Characterization of triticale silage inoculated with homolactic bacteria and exposed to aerobic stress during storage[1,2]

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ABSTRACT

Fresh whole plant spring triticale (x *Triticosecale* spp.) was field wilted and chopped prior to either being sprayed or not with a homolactic bacterial inoculant (HBI). Wilted triticale was ensiled for 120 d at 20 to 23 °C using 16 PVC mini-silos of 3 L capacity fitted with two-way mechanics to vent gas {which imposed aerobic stress (ASTS) when it remained open}, and filled with about 2 kg of the crop containing 35% dry matter (DM) and 5.2% water soluble carbohydrates (WSC) in the DM. Four treatments of a 2x2 factorial were: 1) No HBI/vent closed; 2) HBI/vent closed; 3) No HBI/vent open; 4) HBI/vent open. Upon opening the mini-silos, chemical composition, fermentation characteristics and in vitro 30 h neutral detergent fiber (NDF) digestibility of the silages were determined. Relative to pre-ensiled forage, either sprayed or not with HBI, ensiling increased ($P<0.05$) contents of moisture, inorganic matter, fibrous fractions {acid detergent fiber (ADF) and lignin}, and ether extract (EE), while decreasing contents of WSC and non-fibrous carbohydrates (NFC). However, treatment had no consistent effect on content of silage nutrients. Of the two non-inoculated silages, the one subjected to ASTS was more than 20 percentage points lower (66 vs. 88 %) in DM recovery (DMR), whereas the HBI silage subjected to ASTS was protected from DM losses. Ensiling and ASTS during the 120 d fermentation decreased NDF digestibility, whereas inoculated non-ASTS silage was nearly as digestible (57.5) as the pre-ensiled forages (58.2 and 60.7%, without and with HBI). Inoculation tended to steer fermentation in a homolactic direction. On balance, HBI is recommended because of the benefits in the fermentation pattern, fiber digestibility and DMR, especially in the presence of ASTS.

Key words: silage, triticale, aerobic stress, bacterial inoculant, fermentation

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RESUMEN

Caracterización de ensilaje de triticale inoculado con bacterias homolácticas antes del ensilado y expuesto a estrés aeróbico durante el almacenamiento

Forraje fresco de planta entera de triticale primaveral (x *Triticosecale* spp.) se dejó marchitar a campo, luego se picó antes de ser rociado con un inóculo de bacterias homolácticas (IBH). El triticale marchitado se ensiló durante 120 d, a temperatura de 20 a 23° C, en 16 mini-silos de PVC de capacidad de 3 L y provistos de un mecanismo ventilador de gases bidireccional {el cual impuso estrés aeróbico (EA) al contenido cuando abierto}. Se llenaron los mini-silos con unos 2 kg de forraje conteniendo 35% de materia seca (MS) y 5.2% de carbohidratos acuosolubles (CAS) en la MS. Hubo cuatro tratamientos en un arreglo factorial 2x2: 1) sin IBH/ventilación cerrada (VC); 2) IHB/VC; 3) sin IBH/ventilación abierta (VA); 4) IBH/VA. Al abrir los mini-silos se determinó la composición química, características fermentativas y digestibilidad in vitro a las 30 h de la fibra detergente neutro (FDN) del ensilaje. Relativo a los forrajes preensilados (rociado o no con IBH), el ensilamiento causó un aumento ($P<0.05$) en los contenidos de humedad, materia inorgánica, fracciones fibrosas {fibra detergente ácido (FDA) y lignina} y extracto etéreo (EE), mientras que causó una reducción en los contenidos de CAS y carbohidratos no fibrosos (CNF). En cambio, el tratamiento no ejerció efectos consistentes en los contenidos en los nutrientes de los ensilajes. De los dos ensilajes sin inoculación, el que se sometió a EA tuvo una recuperación de MS (RMS) inferior de más de 20 puntos porcentuales (66 vs 88%); por otro lado, el ensilaje con IBH sometido a EA quedó protegido de la pérdida de MS. El ensilamiento y la EA durante la fermentación fueron adversos a la digestibilidad de la FDN, mientras el ensilaje con IBH y sin AE presentó un valor (57.5%) casi tan alto como los forrajes preensilados (58.2 y 60.7%, sin y con rociado de IBH). El IBH mostró un efecto de encauzar la fermentación en dirección homoláctica. En resumen, se recomienda el uso del IBH en virtud de los beneficios que aporta al patrón de fermentación, digestibilidad de la fibra y RMS, sobre todo bajo condiciones de EA.

Palabras clave: ensilaje, triticale, estrés aeróbico, inoculante bacteriano, fermentación

INTRODUCTION

Aerobic exposure (AE) of silages results in forage deterioration and the loss of nutritional value. Research on these effects has focused on silages at the point of feed-out (Queiroz et al., 2012) but only sporadic research has been conducted to determine the effect of aerobic stress (ASTS) during storage on nutritional qualities of the resulting silage. Gordon et al. (1961) concluded that sealing the silo with plastic to prevent ASTS during storage resulted in more complete recovery of alfalfa hay-crop silage and significantly greater consumption by dairy cows than with unsealed silos. Oelberg et al. (1983) covered alfalfa silages with black plastic and obtained greater dry matter recovery (DMR), dry matter (DM) intake and rate of weight gain by heifers compared with uncovered silages. Bolsen et al. (1993) found that sealing hori-

zontal silos filled with alfalfa, corn and forage sorghum silage crops reduced DM and organic matter losses dramatically in the top 67-cm layer. More recently, research has focused on different types of plastic covers used to diminish the extent of aerobiosis and thus constrain spoilage and DM losses (Borreani et al., 2007). García et al. (1989) observed that aerated silages do not ferment as well as anaerobic silages and their pH takes longer to decline.

