Effects of inoculation with homolactic bacteria on the conservation of wheat silage stored in bunker-silos

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ABSTRACT
This study aimed to determine the effects of a homolactic inoculant on the nutritional value, fermentation pattern, aerobic stability and dry matter (DM) loss of wheat silage stored in farm-scale bunker silos. The experiment was carried out in a completely randomised design, whereas treatments were arranged in a $2^3$ factorial scheme: wheat silage without inoculant (WS) or treated with a homolactic inoculants ($Lactobacillus plantarum$ plus $Pediococcus acidilactici$ at $1 \times 10^5$ cfu/g; WSI), evaluated in three silo layers (bottom, middle and top layers) of a bunker silo. The WS silage had higher levels of acetic, propionic and butyric acids and greater fermentative DM losses. However, the homolactic inoculant (WSI) worsened the aerobic stability. As previously expected, silage stored at the top layer had lower density and higher DM losses. In conclusion, the homolactic inoculant decreased the formation of volatile fatty acids and fermentative losses, but led to worse aerobic stability of wheat silages stored in bunker silos.

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Introduction

Winter cereal crops are recognised and widely spread as good forage sources in subtropical and temperate regions. The advantages of using wheat in silage production are related to its high dry matter (DM) yield per area compared with other winter crops (Chen and Weinberg 2009), availability of a technology package for the farmers (Fontaneli et al. 2009) and the possibility of roughage production without land competition with other important crops like corn and soybean.

Up-to-date, most of the studies evaluating silage inoculants were carried out in laboratory silos (Muck 2010). Imperfect sealing conditions in large-scale silos are more challenging to proper conservation (Mari et al. 2009; Queiroz et al. 2012), hence the silage obtained in lab conditions may not reflect the process in the field. Therefore, it is important to validate those results on large-scale silos, focussing on possible changes that may occur at each silo layer, during the fermentation and after silage feedout.

This work was performed to evaluate the chemical composition, fermentation end-products, aerobic stability and DM losses of wheat silage stored in farm-scale silos, with or without a homolactic inoculant.

Materials and methods

The experiment was carried out at the State University of Middle-West, Guarapuava, Brazil ($25^\circ 24^\prime S, 51^\circ 28^\prime W$). Wheat (cv BRS Umbu) was sown in 3 hectares, using a row spacing of 0.17 m and a stand density of 220 seeds/m$^2$. Whole crop wheat was directly harvested at soft dough stage (40% DM) with a pull-type forage harvester (Pecus 9004 PRN 1200, Oslo, Norway), chopped at 2-cm theoretical cut and packed in two 30-t bunker silos (3.5 m width $\times$ 11 m length $\times$ 1.2 m height), using a tractor weighting approximately 6 tons.

At ensiling, two treatments control (WS) and inoculated silage (WSI), besides three silo layers (lower = 0–40 cm vs. medium = 40–80 cm vs. upper = 80–120 cm layer) were factorially arranged, with 12 replicates per layer (one silo per silage type). The inoculant (Master tropical – Katec Lallemand) contained $Lactobacillus plantarum$ MA 18/SU and $Pediococcus acidilactici$ MA...
18/5M and was applied at a theoretical rate of 1 × 10^8 cfu/g of forage (diluted in water, 4 L/t).

During ensiling, 12 tracer bags (12 × 50 cm, nylon 85 μm pore size) containing 1.5 kg of pre-ensiling forage were allocated throughout each silo layer. Silos were filled simultaneously (every second load for each silo) in one working day and sealed with polyethylene film (black-on-white, 200 μm). After 144 d of storage, silos were unloaded simultaneously at a feedout rate of approximately 14 cm/d and tracer bags were recovered for sampling. Silage density was measured with a 5-cm diameter corer (Holmes and Muck 1999). Dry matter loss was determined as the disappearance of DM within the tracer bags during the storage period (Jobim et al. 2007).

All analyses were performed with silage sampled from tracer bags. A aliquot of 200 g was pre-dried in a forced-air oven at 55 °C for 72 h. Dried samples were ground through a 1-mm screen (Wiley mill). Subsamples were analysed for DM in an air-forced oven at 105 °C, ash and crude protein (AOAC 1990). Ether extract was determined as described by AOAC (2000). NDF, using heat-stable amylase (A3306, Sigma Chemical Co., St. Louis, MO), ADF and lignin content were performed according to Van Soest et al. (1991). The in vitro DM digestibility (IVDMD) was measured using the DAISYII method (Holden 1999).

Water extracts were prepared with fresh silage (1:10) for analyses of pH and fermentation end-products. Lactic, acetic, propionic and butyric acids, and ethanol were analysed by gas chromatography (Erwin et al. 1961), whereas ammonia nitrogen (N-NH3 in total N) was determined according to Chaney and Marbach (1962).

