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Lactational performance, rumen fermentation, and enteric methane emission of dairy cows fed an amylase-enabled corn silage

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ABSTRACT

This study investigated the effects of an amylase-enabled corn silage on lactational performance, enteric CH_4 emission, and rumen fermentation of lactating dairy cows. Following a 2-wk covariate period, 48 Holstein cows were blocked based on parity, days in milk, milk yield (MY), and CH_4 emission. Cows were randomly assigned to 1 of 2 treatments in an 8-wk randomized complete block design experiment: (1) control corn silage (CON) from an isogenic corn without α-amylase trait and (2) Enogen hybrid corn (Syngenta Seeds LLC) harvested as silage (ECS) containing a bacterial transgene expressing α-amylase (i.e., amylase-enabled) in the endosperm of the grain. The ECS and CON silages were included at 40% of the dietary dry matter (DM) and contained, on average, 43.3 and 41.8% DM and (% DM) 36.7 and 37.5% neutral detergent fiber, and 36.1 and 33.1% starch, respectively. Rumen samples were collected from a subset of 10 cows using the ororuminal sampling technique on wk 3 of the experimental period. Enteric CH_4 emission was measured using the GreenFeed system (C-Lock Inc.). Dry matter intake (DMI) was similar between treatments. Compared with CON, MY (38.8 vs. 40.8 kg/d), feed efficiency (1.47 vs. 1.55 kg of MY/kg of DMI), and milk true protein (1.20 vs. 1.25 kg/d) and lactose yields (1.89 vs. 2.00 kg/d) were increased, whereas milk urea nitrogen (14.0 vs. 12.7 mg/dL) was decreased, with the ECS diet. No effect of treatment on energy-corrected MY (ECM) was observed, but a trend was detected for increased ECM feed efficiency (1.45 vs. 1.50 kg of ECM/kg of DMI) for cows fed ECS compared with CON-fed cows. Daily CH_4 emission was not affected by treatment, but emission intensity was decreased with the ECS diet (11.1 vs. 10.3 g/kg of milk, CON and ECS, respectively); CH_4 emission intensity on ECM basis was not different between treatments. Rumen fermentation, apart from a reduced molar proportion of butyrate in ECS-fed cows, was not affected by treatment. Apparent total-tract digestibility of nutrients and urinary and fecal nitrogen excretions, apart from a trend for increased DM digestibility by ECS-fed cows, were not affected by treatment. Overall, ECS inclusion at 40% of dietary DM increased milk, milk protein, and lactose yields and feed efficiency, and tended to increase ECM feed efficiency but had no effect on ECM yield in dairy cows. The increased MY with ECS led to a decrease in enteric CH_4 emission intensity, compared with the control silage.

Key words: amylase-enabled corn silage, enteric methane, dairy cow

INTRODUCTION

Corn silage is a fundamental component of lactating cow diets, with more than 132 million tons produced in 2019 in the United States (USDA National Statistics Service, 2020). Corn silage is a predominant forage source, as it commonly constitutes more than 30% of dietary DM in lactating cow diets in central Pennsylvania and the United States (Jordan and Fourdraine, 1993; Hristov et al., 2015). Moreover, corn silage is a high energy-density feed and yields more DM per hectare than any other forage (USDA National Statistics Service, 2020). Dairy farmers and corn growers strive to produce high-quality corn silage to meet the requirements of high-producing dairy cows. Therefore, developing novel methods and techniques that enhance the nutritive value of corn silage is essential to optimize forage utilization by the cow and achieve greater production efficiencies.

It has been shown that some exogenous enzymes are resistant to ruminal degradation, and their supplementation could aid ruminal bacteria in converting nutrients into animal product (Hristov et al., 1998). Extensive research has been done with feeding exogenous fibrolytic

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enzymes, but supplementation of exogenous amylases has been less investigated. Nevertheless, some studies have demonstrated positive effects on feed efficiency of lactating cows by increasing milk yields (**MY**), reducing DMI, or a combination of both when amylase was supplemented in the diet (Klingerman et al., 2009; Gencoglu et al., 2010; Andreazzi et al., 2018). Nozière et al. (2014) reported that amylase supplementation can improve ruminal degradation of dietary starch. In some dietary situations, this could benefit cow productivity, as it has been suggested that degradation of starch in the small intestine can be hindered by a limited capacity of the pancreas to secrete α-amylases (Huntington et al., 2006). Increases in production efficiencies when supplementing exogenous enzymes could also have potential benefits in reducing enteric CH_4 emission yield and intensity (Hristov et al., 2013). However, responses to amylase supplementation have been inconsistent, as some studies have shown negligible effects on lactational performance of dairy cows (Ferraretto et al., 2011; Weiss et al., 2011).

Enogen brand corn hybrids (Syngenta Seeds LLC) were originally developed by Syngenta to improve corn ethanol production efficiency. These hybrids are characterized by the presence of a bacterial transgene expressing high levels of thermotolerant α-amylase (i.e., amylase-enabled) in the endosperm of the grain. The gene coding for this specific amylase enzyme (AMY797E) is linked to the maize gamma-zein promoter, which causes the protein to be produced and stored primarily in the starchy endosperm of Enogen grain during crop growth, without alteration of the starch or any other nutritional component of the grain (APHIS, 2011). The enzyme is characterized as a liquefying endo-amylase, with the ability to cleave α-1,4-glycosidic bonds within the inner parts of amylopectin molecules in a random-attack pattern, resulting in the production of an array of linear and branched oligosaccharides (Atichokudomchai et al., 2006). Feeding experiments with Enogen corn in growing steers and finishing beef cattle have been inconclusive in terms of production responses. Some have reported improved feed efficiency when feeding Enogen corn as silage or grain source or both (Baker et al., 2019; Johnson et al., 2019), whereas similar studies have reported only marginal responses (Schoonmaker et al., 2014; Brinton et al., 2020a,b). To the best of our knowledge, the current experiment and that of Rebelo et al. (2020) are the only studies that have investigated the effect of Enogen fed either as grain or as silage in dairy cattle diets. Ensiling Enogen corn could offer the dual benefit of an in-silo fermentation and potential ruminal and postruminal effects of exogenous amylase, thus further enhancing nutrient utilization in dairy cattle. Therefore, the objective of this study was to determine the effect of feeding Enogen corn silage (**ECS**) on the lactational performance, rumen fermentation, and enteric gas emission of dairy cows. Our hypothesis was that feeding ECS would exhibit beneficial effects on lactational performance, enteric gas emission, and rumen fermentation in lactating dairy cows.