Homolactic bacterial inoculants (HBI) are often applied in silage making as a means to control and direct fermentation by dominating the epiphytic bacteria present in the crop, thus enhancing lactic fermentation (Weinberg and Muck, 1996). Muck and Kung (1997) outlined the desirable effects of HBI: 1) a rapid reduction in pH to help reduce the activity of plant enzymes and minimize protein losses; 2) a shift in the fermentation products to improve DMR and digestibility of the silage; 3) a rapid drop in pH and a low final pH to minimize the population of detrimental microorganisms that produce high levels of acetic and butyric acids and of spoilage organisms. Harrison et al. (1989) found that the addition of HBI increased the utilization of water-soluble carbohydrates (WSC) and the decline in pH, limited the formation of NH_3-N, and improved the in vitro digestibility of DM and acid detergent fiber (ADF). In other experiments, inoculation with L-lactic acid-producing *lactobacilli* had beneficial effects in decreasing the proportion of D-isomer to total lactic acid and improving silage quality (Cai et al., 1998). Contreras-Govea et al. (2013) reported that *L. plantarum* inoculation preserved more true protein during silage fermentation than a non-inoculated control. These experiments with HBI were conducted under anaerobic conditions, but their effect on the nutritional characteristics of silage improperly stored and subjected to ASTS during storage has not been adequately characterized. Is there value to inoculating silages when they are subjected to ASTS during storage or should silages be inoculated only when storage conditions are nearly ideal? The present study was conducted to address this question and determine the effect of HBI with and without ASTS during 120 d of ensiling on the nutritional and fermentation characteristics and aerobic stability of whole plant spring triticale (x *Triticosecale* spp.).

MATERIALS AND METHODS

Vegetative material

Triticale was grown and harvested at a commercial crop farm in Lafayette County, WI. The whole plant forage was swathed and allowed

to wilt to a moisture content of approximately 65%. Wilted triticale was chopped to a theoretical length of cut (TLC) of 20 mm and transported to Fitchburg, WI, for further processing. Particle size distribution was determined using the Penn State particle size separator (Heinrichs and Kononoff, 1996): the proportions of material that remained on top of sieves with a pore size of 19.04, 7.85, 1.27 mm were 23.9, 43.2 and 31%, respectively, while 2% reached the bottom pan. Particle size distribution of the chopped triticale was similar to that recommended by Heinrichs and Kononoff (1996) for haylage. These authors only provide guidelines for corn silage or haylage and we deemed the latter to be more appropriate to the present situation. One-half of the vegetative material was inoculated using a water soluble HBI at a rate of 1.1 g/t of wilted matter with a product supplying $>9.1x10^{10}$ CFU/g, containing *Pediococcus acidilactici*, *P. pentosacesus*, *Lactobacillus plantarum* and *Lactococcus lactis*. The other half of the vegetative material received the same amount of water. The liquids were applied using a hand sprayer while the forage was mixed manually. Four samples each of inoculated or non-inoculated wilted herbage were collected prior to ensiling and stored at -18° C until analyzed.

Fermentation process

Sixteen 3 L capacity PVC mini-silos fitted with two-way mechanics to vent gas were filled with about 2 kg of the crop containing 35% DM and 5.2% WSC (DM basis). Four mini-silos were assigned to each of four treatments to be evaluated: 1) No HBI with gas vent closed (Non-HBI/Closed); 2) HBI with gas vent closed (HBI/Closed); 3) No HBI with gas vent open (Non-HBI/Open); 4) HBI with gas vent open (HBI/Open). Aerobic stress during the ensiling period was induced by keeping the gas vent open. Triticale was fermented for 120 d at a temperature of 20 to 23° C. Upon opening the mini-silos, silages were weighed; temperature measured using a 12-cm Taylor thermometer (Taylor Precision Products, model 5989)[6] placed in the middle of each mini-silo for 30 s, sampled and subsequently analyzed for nutrient content and fermentation products. Pre-ensiled forage and silage samples were analyzed for DM, crude protein (CP), ADF, amylase-treated neutral detergent fiber (aNDF), ether extract (EE), ash, acid detergent insoluble crude protein (ADICP), lignin, starch, WSC, non-fibrous carbohydrates (NFC) and in vitro determination of 30 h NDF digestion, total tract NDF digestibility (TTNDFD), rate of degradation of NDF ($NDFk_d$) and non-degradable

[6]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

NDF after 240 h incubation (uNDF240; Rock River Laboratory, Inc., Watertown, WI; NIRS technique). Additionally, fermentation characteristics (pH, lactic acid, acetic acid, propionic acid, butyric acid, ethanol, total volatile fatty acids (VFA), and ammonia nitrogen (NH_3-N) were analyzed by wet chemistry at the commercial laboratory cited. The silage DM recovered at silo opening divided by the DM mass ensiled, multiplied by 100 was used to calculate % DMR.