An aerobic stability test was performed for 7 d. Approximately 1.2 kg of fresh silage was loosely added to plastic buckets and exposed to air at 20 ± 1.5 °C. Aerobic stability was defined as the time (hours) until silage temperature reached 2 °C above ambient temperature. Mean daily silage temperatures above ambient temperature were accumulated over 7 d of aerobic exposure and used as a marker of aerobic deterioration (O’Kiely 2009). Maximum temperature and time to reach maximum temperature were also recorded. Silage pH was determined daily across the aerobic exposure.

Data were analysed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC), including the effects of inoculant (WS and WSI), silo layer (upper, medium and lower) and their interaction. Treatment means were compared by Tukey’s test (α = 0.05).

Results and discussion

Inoculation provided a decrease in DM losses (Table 1), which reflected in differences in chemical composition between treatments. Compared with heterolactic bacteria, homolactic bacteria typically present greater efficiency of glucose utilisation (Axelsson 2004). In this way, accumulation of lactic acid and pH drop to levels capable to inhibit the growth of undesirable microorganisms can be provided more efficiently by homolactic fermentation (Muck 2013). In the current trial, the greater oxidation of organic matter in the control silage (mainly soluble sugar) likely increased CP and EE.

Differences were observed in the stratification of the silo panel for DM, DML, density, IVDMD and ash content (Table 2). Silage from upper layer showed higher DM content and reduced density (p < .05). A poor compaction increases the oxygen ingress among the silage particles determining deterioration

| Table 1. Chemical composition, dry matter losses (DML) and density of wheat silages with and without a homolactic inoculant. |
|---------------------------------------------------------------|
| Item | Treatment | Silo layer | p Value |
| DM, % | WS | WSI | UL | ML | LL | SEM | S | L | S × L |
| 40.15 | 40.94 | 42.50 | 41.83 | 37.50 | 0.92 | .2314 | <.0001 | .1112 |
| DML, % | 13.14 | 10.35 | 14.89 | 9.93 | 11.45 | 2.26 | .0828 | <.0001 | .3 |
| Density, DM, kg/m³ | 181.27 | 184.4 | 138.46 | 205.12 | 192.3a | 35.34 | .1162 | <.0001 | .37 |
| Chemical composition, %DM |
| NDF | 52.48 | 53.3 | 53.65 | 51.42 | 2.98 | .3375 | .0819 | .1235 |
| ADF | 32.88 | 32.29 | 32.55 | 32.77 | 32.48 | 1.65 | .3731 | .8463 | .9456 |
| CP | 9.79 | 8.96 | 9.23 | 9.56 | 9.36 | .036 | .0053 | .3202 | .1841 |
| EE | 2.44 | 1.96 | 2.24 | 2.14 | 2.22 | 0.88 | <.0001 | .4198 | .064 |
| Ash | 3.86 | 3.45 | 3.78 | 3.22 | 3.96 | 0.91 | .0004 | <.0001 | .0064 |
| IVDMD | 62.03 | 62.41 | 61.36 | 63.54 | 62.78 | 1.54 | .3731 | .0076 | .2018 |

Means within silage with different superscripts differ (a, b: p < .05).

1WS: wheat silage control and WSI: wheat silage treated with homolactic inoculant.
2UL: top layer; ML: middle layer; LL: bottom layer.
3Probabilities for silage (S), layer (L) and interaction (S × L) effects.
4SME: standard error of the means.
potential during silage storage and feedout (Holmes and Muck 1999; Chen and Weinberg 2009). The presence of oxygen leads to undesirable microorganism's development which consumes nutrients enhancing the DM losses. Velho et al. (2007) indicated that small differences in density have resulted in large changes in silage quality. Thus, compared to medium layer, silage from the upper layer showed higher ash content and lower IVDMD. Weinberg et al. (2010) evaluated different locations on the wheat silos profile and observed variations in ash levels, which were justified by the occurrence of fermentation losses.

Higher moisture content on the bottom part of the silo can be attributed to effluent produced during fermentation. Effluent accumulates in the lower layer reducing the silage DM content leading to an extent of fermentation decreasing silage quality which is shown by lower IVDMD and higher ash content on bottom compared to middle layer. Xie et al. (2012) evaluating whole crop wheat silage harvested at different stages observed higher ash content in silage with higher moisture content.

