Animal Feed Science and Technology 246 (2018) 36–45

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effect of inoculation with *Lactobacillus buchneri* LB1819 and *Lactococcus lactis* O224 on fermentation and mycotoxin production in maize silage compacted at different densities

Antonio Gallo[a,*], Thiago F. Bernardes[b], Giuseppe Copani[c], Paola Fortunati[a], Gianluca Giuberti[a], Sara Bruschi[a], Keith A. Bryan[c], Natasja G. Nielsen[c], Kristian L. Witt[c], Francesco Masoero[a]

[a] *Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, 29122, Piacenza, Italy*
[b] *Department of Animal Science, Federal University of Lavras (UFLA), Lavras, Minas Gerais, 37200000, Brazil*
[c] *Chr. Hansen, Animal Health & Nutrition, Boege Alle 10-12 – 2970 Hoersholm, Denmark*

ARTICLE INFO

Keywords:
Aerobic stability
Density
Heterolactic fermentation
Homolactic fermentation

ABSTRACT

We examined the effectiveness of a combined inoculant of hetero-fermentative *Lactobacillus buchneri* LB1819 and homo-fermentative *Lactococcus lactis* O224 on quality of maize silage at two different densities. The four treatments were: CTR-LD, untreated at low-density (132 ± 6 kg DM/m^3); CTR-HD, untreated at high density (186 ± 6 kg DM/m^3); TRT-LD, inoculated at low density; and TRT-HD, inoculated at high density. The 5 replicates for each treatment were ensiled in 20-L plastic jars and the inoculant was applied at a rate of 250,000 CFU/g ensiled forage. The fermentation profile, aerobic stability, and DM losses were measured after 2, 4, 8, 16, and 32 days of ensiling, and the chemical composition, microbial counts, and concentrations of mycotoxins were assessed in the 32 day samples. Data measured once during experiment were analyzed according to a completely randomized design (CRD), with a 2 × 2 factorial arrangement of treatments (i.e., inoculation, density, and their interaction). Data measured at different time points were analyzed according to a CRD, with a 2 × 2 × 5 factorial arrangement of treatments (i.e., inoculation, density, length of ensiling, and their first or second order interactions). The lactic acid concentration increased ($P < 0.05$) with the duration of ensiling, with no differences among treatments (overall means on day 2 and day 32: 26.7 and 50.9 g/kg DM, respectively). Aerobic stability was greatest ($P < 0.05$) in the TRT-HD silage at 32 days. After 32 days of ensiling, soluble crude protein was greater in the CTR-HD and TRT-LD silages than the CTR-LD and TRT-HD silages ($P < 0.05$, inoculation × density interaction). The treatments had similar chemical parameters, yeast and mould counts (overall mean: 4.53 $\log_{10}$ CFU/g and 1.59 $\log_{10}$ CFU/g, respectively). The overall concentration of aflatoxin B_1 was 0.412 μg/kg DM. The CTR

Abbreviations: ADFom, ADF expressed exclusive of residual ash; aNDFom, NDF assayed with sodium sulphite and a heat stable amylase and expressed exclusive of residual ash; BAs, biogenic amines; CFUs, colony forming units; CP, crude protein; CRD, completely randomized design; CTR, freshly chopped uninoculated maize forage prior to ensiling; CTR-HD, untreated silage at high density; CTR-LD, untreated silage at low-density; DM, dry matter; DON, deoxynivalenol; EE, ether extract; GC, gas chromatography; GLM, general linear model; HD, high density; HPLC, high performance liquid chromatography; LAB, lactic acid bacteria; LD, low density; lignin (sa), lignin determined by solubilization of cellulose with sulphuric acid; Sol CP, soluble CP; TN, total nitrogen; TRT, freshly chopped inoculated maize forage prior to ensiling; TRT-HD, inoculated silage at high density; TRT-LD, inoculated silage at low density; VFA, volatile fatty acids; ZEA, zearalenone

* Corresponding author.
E-mail address: antonio.gallo@unicatt.it (A. Gallo).

https://doi.org/10.1016/j.anifeedsci.2018.09.009
Received 13 April 2018; Received in revised form 12 September 2018; Accepted 18 September 2018
0377-8401/ © 2018 Elsevier B.V. All rights reserved.

groups had higher concentrations of fumonisin B_2 (413 *vs.* 278 μg/kg DM, $P < 0.05$) and roquefortine C (22.81 *vs.* 3.39 μg/kg DM, $P < 0.05$) than the TRT groups. Conversely, the CTR groups had lower concentrations of fusaric acid than the TRT groups after 32 days (2849 *vs.* 4162 μg/kg DM, $P < 0.05$). Both CTR and *Lactobacillus buchneri LB1819* and *Lactococcus lactis* O224 treated samples at a high silage density increased the aerobic stability after 32 days of ensiling ($P < 0.05$, day × density interaction). Results indicated that inoculation and use of a greater silage density enhanced the fermentation and aerobic stability of maize silage.

1. Introduction

Many studies have examined the use of inoculants containing homo- or hetero-fermentative lactic acid bacteria (LAB) on maize forage, and difficult-to-ensile forages, in efforts to improve the fermentation and reduce dry matter (DM) and nutrient losses that occur during ensiling (Ranjit and Kung, 2000; Driehuis et al., 2001; Kleinschmit and Kung, 2006). Inoculation generally has beneficial effects on silage intake, rumen nutrient digestibility, and animal performance (Weiss et al., 2016), although there are some inconsistencies among previous studies, as declared by Driehuis et al. (1997).

Nevertheless, some farmers have doubts about the cost effectiveness of silage inoculants, especially for maize silage, which is considered easy to ensile. Indeed, farmers often use additives for ensiling forages consisting of small-grain, alfalfa or ryegrass crops, in which their benefits are typically more apparent (Borreani and Tabacco, 2010; Bernardes and do Rêgo, 2014). However, suboptimal management of maize silage (*e.g.,* inadequate DM concentration at harvest, slow silo filling, imperfect mass sealing, poor mass compaction, delay of mass pH decline, air penetration, and inappropriate unloading techniques) could lead to air penetration and consequent aerobic deterioration of the ensiled mass (Muck, 1988; Dunière et al., 2013; Brüning et al., 2018). This process could cause the proliferation of yeasts (single-celled fungi), moulds (multi-cellular fungi with extensive hyphal networks), and other undesirable microorganisms, thus increasing nutrient and DM losses (due to heat-damage of nutrients and proteolysis), emission of volatile organic compounds, and production of mycotoxins (Muck, 2013; Gerlach et al., 2014). Several other environmental or biotic factors (*e.g.,* ambient temperature, rainfall, initial populations of yeasts, moulds, and microorganisms) may contribute to an increased forages deterioration (Muck, 1988; Masoero et al., 2013).