Data pertaining to nutrient content of pre-ensiled wilted forage, either sprayed or not with HBI, and silages resulting from the four treatments were analyzed using the GLM procedure of SAS (SAS Institute, 2004) in a completely randomized design (CRD) with four replicates per analyzed material. Silage temperature at opening the mini-silos and DMR were analyzed as a CRD with four treatments replicated four times. Mean separation was conducted using Tukey's Test.

Aerobic stability

Aerobic stability was determined in the resulting silages by monitoring temperature at 6 h intervals during 7 d (Honig, 1986). Approximately 300 g of each silage was loosely placed in Styrofoam containers and exposed to air in thermally insulated chambers. A 12-cm Taylor thermometer (model 5989) was placed in the middle of each sample. Aerobic stability was defined as the time, after opening the mini-silo, for silage temperature to reach 3° C above ambient. Data were analyzed using the GLM of SAS (SAS Institute, 2004) as a split plot design using mini-silo as the repetitive measure replicated four times with a factorial arrangement of treatments: four treatments x 29 time points when temperature was recorded. Mean separation was conducted using Tukey's Test.

RESULTS AND DISCUSSION

Nutritional characteristics

The combined inoculated and non-inoculated wilted vegetative material to be ensiled averaged 35% DM, 17.2% CP, 34.4% ADF, 0.7% lignin, 0% starch, 5.2% WSC and 11.55% ash (Table 1). The only difference ($P<0.05$) in composition due to HBI was a lower aNDF content (54.42 vs. 56.20%). The pH of the wilted material before ensiling was 6.37 and very little VFA or NH3-N was present (Table 2).

Ensiling produced wetter ($P<0.05$) vegetative material by a margin of about 3 percentage units (Table 1). Among the silages, inoculation increased ($P<0.05$) the DM content of silage exposed to ASTS to 33.02%, which exceeded ($P<0.05$) that of the non-inoculated silages, but not of the inoculated silage not exposed to ASTS (31.85%). Ensiling

TABLE 1.—*Chemical composition of wilted triticale forage either sprayed or not with homolactic bacteria inoculant (HBI) prior to ensiling and of silages made from the two forages and exposed to aerobic stress during ensiling or not.*

	Pre-ensiled forage		Silage					
Item[2] %	Wilted/ Non-HBI	Wilted/ HBI	Non-HBI/ Closed[1]	HBI/ Closed	Non-HBI/ Open[1]	HBI/ Open	SD	P<
DM[3]	34.69 a[4]	35.38 a	31.33 c	31.85 bc	31.36 c	33.02 b	0.67	0.0001
CP	16.88 ab	17.57 a	16.60 ab	17.14 ab	16.27 b	16.95 ab	0.45	0.01
ADICP	0.59	0.57	0.68	0.62	0.63	0.56	0.06	0.10
EE	2.55 b	2.60 b	4.01 a	4.03 a	4.06 a	3.96 a	0.08	0.0001
ADF	35.00 c	33.84 c	38.75 ab	37.61 b	38.96 a	38.27 ab	0.55	0.0001
aNDF	56.20 a	54.42 b	56.63 a	55.74 a	56.59 a	56.12 a	0.47	0.0001
Lignin	0.65 b	0.76 b	3.02 a	2.57 a	2.47 a	2.81 a	0.32	0.0001
Starch	0	0	0.29	0.56	0.15	0	0.32	0.13
WSC	4.66 a	5.72 a	0 b	0 b	0 b	0 b	0.44	0.0001
NFC	15.23 a	17.24 a	8.78 b	9.24 b	7.74 b	8.07 b	1.18	0.0001
Ash	12.01 b	11.08 b	14.73 a	14.66 a	16.05 a	15.58 a	0.94	0.0001

[1]Closed = 2-way vent closed not allowing air to enter the mini-silos during silage storage; Open = 2-way vent open allowing air to enter during silage storage.

[2]Dry matter (DM), crude protein (CP), acid detergent insoluble crude protein (ADICP), ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (aNDF), and non-fibrous carbohydrates (NFC)

[3]As is basis; all other DM basis

[4]Within a row, means with different letters differ P<0.05

TABLE 2.—*Fermentation characteristics of triticale silage with or without homolactic bacteria inoculation and with or without aerobic stress during ensiling and corresponding values found in the wilted material prior to ensiling.*

	Pre-ensiled forage		Silage					
Item	Wilted/ Non-HBI	Wilted/ HBI	Non-HBI/ Closed[1]	HBI/ Closed	Non-HBI/ Open[1]	HBI/ Open	SD	P<
pH	6.37 a[2]	6.37 a	5.07 b	4.87 c	5.07 b	4.96 bc	0.07	0.0001
Fermentation profile[3] (%)								
Lactic Acid	0.24 c	0.24 c	3.71 b	5.33 a	3.57 b	3.78 b	0.52	0.0001
Acetic Acid	0.19 c	0.20 c	4.20 ab	3.56 b	4.65 a	4.31 ab	0.44	0.0001
Propionic Acid	0.19 c	0.21 bc	0.28 ab	0.29 a	0.33 a	0.28 ab	0.03	0.0001
Butyric Acid	0	0	0	0	0.04	0	0.03	0.4457
Ethanol	0 c	0 c	0.98 a	0.87 ab	0.76 b	0.83 ab	0.09	0.0001
Total VFA	0.61 c	0.66 c	8.18 b	9.19 a	8.60 ab	8.37 ab	0.38	0.0001
NH_3-N, CP equivalent	0.18 b	0.20 b	1.83 a	1.70 a	1.81 a	1.58 a	0.11	0.0001