MATERIALS AND METHODS

Crop and Silages

The ECS hybrid (E109R3-3000GT-EVT3) and its isogenic counterpart (NK0929-3122-EZ1), without the amylase trait, were provided by Syngenta Seeds LLC. Both hybrids were planted on June 1, 2018, grown in Centre County, Pennsylvania, and harvested on September 9, 2018. During these months, average temperature was 20.1°C, with a maximum of 25.2°C and a minimum of 14.8°C, according to the National Oceanic and Atmospheric Administration (NOAA, 2021). Both crops were planted with a John Deere 1755 no-till planter (John Deere Co.) in 2.02- and 1.01-ha fields. Fields used were 3.75 km apart and were planted to wheat the year before; soil tests indicated silt loam textures, and all P and K requirements were met through historic and current manure applications. The target rate for N application was 185 kg/ha. Corn hybrids were planted with 76-cm row spacing, and seeding population at planting was 79,040 plants/ha. Corn harvest was conducted using a John Deere 6750 self-propelled forage harvester (John Deere Co.). The Farm Operations and Services of the Pennsylvania State University reported that the ECS and CON hybrids were harvested at, on average, 42.2 and 43.4% DM, respectively. Silages were inoculated at a target rate of 0.23 kg/t with Silo King (Agriking) through an applicator on the harvester and ensiled in 3.0-m-diameter plastic silage bags (Up North Plastics). Silo King is a lactic acid bacteria–based inoculant containing 1.65×10^7 cfu/g, based on manufacturer specifications. Both corn hybrids were grown for approximately 130 d, and silages were fermented for approximately 220 d before the beginning of the animal experiment in April 2019.

Animals and Diets

All animals involved in the experiment were cared for according to the guidelines approved by The Pennsylvania State University's Institutional Animal Care and Use Committee. The study was conducted with a total of 48 primi- (25) and multiparous (23) Holstein cows averaging (±SD) DIM 78.81 ± 31.04 d and MY 44.31 ± 10.58 kg/d at the beginning of the covariate period. Cows were housed at The Pennsylvania State Universi-

ty's Dairy Teaching and Research Center freestall barn, equipped with a Calan Broadbent Feeding System (American Calan Inc.) for individual cow monitoring of DMI. Two GreenFeed units (C-Lock Inc.) were used for enteric gas emission measurements.

The experiment was a randomized complete block design with a 2-wk covariate period at the beginning of the study, followed by a 2-wk treatment adaptation period, and a 6-wk experimental period. During the covariate period, cows assigned to the control treatment averaged (mean ± SD) BW 624.4 ± 57 kg, MY 42.6 ± 8.9 kg/d, DMI 25.4 ± 3.71 kg/d, milk fat 3.84 ± 0.51%, milk true protein 2.92 ± 0.26%, and CH_4 emission 391.7 ± 47 g/d; whereas cows assigned to the ECS treatment averaged 619 ± 64.5 kg, 43.4 ± 8.5 kg/d, 25.2 ± 3.82 kg/d, 3.75 ± 0.52%, 2.82 ± 0.25%, and 397.8 ± 57 g/d, respectively. Cows were blocked into 24 blocks of 2 cows each based on parity, DIM and MY, and CH_4 emission during the covariate period. Cows had free access to drinking water, and diets were fed from a Rissler model 1050 TMR mixer (I. H. Rissler Mfg. LLC). Feeding was once daily at approximately 0900 h, and feed was offered for ad libitum intake to approximately 10% refusals. The doors in the Calan Broadbent Feeding System were checked daily to prevent feed waste and ensure consistent intakes by the animals. The cows were milked twice daily at approximately 0645 and 1745 h.

Two different diets were fed to the cows as TMR. The treatment diet included ECS at 40% of dietary DM, whereas the control diet (**CON**) included silage from the genetically nearly identical isoline hybrid, without the amylase trait, also at 40% of dietary DM. Diets were formulated to meet or exceed the NE_L and MP requirements of cows producing 44 kg/d of milk containing 3.50% fat and 3.15% true protein at 26 kg/d of DMI, according to NRC (2001).