Contrary to the expectation, the content of lactic acid did not differ between treatments (Table 3), differently of others studies where inoculation provided higher contents of lactic acid in wheat silages (Weinberg et al. 2002; Filya et al. 2004). This result is associated to the high DM at wheat harvesting. Reduced water availability is associated with inhibition of lactic acid bacteria development (Pahlow et al. 2003). Filya et al. (2000) comparing the effect of bacterial inoculants over fresh and wilted wheat have observed reduced lactic acid content for desiccated forage, which presented 42% of DM, close to DM content observed in this study. It was observed in the silage × layer interaction post analysis (Table 4) that WS presented higher concentrations of acetic, propionic and butyric acids. Fermentation end-products in WS are an indicative of heterofermentative process, due to a lower lactic acid concentration compared to acetic acid.

Addah et al. (2011) have observed that inoculation of barley forage with homolactic bacteria reduced propionic acid concentration. On the contrary, Addah et al. (2012), when evaluating barley silage with heterolactic inoculation, observed that propionic acid concentration was not affected. In the present study, homolactic fermentation in WSI silage possibly provided competitive exclusion of microorganisms during fermentative metabolism, with a predominance of fermentation by lactic acid producing bacteria.

The rapid pH decline reduces Clostridium activity, which is responsible for butyric acid production (Muck 2010). This fact justifies the high butyric acid concentration in WS silage, in which there might have been a prolonged fermentation up to reach a pH sufficient to inhibit Clostridium action.

Inoculation has modified fermentation profile throughout silo layers. WS and WSI silages showed higher propionic acid levels at the lower layer. High propionic acid levels have been linked to fungus and yeast inhibition (Pahlow et al. 2003). In this way, data from the current study indicated that WS has higher antifungal effect on all silo layers.

Higher butyric acid content was observed in WS treatment at the lower layer. This result may indicate that Clostridium, which is the main butyric acid producer, benefited from the higher humidity of this layer to

| Table 2. Slice of silage × layer interaction for ash. |
|------------------------------------------------------|
| Silo layer   |  |  |  |  |  |  |  |  |  |
|             | WS | WSI |
| Upper       |   |   |
| Ash content, % DM | 3.824ab | 3.75a |
| Medium      |   |   |
| Ash content, % DM | 3.65ab | 2.78bc |
| Lower       |   |   |
| Ash content, % DM | 4.11ab | 3.81ab |

Means within silage with different superscripts differ (a, b: p < 0.05).
Means within silo layer with different superscripts differ (A, B: p < 0.05).
1WS: wheat silage; WSI: wheat silage inoculants.

| Table 3. Fermentation products of wheat silage with or without bacterial inoculants. |
|---------------------------------------------------------------|
| Item                  | Treatment1 | Silo layer2 | p Value3 |
|                      | WS         | WSI         | UL   | ML   | LL   | SEM4 | S       | L          | S × L      |
| pH, index            | 4.08       | 4.02        | 4.09 | 4.06 | 4    | 0.95 | 0.0254  | 0.1906     | 0.4312     |
| Fermentation products, %DM |  |  |  |  |  |  |  |  |  |
| Alcohol              | 0.777      | 0.808       | 0.823 | 0.778 | 0.777 | 0.11 | 0.6341  | 0.7935     | 0.9823     |
| Acetic acid          | 1.913      | 0.389       | 1.117 | 1.219 | 1.116 | 0.85 | 0.0001  | 0.006   | 0.002      |
| Propionic acid       | 0.529      | 0.041       | 0.247b | 0.18c | 0.43a | 0.31 | 0.0027  | 0.011    | 0.0011     |
| Butyric acid         | 0.566      | 0.22        | 0.309b | 0.382b | 0.488a | 0.2  | <0.001  | 0.0005    | 0.0027     |
| Lactic acid          | 1.767      | 1.867       | 1.816 | 1.786 | 1.848 | 0.32 | 0.4816  | 0.9379    | 0.9374     |
| N-NH3/TN             | 10.280     | 9.880       | 10.07 | 9.94  | 10.21 | 1.15 | 0.4508  | 0.9148    | 0.856      |

Means within silage with different superscripts differ (a, b, c: p < 0.05).
1WS: wheat silage control; WSI: wheat silage treated with homolactic inoculant.
2UL: top layer; ML: middle layer; LL: bottom layer.
3Probabilities for silage (S), layer (L) and interaction (S×L) effects.
4SME: standard error of the means.
grow, what did not occur in the upper and medium layers, due to their smaller moisture availability. While for WSI, the fast pH reduction may have promoted a suppressive effect on clostridial activity. These results suggest that inoculation changed the fermentation pattern independently of DM density of the layers.

Wheat silage inoculation resulted in less aerobic stability, higher maximum and average temperatures, and maximum difference (Table 5) when compared to silage without inoculants. These results suggest that the aerobic metabolism of inoculated silage increased nutrient oxidation.

In particular, WSI remained steady until the second day of air exposure and with marked pH variation. In contrast, WS kept under room temperature for 128 hours got pH values near those obtained at the end of storage period. The present study confirms that inoculation increases silage susceptibility to aerobic deterioration (Muck 2010). Therefore, temperature and pH raises predisposed silage to a continuous deterioration process by microorganism strains.