[1]Closed = 2-way vent closed not allowing air to enter the mini-silos during silage storage; Open = 2-way vent open allowing air to enter during silage storage.
[2]Within a row, means with different letters differ P<0.05
[3]DM basis

decreased (P<0.05) the content of WSC to zero and markedly reduced NFC content also. One of the perceived benefits of inoculation is the preservation of WSC, but in the present case WSC were completely consumed during the fermentation process regardless of inoculation. Ensiling increased (P<0.05) the content of EE by more than one-percentage unit, but in the silages neither inoculation nor ASTS had an effect on this crude fat fraction. This effect of ensiling may be important as increased EE intake could be involved in the causation of milk fat depression in dairy cows. Chow et al. (2004) also observed an increase in total fatty acids in ryegrass due to ensiling. In that research, the proportion of triglycerides increased during wilting but not any further during ensiling, whereas in reverse fashion, the proportion of free fatty acids did not change during wilting, but strongly increased during ensiling. After ensiling, the unsaturated fatty acids constituted a similar or slightly lower proportion of the total fatty acids; however, the absolute quantity of unsaturated fatty acids increased. This suggests that due to ensiling, the rumen will be presented with a larger amount of unsaturated fatty acids that could alter rumen bio-hydrogenation resulting in the production of trans-10, cis-12 conjugated linoleic acid or other fatty acids that are potent inhibitors of milk fat synthesis (Bauman and Griinari, 2001).

Ensiling also increased (P<0.05) the content of ash, but among the silages neither inoculation nor ASTS had any such effect (Table 1). The highest numerical value (16.05%) was for the Non-HBI/Open silage and is consonant with its decreased DMR. The increase in ash content was likely the result of its concentration as other fractions experienced losses.

Fermentation Process

The purpose of applying HBI is to control and direct fermentation by dominating the epiphytic bacteria present in the crop, thus enhancing fermentation. Compared to the wilted forage, ensiling increased (P<0.05) VFA, ethanol and NH_3-N content while decreasing pH, consonant with the findings of Rodríguez et al. (2014). Inoculation in the absence of ASTS (HBI/Closed) decreased (P<0.05) pH relative to the non-inoculated silages (Table 2), but a similar effect of inoculating was seen also in the presence of ASTS (4.87 vs. 4.96). This small difference in pH could be explained by the respective level of lactic acid achieved by these treatments (5.33% for HBI/Closed vs. 3.78% in HBI/Open). The present results agree with those of Ozduven et al. (2010) who treated triticale with lactic acid bacteria (LAB) resulting in silage with lower pH and higher lactic acid. The HBI/Open silage did not differ (P>0.05) from the non-inoculated silages in terms of pH or lactic

acid content regardless of ASTS or not. The HBI/Closed silage had a lower (P<0.05) acetic acid content compared to the non-HBI/Open silage (3.56 vs. 4.65%), which was expected since the purpose of bacterial inoculation is to encourage a homo-fermentation. In contrast, Bolsen et al. (1993) found that sealing or not sealing sorghum silages did not affect the content of acetic acid. Possibly in the present study ASTS lowered the content of acetic acid of the silages due to volatilization, which prevented its accumulation. The HBI/Closed treatment resulted in a lower (P<0.05) content of acetic acid (Table 2) and higher DMR (Figure 1) compared to the Non-HBI/Open treatment, which suggests that a more efficient fermentation took place. The content of acetic acid relative to lactic acid was greater in all silages except HBI/Closed. This suggests that the tendency of the epiphytic bacteria present in triticale to favor a hetero-fermentation was reversed by inoculation. However, the benefits of inoculation to foster homolactic fermentation were negated by ASTS. The HBI/open silage had numerically higher lactic acid and lower acetic acid content compared to those of the Non-HBI/Open silage, suggesting that the inoculant was not active enough to overcome the effects of ASTS.

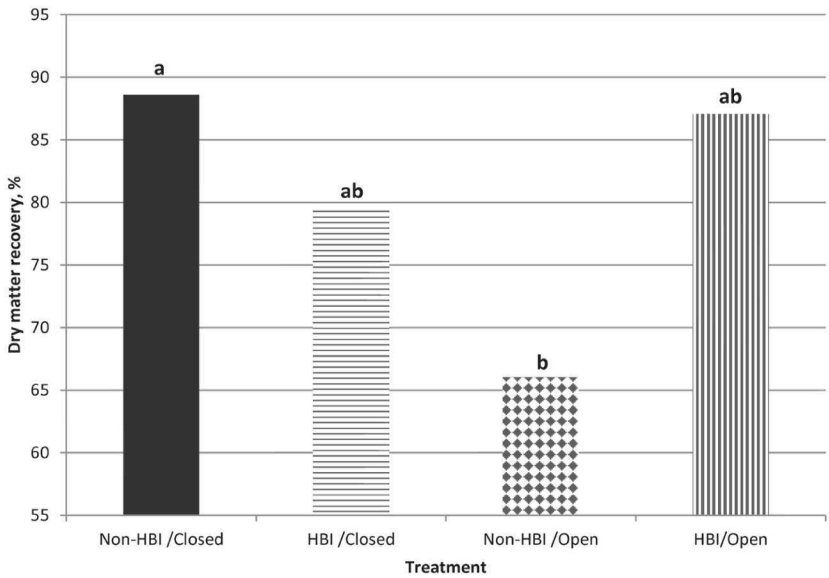

FIGURE 1. Effects of homolactic bacteria inoculation and aerobic stress during ensiling on dry matter recovery of triticale silage[1].