Sampling and Measurements

Diet and Feed Ingredients. The amount of feed offered and refused was weighed individually and recorded daily for each cow at the time of feeding to measure daily as-fed intake during the entire experiment. The weekly DM content of the TMR and refusals were used to calculate daily DMI. Samples of the concentrate feeds were collected weekly, and samples of the forages, TMR, and refusals were collected twice weekly and stored at −20°C. Feed samples were later dried for 72 h at 55°C in a forced-air oven and ground in a Wiley mill (Thomas Scientific) through a 1-mm sieve for further analyses. Samples of corn silages used in the experiment were collected weekly throughout the adaptation and experimental periods for 4 consecutive days each week. Corn silage samples were immediately refrigerated after collection, composited every 2 d, and shipped fresh on ice for chemical composition and fermentation analyses (Table 1 and Table 2, respectively) at Cumberland Valley Analytical Services (**CVAS**; Waynesboro, PA) and Rock River Laboratory (**RRL**; Watertown, WI). Sample DM, CP, ADF, and NDF were analyzed by wet chemistry at CVAS and by near infrared (NIR) at RRL; TDN and NFC of the silages were calculated based on their nutrient analyses (CVAS). Analyses for fermentation acids and NH_3-N concentrations were performed via wet chemistry at RRL, whereas lignin, undigested NDF at 240-h (**uNDF**), and water-soluble carbohydrates were determined by NIR. Corn silage starch digestibility was analyzed using an in vitro procedure at CVAS (Goering and Van Soest, 1970; Richards et al., 1995) and an in situ procedure at RRL (Goeser, 2016). Nutrient compositions of the silages obtained from RRL were used to calculate their organic matter digestibility index (**OMDI**; Penn State Extension, 2020). The specific amylase protein in ECS was confirmed to be present in ECS using a commercially available qualitative test kit (QuickStix for Enogen, EnviroLogix Inc.), whereas the CON silage tested negative for the protein. Composited silage samples collected throughout the experiment (i.e., ensiled for approximately 220 to 300 d) were analyzed for amylase activity using commercially available kits (Phadebas Amylase Test, Phadebas Inc.; incubated at 37°C for 24 h). Separate samples of each silage were collected biweekly for particle size analysis using the Penn State Particle Separator with 19-, 8-, and 4-mm sieves, following the guidelines for corn silage, according to Heinrichs and Kononoff (2002). Face temperature of both silages was measured weekly after feed-out (0900 h) with a Reotemp Heavy Duty Digital Compost stem thermometer. At the end of the animal experiment, 3 kg of each corn silage were collected in 20-L plastic buckets, in triplicate, for analysis of silage aerobic stability (Table 2). The buckets were covered with a double layer of cheesecloth and kept at a room temperature of approximately 23°C and exposed to air for an average of 266 h (Kung et al., 1998). Temperature of each container was recorded hourly (McAllister et al., 1998) using a DS1922L-F5 Thermochron iButton 8K probe (OnSolution Pty Ltd.). Silage samples were shipped fresh, on ice, for mold and yeast enumeration at CVAS (method 997.02; AOAC International, 1997). Samples of the other dietary forage sources and concentrate feeds were composited (on equal DM weight basis) to form one composite sample for the entire experimental period and submitted to CVAS for wet chemistry analyses of CP (method 990.03; AOAC International, 2000), amylase-treated NDF (Van Soest et al., 1991), ether extract (method 2003.05; AOAC International, 2006), ADF (method 973.18; AOAC International,

2000), ash (method 942.05; AOAC International, 2000), minerals (method 985.01; AOAC International, 2000), and estimated NFC (NRC, 2001). Starch was analyzed as described in Hall (2009). The nutrient compositions of the diets (i.e., CP, NDF, ADF, ether extract, starch, ash, Ca, and P) were calculated by using the analyzed composition of the individual feed ingredients and their inclusion in the TMR (Table 3). Balances of RDP, RUP, and NE_L and MP were estimated based on NRC (2001) using average DMI, MY, milk composition, and BW of the cows during the experiment.

Milk Production and Composition. Milk production of the cows was recorded daily at each milking. Milk samples were collected from 2 consecutive milkings (p.m. and a.m.) biweekly during the experimental period. One aliquot of each sample was placed in tubes with a preservative (2-bromo-2-nitropropane-1,3-diol) and submitted to Dairy One Cooperative Inc. (Ithaca, NY) for analysis of milk fat, true protein, lactose, SCC, and MUN using infrared spectroscopy (MilkoScan 4000, Foss Electric). Milk composition data were weighed for the corresponding average MY during p.m. and a.m. milkings. The averaged MY and DMI during each experimental week were used to calculate milk fat, true protein, lactose, and ECM yields; ECM was calculated according to Sjaunja et al. (1990). Separate milk samples were collected during experimental wk 1, 2, and 5, placed in tubes without preservative, and stored at −20°C until composited and analyzed for milk fatty acid (**FA**) composition as described in Rico and Harvatine (2013). For more details on these analyses, see the supplemental material (Supplemental File S1, Supplemental Tables S1–S4; https://doi.org/10.26208/am92-yn24; Cueva et al., 2021).

Body Weight and Body Weight Change. Cow BW was recorded twice daily upon exiting the milking parlor, using an AfiFarm 3.04E scale system (SAE Afikim). Body weight change was calculated as the difference between the average BW during experimental wk 5 and 6, minus the average BW during wk 2 of the covariate period divided by the days on study.

Table 1. Nutrient composition mean ± SE (% of DM or as indicated) of control (CON) and Enogen (ECS, Syngenta Seeds LLC) corn silages

Item	Corn silage[1]	
	CON	ECS
Chemical analyses		
DM, % of as fed	41.8 ± 3.68	43.3 ± 3.56
CP[2]	8.10 ± 0.094	7.59 ± 0.095
NH_3-N[3]	0.73 ± 0.060	0.60 ± 0.062
ADF[2]	22.9 ± 0.35	22.0 ± 0.43
aNDF[2,4]	37.5 ± 0.45	36.7 ± 0.59
30-h NDF digestibility, % NDF[2]	52.9 ± 0.63	54.3 ± 0.59
Lignin[5]	4.19 ± 0.099	3.90 ± 0.130
Undigested NDF, 240-h[5]	10.4 ± 0.57	8.90 ± 0.68
Starch[6]	32.0 ± 0.75	35.2 ± 0.84
Starch[3]	34.1 ± 0.99	37.0 ± 1.36
NFC[5]	48.8 ± 0.60	50.4 ± 0.67
Water-soluble carbohydrates[5]	4.68 ± 0.369	4.59 ± 0.309
7-h starch digestibility, % starch[7]	78.7 ± 2.88	75.5 ± 1.64
7-h starch digestibility, % starch[8]	86.0 ± 2.02	85.9 ± 1.55
Amylase activity, μkat/L	0.55 ± 0.13	7.36 ± 0.70
Ash[2]	4.30 ± 0.169	4.02 ± 0.136
Ca[2]	0.19 ± 0.007	0.18 ± 0.007
P[2]	0.24 ± 0.007	0.23 ± 0.005
Mg[2]	0.14 ± 0.004	0.13 ± 0.003
K[2]	1.09 ± 0.024	1.05 ± 0.031

