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Influence on lactic acid content in maize silage variations by manganese supplementation

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ABSTRACT

Immense research has been performed on influencing silage characteristics by use of biological and chemical additives. For new applications, such as the non-food material use of silage ingredients (e.g. lactic acid) in biorefineries, new ensiling techniques and additives are required. To investigate the influence of manganese supplementation on lactic acid production in maize silage, different concentrations of $MnSO_4$ (0.001 g kg^{-1} & 0.003 g kg^{-1}) and contrast treatments (homofermentative lactic acid bacteria (LAB) mixture & carbonated lime in various combinations) were applied to the raw material maize. Manganese addition had no effect ($r = 0.047$) on the lactic acid content in the silage. In fact the addition of carbonated lime (13.81 g kg^{-1}) increased the lactic acid amount by 169.4% up to 167.3 ± 3.1 g kg^{-1} DM (dry matter). As well the addition of the homofermentative LAB mixture slightly increased the amount of lactic acid in the silage by 12.6%.

1. Introduction

The aim of generating silage in livestock production is to preserve fresh material by natural fermentation under anaerobic conditions, whereby lactic acid bacteria (LAB) ferment water soluble carbohydrates into organic acids, mainly lactic acid (Kandler, 1983; Weinberg and Muck, 1996). This process with its four phases is extensively portrayed in scientific literature (Bolsen et al., 1996; Lindgren et al., 1985; McDonald et al., 1991; Pitt et al., 1985). In the last decades enormous research has been done on improving silage quality of a broad range of feedstock by using bacterial inoculants (e.g. *Lactobacillus plantarum*, *Lactobacillus buchneri*) (Arasu et al., 2014; Holzer et al., 2003; Mouafi et al., 2013; Weinberg et al., 1988), chemical additives (e.g., formic acid, sulphuric acid) (Henderson, 1993; Mayne, 1990; Wanapat et al., 2013) or technical advancements (e.g., temperature effects, effects of particle size, type of silo construction) (Ali et al., 2014; Aoki et al., 2013; Khaing et al., 2014; Pedroso et al., 2014). As lactic acid is the main organic acid in silages and a valuable platform chemical, the management of ensiling may be focussed on enhancing its contents with regard to a future perspective: the biorefinery. A biorefinery uses biomass as a continually renewable source to produce chemicals, bio-based materials and fuels as a substitute for petroleum (e.g., pure lactic acid can be used to produce poly lactic acid, which is an essential building block for biopolymers) (Kamm, 2007). The study by Haag et al. (2015) concluded that the content of lactic acid in maize silage can be increased by 91.9% up to a value of 133.2 ± 3.7 g kg^{-1} DM, in comparison to the standard silage without additives, using homofermentative LAB in addition with carbonated lime. Huenting et al. (Huenting et al., 2012) indeed presented a lactic acid content of up to 175 g kg^{-1} DM in maize silage by the addition of carbonated lime in high amounts (35 g $CaCO_3$ kg^{-1} fresh matter).

In consideration of the many approaches to improve certain characteristics of silages, there is still a lack of knowledge according to trace element (manganese) supplementation in the ensiling process. With particular regards to maize, known as a plant with low concentrations of trace elements due to the missing uptake from the soil, this plant appears to be auspicious for a supplementation of microorganisms with trace elements for ensiling (Kirkham, 1975). The aim of this research was to determine whether manganese (Mn) supplementation has an effect on the production of lactic acid in maize silage. Manganese is important for Mn -dependent enzymes (e.g., the lactate dehydrogenase) and as an enzyme cofactor for the superoxide dismutase. In *Lactobacillus plantarum*, Mn plays a role in following enzymatic functions: Lactate dehydrogenase, the malolactic enzyme, RNA (ribonucleic acid) polymerase,

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Table 1
Treatment variations for maize with different silage additives.

Treatment	$CaCO_3$ (g kg^{-1})	Ho[a] (g kg^{-1})	$MnSO_4$ (g kg^{-1})
Control	–	–	–
Mn 1	–	–	0.001
Mn 2	–	–	0.003
pH	13.81	–	–
Ho[a]	–	0.001	–
Mn 1 + pH	13.81	–	0.001
Mn 2 + pH	13.81	–	0.003
Mn 1 + Ho	–	0.001	0.001
Mn 2 + Ho	–	0.001	0.003
Mn 1 + Ho + pH	13.81	0.001	0.001
Mn 2 + Ho + pH	13.81	0.001	0.003

[a] Ho is a homofermentative lactic acid bacteria mixture consisting of *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Lactobacillus rhamnosus*.

