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Impact of ferulic acid esterase producing lactobacilli and fibrolytic enzymes on conservation characteristics, aerobic stability and fiber degradability of barley silage

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ABSTRACT

The objective of this study was to determine the effects of applying ferulic acid esterase (FAE) producing inoculants or non-FAE inoculants in combination with exogenous fibrolytic enzymes (EFE) at ensiling on silage characteristics and nutritive value of whole barley crop (*Hordeum vulgare*). Barley (330 g DM/kg fresh crop) was harvested at early-dough, chopped and allocated to one of the following four treatments: untreated (Control), FAE producing *Lactobacillus buchneri* mixture inoculants (T1), none-FAE *L. buchneri* mixture inoculants (T2), and T2 plus EFE (T3). Inoculants were applied at 1×10^8 colony forming units/gram forage (cfu/g) and EFE at 424 U (xylanase) and 180 U (endoglucanase)/kg fresh weight. Forage was packed into 12 laboratory silos per treatment and ensiled for 7, 28 and 90 d. Subsamples at 90 d were used for *in vitro*, *in situ* and aerobic stability studies. After 90 d of ensiling, all inoculated silages had less ($P < 0.001$) water soluble carbohydrate, and greater ($P < 0.001$) DM loss as compared to the Control. For T1 and T2, aNDF was greater ($P = 0.003$) than Control and T3 silage. From d 0 to d 7 of ensiling, 16*S rRNA* copy numbers of *L. buchneri* in Control increased from 6.5 to 8.0 $\log_{10}$ copies/g, thereafter remaining stable, whereas it increased from 7.0 to 10.0 $\log_{10}$ copies/g in all inoculated silages, reaching ~10.5 $\log_{10}$ copies/g after 28 d of ensiling. At this time, inoculated silages also had a higher ($P < 0.001$) pH, produced more acetic acid and ethanol, but less ($P = 0.01$) lactic acid than Control. After 21-d aerobic exposure, all inoculated silages remained stable, but Control silage deteriorated as indicated by a reduction ($P < 0.001$) in lactic acid and an increase ($P < 0.001$) in pH, and numbers of yeast. The T3 silage had less ($P < 0.001$) total gas production than other silages in *in vitro* ruminal fermentation. Compared to Control, the soluble fraction (a) of DM was less ($P < 0.001$) in T1 and T2, whereas it was greater ($P < 0.001$) in T3 silage. *In situ* NDF digestibility of T1 silage was greater ($P < 0.01$) than that of Control and T3 silage at 24 and 72 h of incubation. Inoculation of whole crop barley silage with inoculants containing FAE or non-FAE producing *L. buchneri* improved ruminal NDF digestibility, but a combination of EFE with a none-FAE *L. buchneri* reduced NDF degradability.

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DM, dry matter; IVDMD, *in vitro* dry matter digestibility; FAE, ferulic acid esterase; LAB, lactic acid bacteria; aNDF, neutral detergent fiber analyzed with a heat stable amylase and expressed inclusive of residual ash; NDFD, aNDF digestibility; VFA, volatile fatty acid; WSC, cold water soluble carbohydrates.

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Table 1
Treatments, sources and composition of inoculants used in the study.

Silage	Treatment	Composition (g/power)	Source
Control	Ultrapure water		–
T1	*L. buchneri* LN4017 (ATCC PTA-6138)	1.0×10^{11} cfu[1]	Pionner Hi-Bred Ltd., Chathan, ON, Canada
	L. plantarum LP7109 (ATCC PTA-6139)	2.0×10^{10} cfu	
	L. Casei LC3200 (ATCC PTA-6135)	1.0×10^{10} cfu	
T2	*L. buchneri* LN1391 (ATCC PTA-2493)	1.0×10^{11} cfu	Pionner Hi-Bred Ltd., Chathan, ON, Canada
	L. plantarum LP286 (ATCC PTA-53187)	2.0×10^{10} cfu	
	E. faectum EF301 (ATCC PTA-55593)	1.0×10^{10} cfu	
T3	*L. buchneri* LN1391 (ATCC PTA-2493)	1.0×10^{11} cfu	Pionner Hi-Bred Ltd., Chathan, ON, Canada
	L. plantarum LP286 (ATCC PTA-53187)	2.0×10^{10} cfu	
	E. faectum EF301 (ATCC PTA-55593) Adisseo Rovabio Excel LC2[2]	1.0×10^{10} cfu	Adisseo, Alpharetta, GA

[1] cfu, colony forming units.
[2] Adisseo Rovabio Excel LC2 contains xylanase (212 μmol xylose equivalents/min/ml of enzyme) and endoglucanase (90 μmol glucose equivalents/min/ml of enzyme). Enzymatic activities were determined at pH 6.0 at 39 °C.