[1]Samples of both silage treatments were collected weekly throughout the experiment for 4 consecutive days each week. The samples were composited and analyzed by Cumberland Valley Analytical Services Inc. (Waynesboro, PA) and by Rock River Laboratory (Watertown, WI). Pooled data from both laboratories are presented in table.

[2]Average measurements of samples analyzed by wet chemistry (Cumberland Valley Analytical Services Inc. and Rock River Laboratory), n = 32 (n represents number of observations used in the statistical analysis).

[3]Analyzed by wet chemistry at Rock River Laboratory, n = 16.

[4]aNDF = amylase-treated NDF.

[5]Analyzed by near-infrared analysis at Rock River Laboratory, n = 16.

[6]Analyzed by wet chemistry at Cumberland Valley Analytical Services Inc., n = 16.

[7]Samples analyzed in vitro at Cumberland Valley Analytical Services Inc., n = 14.

[8]Samples analyzed in situ at Rock River Laboratory, n = 16.

Table 2. Fermentation characteristics and aerobic stability means ± SE (% of DM or as indicated) of control (CON) and Enogen (ECS, Syngenta Seeds LLC) corn silages

Item	Corn silage[1]	
	CON	ECS
pH[2]	3.85 ± 0.021	3.89 ± 0.028
Fermentation[3]		
Lactic acid	5.09 ± 0.206	4.77 ± 0.361
Acetic acid	1.08 ± 0.064	0.93 ± 0.043
Temperature, °C		
Silage face[4]	24.7 ± 0.44	24.1 ± 0.45
Aerobic exposure[5]	32.7 ± 0.42	33.0 ± 0.43
Ambient difference[6]	10.3 ± 0.42	10.6 ± 0.42
Mold,[7] × 10^5 cfu/g	45.5 ± 19.13	5.35 ± 5.32
Yeast,[7] × 10^9 cfu/g	3.96 ± 1.133	6.85 ± 1.054

[1]Samples of both silage treatments were collected weekly throughout the experiment for 4 consecutive days each week. The samples were composited and analyzed by Cumberland Valley Analytical Services Inc. (Waynesboro, PA) and by Rock River Laboratory (Watertown, WI). Pooled data from both laboratories are presented in table.

[2]Read with a combination pH electrode (Cumberland Valley Analytical Services Inc. and Rock River Laboratory), n = 32 (n represents number of observations used in the statistical analysis).

[3]Analyzed by wet chemistry at Rock River Laboratory, n = 16.

[4]Temperature was recorded after both silages were fed out (daily at 0900 h) with a Reotemp Heavy Duty Digital Compost stem thermometer every week during sample collection, n = 64.

[5]Average silage temperature of both silages after 266 h of aerobic exposure as part of an aerobic stability test, n = 544.

[6]Average silage temperature of both silages after aerobic exposure relative to the average room temperature [Σ(treatment silage temperature − room temperature)/n of treatment].

[7]For details, see Materials and Methods; n = 32.

Enteric Gas Emissions. During the covariate and experimental periods, 2 GreenFeed units were permanently available for individual cows to visit, and, during visits, enteric gas emissions (CH_4, CO_2, and H_2) were measured. Details of the procedure were given in Melgar et al. (2021). A pelletized bait feed (Stocker Grower 14, Purina Animal Nutrition LLC) was used to attract the cows to the GreenFeed units, and the weight of pellets dispensed was recorded and included in the daily DMI estimation. Cows were allowed a maximum of 6 visits in 24 h, with 4-h intervals between visits, and no more than 12 feed drops of approximately 33 g each per visit. Cows were identified with a radio-frequency identification ear tag and were adapted to GreenFeed before the beginning of the experiment. The GreenFeed units were calibrated daily, and CO_2 recovery tests were performed monthly following the manufacturer's recommendations (http://greenfeed.c-lockinc.com). Emissions were measured during the covariate period and throughout the adaptation and experimental periods and averaged weekly. A total of 288 observations, including a total of 17,136 GreenFeed visits (an average of 5 ± 1.1 visits/cow per day), were collected and processed for this experiment. Weekly average DMI and milk and ECM yields were used to estimate weekly averages of CH_4 and CO_2 yields (i.e., g/kg of DMI) and intensity (i.e., g/kg of milk or ECM).

Table 3. Ingredient and nutrient composition of the diets fed to the cows during the experiment

Item	Diet[1]	
	CON	ECS
Ingredient, % of DM		
Control corn silage	40.0	—
Enogen corn silage	—	40.0
Alfalfa haylage[2]	15.4	15.4
Straw-hay mix	3.8	3.8
Cottonseed, whole	5.2	5.2
Corn grain, finely ground[3]	13.8	13.8
Canola meal	13.6	13.6
SoyPLUS[4]	4.7	4.7
Molasses[5]	2.0	2.0
Vitamin and mineral premix[6]	1.5	1.5
Composition, % of DM (or as indicated)		
CP[7]	16.7	16.5
RDP[8]	9.7	9.4
RUP[8]	7.0	7.1
NDF[9]	33.9	33.6
ADF[9]	22.9	22.6
Ether extract[7]	3.39	3.63
NFC[8]	44.1	44.4
Starch[7]	24.0	25.2
Ca[7]	0.72	0.71
P[7]	0.42	0.42
NE_L,[8] Mcal/kg	1.51	1.52
NE_L balance,[9] Mcal/d	0.6	0.0
MP balance,[9] g/d	235	136

[1]Average diet composition for adaptation and experimental periods (i.e., wk 2 to 8). CON = control; ECS = Enogen corn silage, Syngenta Seeds LLC.