An extensive review by Dunière et al. (2013) concluded that facultative anaerobic yeasts are the major microorganisms responsible for aerobic deterioration during early phase of ensiling and during the feed-out stage (Oude Elferink et al., 2000). In addition, several moulds can produce secondary metabolites, such as mycotoxins, that remain in the silage even after the moulds themselves have disappeared. Species in the genera of *Penicillium, Fusarium,* and *Aspergillus* are the most common mycotoxin-producing moulds isolated from maize silage (Gallo et al., 2015a). Both yeasts and moulds are thus responsible for silage degradation and have potentially negative effects on animal and human health. In addition, silage deterioration processes are often more severe in specific areas of silage silos, especially the lateral and upper parts, which are generally difficult to pack and seal, and thus have suboptimal mass compression (Vissers et al., 2007; Borreani and Tabacco, 2010; Dunière et al., 2013; Gallo et al., 2016a). As recently detailed by Brüning et al. (2018), a low silage density, from inadequate compaction during forage filling and storage, could increase silage porosity and the penetration of air before the silo is sealed. This could lead to rapid oxidation of water-soluble carbohydrates, causing an excessive increase in silage temperature (due to plant respiration) and increased populations of undesirable microorganisms. It is well known that homofermentative LAB, alone or in combination with heterofermentative LAB, rapidly decreases the pH of silage due to the production of lactic acid (Muck et al., 2018). Inoculation with these bacteria may therefore reduce aerobic and facultative anaerobic microorganisms at the beginning of silage storage. However, to the best of our knowledge, no studies have yet examined the benefits of LAB in poorly compacted silage.

The main objective of this work was to assess the effect of a commercial silage inoculant containing the hetero-fermentative *Lactobacillus buchneri* LB1819 (DSM22501/1k20738) and the homo-fermentative *Lactococcus lactis* O224 (DSM11037/1k2081) on fermentation, DM loss, aerobic stability, microbial counts, and mycotoxin contamination of maize ensiled at two different densities, as a simulation of differences in silo management.

2. Materials and methods

2.1. Silage preparation and treatments

A late-season maize hybrid (Pioneer Hi-Bred International, Inc., Des Moines, IA) was grown at the Bersani farm, Gragnano Trebbiense, Piacenza, Italy, harvested at the ½ milk line stage of maturity on September 2, 2016, and then chopped to a 6–8 mm theoretical cut with a self-propelled forage harvester (John Deere 7780) that had a pick up mechanical processor for maize. A total mass of approximately 20 tonnes from a homogenous field (45°01′29.2″N 9°34′23.7″E) was used. Approximately 4 tonnes of freshly chopped maize forage was sampled from different plots of experimental field and each of them was split into two equal amounts; one mass was untreated (CTR, without any inoculant) and the other mass was treated (TRT) with an inoculant containing *Lactobacillus buchneri* LB1819 and *Lactococcus lactis* O224 (SiloSolve® FC, Chr. Hansen A/S, Hørsholm, Denmark), used according to the manufacturer's recommendations. In particular, the inoculant (freeze dried powder) was diluted in heated tap water and applied at the rate of 2 mg/kg of fresh forage with a sprayer, to reach a final dose of approximately 250,000 CFU/g freshly-chopped forage. An equal

amount of heated tap water without inoculant was sprayed on the CTR mass. The CTR and TRT freshly chopped forages were thoroughly mixed with a wheel loader and shovels. Three representative samples (about 1 kg each) were taken from different parts of each forage pile, pooled into one sample, stored in airtight bags, and used for analysis of chemical characteristics and mycotoxin contamination, as described below.

Both CTR and TRT freshly-chopped maize were then placed into 20 L plastic jars ("mini-silos"), compacted to a low density (132 ± 6 kg DM/m^3) or a high density (186 ± 6 kg DM/m^3), and then carefully sealed with plastic lids and stored in a barn (ambient temperature: 20.3 ± 0.6 °C). Accordingly, the four different treatments were: CTR-low density (CTR-LD), CTR-high density (CTR-HD), TRT-low density (TRT-LD), and TRT-high density (TRT-HD). A total of 100 mini-silos were prepared. In particular, 20 mini-silos for each treatment and each duration of ensiling (2, 4, 8, 16, or 32 days) were prepared from each forage pile. The mini-silos were weighed immediately after closing and before opening to determine DM loss.

2.2. Sample preparation and analysis

Each mini-silo was opened after 2, 4, 8, 16, or 32 days of ensiling, the mass was mixed thoroughly, and representative samples (about 2 kg) were collected. Each mini-silo sample was then split into 2 homogeneous subsamples (about 1 kg). One of the subsamples was used for chemical analysis and mycotoxin analysis, as described by Gallo et al. (2016b). Briefly, samples were dried at 60 °C in a ventilated oven for 48 h until a constant weight was obtained, milled through a 1-mm screen using a laboratory mill (Thomas-Wiley, Arthur H. Thomas Co., Philadelphia, PA, USA), and then stored for subsequent analysis. Uncorrected DM was determined by the gravimetric loss of free water after heating at 105 °C for 3 h (AOAC (Association of Official Analytical Chemists), 1995; method 945.15). Then, the DM concentration was corrected for the volatile losses that occurred during oven drying using the equations adopted by NorFor (2011). Ash concentration was determined as gravimetric residue after incineration at 550 °C for 2 h (AOAC (Association of Official Analytical Chemists), 1995; method 942.05), an ether extract (EE) was obtained using the AOAC method 920.29 (AOAC (Association of Official Analytical Chemists), 1995), and crude protein (CP; N × 6.25) was determined using the Kjeldahl method (AOAC (Association of Official Analytical Chemists), 1995; method 984.13). The soluble fraction of CP (expressed on a DM basis) was determined according to Licitra et al. (1996). Neutral detergent (ND), acid detergent (AD), and lignin sulphuric acid (sa) fiber fractions were sequentially determined using the Ankom^{II} Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA), as described by Van Soest et al. (1991). The ND solution contained sodium sulphite and a heat stable amylase (activity: 17.400 Liquefon-U/mL, Ankom Technology). All fiber fractions were corrected for residual ash (aNDFom, ADFom, lignin [sa]). Starch content was determined by polarimetry (Polax 2 L, Atago®, Tokyo, Japan) and the free sugar concentration was determined following phosphate buffer and acetonitrile extraction, and quantification using a high-performance liquid chromatography (HPLC) system (Jasco, Tokyo, Japan), that was equipped with an LU-980 pump and an RI-2031 refractive index detector.

Mycotoxins produced by *Fusarium* spp. (zearalenone [ZEA] and deoxynivalenol [DON]), *Penicillium roqueforti* (PR toxin, mycophenolic acid, and roquefortine C), and *Aspergillus fumigatus* (gliotoxin) were extracted simultaneously from 3 g of a dried silage sample using a 30 mL extraction solution, as described by Gallo et al. (2016b). The mixture was shaken for 45 min, and 4.0 g of anhydrous magnesium sulfate was then added to obtain phase separation. After shaking for 2 min and centrifugation at 4500 × *g* for 10 min, the upper acetonitrile phase was recovered. Then, the extract was diluted 5-fold (0.1 mL brought to 0.5 mL) by addition of acetonitrile:water 30:70 (v/v), and passed through a filter (Millipore Corporation, Bedford, Massachusetts, USA, HV 0.45 μm). A 5 μL sample was injected into the HPLC-MS/MS system, using the system and procedures previously described by Gallo et al. (2015b). All analyses were performed in duplicate.

