Journal of Integrative Agriculture 2016, 15(7): 1592–1600

Available online at www.sciencedirect.com

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RESEARCH ARTICLE

Effect of lactic acid bacteria and propionic acid on conservation characteristics, aerobic stability and *in vitro* gas production kinetics and digestibility of whole-crop corn based total mixed ration silage

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Abstract

This study was conducted to evaluate the effect of lactic acid bacteria and propionic acid on the fermentation quality, aerobic stability and *in vitro* gas production kinetics and digestibility of whole-crop corn based total mixed ration (TMR) silage. Total mixed ration was ensiled with four treatments: (1) no additives (control); (2) an inoculant (*Lactobacillus plantarum*) (L); (3) propionic acid (P); (4) propionic acid+lactic acid bacteria (PL). All treatments were ensiled in laboratory-scale silos for 45 days, and then subjected to an aerobic stability test for 12 days. Further, four TMR silages were incubated *in vitro* with buffered rumen fluid to study *in vitro* gas production kinetics and digestibility. The results indicated that all TMR silages had good fermentation characteristics with low pH (<3.80) and ammonia nitrogen (NH_3-N) contents, and high lactic acid contents as well as Flieg points. Addition of L further improved TMR silage quality with more lactic acid production. Addition of P and PL decreased lactic acid and NH_3-N contents of TMR silage compared to the control (P<0.05). After 12 days aerobic exposure, P and PL silages remained stable, but L and the control silages deteriorated as indicated by a reduction in lactic acid and an increase in pH, and numbers of yeast. Compared to the control, addition of L had no effects on TMR silage in terms of 72 h cumulative gas production, *in vitro* dry matter digestibility, metabolizable energy, net energy for lactation and short chain fatty acids, whereas addition of PL significantly (P<0.05) increased them. L silage had higher (P<0.05) *in vitro* neutral detergent fiber digestibility than the control silage. The results of our study suggested that TMR silage prepared with whole-crop corn can be well preserved with or without additives. Furthermore, the findings of this study suggested that propionic acid is compatible with lactic acid bacteria inoculants, and when used together, although they reduced lactic acid production of TMR silage, they improved aerobic stability and *in vitro* nutrients digestibility of TMR silage.

Keywords: lactic acid bacteria, propionic acid, fermentation quality, aerobic stability, *in vitro* digestibility, total mixed ration silage

Received 21 June, 2016 Accepted 8 September, 2016

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doi: 10.1016/S2095-3119(16)61482-X

1. Introduction

Tibet is one of the five most important pastural areas in China, in which dairy cow industry is one of the main livestock industries and plays important roles in economic and social development (Chen *et al*. 2014). However, in this region,

dairy cows have long been suffering from low production performance under the traditional grazing feeding system due to the harsh environment and feed unavailability during dry and/or cold seasons. In this respect, an efficient use of feed resources that are cultivated and harvested during the rainy season might improve dairy cow production. Hence, conservation of feeds produced during the rainy season, notably as silage, is a key element in dairy cow production as it prevents nutrient deficits during periods of feed shortage. Over the past few years, total mixed ration (TMR) silage feeding system has been widely adopted by dairy cows' feedlots and cooperatives in Tibet, particularly based on whole crop corn as roughage because it has good agronomic characteristics, yields high concentrations of nutrients, ensiles well, and incorporates easily into TMR (Neylon and Kung 2003). From the production and environmental point of view, TMR silage feeding system could not only facilitate year-round fodder provision and improve the efficiency of the dairy cow production, but also alleviate the intensive grazing burden posed by livestock on the fragile alpine rangeland ecosystems. Moreover, this is also in accordance with the national policies of promoting agricultural structure regulation and establishment of intensive and large scale husbandry in Tibet. Therefore, improving the nutritional characteristics and feeding value of whole-crop corn TMR silage could have a positive impact on the profitability of dairy cow operations.

