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Effects of an inoculant containing a *Lactobacillus buchneri* that produces ferulate-esterase on fermentation products, aerobic stability, and fibre digestibility of maize silage harvested at different stages of maturity

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ABSTRACT

The aim of this research was to study the effects of a commercial inoculant containing *Lactobacillus casei* and *Lactobacillus buchneri* that produces ferulate esterase enzymes on fermentation products, aerobic stability, microbial status, dry matter (DM) losses, and digestibility of neutral detergent fibre (aNDF-D) of maize silages ensiled at four stages of maturity. The kernel milk line (ML) was used to time the forage harvest, and 1/6 ML, 2/5 ML, 3/4 ML and black layer (BL) were observed, for harvest stages I, II, III and IV, respectively. Chopped whole plant maize was untreated or treated with *L. casei* LC32909 and *L. buchneri* LN40177, which were applied to achieve a final application rate of 1×10^4 cfu/g and 1.0×10^5 cfu/g of fresh forage, respectively. The maize was ensiled in laboratory silos for 260 days before opening. The DM content, starch and ether extract concentrations and mould count increased, whereas water activity, nitrate, ash, water soluble carbohydrates (WSC) and crude protein (CP) contents progressively decreased with increasing maturity at harvest. The 24-h and 48-h aNDF-D were similar for harvest stages I, II and III, whereas they were the lowest in harvest stage IV. The effect of inoculation decreased with increasing DM content at ensiling, and the inoculum was ineffective at the last stage of maturity, probably due to the high epiphytic lactic acid bacteria (LAB) count, low water activity and low sugar content that could have negatively influenced the inoculation outcome. The inoculation lowered the lactic acid, yeast and mould counts and increased acetic acid, 1,2-propanediol, pH, DM losses and aerobic stability in the first three harvest stages, whereas no differences were observed between the treated and untreated silages harvested at the last stage of maturity. Regardless of the treatment, the yeast count fell under the detection limit and

Abbreviations: ADF, acid detergent fibre; aNDF, neutral detergent fibre; aNDF-D, digestibility of neutral detergent fibre; a_w, water activity; BL, black layer; C, untreated control; cfu, colony-forming units; DM, dry matter; d*T*, difference between silage temperature and ambient temperature; FE, ferulate esterase; H, harvest stage; LAB, lactic acid bacteria; ML, milk line; T, treated with *Lactobacillus casei* LC32909 and *L. buchneri* LN40177; WSC, water soluble carbohydrates.

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the aerobic stability of the silage increased to over 200 h when the acetic acid content exceeded 25 g/kg DM. Furthermore, the DM losses were closely correlated to the acetic acid production and increased to 80 g/kg of DM in the treated silages harvested at the earliest stage of maturity. The potential milk production, estimated with MILK2006 model (Shaver et al., 2006, http://www.uwex.edu/ces/dairynutrition/spreadsheets.cfm), showed that the greater aNDF-D of the treated silage, which was observed in harvest stages I and III, did not counterbalance the higher DM losses attributable to the *L. buchneri* activity during ensiling, in terms of milk per Mg of original ensiled DM.

1. Introduction

Whole-crop maize silage is the major forage fibre source in dairy cow diets in Europe and in the United States, but its aerobic instability could decrease its nutritive value (Wilkinson and Davies, 2013). This problem could be prevented by the use of an inoculant containing *Lactobacillus buchneri*, a heterofermentative LAB, which could improve the aerobic stability of silages through the production of acetic acid from lactic acid during the anaerobic phase of silage conservation (Oude Elferink et al., 2001). The positive effect of this organism on aerobic stability, when added to maize silage, has been evaluated extensively on maize silage in laboratory experiments (Kleinschmit and Kung, 2006), in farm-scale experiments (Kristensen et al., 2010) and in farm surveys (Mari et al., 2009; Tabacco et al., 2011a). However, treating silages with inoculants containing *L. buchneri* alone has often led to an increase in DM losses and a slight increase in the final silage pH (Reich and Kung, 2010; Tabacco et al., 2009). Thus, selected homofermentative LAB have traditionally been used to rapidly produce lactic acid, lower pH and, consequently, improve the efficiency of the fermentation process and minimize DM and nutrient losses over conservation (Muck, 2004). Dual-purpose inoculants containing homofermentative and heterofermentative LAB have recently been developed to overcome the limitations of inoculants containing either type of bacteria alone. Beneficial effects of dual-purpose inoculants on the aerobic stability of maize silage have also been reported (Queiroz et al., 2012).

Recent studies have indicated that some isolates of *L. buchneri*, apart from producing acetic acid, can produce ferulate-esterase (FE) enzyme, which can hydrolyses feruloyl ester linkages between lignin and hemicellulose, and they are advocated to potentially improve fibre digestibility of forages during ensiling (Nsereko et al., 2008). Only a few studies have assessed the effects of the third-generation FE-producing inoculants containing *L. buchneri* on aerobic stability and on the improvement of fibre digestibility of maize (Nsereko et al., 2008; Kang et al., 2009), barley (Addah et al., 2012) and grass silages (De Boever et al., 2013). However, to the authors' knowledge, no research has been conducted to investigate the effects of these third-generation inoculants on the aerobic stability and fibre digestibility of maize silage harvested at different stages of maturity.

In US and Europe, corn is commonly harvested for silage in horizontal silos when the milk line (ML) ranges from 3/5 to 1/4. In agricultural practice, however, forage maize is often harvested earlier or later than the optimum time for harvesting (Herrmann et al., 2005; Windle et al., 2014). More common reasons for harvest to occur outside of the recommended range of maturity are unfavourable weather conditions, inadequate capacity to harvest large amounts of forage in a short period of time, unavailability of custom harvest equipment at the optimal harvest time, errors when choosing the maturity class of hybrids, or lack of monitoring whole plant moisture or kernel milk line during the filling period (Windle et al., 2014). Harvesting too early can be unfavourable, because the energy content of maize silage is lower due to incomplete starch accumulation in the kernels (Wiersma et al., 1993). In contrast, late harvesting at the black layer (BL) stage may result in a higher proportion of starch, but in reduced fibre digestibility (Wiersma et al., 1993). The NDF degradability of whole crop maize decreases progressively from the early to late maturity stages, despite the decline in NDF content (Johnson et al., 1999; Opsi et al., 2013), and this is due to the decline in digestibility of the stover with progressive maturity, which is associated with decreasing non-structural carbohydrates and increasing fibre and lignin concentrations (Bal et al., 2000). Furthermore, as maturity advances, the ensilability characteristics become progressively worse (*i.e.* higher DM content and more difficulties in achieving high packing density, and lower fermentable sugars that make LAB activity more difficult; Buxton and O'Kiely, 2003) and maintaining aerobic stability of silage during consumption becomes more challenging (Hu et al., 2009). Since energy is the primary contribution of maize silages to dairy cattle rations, the availability of a LAB inoculum that enhances fermentation increases aerobic stability after silo opening, and increases the proportion of digestible NDF that could be beneficial to ensile maize at more advanced stages of maturity and thus maximizes starch production (Opsi et al., 2013).

Therefore, the objective of this research was to evaluate whether the use of an FE-producing silage inoculant could increase aerobic stability and fibre digestion in maize silage, especially when its harvest was delayed to more advanced stages of maturity than 1/2 kernel milk line.