Immediately after collection, the remaining mini-silo subsamples and samples (about 1 kg) that were collected from freshly-chopped whole-plants before ensiling (with the subsamples only used for analysis of mould and yeast counts), were stored while wet at 4 °C, and analyzed within 24 h for determination of lactic acid, ethanol, volatile fatty acids (VFAs), and ammonia nitrogen concentrations, and to begin the aerobic stability test (Gallo et al., 2016b). About 100 g of each treatment and replicate was frozen at −18 °C before analysis of the concentrations of biogenic amines (BAs).

To quantify VFAs, lactic acid, 1,2-propanediol, and alcohols (ethanol and 1-propanol), a wet sample (about 50 g) was extracted using a Stomacher blender (Seward Ltd., West Sussex, UK) for 3 min in distilled water (water:sample fresh weight ratio: 3:1). The mixture was filtered through gauze, and then two aliquots (2 mL each) of the resulting solution were added to 1 mL of an oxalic acid solution (15.2 g/L) and 1 mL of a mixture of pivalic acid (internal standard, 1 g/L) and formic acid (50 mL/L). To remove solid impurities, the mixtures were shaken for 10 min, centrifuged at 4500 × *g* for 10 min, and the liquid phase was retained for determination of VFAs and alcohols using gas chromatography (GC). The GC system was equipped with a flame ionization detector, and the procedures were as reported by Gallo et al. (2015b). Determinations of lactic acid and 1,2-propanediol were performed using HPLC (Carvalho et al., 2012). When a compound was undetectable, its detection limit was reported (*i.e.* 0.1 g/kg DM).

Ammonia nitrogen concentration was determined in about 20 g of fresh sample in a slurry mix composed of 150 mL of distilled water and magnesium oxide (10 g/sample; NorFor, 2011). The ammonia nitrogen concentration, expressed on a total nitrogen (TN) basis, was determined after steam distillation of this solution using the Kjeldahl method, as described above. The pH of the previously filtered water mixture was also measured.

Aerobic stability was determined using the methods of Ranjit and Kung (2000) and reported in units of days, except that the ensiled mass was considered aerobically unstable when the difference between environmental and silage temperatures was 3 °C or more (Jatkauskas et al., 2013). About 3 kg of each sample was placed in 20 L pails and allowed to aerobically deteriorate at room temperature (20.2 ± 0.8 °C). During the test, environmental and silage mass temperatures were recorded every 6 h by a data logger (Microlite, Dostmann Electronic GmbH, Wertheim, DE). The ensiled mass temperature was also monitored daily using a digital

thermometer (88260 Digitron, Digitron Italia S.r.l., Ferentino, FR, Italy). A double layer of cheesecloth, which allowed penetration of air, was placed over each mini-silo to prevent drying and contamination of the sample. On each day of opening the mini-silos, a sample of aerobically unstable silage was collected until all silages lost aerobic stability. At this time, samples were analyzed for DM concentration (as described above) to verify DM loss at the end of aerobic stability.

Microbial counts were determined as previously reported (Borreani and Tabacco, 2010). Briefly, a sample of about 30 g was transferred into a sterile homogenization bag, suspended (1:10, w/v) in a peptone physiological salt solution (1 g/L of neutralized bacteriological peptone and 9 g/L of sodium chloride), and homogenized for 3 min in a laboratory stomacher blender. Serial dilutions were prepared, and yeast and mould numbers were determined using the pour plate technique, with 40 g/L of rose-bengal chloramphenicol agar (CM0549, Oxoid, Basingstoke, Hampshire, UK) after incubation at 25 °C for 3 days (yeast) and 5 days (mould). The yeast and mould colony forming units (CFUs) were enumerated separately, according to their macro-morphological features. The mean CFUs of duplicate subsamples were recorded for total yeasts and moulds on plates that yielded 10–100 CFUs per Petri dish.

The BAs were determined as described by Smith and Davies (1985). Briefly, 20 g of fresh core maize samples were added to 100 mL of a 0.10 v/v perchloric acid solution and 2 mL of an internal standard (1,7-diaminoheptane; concentration 2 μg/mL). Samples were homogenized for 1 min at about 3000 *g*, and the suspension was incubated for 1 h at 4 °C. Then, the solution was centrifuged for 20 min at 2 °C and the supernatant was collected. The precipitate was extracted as described above, and the two resulting supernatants were pooled to reach a final volume of about 200 mL. Samples were analyzed using an HPLC system consisting of two solvent metering pumps programmed with a microprocessor controller. Samples were injected into a fixed 20 μL loop for loading onto a reverse-phase C18 column, and eluted from the column with a programmed water:methanol (v/v) solvent gradient, which changed from 0.60 to 0.95 in 23 min at a flow rate of 1 mL/min.

2.3. Statistical analysis

The yeast CFUs, mould CFUs, and mycotoxin concentrations were $\log_{10}$-transformed prior to statistical analysis. The microbial data are presented as $\log_{10}$ values; the mycotoxin concentrations are reported as their natural values, but all terms in the ANOVA (standard errors of the means and P values) refer to $\log_{10}$-transformed values. Data measured at the same time point were analyzed according to a completely randomized design, with a 2 × 2 factorial arrangement of treatments, using the general linear model (GLM) procedure of SAS (2003), with the following model:

$$Y_{ijk} = \mu + T_i + Cj + (T_i \times Cj)_{ij} + e_{ijk} \quad (1)$$

where Y_{ijk} is the dependent variable; μ is the overall mean; T_i is the fixed effect of microbial inoculum (i = 2, CTR *vs.* TRT); Cj is the fixed effect of density (j = 2, Low *vs.* High); $(T \times Cj)_{ij}$ is the first order interaction, and e_{ijk} is the residual error.

Data measured at different time points were analyzed according to a completely randomized design, with a 2 × 2 × 5 factorial arrangement of treatments, using the GLM procedure of SAS (2003), with the following model:

$$Y_{ijkl} = \mu + T_i + Cj + D_k + (T_i \times Cj)_{ij} + (T_i \times D_k)_{ik} + (Cj \times D_k)_{jk} + (T_i \times Cj \times D_k)_{ijk} + e_{ijkl}$$

Table 1
Characteristics of uninoculated (CTR) and inoculated (TRT) maize forage prior to ensiling.

Analyte	Dimension	CTR (n = 3)	TRT (n = 3)
Dry matter	g/kg as-fed	363 ± 1.1	370 ± 2.0
Ash	g/kg DM	37 ± 1.2	36 ± 1.1
EE	g/kg DM	15 ± 3.1	15 ± 2.2
CP	g/kg DM	71 ± 6.0	72 ± 5.2
Sol CP	g/kg DM	46 ± 2.0	46 ± 2.1
Starch	g/kg DM	323 ± 2.2	321 ± 2.1
aNDFom	g/kg DM	407 ± 11.0	414 ± 5.1
ADFom	g/kg DM	197 ± 8.1	208 ± 8.0
Lignin (sa)	g/kg DM	25 ± 3.2	27 ± 3.1
Sugars	g/kg DM	91 ± 11.0	89 ± 9.2
pH	–	5.84 ± 0.2	5.82 ± 0.2
Yeasts	Log_{10} CFU/g	6.9 ± 0.1	6.9 ± 0.1
Moulds	Log_{10} CFU/g	6.5 ± 0.1	6.4 ± 0.2
Mycotoxins[a]			
Aflatoxin B1	μg/kg DM	1.01 ± 0.24	0.17 ± 0.40
Fumonisin B1	μg/kg DM	4415 ± 67	5276 ± 587
Fumonisin B2	μg/kg DM	2188 ± 45	3038 ± 120

Abbreviations here and below: ADFom, acid detergent fiber on an organic manner basis; CFU, colony forming unit; CP, crude protein; CTR, uninoculated maize forage; DM, dry mass; EE, ether extract; aNDFom, neutral detergent fiber on an organic manner basis; Lignin sa, lignin determined by solubilization of cellulose with sulphuric acid; Sol CP, soluble crude protein; TRT inoculated maize forage.