[1]COLUMNS WITH DIFFERENT LETTERS DIFFER P<0.05

Ensiling increased ($P<0.05$) the content of propionic acid over that of the pre-ensiled forage, but the silages did not differ one from another (Table 2). It is interesting to note that butyric acid was detected in a small concentration only in the Non-HBI/Open silage, which also had the greatest content of ash (16.05%). Butyric acid is produced by *Clostridium* spp., soil being a main source of these bacteria (Julien et al., 2008). Therefore, silages that were contaminated with soil at harvest may have contained Clostridia as well. Clostridia are the principal anaerobic microorganisms detrimental to silage quality (Muck, 1988); in the present study, butyric acid was detected in one of the silages exposed to ASTS. This same treatment produced silage with the lowest ($P<0.05$) content of ethanol, which differed only from that of the Non-HBI/Closed silage. Again, ethanol volatilized is suspected to be involved in this result. The use of HBI during ASTS had little effect on the fermentation parameters reported herein, whereas this practice in the absence of ASTS improved the fermentation characteristics of the silage as indicated by a lower pH, a higher content of lactic acid and total VFA. Although only numerical, similar trends were observed for the HBI in the presence of ASTS.

NDF digestibility characteristics

Aerobic stress during the storage period decreased ($P<0.05$) NDF digestibility compared to silages not exposed to ASTS (Table 3). The silage resulting from the HBI/Closed treatment had the highest 30 h NDF digestibility, which surpassed ($P<0.05$) the ASTS silages and was comparable to the digestibility of the pre-ensiled wilted forage. McAllister et al. (1998) reported increases in DM and organic matter digestibility in feedlot steers fed alfalfa silage inoculated with *L. plantarum*, whereas Lynch et al. (2014) failed to find effects due to microbial inoculation on fiber digestibility of haylage. The present results are in agreement with the findings of Thomas-Moen et al. (2014) of increased fiber digestibility of wheat or oat silages treated with a bacterial inoculant, while chemical composition was not affected. Weinberg et al. (2007) also found that HBI of wheat or corn silages resulted in improved DM and NDF in vitro digestibility compared to a non-inoculated control. Ozduven et al. (2010) reported that LAB inoculation alone did not improve DM digestibility of triticale silage, but a combination of LAB+enzymes was successful. The beneficial effects of bacterial inoculation on fiber digestibility might be expected to vary among plant species (for example, *gramineae* vs. *leguminosae*) of different levels and types of structural carbohydrates and lignin content, and to depend also on the bacterial species used to inoculate the silage. The mechanism by which HBI improves NDF digestibility is not well understood.

TABLE 3.—*Neutral detergent fiber digestion characteristics of wilted forage either sprayed or not with homolactic bacteria inoculant prior to ensiling and of silages made from the two forages and exposed to aerobic stress during fermentation or not.*

	Pre-ensiled forage		Silage[1]					
Item[2]	Wilted /Non-HBI	Wilted / HBI	Non-HBI / Closed	HBI/ Closed	Non-HBI / Open[1]	HBI/ Open	SD	P<
30 h NDF digestibility, %	58.19 ab[3]	60.74 a	54.11 bc	57.52 ab	52.83 c	52.51 c	1.83	0.0001
TTNDFD, %	53.25 a	55.31 a	46.14 b	47.51 b	45.52 b	47.21 b	0.92	0.0001
NDF k_d, %/h	4.27 ab	4.48 a	4.20 ab	4.25 ab	4.02 b	4.28 ab	0.13	0.0045
uNDF240, %	8.80 c	8.10 c	13.00 ab	12.00 b	12.70 ab	13.20 a	0.47	0.0001

[1]Closed = 2-way vent closed not allowing air o enter the mini-silos during silage storage; Open = 2-way vent open allowing air to enter during silage storage.
[2]Neutral detergent fiber (NDF), total tract NDF digestibility (TTNDFD), rate of degradation of NDF ($NDFk_d$), non-degradable NDF after 240 h (uNDF240)
[3]Within a row, means with different letters differ P<0.05

Fibrous carbohydrates are a potential source of fermentable substrate for LAB; however, in order for the LAB to utilize this source, hydrolytic enzymes are also required (Rooke and Hatfield, 2003). In the present case, bacteria of the HBI might have produced compounds during fermentation that served as substrate for cellulolytic bacteria that were part of the epiphytic microflora during ensiling. Baranowski and Russel (1993) inoculated grass silage with cellulolytic bacteria prior to ensiling and succeeded in improving the fermentation characteristics while decreasing the content of ADF, NDF and cellulose compared to a non-inoculated control; however, these authors did not report their silage digestibility data. To the contrary, Weinberg et al. (2007) hypothesized that the LAB inoculants might compete with lactate producers in the rumen (e.g., *Streptococcus bovis*) for substrates released from starch hydrolysis or other nutrients. This type of competition might reduce the substrate available for lactate production by rumen bacteria and consequent pH decline, resulting in higher activity of cellulolytic rumen populations.

