینوزو دا سیلویرا، س. لوییس اسچونز، ت. جواو تونین، ف. ریمان اسکونیسکی، و ج. وگاس

چکیده

هدف این پژوهش، ارزیابی بهره‌دهی و ویژگی‌های غذایی وتخمیری و پایداری هوازی مواد سیلویی چاودار سفید، جو، و گندم پیشاخشک (pre-drying) در مراحل مختلف فنولوژیکی با و بدون مواد افزودنی بود. آزمایش با طرح کاملا تصادفی با فاکتوریل 3x3 x2 (3 گونه علوفه، 3 ماده افزودنی، و 2 مرحله فنولوژیک) در 3 تکرار اجرا شد. بعداز برداشت، غلات مزبور در تابش خورشید قرار داده شد و قبل از سیلو کردن، با ماده تلقیح کننده حاوی مخلوطی از باکتری های تخمیری و آنزیم ها و/یا

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پروپیونیک اسید تیمار شد (یا نشد). با مقایسه غلات برداشت شده در مراحل فنولوژیک رشد سبزینه ای و مرحله زایشی آشکار شد که در برداشت مرحله رشد سبزینه ای درصد پروتئین خام بیشتر بود، ولی مقدار فیبرشوینده خنثی (neutral detergent fiber)، فیبر شوینده اسیدی، و لینگنین کمتر بود، هرچند که مقدار قابلیت هضم فیبرشوینده خنثی در تیمار30 ساعت بعد از انکوباسیون بیشتر بود. افزودن ماده تلقیح کننده حاوی باکتری‌های هموفرمنتاتیو و هتروفرمنتاتیو منجر به مواد سیلویی با مقادیر بیشتر لاکتیک اسید، pH کمتر، و تلفات نیتروژن آمونیاکی شد. نیز، درمقایسه با تیمار گروه شاهد، افزایش پروپیونیک اسید منجر به بهبود ویژگی‌های تخمیری نشد. غلات برداشت شده در مرحله رشد سبزینه ای، مواد سیلویی با بهترین ترکیب بروماتولوژیک (bromatological) را تولید کرد. هرچند کاربرد مواد افزودنی زیستی تغییری در ترکیب بروماتولوژیک مواد سیلو شده ایجاد نکرد، اما بیشتر پارامترهای تخمیری در اثر تیمار کردن با مواد تلقیحی باکتریایی بهبود یافت، با این همه، این کار در بهبود پایداری هوازی مواد سیلو شده بعد از قرار گرفتن در معرض هوا موثر نبود.

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