[a] The 3 mycotoxins detected in at least in one sample are indicated. Mycotoxins analyzed, but not detected were deoxynivalenol, zearalenone, fusaric acid, PR toxin, mycophenolic acid, and roquefortine C.

Table 2
Effect of storage duration (2–32 days), inoculation (CTR *vs.* TRT), and forage density (low *vs.* high) on changes during fermentation of maize silage.

Items[a]	Dimension	Storage duration					Inoculation		Density		√MSE	Effects of model (P < 0.05)						
		2	4	8	16	32	CTR	TRT	Low	High		Days[b]	Inoculation	Density	Days ×Inoculation	Inoculation ×Density	Days ×Density	Days ×Inoculation ×Density
DM	g/kg as-fed	353	353	352	347	347	349	352	350	351	6.8	< 0.05[L]	< 0.05	0.751	0.070	0.199	0.628	0.515
DM loss	g/kg DM	41	46	45	59	61	47	54	52	49	17.4	< 0.05[L]	0.078	0.399	0.148	0.259	0.465	0.141
pH	–	3.90	3.67	3.67	3.57	3.45	3.64	3.66	3.66	3.64	0.028	< 0.05	< 0.05	< 0.05	< 0.05	0.748	< 0.05	< 0.05
Lactate	g/kg DM	26.7	30.4	38.4	44.6	50.9	38.6	37.8	37.2	39.2	6.91	< 0.05	0.541	0.147	0.501	0.076	0.484	0.969
Acetate	g/kg DM	10.4	10.0	12.5	13.8	13.0	11.4	12.4	12.8	11.0	1.24	< 0.05	< 0.05	< 0.05	< 0.05	0.760	< 0.05	0.787
Ethanol	g/kg DM	5.2	5.4	6.5	7.7	5.8	6.5	5.8	7.3	4.9	0.093	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.435
1,2-propanediol	g/kg DM	0.25	0.18	0.07	0.06	0.05	0.11	0.14	0.12	0.12	0.036	< 0.05[Q]	< 0.05	0.246	0.105	0.381	0.714	0.816
Ammonia nitrogen	g/kg TN	21.9	27.6	29.8	33.6	35.9	30.7	28.8	29.2	30.3	2.59	< 0.05	< 0.05	< 0.05	< 0.05	0.275	< 0.05	0.108
Lactate:Acetate	–	2.64	3.05	3.12	3.32	4.03	3.42	3.05	2.91	3.55	0.330	< 0.05	< 0.05	< 0.05	< 0.05	0.138	< 0.05	< 0.05
Aerobic stability	days	5.7	8.7	9.5	13.9	20.1	10.5	12.6	9.6	13.6	1.87	< 0.05	< 0.05	< 0.05	0.078	0.831	< 0.05	0.147
DM loss after aerobic stability	g/kg DM	88	81	83	89	216	116	108	114	109	33.1	< 0.05	0.220	0.420	< 0.05	0.263	0.208	< 0.05
Biogenic amines	mg/kg as-fed	127	162	211	198	216	182	184	183	183	6.2	< 0.05	0.189	0.992	< 0.05	< 0.05	< 0.05	< 0.05

Abbreviations here and below: TN, total nitrogen.
[a] The butyric, isobutyric, valeric and isovaleric acids were not reported being undetected in all samples.
[b] When no interactions were significant (P < 0.05), orthogonal polynomial analysis was used to evaluate linear (L) or quadratic (Q) effects of the duration of ensiling.

(2)

where Y_{ijk} is the dependent variable; μ is the overall mean; T_i is the fixed effect of microbial inoculum (i = 2, CTR *vs.* TRT); Cj is the fixed effect of density (j = 2, Low *vs.* High); D_k is the fixed effect of length of ensiling (k = 5, 2, 4, 8, 16, or 32 days of ensiling); the $(T_i \times Cj)_{ij}$, $(T_i \times D_k)_{ik}$, and $(Cj \times D_k)_{jk}$ terms are the first order interactions; the $(T_i \times Cj \times D_k)_{ijk}$ term is the second order interaction; and e_{ijkl} is the residual error.

If the interactions were not significant (P > 0.05), data were averaged for the main effects tested, and reported accordingly. Further, when no interactions were significant, orthogonal polynomial contrast analysis was used to evaluate the effect of duration of ensiling on specific parameters. The IML procedure of SAS (2003) was used to generate unequally spaced linear and quadratic contrast coefficients, and post-hoc comparisons were performed using the pdiff and stderr options of the LSMEANS statement of SAS (2003). When a second-order interaction was significant, differences among specific means of interest were verified and reported in the text.

3. Results

Before ensiling, the freshly chopped uninoculated (CTR) and inoculated (TRT) whole-plant maize used prior to ensiling in mini-silos had similar chemical compositions as well as microbial traits (Table 1). In particular, the average DM concentration was 363 g/kg as fed for CTR and 370 g/kg as fed for TRT freshly chopped whole-plants. Furthermore, average starch and NDFom values of 322 g/kg DM and 411 g/kg DM were obtained, respectively. The freshly chopped whole plant maize was contaminated with some mycotoxins, including aflatoxin B_1 (on average, 0.59 μg/kg DM) and fumonisins B1 and B2 (on average, 4846 μg/kg DM and 2613 μg/kg DM, respectively).

Table 2 shows that silage DM decreased with ensiling duration (linear trend: P < 0.05), and was 353 g/kg as-fed on day 2 and 347 g/kg as-fed on day 32. In addition, the DM concentration was 3 g/kg as-fed lower in the CTR silage than in the TRT silage (P < 0.05). Inoculation and silage density had no effect on DM loss, but DM loss increased almost linearly with ensiling duration (P < 0.05), from 41 g/kg DM on day 2–61 g/kg DM on day 32.