DM recovery and silage temperature upon opening mini-silos

The Non-HBI/Closed treatment gave the highest DMR, which exceeded ($P<0.05$) that of the Non-HBI/Open treatment (Figure 1), but not ($P>0.05$) those of the inoculated silages (HBI/closed or HBI/Open). The other silages, excluding HBI/Closed, did not differ ($P<0.05$) among themselves, despite large numerical differences. Aerobic stress during ensiling resulted in 25% greater DM loss compared with the non-ASTS silage. These results indicate that DMR of triticale silage is highly variable and four replicates were not enough to detect statistically significant effects. Published information regarding the DMR of triticale silage is scarce. Muck and Kung (1997) indicated that the use of inoculants improved silage DMR in fewer than half of the studies surveyed between 1990 and 1995. Bumbieris Junior et al. (2010) attempted unsuccessfully to increase DMR of triticale silage by co-ensiling it with oats or legumes. Santos et al. (2014) succeeded in increasing the DMR of ensiled *Pennisteum purpureum* by adding a source of starch prior to ensiling. Adding starch or sugar at ensiling could also prove beneficial in improving the DMR of triticale silage.

Martin et al. (2004) reported that the average DM invisible loss for forage ensiled in bags was 9.5% and total losses were 16.5%, while McGechan (1990) reported total DM losses of 20% or more. Martin et al. (2004) defined invisible plus uncollectable losses as the difference between the amount ensiled and the total amount (good and bad silage) removed from the bag, which represents the sum of gaseous and seepage losses and silage left on the ground during filling and empty-

ing. The total losses observed in the present study ranged from 11.4 to 34% of the DM and they were almost entirely gaseous losses, as there was no visible seepage and all of the vegetative material was accounted for between weighing the mini-silo before starting fermentation and before emptying post-fermentation.

The temperature of the HBI/Open silage was higher ($P<0.05$) than that of the other three treatments (Figure 2). This finding is probably of little biological importance as the temperature of all the silages was below ambient temperature (21 to 23° C).

Aerobic Stability

After silages are removed from a silo and exposed to air, aerobic microorganisms such as yeasts oxidize substrates, including fermentation acids, resulting in deterioration of the silage prior to it being fed. Aerobic stability is an important criterion in judging the fermentation quality and success of forage conservation (Honig, 1986). Due to the simplicity of measuring silage temperature, an increase in temperature is a convenient indicator of aerobic deterioration (Honig, 1986). In the present study, all silages were aerobically stable when exposed to air after

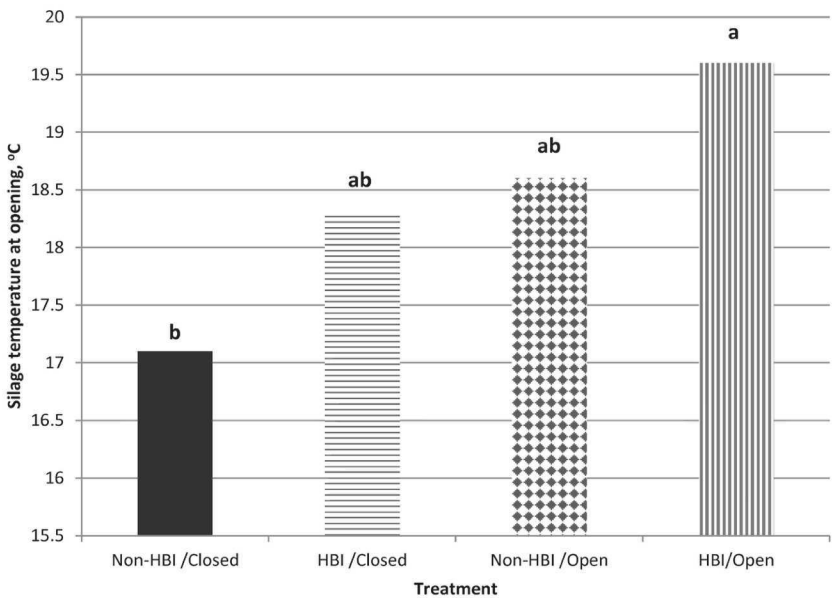

FIGURE 2. Effects of homolactic bacteria inoculation and aerobic stress during ensiling on the temperature of triticale silage when the mini-silo was opened[1].

[1]COLUMNS WITH DIFFERENT LETTERS DIFFER $P<0.05$

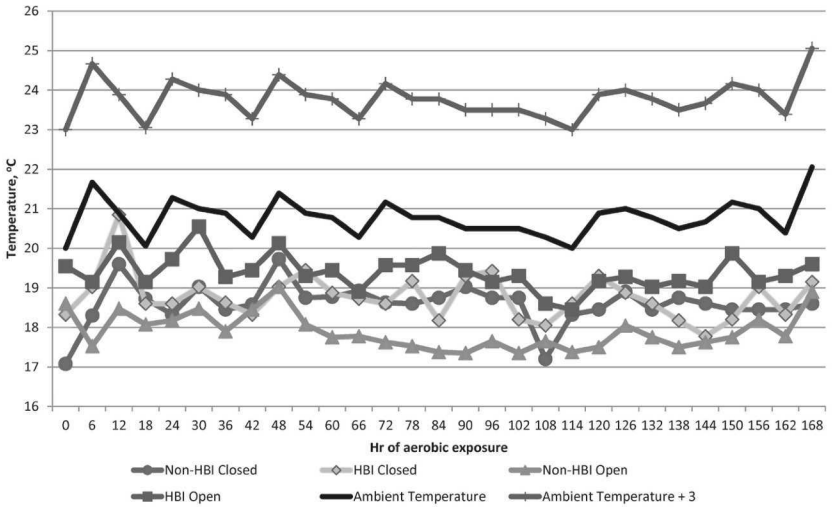

FIGURE 3. Effects of homolactic bacteria inoculation and aerobic stress during ensiling on the temperature of triticale silage during 7 d of aerobic stability testing.