[2]Haylage was 47.7% DM and contained (on DM basis): 19.9% CP and 44.8% NDF.

[3]Corn grain was 89.2% DM and contained (on DM basis): 7.70% CP and 73.1% starch.

[4]SoyPLUS is a soybean meal product (Landus Cooperative).

[5]Liquid molasses from Westway Feed Products.

[6]The mineral and vitamin premix (Cargill Animal Nutrition, Cargill Inc.) contained (as-is basis) trace mineral mix, 0.86%; MgO (56% Mg), 8.0%; NaCl, 6.4%; vitamin ADE premix (Cargill Animal Nutrition, Cargill Inc.), 0.48%; limestone, 37.2%; selenium premix (Cargill Animal Nutrition, Cargill Inc.), 0.07%; and dry corn distillers grains with solubles, 46.7%. Ca, 14.1%; P, 0.39%; Mg, 4.59%; K, 0.44%; S, 0.39%; Se, 6.91 mg/kg; Cu, 362 mg/kg; Zn, 1,085 mg/kg; Fe, 186 mg/kg, vitamin A, 276,717 IU/kg; vitamin D, 75,000 IU/kg; and vitamin E, 1,983 IU/kg.

[7]Values calculated using the chemical analysis (Cumberland Valley Analytical Services Inc.) of individual feed ingredients and their inclusion rate in the diets.

[8]Estimated based on NRC (2001) by Cumberland Valley Analytical Services Inc.

[9]Estimated based on NRC (2001) using actual DMI, milk yield, milk composition, and BW of the cows throughout the experiment.

Ruminal Contents. Rumen samples were collected from 10 cows (5 CON and 5 ECS-fed cows) on d 3 of wk 3 of the experimental period (i.e., following 2 wk of adaptation to treatment diets), 3 h after feeding, using the ororuminal tubing technique (Lage et al., 2020). Briefly, the sampling device consisted of a 244-cm-long polyethylene orogastric tubing with a 15-mL perforated conical tube attached. An electric vacuum pump (Gast model 0823-v131q-g608nex, Septic Solutions Inc.) was used to obtain rumen contents. During the sampling event, rumen fluid was collected by placing an oral speculum in the mouth of the animal and pushing the orogastric tube down the esophagus, through the fiber mat, into the rumen. After discarding the first 200 mL to avoid saliva contamination, approximately 500 mL of rumen contents were collected for further analyses. Rumen contents were filtered through 2 layers of cheesecloth and immediately analyzed for pH (59000-60 pH Tester, Cole-Parmer) and processed for VFA (Yang and Varga, 1989), NH_3 (Chaney and Marbach, 1962), and total protozoal count analyses (Hristov et al., 2011). Aliquots of rumen samples were stored frozen in a −80°C freezer for bacterial population analysis. For more details on these analyses, see Supplemental File S1 (https://doi.org/10.26208/am92-yn24; Cueva et al., 2021). Separate aliquots of whole ruminal contents samples were frozen at −20°C, then transferred to a −80°C freezer, and later analyzed for α-amylase activity against insoluble wheat starch. Samples were thawed and kept on ice at all times for further processing. After thawing, samples were sonicated for 45 s (Ultrasonic Cleaner, W. W. Grainger), centrifuged at 38,300 × *g* (10 min; 4°C), and the supernatant was

analyzed for α-amylase activity as described in Hristov et al. (1998).

Apparent Total-Tract Digestibility and Nitrogen Utilization. Spot urine and fecal samples (approximately 300 mL and 500 g per sample, respectively) were collected 3 and 8 h after feeding for 2 d during experimental wk 4 for estimation of N utilization and apparent total-tract digestibility of dietary nutrients. A full description of the urine and fecal sample processing and analyses can be found in Lee et al. (2012) and Oh et al. (2013). Briefly, raw urine from each sampling was acidified, diluted, and composited by cow, and the diluted samples were frozen at −20°C for later analysis of allantoin, uric acid, creatinine, urea, and total N. Allantoin was analyzed following the procedure by Chen et al. (1992). Stanbio Laboratory kits were used to analyze uric acid (Uric Acid Kit 1045), creatinine (Creatinine Kit 420), and urinary urea N (Urea Nitrogen Kit 580). Total N was analyzed in freeze-dried, 1:10 diluted, and acidified urine samples using a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technologies Inc.). Daily volume of excreted urine was estimated based on urinary creatinine concentration, assuming a creatinine excretion rate of 29 mg/kg of BW based on unpublished total urine collection data from Hristov et al. (2011). Daily total N, urinary urea N, and purine derivatives excretions were calculated using the estimated urine output.

Fecal samples were oven-dried at 65°C, ground through a 1-mm screen in a Wiley mill (Thomas Scientific), and analyzed for DM, OM, CP, starch, NDF, and ADF. Analysis of OM was conducted by ashing the TMR samples for 4 h at 600°C. A Mixer Mill MM 200 (Retsch GmbH) was used to pulverize a 0.5-g aliquot of fecal sample for CP analysis (N × 6.25) using the Costech ECS 4010 C/N/S elemental analyzer. The NDF and ADF were analyzed with an Ankom 200 fiber analyzer (Ankom Technology Corp.) based on the procedures of Van Soest et al. (1991) using α-amylase and sodium sulfite in the NDF analysis. Starch analysis of fecal DM was according to Hall (2009). Apparent total-tract digestibility of nutrients was estimated using indigestible NDF as an intrinsic digestibility marker (Schneider and Flatt, 1975). Fecal and TMR samples were analyzed for indigestible NDF after a 12-d ruminal incubation in situ, according to Huhtanen et al. (1994), with the exception that filter bags of 25-μm pore size (F57; Ankom Technology) were used for the rumen incubation (Lee et al., 2012).