Silage pH decreased with ensiling duration, from 3.90 on day 2 to 3.45 on day 32. On day 32, the pH in the TRT-HD silage reached it maximal value of 3.49 (P < 0.05, days × inoculation × density interaction). Lactate concentration increased with ensiling duration (P < 0.05), from 26.7 g/kg DM on day 2 to 50.9 g/kg DM on day 32. Acetate concentration also increased with ensiling duration, and was higher in the TRT silage than the CTR silage on day 32 (14.0 g/kg DM *vs.* 11.9 g/kg DM, P < 0.05, days × inoculation interaction, Fig. 1), and higher in the LD than HD silage on days 16 and 32 (P < 0.05, days × density interaction, Fig. 2). At day 32, ethanol was lower in the TRT than CTR silage (P < 0.05, days × inoculation interaction, Fig. 1), and was also lower in the HD than LD silage (P < 0.05, days × density interaction, Fig. 2). The 1,2-propanediol concentration decreased (P < 0.05), and was slightly higher in the TRT than CTR silage (0.14 *vs.* 0.11 g/kg DM, P < 0.05). The ammonia nitrogen level was greater in the CTR than TRT silage on days 16 and 32 (P < 0.05, days × inoculation interaction, Fig. 1). On day 8, the ammonia nitrogen level was greater in the HD than LD silage (3.22 *vs.* 2.73 g/100 g TN, P < 0.05, days × density interaction, Fig. 2). The lactate:acetate ratio

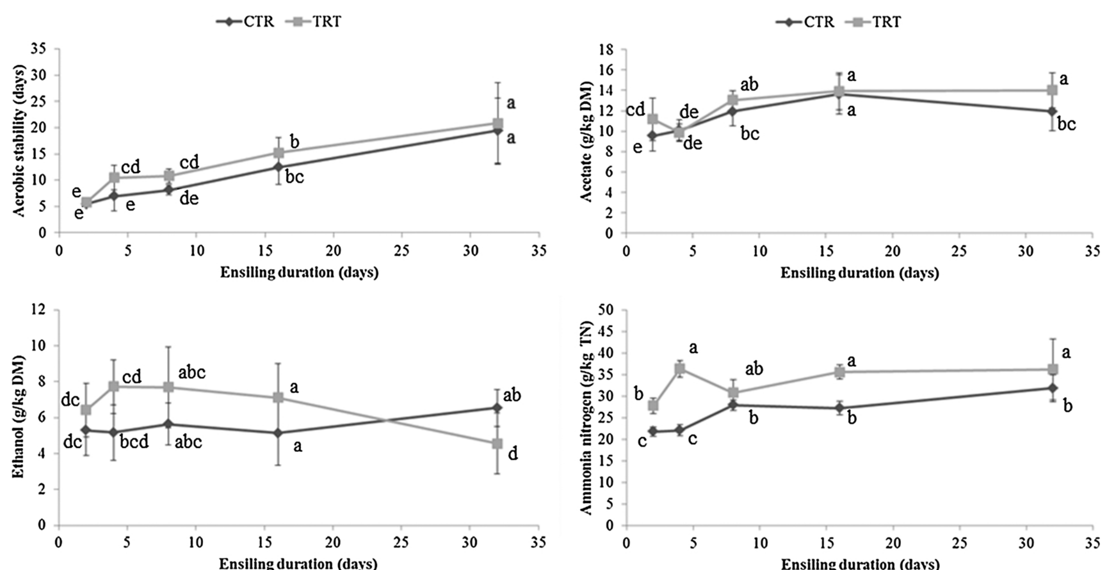

Fig. 1. Study of first order interaction between effects of inoculation (*i.e.*, CTR *vs.* TRT) and storage length (*i.e.*, 2, 4, 8, 16, or 32 days) for the parameters: aerobic stability (days), acetate (g/kg DM), ethanol (g/kg DM) and ammonia nitrogen (g/kg total nitrogen) of maize silages (see √MSE in Table 2). CTR, untreated silages; TRT, silages treated with *Lactobacillus buchneri* and *Lactococcus lactis.* Graphical values were mean ± standard error. Means with different superscript letters differed at a P < 0.05. The aerobic stability showed an interaction significant at P = 0.078.

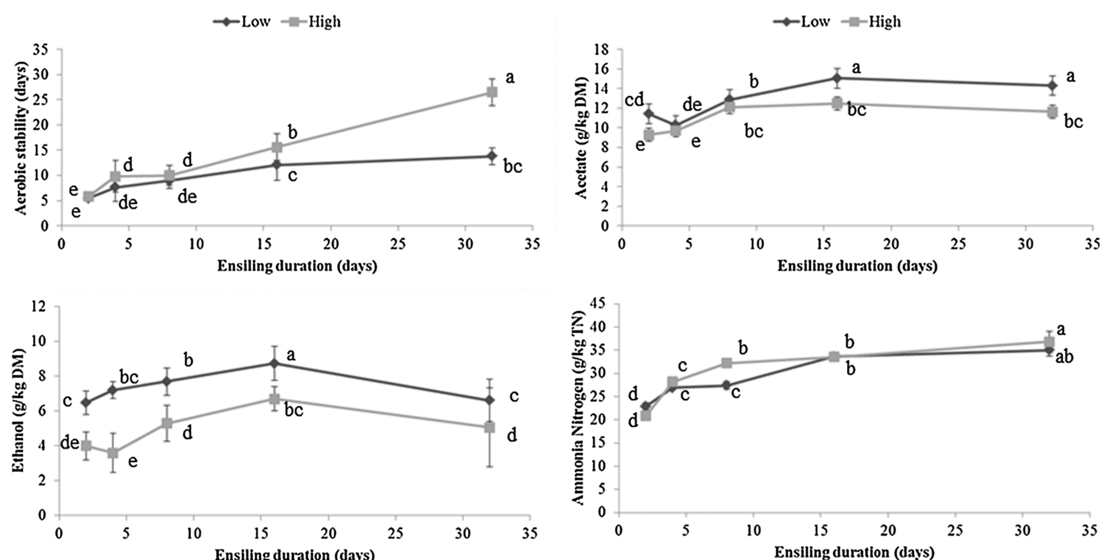

Fig. 2. Study of first order interaction between effects of density (*i.e.*, low *vs.* high) and storage length (*i.e.*, 2, 4, 8, 16, or 32 days) for the parameters: aerobic stability (days), acetate (g/kg DM), ethanol (g/kg DM) and ammonia nitrogen (g/kg total nitrogen) of maize silages (see √MSE in Table 2). Low, low density silages; High, high density silages. Graphical values were mean ± standard error. Means with different superscript letters differed at a P < 0.05.

was similar for the different treatments on days 2 and 32, but differed on days 4, 8, and 16 ($P < 0.05$, days × inoculation × density interaction).

The aerobic stability of the CTR and TRT silage increased with ensiling duration ($P < 0.05$), but lower values were present in the LD than HD silage on days 16 and 32 ($P < 0.05$, days × density interaction, Fig. 2). Overall, the aerobic stability was higher in the TRT than CTR silage ($P < 0.05$), with values of 12.6 and 10.5 days, respectively. The DM loss after the aerobic stability test did not differ among treatments from days 2 to 16, but was higher than 230 g/kg DM at 32 days in the CTR-HD and TRT-LD silages ($P < 0.05$, days × inoculation × density interaction).

Table 3
Effect of inoculation (CTR *vs.* TRT) and forage density (low *vs.* high) on chemical composition, microbe counts, and the concentration of mycotoxins of maize silage after 32 days.