Xylose isomerase, Manganicatalase, Manganisuperoxide dismutase and the NADH oxidase. Nonenzyme related Mn plays a role against oxygen damage (O_2^-) in *Lactobacillus plantarum,*which actively excludes iron and contains high levels of manganese instead (Archibald, 1986). Fitzpatrick et al. (2001) observed a beneficial effect of manganese sulfate ($MnSO_4$) addition on the fermentation of whey permeate supplemented with yeast extract by *Lactobacillus casei*. Whereat fermentations performed at addition of 0.005 g l^{-1} $MnSO_4$ gave a similar performance to an addition of 0.03 g l^{-1} $MnSO_4$. He showed that Mn has a major beneficial effect on cell growth rate and the maximum cell concentration obtained (Fitzpatrick et al., 2001). However, Fitzpatrick et al. (2001) showed the importance of Mn for optimal cell growth rate and production of lactic acid by *Lactobacillus casei*, which is an important LAB for cheese ripening but not a LAB used in common ensiling additives. Consequently the supply of lactic acid bacteria with manganese is important to guarantee high reproduction rates and the optimal generation of lactic acid. Therefore, maize was charged with $MnSO_4$ in different concentrations. Additionally the effect of Manganese in combination with a homofermentative LAB mixture (*Lactobacillus plantarum, Lactobacillus buchneri and Lactobacillus rhamnosus*) and carbonated lime was investigated owing to the results of Haag et al. (2015) which have shown that these are promising treatments for maize silage to create high lactic acid contents.

2. Material and methods

2.1. Silage additives

Different silage additives were used for treating the material (Table 1). Ho (SILASIL ENERGY.BG, Schaumann Bioenergy GmbH, Germany) is a homofermentative ensiling additive composed of *Lactobacillus plantarum*, *Lactobacillus buchneri*, and *Lactobacillus rhamnosus*. Although *Lactobacillus buchneri* is a heterofermentative LAB which produces both lactic acid and acetic acid the LAB mixture is dominantly homofermentative. *Lactobacillus buchneri* was only added to enhance the aerobic stability after opening the silos, with respect to practical scale conditions. The application amount was given by the producer. Carbonated lime was added to increase the amount of lactic acid in silage due to buffering the pH-value and hence preventing the lactobacilli from being inhibited (Huenting et al., 2012). $MnSO_4$ was added as manganese(II) sulfate monohydrate.

2.2. Silage preparation

The raw material maize (*Zea mays*) was received from a farm in the federal state of Schleswig Holstein (Germany) in the surrounding area of Neumünster. The whole plants were harvested at dough ripe stage of maturity and chopped with a precision forage harvester (Jaguar Speedstar 870, Claas, Germany) and further reduced down to 8 mm theoretical length of cut. Maize silages were prepared in 1.5 l laboratory scale glass jars (Weck, Germany). All additives were dissolved in sterile tap water and applied equally with a precision nozzle on 20 kg of the raw material. Afterwards the jars were filled with the treated raw substrate and compacted with a pneumatic compacting device to a density of 0.9 kg l^{-1}. After sealing airtight with a rubber ring, a glass lid and three brackets, the jars were stored in a tempered and dark room at a constant temperature of 20 °C. For all treatments a storage duration of 13, 27, and 90 days was defined to determine the ingredient contents over time. The jars were weighed at the beginning as well as at the end of the storage periods to determine preservation losses. All treatments and storage durations were performed in triplicates.

2.3. Analytics

Samples of the raw material and the silages were analysed before filling the jars with the raw material and directly after the specified opening dates of the jars respectively. The DM content was determined in accordance to DIN EN 12880 (German Institute for Standardization, 2001). With the loss of volatile compounds (Mukengele et al., 2006), all DM values and all referring values were corrected according to Weissbach and Strubelt (Weissbach and Strubelt, 2008). DM losses during the ensiling process were measured using the formula of Weissbach (Weissbach, 1998). The concentrations of crude ash (XA), crude protein (XP), crude fat (XL), crude fiber (XF), and the concentrations of cell wall fractions, neutral detergent fiber (NDF), acid detergent fiber (ADF) and the total content of starch were determined with near infrared spectroscopy (MPA Multi Purpose FT-NIR Analyzer, Bruker, USA) according to (Federation of German Agricultural Investigation and Research Institutes, 2007). With high performance liquid chromatography (HPLC) the contents of lactic acid, acetic acid, propionic acid, butyric acid (n-butyric acid), 1,2 Propanediol, 1,3 Propanediol, ethanol, propanol and the sum of sugars (fructose, glucose, saccharose) were determined after calibration with an adequate standard. Therefore 50 g of the sample material was combined with 250 ml distilled water in a PVC stomacher bag ("Model 400 Bags", Seward, United Kingdom) and was treated for 4 min in a stomacher (Stomacher 400, Seward, United Kingdom). After the measurement of the pH-value in the extract with an electrode (Memosens CPS31D, Endress + Hauser, Germany), 10 ml of the received extract were centrifuged for 10 min at 10.000 Rpm (Centrifuge 5415 D, Eppendorf, Germany). Proceedingly 4 ml were filtrated, using a filter with a 0.2 μm pore diameter (SPARTEN 30/A, Whatman, USA), in a PVC-test tube. The solution was diluted at the ratio of 1:1 and H_2SO_4 was added to achieve a 0.02 n H_2SO_4 solution. Subsequently the samples were placed in the autosampler of a high pressure liquid chromatograph (HPLC Smartline, Knauer Wissenschaftliche Geräte GmbH, Germany). The statistical analytics were performed using the statistical software R (R Core Team, 2012) to determine significant differences in silage characteristics for the different treatment variations. The significance test was based on Tukey's studentized range test. Correlations were based on the BLUEs, Pearson's correlation coefficient.

3. Results and discussion

3.1. Chemical composition

The chemical compositions of the fresh maize and treated silages are displayed in Table 2. The DM content of the fresh material was measured at 286 g kg^{-1}, which lies slightly below the bottom range of the optimal DM content for ensiling (Gerighausen,