Usually, acetic acid prolongs silage aerobic stability by inhibit fungus and yeast. The effect of acetic acid on aerobic stability is related to the undissociated concentration in solution (Muck 2010). Furthermore, propionic acid increases silage aerobic stability (Pahlow et al. 2003) when inhibiting yeast and mould growth. In the present study, high concentrations of acetic and propionic acid probably provided the greatest aerobic stability of WS.

The post analysis of silage × silo layer interaction demonstrated that WSI had the highest sum of temperature, average difference in °C and pH average at all layers (Table 6). This effect can be attributed to the strong antifungal effect of acetic acid (Kung Jr. 2013).

### Table 4. Post analysis of silage × layer interaction for the variables acetic acid, propionic acid and butyric acid.

| Silo layer | Silages
|------------|-----------------
|            | WS | WSI |
| Acetic acid, % DM |    |     |
| Upper       | 1.846±b     | 0.389±b |
| Medium      | 2.068±b     | 0.71±b |
| Lower       | 1.825±b     | 0.40±b |
| Butyric acid, % DM |    |     |
| Upper       | 0.453±b     | 0.165±b |
| Medium      | 0.504±b     | 0.259±b |
| Lower       | 0.740±a     | 0.235±b |
| Propionic acid, % DM |    |     |
| Upper       | 0.465±ab    | 0.028±b |
| Medium      | 0.325±c     | 0.034±b |
| Lower       | 0.798±a     | 0.06±b |

Means within silage with different superscripts differ (a, b: p<.05).
Means within silo layer with different superscripts differ (A, B, C: p<.05).
WS: wheat silage; WSI: wheat silage inoculants.

### Table 5. Aerobic stability of wheat silage with/without bacterial inoculants.

| Variables | Treatment | Silo layer | SEM | p Value |
|-----------|-----------|------------|-----|---------|
| Stability, h | WS | WSI | UL | ML | LL | S | L | S × L |
| Maximum T, °C | 27.8 | 33.5 | 31.5 | 28.9 | 31.5 | 4.52 | <.0001 | 0.0974 | 0.2802 |
| Average T, °C | 19.7 | 25.9 | 22.7 | 22.0 | 23.8 | 2.67 | <.0001 | <.0001 | <.0001 |
| Sum of T, °C | 136.6 | 176.2 | 158.8 | 153.1 | 157.3 | 0.97 | <.0001 | <.0001 | <.0001 |
| Maximum dif.°C | 150 | 12.2 | 9.4 | 12.5 | 1.83 | <.0001 | 0.0032 | 0.0641 |
| Average dif.°C | 1.44 | 6.63 | 4.54 | 3.68b | 3.89b | 0.03 | <.0001 | <.0001 | 0.0001 |
| Average pH | 4.2 | 5.8 | 5.2 | 4.6 | 5.3 | 0.10 | <.0001 | 0.0055 | 0.0035 |

Means within silage with different superscripts differ (a, b: p<.05).
WS: wheat silage; WSI: wheat silage with inoculants.
UL: upper layer; ML: medium layer; LL: lower layer.
Interaction effect: silage (S), layer (ES) and interaction (S × E).
SEM: standard mean error.
Maximum temperature in °C.
Average temperature in °C.
Sum of temperature in °C.
Maximum dif.: maximum temperature difference in °C.
Average dif.: average temperature difference in °C compared to room temperature.

### Table 6. Post analysis of silage × layer interaction for the variables temperature sum, average difference, average pH and maximum pH.

| Silo layer | Silages
|------------|-----------------
|            | WS | WSI |
| Temperature sum, hours | 128.7 | 42.0 | 85.0ab | 93.0a | 78.0b | 58.66 | <.0001 | 0.0017 | 0.1677 |
| Average difference, hours | 2.175±a | 6.92±a |
| pH average, index | 4.16±b | 6.16±a |
| Maximum pH, index | 4.33±b | 8.31±a |

Means within silage with different superscripts differ (a, b: p<.05).
Means within silo layer with different superscripts differ (A, B, C: p<.05).
WS: wheat silage; WSI: wheat silage inoculants.
since this silage showed a reduced concentration of this acid (Table 3).

Comparing silo layers, the upper layer of the WS treatment showed higher temperature accumulation, average difference and maximum pH. This fact justifies the highest DM losses at this layer associated with the lowest silage density.

**Conclusions**

The homolactic inoculant reduced the formation of volatile fatty acids and DM loss during the fermentation. Therefore, the homolactic inoculant worsened the aerobic stability of wheat silages stored in bunker silos. Lower silage density at top layer was associated with higher DM losses.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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