Items	Dimension	CTR		TRT		√MSE	P of the model		
		Low	High	Low	High		Inoculation	Density	Inoculation × Density
DM	g/kg as-fed	347	350	346	346	4.5	0.230	0.388	0.396
Ash	g/kg DM	41	42	41	42	2.3	0.814	0.665	0.7315
EE	g/kg DM	13	13	14	13	0.9	0.605	0.315	0.753
CP	g/kg DM	67	68	70	66	3.8	0.599	0.446	0.218
Sol CP	g/kg DM	28	33	32	29	2.9	0.869	0.676	< 0.05
Starch	g/kg DM	330	327	317	317	14.2	0.092	0.801	0.801
aNDFom	g/kg DM	424	409	440	432	24.5	0.095	0.318	0.756
ADFom	g/kg DM	202	196	201	207	11.8	0.372	0.989	0.305
Lignin (sa)	g/kg DM	21	25	26	27	61	0.222	0.364	0.596
Sugars	g/kg DM	10	5	10	17	10.9	0.248	0.896	0.246
Microbes									
Yeasts	log_{10} CFU/g	4.79	4.82	4.21	4.31	1.651	0.472	0.929	0.966
Moulds	log_{10} CFU/g	1.65	1.20	1.74	1.75	1.176	0.546	0.688	0.666
Mycotoxins[a]									
Aflatoxin B1	μg/kg DM	0.632	0.506	0.225	0.285	1.868	0.143	0.694	0.408
Fumonisin B1	μg/kg DM	4263	4007	3906	3380	0.181	0.081	0.286	0.739
Fumonisin B2	μg/kg DM	491	334	312	244	0.348	< 0.05	0.071	0.455
Fusaric acid	μg/kg DM	2915	2783	5187	3163	0.291	< 0.05	< 0.05	0.069
Mycophenolic acid	μg/kg DM	13.13	5.53	2.45	0	3.253	0.259	0.306	0.938
Roquefortine C	μg/kg DM	16.90	28.72	2.15	4.63	3.158	< 0.05	0.854	0.685

[a] Mycotoxins detected in at least in one sample are indicated. Mycotoxins analyzed, but not detected were: deoxynivalenol, zearalenone, and PR toxin. For each treatment, the indicated concentration is the average for each mycotoxin, including samples in which the mycotoxin was not detected (in which case, the instrumental detection limit was used). Mycotoxin levels were analyzed as log_{10}-transformed data, and the ANOVA terms (√MSE and P of the model) refer to the log_{10}-transformed data, but the means are in the natural scale.

The BA levels were lower than 250 mg/kg in all silages.

Table 3 shows that the chemical composition of CTR and TRT silages were similar on day 32, except that soluble CP was higher in the CTR-HD and TRT-LD silages than the CTR-LD and TRT-HD silages ($P < 0.05$, inoculation × density interaction). Yeast (average: 4.53 $\log_{10}$ CFU/g) and mould (average:1.59 $\log_{10}$ CFU/g) counts were similar among all silages.

We detected aflatoxin B1 in all CTR samples and in 0.6 of TRT silages, and the contamination levels were similar (0.412 μg/kg DM). We also detected fumonisin B_1, fumonisin B_2, and fusaric acid in all samples. The fumonisin B_2 level was greater in the CTR than TRT silage (413 *vs.* 278 μg/kg DM, $P < 0.05$), but the fusaric acid level was lower in the CTR than TRT silage (2849 *vs.* 4162 μg/kg DM, $P < 0.05$). Despite the overall low levels of mycotoxin contamination, a 10-fold higher level of roquefortine C ($P < 0.05$) was present in the CTR than TRT silage.

4. Discussion

Freshly chopped whole-plant maize that is used prior to ensiling could be considered typical for normal maize silage (Arriola et al., 2011; Carvalho et al., 2012; Oliveira et al., 2017). Our measurements of DM concentration and other chemical characteristics indicated the freshly chopped whole-plant maize was harvested slightly past the ½ milk line stage of maturity. However, the sugar concentration (90 g/kg DM) of this mass indicated it was adequate for ensiling (Oliveira et al., 2017). Before ensiling, the yeast and mould counts were greater than 6 $\log_{10}$ CFU/g herbage, in agreement with previous results (Ranjit and Kung, 2000; Borreani and Tabacco, 2010).

In general, when a forage is ensiled, two conditions are required to improve aerobic stability and avoid the proliferation of spoilage microorganisms: low pH (for inhibition of bacterial growth) and absence of oxygen (for reducing yeast and mould proliferation) (Ranjit and Kung, 2000). Addition of homo-fermentative LAB to forage at the initiation of ensiling of freshly chopped-whole plant maize increases the production of lactic acid, thus causing a drop in pH (Weiss et al., 2016; Romero et al., 2017). Hetero-fermentation is less efficient than homo-fermentation in the rapid reduction of pH, even though a high level of acetic acid, rather than lactic acid, better inhibits growth of acid-tolerant yeasts (Ranjit and Kung, 2000; Kleinschmit and Kung, 2006). The inoculum we used had facultative hetero- and homo-fermentative LAB. At the tested level of inoculation (approximately 250,000 CFU/g freshly-chopped forage), we found that the CRT and TRT silages had no significant differences in the pH, VFA profile, and other fermentative traits, except that the ethanol level that was lower in the TRT silage at day 32. Consistent with the results of Sadeghi et al. (2012), we found that the pH dropped rapidly in the CTR and TRT silage. Conversely, other studies of the long-term ensilage of maize reported pH values above 4.0 (Arriola et al., 2011; Kim et al., 2015). Further, the CTR and TRT silages had similar lactic acid concentrations, but the TRT silage had a 0.08 g/kg DM higher level of acetic acid and a 0.27 g/kg DM higher level of 1,2-propanediol. Conversely, other authors reported that inoculation of ensiled mass with doses of facultative hetero-fermentative LAB that were similar to our doses led to a doubling of the concentration of acetate relative to the untreated controls (Filya, 2003; Arriola et al., 2011). In addition, we also found that the yeast and mould counts did not differ for the CTR and TRT silages at day 32, thus suggesting that all treatments led to complete or nearly complete fermentation.

Nevertheless, we observed greater aerobic stability in the TRT than CTR silage. Other authors reported similar changes in the fermentative profiles (Addah et al., 2012; Weiss et al., 2016) and in the yeast and mould counts (Weinberg et al., 1993; Driehuis et al., 2001) of ensiled mass, and that these changes are associated with improved silage stability after air penetration, even though there were some discrepancies among these experiments (Oliveira et al., 2017). Aerobic stability is a measure of the time that silage remains stable after exposure to air. Thus, Kung (2010) said that "*exposure to air is the first domino to fall that causes a chain reaction ultimately resulting in spoiled feed*"; this spoiled feed is due to the yeast-mediated degradation of lactic acid. Yeast species in multiple genera (*Candida*, *Hansenula*, *Pichia*, *Issatchenkia*, and *Saccharomyces*) can degrade lactic acid and cause a concomitant increase of pH that may allow the growth of opportunistic bacteria (*e.g.* Bacilli, *Acetobacter* spp.) and moulds (*e.g. Aspergillus*, *Fusarium*, and *Penicillium* spp.), thus further reducing silage quality (Kung, 2010).

