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Effect of ensiling time and exogenous protease addition to whole-plant corn silage of various hybrids, maturities, and chop lengths on nitrogen fractions and ruminal in vitro starch digestibility

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ABSTRACT

The objective of this study was to evaluate the effects of ensiling time and exogenous protease addition on soluble CP (% of CP), ammonia-N (% of N), and ruminal in vitro starch digestibility (ivSD) of whole-plant corn silage (WPCS) from 3 hybrids, 2 maturities, and 2 chop lengths. Samples from 3 nonisogenic hybrids [brown midrib containing the *bm3* gene mutation (BM3), dual-purpose (DP), or floury-leafy (LFY)] at 2 harvest maturities [2/3 kernel milk line (early) or 7 d later (late)] with 2 theoretical lengths of cut settings (0.64 or 1.95 cm) on a forage harvester were collected at harvest, treated with or without exogenous protease, and ensiled in triplicate in vacuum heat-sealed plastic bags for 0, 30, 60, 120, and 240 d. Thus, the experiment consisted of 120 treatments (3 hybrids × 2 maturities × 2 chop lengths × 2 protease treatments × 5 time points) and 360 mini-silos (3 replications per treatment). Vitreousness, measured by dissection on unfermented kernels on the day of harvest, averaged 66.8, 65.0, and 59.0% for BM3, DP, and LFY, respectively. A protease × maturity interaction was observed with protease increasing ivSD in late but not early maturity. Ensiling time × hybrid interactions were observed for ammonia-N and soluble CP concentrations with greater values for FLY than other hybrids only after 120 d of ensiling. Ensiling time × hybrid or protease × hybrid interactions were not observed for ivSD. Measurements of ivSD were greatest for FLY and lowest for BM3. Length of the ensiling period did not attenuate negative effects of kernel vitreousness or maturity on ivSD in WPCS. Results suggest that the dosage of exogenous protease addition used in the present study may reduce but not overcome the negative effects of maturity on ivSD in WPCS. No interactions between chop length and ensiling time or exogenous protease addition were observed for ivSD.

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INTRODUCTION

Starch digestibility of whole-plant corn silage (**WPCS**) may be affected by several factors. First, the starch endosperm is protected by the pericarp, which, if intact, is highly resistant to microbial attachment (McAllister et al., 1994); thereby, breakage of the seed coat can increase starch digestibility (Ferraretto and Shaver, 2012a,b). However, even the exposed endosperm is not fully digested due to existence of starch-protein matrices formed by the chemical bonds of zein proteins with starch granules (Kotarski et al., 1992; McAllister et al., 1993). The concentration of zein proteins in corn kernels may be affected by several factors, including nitrogen fertilization rates (Masoero et al., 2011), maturity at harvest (Ferraretto and Shaver, 2012b), fermentation (Hoffman et al., 2011), and endosperm type (Philippeau and Michalet-Doreau, 1997; Correa et al., 2002).

Recently, greater in vitro starch digestibility (**ivSD**) was observed for WPCS stored for a longer length of time (Der Bedrosian et al., 2012; Ferraretto et al., 2015d). The greater concentration of soluble CP (% of CP) and ammonia-N (% CP) for WPCS with extended ensiling time in these trials imply the occurrence of proteolysis of zein proteins (Hoffman et al., 2011) or an effect of fermentation on particle size (Ferraretto et al., 2015a). Although improvement in ruminal ivSD with extended ensiling time is well established, its effects on WPCS of various hybrids, maturity, and chop length are not well understood. When WPCS was harvested at 41% DM, brown midrib containing the *bm3* gene mutation (**BM3**) WPCS had similar ruminal ivSD compared with conventional WPCS as fresh forage but lower ivSD when compared after 45 to 360 d of ensiling (Der Bedrosian et al., 2012). In contrast, these authors observed that when WPCS was harvested at 32% DM the BM3 took 270 d to reach the same ivSD as conventional WPCS. To our knowledge, no studies

have evaluated the effects of ensiling time on WPCS of various chop lengths.

Addition of exogenous protease to WPCS at ensiling has increased ivSD (Young et al., 2012). Windle et al. (2014) reported increased ivSD with both early and late maturity WPCS. However, this is the sole study found in the literature evaluating exogenous protease addition at ensiling in WPCS of varied maturities. Perhaps exogenous protease addition at ensiling to WPCS of varied hybrids may attenuate the negative effects of vitreous endosperm on starch digestibility, but to our knowledge no such studies have been published.

