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Laboratory silo type and inoculation effects on nutritional composition, fermentation, and bacterial and fungal communities of oat silage

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ABSTRACT

The objectives were to evaluate (1) the use of 2 types of experimental silos (S) to characterize whole-crop oat (*Avena sativa* L.) silage with or without addition of an inoculant (I), and (2) the effect of inoculation on the microbial community structure of oats ensiled using only plastic bucket silos (BKT). From each of 6 sections in a field, oats were harvested, treated (INO) or not (CON) with inoculant, packed into 19-L BKT or vacuum bags (BG), and ensiled for 217 d. The inoculant added contained *Lactobacillus buchneri* and *Pediococcus pentosaceus* (4×10^5 and 1×10^5 cfu/g of fresh oats, respectively). The experimental design was a complete randomized design replicated 6 times. Treatment design was the factorial combination of 2 S × 2 I. Some differences existed between BG versus BKT at silo opening (217 d), including a decreased CP (7.73 vs. 7.04 ± 0.247% of DM) and ethanol (1.93 vs. 1.55 ± 0.155) and increased lactic acid (4.28 vs. 3.65 ± 0.241), respectively. Also, WSC and mold counts were reduced in BG versus BKT for CON (1.78 vs. 2.70 ± 0.162% of DM and 0.8 vs. 2.82 ± 0.409 log cfu/fresh g) but not for INO (~1.53 and 1.55), respectively. Application of INO increased DM recovery (96.1 vs. 92.9 ± 0.63%), aerobic stability (565 vs. 133 ± 29.2 h), acetic acid (2.38 vs. 1.22 ± 0.116% of DM), and reduced NDF (65.0 vs. 67.0 ± 0.57), ADF (36.7 vs. 38.1 ± 0.60), ethanol (0.63 vs. 2.85 ± 0.155), and yeast counts (1.10 vs. 4.13 ± 0.484 log cfu/fresh g) in INO versus CON, respectively. At d 0, no differences were found for S and I on the nutritional composition and background microbial counts. *Leuconostocaceae* (82.9 ± 4.27%) and *Enterobacteriaceae* (15.2 ± 3.52) were the predominant bacterial families and unidentified sequences were predominant for fungi. A higher relative abundance of the *Davidiellaceae* fungal family (34.3 vs. 19.6 ± 4.47) was observed in INO versus CON. At opening (217 d), INO had a lower relative abundance of *Leuconostocaceae* (42.3 vs. 95.8 ± 4.64) and higher *Lactobacillaceae* (57.4 vs. 3.9 ± 4.65) versus CON. Despite several differences were found between BKT and BG, both techniques can be comparable for characterizing effects of INO on the most basic measures used in silage evaluation. The use of inoculant improved oat silage quality partially by a shift in the bacterial community composition during ensiling, which mainly consisted of an increased relative abundance of *Lactobacillaceae* and reduction of *Leuconostocaceae* relative to CON.
Key words: silage, inoculant, mini-silo type, 16S and ITS1 sequencing

INTRODUCTION

Silages are an important source of forage in the United States, representing ~44.2% of the total forage harvested in 2014 (USDA, 2015). Evaluation of additives effects on silage microbial composition and nutritional value using field-scale silos is challenging because of the difficulty of producing homogeneous silage for treatment evaluation (Cherney and Cherney, 2003). On the other hand, laboratory silos provide a more complete control of the ensiling conditions and have been widely used when multiple treatments are tested (Cherney and Cherney, 2003).

Vacuum bags and fixed volume vessels are common methods for screening additives and management practices in silage research (Johnson et al., 2005). Fixed volume vessels, with some limitations, can adequately represent on-farm silos (Cherney and Cherney, 2003). On the other hand, vacuum bags are reported to have a more consistent fermentation and are easier to prepare compared with fixed volume vessels (Johnson et al., 2005; Hoedtke and Zeyner, 2011). In contrast to studies with corn (*Zea mays* L.; Cherney et al., 2004), perennial ryegrass (*Lolium perenne* L.; Johnson et al., 2005; Hoedtke and Zeyner, 2011), and red clover (*Trifolium*

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pretense L.; Johnson et al., 2005), little is known about silo type effects on nutritional value, fermentation profile, and bacterial and fungal communities of small-grain cereals such as oats (*Avena sativa* L.).