We also recorded the presence of different mycotoxins. The results show that some mycotoxins associated with *Aspergillus* and *Fusarium* spp. (aflatoxin B1, and fumonisin B1, fumonisin B2) were present in the freshly-chopped forage. Ma et al. (2017) recently reported that application of aflatoxin B1 to ensiled maize forage reduced the activity of silage microbiota, and thereby reducing the rate of acidification during the first 3 days of ensiling, and also reducing CP concentration, but increasing the rate of DM reduction and the concentrations of butyric acid and NDF. However, we found that the levels of fumonisins B1 and B2 decreased during ensiling, thus corroborating previous findings (Boudra and Morgavi, 2008; Cheli et al., 2013). Ma et al. (2017) also reported that some bacterial silage inoculants had the ability to bind aflatoxin B1 *in vitro*, although this varied among different strains and populations, and depended on strain viability and medium acidity. In fact, these authors stated that inoculation of maize silage with bacteria could prevent the adverse effects of aflatoxin B1. Our previous study (Gallo and Masoero, 2009) discussed the roles of cell walls of several bacteria (*Lactobacillus, Propionibacterium* or *Bifidobacterium* strains) and yeast (*Saccharomyces cerevisiae*) in the sequestering of low molecular weight mycotoxins. Thus, it is likely that multiple physical, chemical, and/or microbiological factors contribute to the decline of mycotoxin contamination during ensiling (Boudra and Morgavi, 2008; Gallo et al., 2015a), so this topic requires further investigation.

Our analysis of other mycotoxins produced by *P. roqueforti* and *P. paneum*, such as mycophenolic acid and roquefortine C, indicated no detectable levels in freshly-chopped forage, but the appearance after 32 days. Although these moulds can grow in typical conditions of silage (low pH, low oxygen, and high carbon dioxide levels [Gallo et al., 2015a]), to the best of our knowledge, this is the first report of production of these mycotoxins during ensiling. Notably, the TRT silages had lower contamination levels of these *Penicillium*-related mycotoxins than the CTR silages. Conversely, we found greater contamination with fusaric acid (a mycotoxin

produced by *Fusarium* spp.) in the TRT than CTR silages. This suggests that applied commercial inoculant impacted the growth of different moulds. We were unable to characterize silage microbiology before or during silage; DNA-based techniques, such as real-time PCR, have only recently been used to characterize silage microbiology (Muck, 2013; Dunière et al., 2017). Further research is needed to determine the mode of action and the effect of different inoculant levels on the populations of specific microbiological populations in different types of silage.

One of the purposes of our study was to test the ability of an inoculant to improve the fermentative quality of maize silage at LD. Ensiling at LD permits air penetration and proliferation of undesirable microorganisms (Vissers et al., 2007; Dunière et al., 2013; Bonifacio et al., 2017). In addition, aerobic spoilage microorganisms could have a more pronounced effect on the peripheral than the central area of ensiled masses (Gallo et al., 2016a). Despite the mass compression difference of about 0.30 between our HD and LD silages, there were only slight differences in their fermentative profiles, and in their yeast and mould counts. In particular, pH was similar in the HD and LD silages, and the lactic acid content was only about 0.05 greater in the HD silages. As expected, the aerobic stability upon exposure to air was greater for the HD than LD silages, and these differences were more evident after 32 days.

5. Conclusions

This study confirms that inoculation of maize silage with *Lactobacillus buchneri* LB1819 and *Lactococcus lactis* O224 improved its aerobic stability, and that the improvement was greater at a high silage density. Our analysis of mycotoxin contaminants indicated that the inoculation protocol reduced the levels of fumonisin B2 and roquefortine C, but increased the level of fusaric acid. However, under the conditions of the current experiments, inoculation of maize silage provided no benefits to other classically studied parameters, including the fermentation profile.

Conflict of interest

The authors Giuseppe Copani, Keith A. Bryan, Natasja G. Nielsen, Kristian L. Witt are employees at Chr Hansen A/S and they played no role in collection, analysis and interpretation of data. All authors declare no potential conflicts of interest.

Acknowledgments

The authors are grateful to Maurizio Van Huegten, and Gianluca Ungari (Cima s.r.l., Castiglione delle Stiviere, Mantova, Italy) for their assistance in the field trial organization and Annalisa Mulazzi, Samantha Sigolo, and Silvia Rastelli for their valuable help in laboratory analysis. Financial support for this study was provided by Chr. Hansen (Hoersholm, Denmark).

References

Addah, W., Baah, J., Okine, E.K., McAllister, T.A., 2012. A third-generation esterase inoculant alters fermentation pattern and improves aerobic stability of barley silage and the efficiency of body weight gain of growing feedlot cattle. J. Anim. Sci. 90, 1541–1552. https://doi.org/10.2527/jas.2011-4085.

AOAC (Association of Official Analytical Chemists), 1995. Official Methods of Analysis, 15th edition. Association of Official Analytical Chemists, Arlington, VA.

Arriola, K.G., Kim, S.C., Adesogan, A.T., 2011. Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage. J. Dairy Sci. 94, 1511–1516. https://doi.org/10.3168/jds.2010-3807.

Bernardes, T.F., do Rêgo, A.C., 2014. Study on the practices of silage production and utilization on Brazilian dairy farms. J. Dairy Sci. 97, 1852–1861. https://doi.org/10.3168/jds.2013-7181.

Bonifacio, H.F., Rotz, C.A., Hafner, S.D., Montes, F., Cohen, M., Mitloehner, F.M., 2017. A process-based emission model of volatile organic compounds from silage sources on farms. Atmos. Environ. 152, 85–97. https://doi.org/10.1016/j.atmosenv.2016.12.024.

Borreani, G., Tabacco, E., 2010. The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. J. Dairy Sci. 93, 2620–2629. https://doi.org/10.3168/jds.2009-2919.

Boudra, H., Morgavi, D.P., 2008. Reduction in Fusarium toxin levels in corn silage with low dry matter and storage time. J. Agric. Food Chem. 56, 4523–4528. https://doi.org/10.1021/jf800267k.

Brüning, D., Gerlach, K., Weiß, K., Südekum, K.H., 2018. Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. Grass Forage Sci. 73, 53–66. https://doi.org/10.1111/gfs.12288.

Carvalho, B.F., Bernardes, T.F., Paula, A., Piza, D.T., Henrique, C., Rabelo, S., Ruggieri, A.C., Reis, R.A., 2012. Revista Brasileira De Zootecnia Short Communication Fermentation and Aerobic Stability of High-moisture Corn Silages Inoculated With Different Levels of Lactobacillus buchneri. pp. 2369–2373.

Cheli, F., Campagnoli, A., Dell'Orto, V., 2013. Fungal populations and mycotoxins in silages: from occurrence to analysis. Anim. Feed Sci. Technol. 183, 1–16. https://doi.org/10.1016/j.anifeedsci.2013.01.013.

Driehuis, F., van Wikselaar, P.G., van Vuuren, A.M., Spoelstra, S.F., 1997. Effect of a bacterial inoculant on rate of fermentation and chemical composition of high dry matter grass silages. J. Agric. Sci. 128, 323–329. https://doi.org/10.1017/S0021859696004157.

Driehuis, F., Oude Elferink, S.J.W.H., Van Wikselaar, P.G., 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. Grass Forage Sci. 56, 330–343. https://doi.org/10.1046/j.1365-2494.2001.00282.x.

Dunière, L., Sindou, J., Chaucheyras-Durand, F., Chevallier, I., Thévenot-Sergentet, D., 2013. Silage processing and strategies to prevent persistence of undesirable microorganisms. Anim. Feed Sci. Technol. 182, 1–15. https://doi.org/10.1016/j.anifeedsci.2013.04.006.