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS, version 9.4 (SAS Institute Inc.). Descriptive statistics of the nutrient composition of the silages were calculated using the PROC MEANS procedure of SAS. The animal data were analyzed as a randomized complete block design experiment. Data were tested for normality using the UNIVARIATE procedure of SAS and were processed for outlier identification using PROC REG of SAS, based on an absolute studentized residual value >3. Log-transformed data were analyzed when the W statistic of the Shapiro-Wilk test was less than 0.05 (i.e., SCC, protozoal counts, and rumen microbial diversity data). The statistical model for production (DMI, MY, milk composition, BW, feed efficiency, and ECM feed efficiency) and CH_4 emission data included the fixed effects of treatment, week-period, treatment × week-period interaction, and a covariate measurement. Block and block × treatment were random effects. Feed DMI, MY, BW, estimated feed efficiency, and enteric gas (CH_4, CO_2, and H_2; g/d per animal) emission data were averaged by week, and the average values were used in the statistical analysis. The individual cow average daily MY and milk compositions during each experimental week were used to calculate yields of milk fat, true protein, lactose, ECM, and ECM feed efficiency. Average DMI, MY, and ECM yield during the experimental weeks were used to estimate CH_4 and CO_2 yield (per kilogram of DMI) and intensity (per kilogram of MY or ECM yield). Data were analyzed using AR(1) covariance structure; week was the repeated term, and block × treatment was the subject.

Milk FA, BW change, rumen fermentation, nutrient intake and apparent digestibility, N utilization, and data were analyzed with treatment in the statistical model. Block and block × treatment were random effects, and all others were fixed.

Statistical differences were considered significant at $P \leq 0.05$, and a trend was declared at $0.05 < P \leq 0.10$. Unless otherwise indicated, data are presented as least squares means.

RESULTS AND DISCUSSION

Corn Silage and Diet Characteristics

In the conditions of the current experiment, the ECS hybrid yielded 17.6 t of DM/ha, whereas its isogenic counterpart yielded 18.7 t of DM/ha. Chemical composition and fermentation profiles of the silages are presented in Table 1 and Table 2, respectively. Overall, with the exception of the high DM, nutrient composition of the silages fell within the ranges of silages used by Ferraretto and Shaver (2015). Silage DM was similar between CON and ECS. The ECS had 6% lower CP, 3.9% lower ADF concentration, and 2.6% greater 30-h NDF digestibility than the control silage. Both

lignin and 240-h uNDF were approximately 7% and 14% lower for ECS than for control silage. Further, analyses from both CVAS and RRL revealed a higher starch content for ECS than for the control (8.5 and 10% higher, respectively). Concentrations of NFC were approximately 3% greater for ECS, compared with control silage. Amylase activity was approximately 13-fold greater for ECS than for CON silage. Previous research has shown that enhanced amylase activity in ECS is reflected in increased 7-h in situ starch digestibility, with concomitant increases in the amount of washout particles, compared with silages without the amylase trait (Shaver, 2019). Although the silage hybrids were genetically nearly identical and harvested at the same stage and date, some differences in their nutritional composition were detectable. This emphasizes the importance of investigating the effects of environmental conditions on ECS nutrient composition.

Particle size distribution of the samples (as percent of total sample DM) processed for both silages averaged 3, 49.3, 41.3, and 6.5% for the top, medium, and lower sieves and bottom pan (respectively). No substantial differences between silages were observed in particle size distribution. We also detected no differences in fermentation acids between silages, except for a 13.8% lower acetic acid concentration in ECS compared with CON silage (Table 2). Acetic acid has antimicrobial properties and is beneficial for silage aerobic stability (Danner et al., 2003). In this context, mold count was lower, but yeast count was greater for ECS compared with control silage. Butyric acid was undetected in both silages, indicating no clostridial proliferation and, overall, good silage fermentation. We found no major differences in the face temperature or rate of temperature change after aerobic exposure between silages. Additionally, pH was below 4.0 for both silages, and the concentration of fermentation end products were within normal values as suggested by Ward and de Ondarza (2008) and Kung et al. (2018). Average OMDI of ECS was 73%, whereas CON silage had an OMDI of 71% (data were not analyzed statistically). The OMDI was developed as a tool to help dairy producers and nutritionists in evaluating overall digestibility of corn silage hybrids (Penn State Extension, 2020). In the case of the current experiment, differences in OMDI are reflective of differences or trends in starch and lignin concentrations and NDF degradability between silages.

Control corn silage was replaced on a DM basis by ECS, and all other ingredients were the same between the diets; therefore differences in dietary nutrient composition (Table 3) resulted from the nutritional differences between the corn silages. Crude protein concentrations of the diets were similar despite ECS being lower in CP concentration relative to CON silage. Further, the difference in starch concentration between the silages resulted in 1.2 percentage units greater dietary starch content of the ECS diet compared with CON. Neutral detergent fiber and ADF concentrations were similar between diets. Both diets met or exceeded NE_L and MP requirements of the cows, according to NRC (2001).

