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Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage

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ABSTRACT

Ensiling conditions strongly influence fermentation characteristics, yeast count, and aerobic stability. Numerous volatile organic compounds including esters are produced, which may negatively affect feed intake and animal performance and air quality. In addition to a farm survey, 3 laboratory experiments were carried out to study the effects of air (by delayed sealing or by air infiltration during anaerobic storage), temperature (20 and 35°C), and various types of additives [blends of either sodium benzoate and sodium propionate (SBSP) or of sodium benzoate and potassium sorbate (SBPS); buffered mixture of formic and propionic acids (FAPA); homofermentative inoculant (LAB)]. After additive treatment, chopped whole corn plants were packed into 1.5-L glass jars and stored for several months. For treatments with air infiltration, glass jars with holes in the lid and body were used. The farm survey in 2009 revealed large variation in lactate, acetate, ethanol, n-propanol, and 1,2-propanediol concentrations. Whereas ethyl esters were detected in all silages, the mean ethyl lactate concentrations were higher than those for ethyl acetate (474 vs. 38 mg/kg of dry matter). In the ensiling experiments, few unequivocal effects of the tested factors on the analyzed parameters were observed due to many interactions. Delayed ensiling without additives decreased lactic acid production but, in one trial, increased acetic acid and had no effect on ethanol. The effect of delayed sealing on yeast counts and aerobic stability differed widely among experiments. Air infiltration during fermentation tested in one trial did not alter lactic acid production, but resulted in more acetic acid in delayed and more ethanol than in promptly sealed untreated silages. Greater ethanol production was associated with increased yeast numbers. Storage at high temperature resulted in lower lactic acid and n-propanol, and a trend toward reduced ethanol production was observed. The additive FAPA consistently caused increased ethanol and reduced n-propanol levels with no effect on yeast counts and aerobic stability. When the additives SBSP and SBPS decreased n-propanol and ethanol, reduced yeast counts were also found. Ethyl ester formation was strongly correlated with those of ethanol and to a lesser degree with those of the respective acid.
Key words: air ingress, storage temperature, silage additives, volatile organic compounds

INTRODUCTION

The occurrence of volatile organic compounds (**VOC**) in grass and corn silages was first reported more than 50 yr ago (Morgan and Pereira 1962), but has only recently attracted significant attention (Hafner et al., 2010, 2012; Howard et al., 2010; Malkina et al., 2011). Ethanol, and to a lesser extent aldehydes, can significantly contribute to air pollution by photochemical reactions with oxides of nitrogen (Howard et al., 2010). In their recent review, Hafner et al. (2013) associated the highest VOC emissions from corn and the resulting ozone formation to various alcohols. Other volatiles (e.g., aldehydes, esters, and acids) may also contribute significantly to emissions under some conditions. Anecdotal evidence has been provided from farmers in Germany (Weiss et al., 2009a), Denmark (Raun and Kristensen, 2010), and the United States (Richard Muck, US Dairy Forage Center, Madison, WI, personal communication) on negative effects of odd (atypically)-smelling corn silages on feed intake and performance by dairy cows. Based on the routinely measured indices of silage fermentation quality—pH, ammonia-N, organic acids, and alcohols—this phenomenon cannot be explained. According to Weiss et al. (2009a), suspect corn silages of German origin were well compacted and well fermented, had low pH and yeast counts, and were stable upon exposure to air. As

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even high concentrations of added acetic acid (Daniel et al., 2013a) or ethanol (Randby et al., 1999; Daniel et al., 2013a,c) and increased acetic acid levels (≥4% of DM) by inoculation with heterofermentative inoculants do not appear to adversely affect feed intake (Ranjit et al., 2002; Kleinschmitt et al., 2013), other VOC (e.g., ethyl and propyl esters) may be considered to explain the observations on dairy farms.