Dunière, L., Xu, S., Long, J., Elekwachi, C., Wang, Y., Turkington, K., Forster, R., McAllister, T.A., 2017. Bacterial and fungal core microbiomes associated with small grain silages during ensiling and aerobic spoilage. BMC Microbiol. 17, 50. https://doi.org/10.1186/s12866-017-0947-0.

Filya, I., 2003. The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. J. Appl. Microbiol. 95, 1080–1086. https://doi.org/10.1046/j.1365-2672.2003.02081.

Gallo, A., Masoero, F., 2009. In vitro models to evaluate the capacity of different sequestering agents to adsorb aflatoxins. Ital. J. Anim. Sci. 9, 109–116. https://doi.org/10.4081/ijas.2010.e21.

Gallo, A., Giuberti, G., Frisvad, J., Bertuzzi, T., Nielsen, K., 2015a. Review on mycotoxin issues in ruminants: occurrence in forages, effects of mycotoxin ingestion on health status and animal performance and practical strategies to counteract their negative effects. Toxins (Basel) 7, 3057–3111. https://doi.org/10.3390/toxins7083057.

Gallo, A., Giuberti, G., Bertuzzi, T., Moschini, M., Masoero, F., 2015b. Study of the effects of PR toxin, mycophenolic acid and roquefortine C on in vitro gas production

parameters and their stability in the rumen environment. J. Agric. Sci. 153, 163–176.
Gallo, A., Bertuzzi, T., Giuberti, G., Moschini, M., Bruschi, S., Cerioli, C., Masoero, F., 2016a. New assessment based on the use of principal factor analysis to investigate corn silage quality from nutritional traits, fermentation end products and mycotoxins. J. Sci. Food Agric. 96, 437–448. https://doi.org/10.1002/jsfa.7109.
Gallo, A., Giuberti, G., Bruschi, S., Fortunati, P., 2016b. Use of principal factor analysis to generate a corn silage fermentative quality index to rank well- or poorly preserved forages. J. Sci. Food Agric. 96, 1686–1696. https://doi.org/10.1002/jsfa.7272.
Gerlach, K., Roß, F., Weiß, K., Büscher, W., Südekum, K.H., 2014. Aerobic exposure of grass silages and its impact on dry matter intake and preference by goats. Small Rumin. Res. 117, 131–141. https://doi.org/10.1016/j.smallrumres.2013.12.033.
Jatkauskas, J., Vrotniakiene, V., Ohlsson, C., Lund, B., 2013. The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. Agric. Food Sci. 22, 137–144.
Kim, S.D., Lee, M.A.H., Hee, Y.J., Song, S.L.Y., Kim, H.K.S., 2015. Effects of L. Plantarum application on chemical composition, fermentation indices and fatty acid profiles of barley silage. J. Agric. Life Sci. 49, 157–167.
Kleinschmit, D.H., Kung, L., 2006. A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89, 4005–4013. https://doi.org/10.3168/jds.S0022-0302(06)72444-4.
Kung, L.J., 2010. Aerobic stability of silage. In Proceedings of California Alfalfa Forage Symposium and Corn/Cereal Silage Conference 95616.
Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3.
Ma, Z.X., Amaro, F.X., Romero, J.J., Pereira, O.G., Jeong, K.C., Adesogan, A.T., 2017. The capacity of silage inoculant bacteria to bind aflatoxin B 1 in vitro and in artificially contaminated corn silage. J. Dairy Sci. 1–13. https://doi.org/10.3168/jds.2016-12370.
Masoero, F., Gallo, A., Giuberti, G., Fiorentini, L., Moschini, M., 2013. Effect of water-saving irrigation regime on whole-plant yield and nutritive value of maize hybrids. J. Sci. Food Agric. 93, 3040–3045. https://doi.org/10.1002/jsfa.6137.
Muck, R.E., 1988. Factors influencing silage quality and their implications for management. J. Dairy Sci. 71, 2992–3002. https://doi.org/10.3168/jds.S0022-0302(88)79897-5.
Muck, R.E., 2013. Recent advances in silage microbiology Recent microbial techniques. Agric. Food Sci. 22, 3–15.
Muck, R.E., Nadeau, E.M.G., Mcallister, T.A., Santos, M.C., K Jr., L., 2018. Silage review: recent advances and future uses of silage additives. J. Dairy Sci. 101, 3980–4000. https://doi.org/10.3168/jds.2017-13839.
NorFor, 2011. NorFor-The Nordic Feed Evaluation System, 1st edition. Wageningen Academic Publishers, Wageningen, the Netherlands.
Oliveira, A.S., Weinberg, Z.G., Ogunade, I.M., Cervantes, A.A.P., Arriola, K.G., Jiang, Y., Kim, D., Li, X., Gonçalves, M.C.M., Vyas, D., Adesogan, A.T., 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J. Dairy Sci. 100, 1–17. https://doi.org/10.3168/jds.2016-11815.
Oude Elferink, S.J.W.H., Driehuis, F., Gottschal, J., Spoelstra, S., 2000. Paper 2.0: silage fermentation processes and their manipulation. FAO Plant P 161, 17–30.
Ranjit, N.K., Kung, L., 2000. The effect of Lactobacillus buchneri, Lactobacillus plantarum, or a chemical preservative on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 83, 526–535. https://doi.org/10.3168/jds.S0022-0302(00)74912-5.
Romero, J.J., Zhao, Y., Balseca-Paredes, M.A., Tiezzi, F., Gutierrez-Rodriguez, E., Castillo, M.S., Chase, L.E., Overton, T.R., Mills, J.K., Foskolos, A., Schlüter, A., Kang, J.-C., Jones, E.B.G., Hyde, K.D., 2017. Laboratory silo type and inoculation effects on nutritional composition, fermentation, and bacterial and fungal communities of oat silage. J. Dairy Sci. 100, 1812–1828. https://doi.org/10.3168/jds.2016-11642.
Sadeghi, K., Khorvash, M., Ghorbani, G.R., Forouzmand, M.A., Boroumand, M., Hashemzadeh-Cigari, F., 2012. Effects of homo-fermentative bacterial inoculants on fermentation characteristics and nutritive value of low dry matter corn silage. Iran. J. Vet. Res. 13, 303–309.
SAS, 2003. User's Guide: Statistics (version 9.13). SAS Institute Inc., Cary, NC.
Smith, M., Davies, P., 1985. Separation and quantitation of polyamines in plant tissue by high performance liquid chromatography of their dansyl derivatives. Plant Physiol. 78, 89–91.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
Vissers, M.M.M., Driehuis, F., Te Giffel, M.C., De Jong, P., Lankveld, J.M.G., 2007. Concentrations of butyric acid bacteria spores in silage and relationships with aerobic deterioration. J. Dairy Sci. 90, 928–936. https://doi.org/10.3168/jds.S0022-0302(07)71576-X.
Weinberg, Z.G., Ashbell, G., Hen, Y., Azrieli, A., 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. J. Appl. Bacteriol. 75, 512–518. https://doi.org/10.1111/j.1365-2672.1993.tb01588.x.
Weiss, K., Kroschewski, B., Auerbach, H., 2016. Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. J. Dairy Sci. 99, 8053–8069. https://doi.org/10.3168/jds.2015-10323.