1. Introduction

Whole crop barley is the major annual cereal forage used for silage production for both feedlot and dairy cattle in western Canada (Juskiw et al., 2000; Strydhorst et al., 2008). Therefore, improving the nutritional characteristics and feeding value of barley silage could have a positive impact on the profitability of both beef and dairy cattle operations.

Homofermentative inoculants produce lactic acid as the principal end-product of hexose carbohydrate fermentation and are known to accelerate pH decline during the ensiling process (Lesins and Schulz, 1968). However, these inoculants are also associated with a reduction in the aerobic stability of silage owing to their conservation of water soluble carbohydrates and lack of production of acetic acid as an inhibitor of yeast and mold (Weinberg et al., 1993). To promote aerobic stability, *Lactobacillus buchneri*, a heterofermentative lactic acid bacteria (LAB) has been used to produce acetic acid, propionic acid, and other organic end-products during the ensiling process that has consistently improved the aerobic stability of small-grain silage (Kleinschmit and Kung, 2006b). However, the effect of these homofermentative and heterofermentative inoculants on fiber degradability has been inconsistent (Weinberg et al., 2007). Efforts to consistently improve fiber degradability have been made by developing inoculants that contain *L. buchneri* that produce ferulic acid esterase (FAE), a trait that has been linked to improved fiber digestibility and feed efficiency (Addah et al., 2012). The FAE can break the esterase linkages between lignin and hemicellulose and as result improve the digestibility of neutral detergent fiber in silage (Nsereko et al., 2008; Kang et al., 2009; Addah et al., 2012).

Addition of exogenous fibrolytic enzyme (*i.e.*, cellulases and xylanases) along with inoculants has been explored as another strategy to improve the digestibility of fiber in silage (Jaster and Moore, 1988; Nadeau et al., 1996; McAllister et al., 2001). To date, few studies have investigated the combination of exogenous enzymes and *L. buchneri* containing inoculants on silage fermentation (Kung and Ranjit, 2001; Lynch et al., 2014). It was hypothesized that application of non-FAE producing inoculants plus exogenous fibrolytic enzyme or FAE producing inoculants alone would improve ensiling fermentation and aerobic stability of the silage and increase fiber digestibility in the rumen. The objective of this study was to determine the effect of FAE producing inoculants or non-FAE inoculants in combination with exogenous fibrolytic enzymes on the ensiling, aerobic stability and *in situ* digestibility of whole-crop barley silage.

2. Materials and methods

2.1. Forage

Barley (*Hordeum vulgare*, L.; Agri-Core United, Winnipeg, MB, Canada) was planted on May 2012 at the Lethbridge Research Center, AB Canada (49°41′N, 112°49′W). The crop was fertilized with 125 kg N/ha pre-seeding, and 12.5 kg N/ha and 57 kg P_2O_5/ha post-seeding. Soil analysis indicated the fertilizer applied to the field satisfied nutrient requirements of crop. Whole barley crop was swathed at the early-dough stage of maturity (330–350 g/kg DM) from three locations within a single plot and chopped to a theoretical 1-cm length using a forage harvester (John Deere 6610; Moline, IL, USA). The forage from each plot location was manually mixed and four 25 kg subsamples were randomly collected for the ensiling process.

2.2. Mini silos experiment

The four subsamples from one location were spread out on separate polyethylene sheets and hand-sprayed with: (1) 75 ml (3 ml/kg fresh forage) deionized water (Control), (2) a FAE producing inoculant (1.0×10^8 cfu/kg fresh forage, T1), (3) a none-FAE producing inoculant (1.0×10^8 cfu/kg fresh forage, T2), or (4) a combination of T2 and an exogenous fibrolytic enzyme (Rovabio Excel LC2, T3). The bacteria strains of each inoculant are described in Table 1. The T3 was made from 75 ml