being removed from the mini-silos (Figure 3). Treatment had no effect (P>0.05) on the average temperature during the 7 d of aerobic exposure, the values being 18.6, 18.8, 17.9, and 19.4° C for the Non-HBI/Closed, HBI/Closed, Non-HBI/Open and HBI/Open treatments, respectively. It is noteworthy that none of the silages reached ambient temperature during the 7 d test. Ozduven et al. (2010) reported that LAB inoculants impaired the aerobic stability of triticale silages, in contrast to the results of this study. Although lactic acid was present (3.6-5.3% of the DM) in the silages and could have served as a substrate for spoilage organisms, there was little starch or WSC available; also the presence of acetic acid (3.6 to 4.7% of the DM) may have deterred the development of spoilage organisms. Hetero-fermentative bacterial inoculants may be used to improve the shelf life of silage by inhibiting the growth of yeasts, thus reducing spoilage and the associated energy losses (Queiroz et al., 2012). Martínez-Fernández et al. (2010) attributed the aerobic stability of triticale-fava silage to the very high content of acetic acid (at least double that observed in the present study) produced by *L. buchneri* in their silages. Another possibility for explaining the aerobic stability of the present silages could be that the ambient temperature (~20° C) during the 7 d of aerobic exposure was too low for rapid growth of spoilage organisms (Martínez-Fernández et al., 2010).

In summary, some of the most interesting findings of this study, such as ensiling triticale increased the content of EE, could have im-

plications for milk fat depression in dairy cows and warrant further research. A second speculation is that the bacteria present in HBIs might have produced compounds during fermentation that served as a substrate for the epiphytic cellulolytic bacteria that were present during ensiling. The inoculation in question was shown to produce benefits in terms of fermentation patterns, fiber digestibility and DMR, especially in the presence of ASTS.

CONCLUSION

Although ensiling increased the EE content of triticale, treatment (HBI or ASTS) did not affect silage composition. Inoculation with HBI steered fermentation in a homolactic direction. Silage that was inoculated and not ASTS had a higher NDF digestibility than the other silages and was comparable in digestibility to the pre-ensiled forage. Aerobic stress during storage decreased NDF digestibility and also resulted in 25% greater DM losses compared with silage that was not exposed to ASTS. Inoculation diminished the negative effect of ASTS on silage DMR.

LITERATURE CITED

Baranowski, A. and S. Russel, 1993. Quality of meadow grass silage inoculated with cellulolytic bacteria. *J. Anim. Feed Sci.* 2: 59-65.

Bauman, D. E. and J. M. Griinari, 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Lives. Prod. Sci.* 70 (1-2): 15-29.

Bolsen, K. K., J. T. Dickerson, B. E. Brent, R. N. Sonon, Jr., B. S. Dalke, C. Lin and J. E. Boyer, Jr., 1993. Rate and extent of top spoilage losses in horizontal silos. *J. Dairy Sci.* 76: 2940-2962.

Borreani, G., E. Tabacco and L. Cavallarin, 2007. A new oxygen barrier film reduces aerobic deterioration in farm-scale corn silage. *J. Dairy Sci.* 90: 4701-4706.

Bumbieris Junior, V. H., C. C. Jobim, J. C. Emile, J. Roman and M. S. da Silva, 2010. Aerobic stability of triticale silage in single culture or in mixtures with oat and/or legumes. *R. Bras. Zootec.* 39(11): 2349-2356.

Cai, Y., Y. Benno, M. Ogawa, S. Ohmomo, S. Kumai and T. Nakase, 1998. Influence of *Lactobacillus* spp. from an inoculant and of *Weissella* and *Leuconostoc* spp. from forage crops on silage fermentation. *Appl. Environ. Microbiol.* 64 (8): 2982-2987.

Chow, T. T., V. Fievez, M. Ensberg, A. Elgersma and S. De Smet, 2004. Fatty acid content, composition and lipolysis during wilting and ensiling of perennial ryegrass (*Lolium perenne* L.): Preliminary findings. *Grassland Sci. in Europe* 9: 981-983.

Contreras-Govea, F. E., R. E. Muck, G. A. Broderick and P. J. Weimer, 2013. *Lactobacillus plantarum* effects on silage fermentation and in vitro microbial yield. *Anim. Feed Sci. Technol.* 179: 61-68.

García, A. D., W. G. Olson, D. E. Otterby, J. G. Linn and W. P. Hansen, 1989. Effects of temperature, moisture, and aeration on fermentation of alfalfa silage. *J. Dairy Sci.* 72: 93-103.