DMI and Milk Production

Dry matter intake, lactational performance, and BW data are presented in Table 4. Enogen corn silage inclusion in the diet did not affect DMI, but it increased ($P < 0.01$) MY by 2 kg/d compared with CON, resulting in improved ($P < 0.01$) feed efficiency and a tendency ($P = 0.09$) for improved ECM feed efficiency. Differences in MY were consistent throughout the experiment (Figure 1), and no treatment × week interactions ($P \geq 0.13$) were observed for any of the production variables. To the best of our knowledge, the only other study to date that has investigated the effects of ECS in dairy cattle is that of Rebelo et al. (2020), who reported increased DMI and MY but no effect on ECM when ECS was included in the diet of lactating dairy cows, in a replicated 3 × 3 Latin square experiment, at a rate of 48% of dietary DM. However, improved feed efficiency in response to ECS inclusion at 40% of diet DM was previously reported by Johnson et al. (2019) in growing steers, in which an increase in ADG and a tendency for increased DMI were also observed. The production responses from the latter experiment are in line with research with beef steers when Enogen was fed in the diet as either grain or silage, or both (Baker et al., 2019; Johnson et al., 2020). Johnson et al. (2020) reported that increases in feed efficiency could be a result of improved dry and organic matter digestibility when feeding Enogen corn to growing calves. In other studies, however, few to no production effects were reported when ECS was included at 12, 24, or 80% of dietary DM or when included as grain in both steer and finishing beef cattle diets (Schoonmaker et al., 2014; Brinton et al., 2020a; Rusche et al., 2020). Discrepancies in data from these studies indicate that corn processing, animal growth stage, dietary characteristics, and inclusion rate of Enogen corn in the diet are all factors that can affect animal responses.

Improved feed efficiency has been observed in response to dietary supplementation of exogenous amylases (Gencoglu et al., 2010; Andreazzi et al., 2018). Andreazzi et al. (2018) reported no effects of exogenous amylase on ECM but observed an increase in feed efficiency and ECM feed efficiency due to increases in MY and a reduction in DMI in mid-lactation Holstein cows. In contrast, Gencoglu et al. (2010) observed no

Table 4. Effect of an amylase-enabled (Enogen, Syngenta Seeds LLC) corn silage on feed DM intake, lactation performance, and BW in dairy cows

	Treatment[1]			
Item	CON	ECS	SEM[2]	*P*-value[3]
DMI, kg/d	26.5	26.3	0.34	0.70
Milk yield, kg/d	38.8	40.8	0.50	<0.001
Feed efficiency,[4] kg/kg	1.47	1.55	0.027	<0.001
Milk fat, %	4.00	3.82	0.080	0.17
Yield, kg/d	1.54	1.55	0.036	0.92
ECM,[5] kg/d	38.1	39.5	0.63	0.12
ECM feed efficiency,[6] kg/kg	1.45	1.50	0.021	0.09
Milk true protein, %	3.11	3.07	0.025	0.22
Yield, kg/d	1.20	1.25	0.016	0.05
Milk lactose, %	4.86	4.92	0.020	0.02
Yield, kg/d	1.89	2.00	0.033	<0.001
MUN, mg/dL	14.0	12.7	0.257	0.002
SCC,[7] $\times 10^3$ cells/mL	72.0	135.4	92.64	0.63
BW, kg	634	637	2.1	0.13
BW change,[8] g/d	298	389	59.2	0.29

[1]Treatments were control (CON) and Enogen (ECS) corn silages, both at a 40% inclusion rate on DM basis. Means are covariate-adjusted LSM.

[2]Largest SEM published in table; n = 282 (n represents number of observations used in the statistical analysis).

[3]Main effect of treatment.

[4]Milk yield ÷ DMI.

[5]Energy-corrected milk (kg/d) = kg of milk × [(38.3 × % fat × 10 +24.2 × % true protein × 10 + 16.54 × % lactose × 10 + 20.7) ÷ 3,140]; Sjaunja et al. (1990).

[6]ECM yield ÷ DMI.

[7]Statistical analysis was performed on log-transformed data.

[8]BW change: (average BW experimental wks 5 and 6 − average BW covariate wk 2) ÷ days on study.

effect on MY or ECM when liquid amylase was applied to feed, but reported greater feed efficiency due to reduced DMI in Holstein cows. Other studies found no production effects when feeding exogenous amylases mixed with the TMR of lactating cows (Ferraretto et al., 2011; Vargas-Rodriguez et al., 2014). Nozière et al. (2014) suggested that starch concentration in the diet may affect animal response to amylase supplementation. In this context, researchers reported that exogenous amylase added to TMR with moderate dietary starch concentrations (27 and 26%) increased or tended to increase FCM compared with their control (Tricarico et al., 2005, and Klingerman et al., 2009, respectively). However, Weiss et al. (2011) observed no production effects in Holstein cows when liquid amylase was added to a TMR formulated with coarsely ground corn, with a dietary starch concentration of 26%. Results from these experiments highlight the confounding effect that type, processing, and amount of dietary starch may have on animal response to exogenous amylases.

In the current experiment, ECS-fed cows had a greater starch intake compared with CON during the experiment (0.27 kg/d). It has been previously reported that MY tends to increase with increasing starch content in dairy cattle diets (Ferraretto et al., 2013). In their meta-analysis, Ferraretto et al. (2013) reported that MY tended to increase at a rate of 0.085 kg/d per percentage unit increase in dietary starch concentration. Assuming that the data of Ferraretto et al. (2013) are applicable to the current study, it can be estimated that the difference in starch intake between cows fed CON and ECS would correspond with a difference in MY of 0.1 kg/d. Other data, however, suggest a much greater MY response to differences in dietary starch concentration. In a study by Agle et al. (2010), MY of Holstein cows increased by 0.34 kg/d per percentage increase in dietary starch (21.3 vs. 29.6%), which, if applied to the current experiment, would explain about 20% (or 0.40 kg/d) of the increase in MY for cows fed ECS. Thus, it appears, the difference in starch intake between treatments in the current experiment can only partially explain the increased milk production by cows fed the ECS diet relative to CON.