Understanding the microbial ecology of silages is critical to identify novel microorganisms related to optimal silage making and to prevent the growth of pathogens that can compromise the animal food safety chain (Driehuis, 2012; Muck, 2013; Nishino, 2015). In this context, next-generation sequencing (**NGS**) provides a practical way to conduct amplicon specific [e.g., 16S rRNA for bacteria or the internal transcribed spacer (**ITS**) unit for fungi] high-throughput sequencing studies of microbial populations coming from a wide array of environments (Adams et al., 2009), including silages (Nishino, 2015). Expanding the use of NGS techniques to silage experiments also provides the opportunity to estimate the effect of environment and management practices (e.g., type of crop, addition of inoculants, DM concentration) on microbial population shifts. Currently, only a few reports are available on the use of NGS in characterizing silage ecology. Reports include description of the microbiome of alfalfa silage (*Medicago sativa* L.) and an unspecified grass silage (McGarvey et al., 2013; Eikmeyer et al., 2013).

The objectives of the present study were to (1) compare the effect of silo type (vacuum bags vs. plastic bucket) with or without a combination inoculant on the nutritional value, fermentation profile, and population of bacteria and fungi of whole-crop oats and (2) characterize via microbial community analysis the effects of a combination inoculant on whole-crop oats silage using plastic bucket silos. We hypothesized that (1) estimates of nutritional value, chemical analysis, fermentation profiles, and the population of bacteria and fungi are similar between vacuum bags and 19-L plastic buckets and (2) adding a combination silage inoculant improves nutritional value, preservation, and aerobic stability of the ensiled oats by causing a large shift in the composition and structure of the bacterial and fungal communities compared with untreated control oat silage.

MATERIALS AND METHODS

Experimental Site, Design, and Treatments

The experimental site (~3 ha) was located at the Center for Environmental Farming Systems in Goldsboro, North Carolina (35°23′ N; 78°1′ W). Oat (cv. Brooks) was planted in clean-tilled seedbed on October 8, 2013, at a rate of 112 kg/ha. Fertilization followed the soil test and recommendation of the North Carolina Department of Agriculture and Consumer Services Soil Testing laboratory. A total of 90 kg/ha of N was split applied in halves (at planting and on March 18, 2014) using 30% liquid N solution.

Six plots were randomly located within the experimental site when oats were at 26% DM concentration and at heading stage. Oats were mowed with a New Holland 7450 disc mower/conditioner (New Holland Agriculture, Turin, Italy) to 7-cm stubble height on May 4, 2014, allowed to wilt in the field for 21 h to 45% DM concentration, and chopped to a theoretical cut length of 1.3 cm with a John Deere 3950 forage harvester (Moline, IL). Material collected from each plot (60 kg, fresh basis) was divided into 4 piles for a total of 24 piles.

Treatments were randomly assigned to one forage pile. Treatments were 2 mini-silo types (**S**) and 2 inoculations (**I**) in a 2 × 2 factorial arrangement replicated 6 times. For S, 0.3 kg (fresh basis) of chopped whole-crop oats were packed into 0.09 mm nylon-polyethylene (66 cm^3/m^2 of film per d O_2 permeability measured at 23°C and 0% relative humidity) embossed bags (15.2 × 30.5 cm, Doug Care Equipment Inc., Springville, CA), vacuumed and sealed with a Fast Vac vacuum machine (113 mmHg vacuum level, distributed by Doug Care Equipment Inc., Springville, CA; **BG**) or 8 kg (fresh basis) were packed into 19-L plastic buckets using an A-frame 11-Mg hand press and sealed with a rubber gasket lid and duct tape (186 kg of DM/m^3; **BKT**). For I, sterile double distilled water (**CON**) or inoculant (**INO**) Biotal Buchneri 500 (Lallemand Animal Nutrition, Milwaukee, WI) dissolved in the same water was applied at a rate of 1 mL/kg of fresh oats. Inoculant application followed the manufacturer's suggested dose of log 5.6 cfu/g of fresh oats for *Lactobacillus buchneri* ATCC number 40788 and log 5 cfu/g of fresh oats for *Pediococcus pentosaceus* plus fibrolytic enzymes from *Trichoderma reesei* (1,103, 3,145, and 50 mg of sugar released/min per g for β-glucanase, xylanase, and galactomannanase activities, respectively; FCC, 2015). Silos were stored at 23°C (±1°C) for 217 d, and weights were recorded individually at d 0 and 217 for determination of DM recovery following the Arriola et al. (2011) procedure.

Sampling Procedure

At d 0 and 217, samples (250 g, fresh basis) were taken from each individual replicate for the determination of nutritional composition, fermentation profile, and the bacterial and fungal population via standard plating techniques. In the case of d 0, samples were obtained immediately after treatment application. Additional sample subsets were collected only from BKT treatment to determine aerobic stability and the composition and structure of the bacterial and fungal