Gordon, C. H., J. C. Derbyshire, W. C. Jacobson, E. A. Kane, C. G. Melin and J. R. McCalmont, 1961. Comparisons of unsealed and plastic sealed silages for preservation efficiency and feeding value. *J. Dairy Sci.* 44 (6): 1113-1121.

Harrison, J. H., S. D. Soderlund and K. A. Loney, 1989. Effect of inoculation rate of selected strains of lactic acid bacteria on fermentation and in vitro digestibility of grass-legume forage. *J. Dairy Sci.* 72: 2421-2426.

Heinrichs, J. and P. Kononoff, 1996. Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. DAS 02-42.

Honig, H., 1986. Evaluation of aerobic stability. Proc. Eurobac Conference, Uppsala, Sweden, pp 76-82.

Julien, M. C., P. Dion, C. Lafrenière, H. Antoun and P. Drouin, 2008. Sources of Clostridia in raw milk on farms. *App. Environ. Microbiol.* 74 (20): 6348-6357.

Lynch, J. P., D. Prema, J. D. Van Hamme, J. S. Church and K. A. Beauchemin, 2014. Fiber degradability, chemical composition and conservation characteristics of alfalfa haylage ensiled with exogenous fibrolytic enzymes and a ferulic acid esterase-producing inoculant. *Can. J. Anim. Sci.* 94: 69-704.

Martin, N. P., R. E. Muck and B. J. Holmes, 2004. Silage density and dry matter loss of bag and bunker silos. *In:* Proc. Idaho Alfalfa and Forage Conference 23-24 February 2004, Twin Falls, ID, University of Idaho Cooperative Extension. 12 pp.

Martínez-Fernández, A., A. Soldado, F. Vicente, A. Martínez and B. de la Roza-Delgado, 2010. Wilting and inoculation of *Lactobacillus buchneri* on intercropped triticale-fava silage: Effects on nutritive, fermentative and aerobic stability characteristics. *Agric. Food Sci.* 19: 302-312.

McAllister, T. A., R. Feniuk, Z. Mir, P. Mir, L. B. Selinger and K.-J. Cheng, 1998. Inoculants for alfalfa silage: Effects on aerobic stability, digestibility and the growth performance of feedlot steers. *Livest. Prod. Sci.* 53(2): 171-181.

McGechan, M. B., 1990. A review of losses arising during conservation of grass forage: Part 2, storage losses. *J. Ag. Engineering Res.* 45(1): 1-30.

Muck, R. E., 1988. Factors influencing silage quality and their implications for management. *J. Dairy Sci.* 71: 2992-3002.

Muck, R. E. and L. Kung, Jr., 1997. Effects of silage additives on ensiling. *In*: Silage: field to feedbunk. BRAES-99-NRAES, Ithaca, NY. pp:187-199.

Oelberg, T. J., A. K. Clark, R. K. McGuffey and D. J. Shingoethe, 1983. Evaluation of covering, dry matter, and preservative at ensiling of alfalfa in bunker silos. *J. Dairy Sci.* 66: 1057-1068.

Ozduven, M. L., Z. Kursun Onal and F. Koc, 2010. The effects of bacterial inoculants and/or enzymes on the fermentation, aerobic stability and in vitro dry and organic matter digestibility characteristics of Triticale silages. *Kafkas Univ Vet Fak Derg* 16(5): 751-756.

Queiroz, O. C. M., A. T. Adesogan, K. G. Arriola and M. F. S. Queiroz, 2012. Effect of dual purpose inoculant on the quality and nutrient losses from corn silage produced in farm-scale silos. *J. Dairy Sci.* 95: 3354-3362.

Rodríguez, A. A., V. Rivera, L. C. Solórzano and P. F. Randel, 2014. Utilización de inóculos comerciales conteniendo bacterias productoras de ácido láctico sobre las características fermentativas y estabilidad aeróbica de ensilaje de maíz. *J. Agric. Univ. P.R.* 98 (1-2): 31-48.

Rooke, J. A. and R. D. Hatfield, 2003. Biochemistry of ensiling. *In:* Silage Science and Technology. Agronomy Monograph No. 42. D. R. Buxton, R. E. Muck, and J. H. Harrison, (eds) Am. Soc. Agron., Madison, WI. pp. 95-159.

Santos, T. M., L. L. Neto, B. L. Portella, K. F. Gobb and A. F. Garces Neto, 2014. Losses and dry matter recovery of Pioneiro grass (*Pennisetum purpureum*) and maize silages in mixtures. *Trop.Grassl. – Forrajes Tropicales* 2: 140-141.

SAS Institute. 2004. SAS/STAT 9.1. User's Guide: SAS Institute, Inc. Cary, NC 27513-2414, USA.

Thomas-Moen, M. E., J. L. Foster, K. C. McCuistion, R. W. Duncan, L. A. Redmon, A. M. Franks, R. W. Jessup and V. A. Olson, 2014. Inoculants to enhance the ruminal degradation of small-grain forage. Forage and Grazinglands DOI: 10.2134/FG-2014-0006-RS 7 pp.

Weinberg, Z. G. and R. E. Muck, 1996. New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiology Reviews* 19: 53-68.

Weinberg, Z. G., O. Shatz, Y. Chen, E. Yosef, M. Nikbahat, D. Ben-Ghedalia and J. Miron, 2007. Effect of lactic acid bacteria inoculants on in vitro digestibility of wheat and corn silages. *J. Dairy Sci.* 90: 4754-4762.