Ethyl and propyl esters of lactate and acetate, respectively, have been found in farm silages and in numerous ensiling experiments with corn (Kristensen et al. 2010; Raun and Kristensen, 2010; Weiss and Auerbach, 2012a). However, the knowledge on the effects of specific esters on feed intake by ruminants is still very limited and conflicting. Krizsan et al. (2007) and Gerlach et al. (2013) overserved negative correlations between some VOC and feed intake, whereas Daniel et al. (2013c) reported no difference when fresh sugar cane silage was compared with oven-dried material, resulting in the loss of volatiles, which was reconstituted with water before feeding. Knowledge is still scarce as to the effects of ensiling conditions (e.g., delayed sealing and air infiltration during the ensiling process, storage temperature) and silage additive type on the formation of VOC, especially that of esters. In previous studies, air infiltration resulted in a decline of lactic acid concentration (Bolsen et al. 1993; Mills and Kung, 2002; Moshtaghi Nia and Wittenberg, 2000). Conflicting results were described on the effects of delayed sealing and air infiltration on acetic acid and production of VOC, especially ethanol (Moshtaghi Nia and Wittenberg, 2000; Mills and Kung, 2002; Kim and Adesogan, 2006). Kim and Adesogan (2006) observed lower lactate and acetate production in corn silage by storage at higher temperature, whereas Weinberg et al. (2001) found no effect of temperature on ethanol or acetate contents. Data on the effects of temperature on ester formation were not available.

Silage additives affect fermentation pattern and aerobic stability in different ways according to their specific mode of action (Kung et al., 2003). Hafner et al. (2014) and Savage et al. (2014) observed increased ethanol and ethyl acetate accumulation by the use of homofermentative lactic acid bacteria, whereas the application of heterofermentative lactic acid bacteria containing *Lactobacillus buchneri* in sorghum silages (Auerbach and Weiss, 2012) resulted in decreased concentrations of ethanol and its esters of lactic and acetic acids. Kristensen et al. (2010) found an increase in n-propanol and propyl acetate in corn silages treated with *Lactobacillus buchneri*. The application of sodium benzoate and potassium sorbate to corn and sorghum at ensiling, alone or in combination, has consistently reduced ethanol contents in silages (Weiss and Auerbach, 2012b; Bernardes et al., 2014; Da Silva et al., 2014). When reported, concentrations of ethyl esters have also been reduced (Auerbach and Weiss, 2012; Hafner et al., 2015; Weiss and Auerbach, 2012b). Interestingly, buffered acid mixtures, mainly containing formic and propionic acids, stimulated ethanol (Auerbach et al., 2012) and ethyl ester production in corn silage (Weiss and Auerbach, 2012b). However, which individual factors increase the production of VOC has not been thoroughly studied with particular focus on esters in corn silage and how they may interact. Therefore, the objective of our experiments was to evaluate the effects of air by delayed sealing or repeated air infiltration during the ensiling process, storage temperature, additive type, and their interactions on selected fermentation characteristics, yeast count, aerobic stability, and the formation of ethyl esters of lactate and acetate in corn silage. These ethyl esters were considered indicator substances for the total VOC production in corn silage (Weiss et al., 2009a) and can be determined by routine analytical procedures, such as GC. Different additive types were used to evaluate the effect of the additives on VOC formation and, more so, to induce large variations in the concentrations of fermentation end-products, which may play a role in esterification reactions.

MATERIALS AND METHODS

Experimental Design of the Ensiling Experiments

Farm Survey. Corn silage samples were taken from the freshly prepared face in the core sections of 11 bunker silos on dairy farms located in the German State of Brandenburg in 2009 using a hollow drill. All farms had reported problems with feed intake and performance by dairy cows. Silages had been made either without additives (4 silos) or treated with different types of commercially available inoculants (3 silos with pure homofermentative inoculants; 1 silo with pure heterofermentative inoculants; 3 silos with inoculants composed of homo- and heterofermentative lactic acid bacteria).

Laboratory Ensiling Experiments. A total of 3 laboratory ensiling experiments were carried out with corn, which was treated with various additives and subjected to different storage conditions with regard to temperature and air exposure (Table 1). In all experiments, a portion of the total forage mass was either immediately ensiled (prompt) or left loosely piled on a clean concrete floor in a barn at ambient temperature of approximately 16 to 18°C for 16 (trial 1 and 2) or 24 h (trial 3) before being filled in the silos (delay) and stored in a temperature-controlled room at 20°C. Silages were packed in 1.5-L glass jar (Weck, Öfingen, Ger-