Silage conservation depends on anaerobic conditions and on the acidification that occurs when a sufficient amount of lactic acid is produced by epiphytic lactic acid bacteria present on the plant surface at harvesting (Weinberg *et al*. 2010). Hence, homofermentative lactic acid bacteria (e.g., *Lactobacillus plantarum*) are always selected for the purpose of rapidly producing lactic acid and lowering the final silage pH, to preserve nutrients by inhibiting plant enzymes and undesirable microorganisms (Schmidt and Kung 2010; Tabacco *et al*. 2011). However, homofermentative inoculants are also associated with reducing the aerobic stability or the bunk life of silages, especially for whole-crop corn and small grain silages, because of their conservation of substrate for aerobic microorganisms growth and a lack of volatile fatty acids (VFAs) production that have antifungal activity (Kleinschmit *et al*. 2005; Hu *et al*. 2009). Aerobic deterioration of silage is unfavorable as a consequence of the nutritional losses, and the proliferation of potentially pathogenic or otherwise undesirable microorganisms and mycotoxin synthesis, which can pose health hazards to animals and humans (Richard *et al*. 2009). Propionic acid has been used to inhibit yeasts that assimilate lactic acid when silages are exposed to air and thus, they improve aerobic stability (Han *et al*. 2015). Kung *et al*. (1998, 2000) found that weak organic acid with strong antifungal properties, such as propionic acid, applied at 0.2–0.3% of fresh forage weight, could improve the stability of corn silage, but this additive showed minor efficiency of fermentation. Moreover, feedback from the field suggested that some silage producers have used the practice of adding both propionic acid and homofermentive lactic acid bacteria inoculants to the same TMR silage because they realized that each often has a shortcoming (Chen *et al*. 2016), but the scientific evidence for this practice is limited. To the best of our knowledge, few studies have investigated the effect of currently available additives on fermentation quality, *in vitro* digestibility and aerobic stability of whole-crop corn based TMR silage in Tibet.

The objective of the present study was to determine the fermentation quality, aerobic stability and *in vitro* digestibility of whole-crop corn based TMR silages with or without application of lactic acid bacteria and propionic acid.

2. Materials and methods

2.1. Silage preparation

Whole-crop corn was cultivated on 18 May 2013 in the experimental field of the Grassland Station of Rikaze (29.27°N, 88.88°E, Tibet, China) on a fertilized (cow dung). Whole-crop corn was harvested at around the half milk-line stage of maturity (227 g kg^{-1} dry matter (DM)), and cut at approximately 10 cm above the ground with a sickle, and then chopped to a theoretical length of 2–3 cm with a paper cutter. As shown in Table 1, TMR was prepared with whole-crop corn and mixed concentrate (7.5% crack corn, 20% rape cake meal, 20% cotton seed, 27.5% distillers dried grains with soluble (DDGS), 20% wheat bran, 5% vitamin-mineral). The TMR (354 g kg^{-1} DM) was ensiled with four different treatments: (1) no additives (control), (2) lactic acid bacteria (*L. plantarum,* Ecosyl MTD/1, Ecosyl Products Ltd., Stokesley, North Yorkshire, UK) addition at 10^6 colony-forming units (cfu) g^{-1} (L), (3) propionic acid addition at 0.3% (P), (4) 0.3% propionic acid+10^6 cfu g^{-1} lactic acid bacteria (PL) on a fresh matter (FM) basis of TMR. Additives were diluted with deionized water to an equivalent of 10 mL kg^{-1} fresh weight and applied using a hand sprayer. An equal volume of deionized water was applied to the control TMR. The TMR material and additives were homogenized. From each treatment of TMR, samples of 0.76 kg were packed into a laboratory silos (polyethylene bottle, 2 L capacity) and compacted by hand (with the aid of a rod), ensuring expulsion of the air and to obtain a density of 380 g L^{-1}. The silos were weighed immediately before and after being filled and were then sealed with a screw top and plastic tapes, and then placed indoors at the ambient temperature (18–22°C). After 45 days of ensiling, the silos