Although the effects of ensiling time and exogenous protease on ivSD are well established, their use as a tool to attenuate the negative effects of maturity, endosperm type, and lack of kernel processing is uncertain. Therefore, the objective of the present study was to evaluate the effects of ensiling time and exogenous protease addition on soluble CP (% of CP), ammonia-N (% of N), and ivSD in WPCS of various hybrids, maturities, and chop lengths. We hypothesized that ensiling WPCS for an extended time or adding an exogenous protease at ensiling would attenuate the differences in ivSD between various hybrids, maturities, and chop lengths.

MATERIALS AND METHODS

Silage Production and Treatments

A brown midrib containing the *bm3* gene mutation (**BM3**; F2F485, Mycogen Seeds, Dow AgroSciences LLC, Indianapolis, IN), a dual-purpose (**DP**; P960, DuPont Pioneer, Johnston, IA) and an experimental leafy-floury (**LFY**; GLF97, Glenn Seed Ltd., Blenheim, ON, Canada) WPCS hybrid were grown in separate field plots (6.5 ha planted at 69,000 seeds/ha for LFY, 5.4 ha planted at 86,000 seeds/ha for DP, and 5.8 ha planted at 79,000 seeds/ha for BM3) at the University of Wisconsin–Madison Agricultural Research Station (Arlington, WI) under the same tillage, fertilizer application, and weed control practices for a companion feeding trial (Ferraretto et al., 2015b). Plant population, reported as seeds planted, was targeted as recommended by respective seed company representatives. The 3 hybrids were harvested at 2 maturities, two-thirds kernel milk line (early) and 7 d later (late), and whole-plants were chopped at 2 theoretical lengths of cut (**TLOC**) settings [0.64-cm (short) or 1.95-cm (long)] on the self-propelled forage harvester (JD 6910, John Deere, Moline, IL) fitted with a conventional kernel processor (2-mm roll gap). Harvest dates were September 7, 10, and 14, 2012, for LFY, DP, and BMR, respectively, at early maturity based on kernel milk line and 7 d later at late maturity stage. The day of harvest, samples of the 3 hybrids, at both maturities and for both chop lengths, were treated with an experimental exogenous bacterial protease produced in *Bacillus licheniformis* (guaranteed protease activity against *p*-nitroaniline of 75,000 PROT units/mL; DSM Nutritional Products, Basel, Switzerland/Novozymes, Bagsvaerd, Denmark) or with an equivalent amount of distilled water that served as a control. Exogenous protease was added at a rate of 1.825 mL (1,825 mg) of protease per kg of corn DM to PROT treatments, which is equivalent to 136.9 PROT units per kg of WPCS of DM. One PROT unit is the amount of enzyme that releases 1 μmol of *p*-nitroaniline from 1 μ*M* of substrate (Suc-Ala-Ala-Pro-Phe-pNA) per minute at pH 9.0 and 37°C. This dosage matched supplier recommendations and was previously demonstrated to increase ivSD of rehydrated and high-moisture corn (Ferraretto et al., 2015c). Samples were vacuum sealed in triplicate in nylon-polyethylene standard barrier vacuum pouches (3.5-mil thickness, 25.4 × 35.6 cm; Doug Care Equipment Inc., Springville, CA) containing 600 g of as-fed WPCS using an external clamp vacuum machine (Bestvac; distributed by Doug Care Equipment Inc., Springville, CA) for each time point and ensiled for 0, 30, 60, 120, and 240 d. Thus, the experiment consisted of 120 treatments (3 hybrids × 2 maturities × 2 chop lengths × 2 protease treatments × 5 ensiling time points) and 360 mini-silos (3 replications per treatment). Mini-silos were stored at room temperature (approximately 20°C) in the dark until reaching the targeted ensiling time. After the ensiling time was reached, the bags were immediately frozen and stored at −20°C to stop fermentation until being processed for analysis. All samples, including 240 d, were frozen for at least 21 d to ensure protocol similarity among all samples. Prior to exogenous protease addition and ensiling, 2 sub-samples for each hybrid at both maturities and each chop length were collected and immediately frozen for further physical and nutrient characterization. Throughout the manuscript these sub-samples will be referred to as fresh samples, whereas samples ensiled but frozen immediately will be referred to as d 0 or whole-plant corn fresh forage.

Fermentation Profile, Physical Characteristics, Nutrients, and Digestibility Analysis

Fresh samples were analyzed undried and unground for particle size as described by Kononoff et al. (2003), whereas dried (at 60°C for 48 h in a forced-air oven) and unground samples were analyzed for corn silage processing score (Ferreira and Mertens, 2005) at the University of Wisconsin–Madison. Samples were composited by hybrid and maturity and sent to Dairyland