Milk Composition and BW

In the current experiment, we detected no effect of treatment on milk true protein concentration, but true protein yield was increased ($P = 0.05$) by the ECS diet, due to increased MY, relative to CON. Increased milk protein yield in response to ECS inclusion in the diet was also reported by Rebelo et al. (2020). A similar ef-

fect on milk protein yield was reported by Klingerman et al. (2009) for liquid amylases sprayed onto the feed of lactating dairy cows. McCarthy et al. (2013) and Gencoglu et al. (2010) observed an increase and a tendency for increase (respectively) in milk protein concentration but reported no effect on milk protein yield. Milk lactose concentration and yield were increased ($P \leq 0.02$) in response to the ECS diet in the current experiment. The effect on lactose yield induced by ECS agrees with findings by Andreazzi et al. (2018) when amylase was mixed with ground corn and added to the TMR fed to Holstein cows. Conversely, McCarthy et al. (2013) reported lower lactose yields in response to blending a dry form of amylase into the TMR of Holstein cows. The differential response reported by McCarthy et al. (2013) and Andreazzi et al. (2018) may suggest that dietary starch concentration (23 vs. 32% DM, respectively) could be a factor in the effects of the exogenous amylase on milk composition. In the current experiment, milk fat concentration and yield did not differ between treatments. Only minor changes in milk FA concentrations due to treatment were detectable, and details on this analysis are presented as Supplemental Table S4 (https://doi.org/10.26208/am92-yn24; Cueva et al., 2021). It has been observed that enhanced starch degradation in the rumen may increase rumen microbial protein yield and liver oxidation of propionate (Allen et al., 2009), which is the main glucogenic VFA in ruminants. This hypothesis, however, is contradictory to the lack of effect of ECS on rumen propionate in the current experiment (see later discussion). In the current study, cows on the ECS diet had a 9.3% lower ($P < 0.01$) MUN concentration relative to CON cows. Concentration of MUN was not different between the Enogen and control diets in the study by Rebelo et al. (2020). In studies with lactating cows where exogenous amylases were supplemented, MUN was not affected (Ferraretto et al., 2011; Weiss et al., 2011; Nozière et al., 2014). However, in another exogenous amylase study, Gencoglu et al. (2010) observed a reduction in MUN at the end of their 12-wk experiment. One likely explanation for the lower MUN in cows fed the ECS diet relative to CON in the current experiment is enhanced utilization of NH_3 by ruminal microbes for protein synthesis as a result of increased starch intake, which led to more starch degraded and available energy in the rumen (NRC, 2001). This hypothesis, however, was not supported by the similarities in rumen NH_3 data (as will be discussed). In the current experiment, treatment had no effect on BW or BW change of the

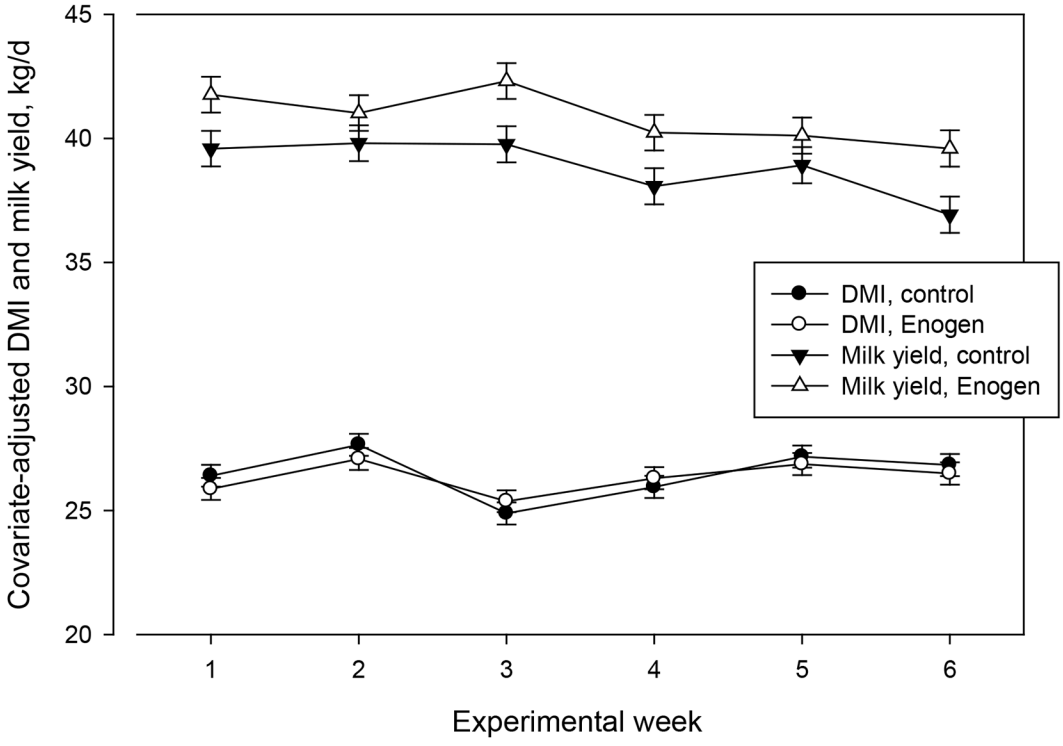

Figure 1. Effect of an amylase-enabled (Enogen feed corn, Syngenta Seeds LLC) corn silage on DMI and milk yield (MY) in dairy cows over the course of the experiment. Treatments were control and Enogen corn silages, both fed at 40% of DM. Data are covariate-adjusted LSM, and error bars represent SEM. Main effect of treatment: $P < 0.001$ for MY and $P = 0.70$ for DMI. Treatment × week: $P \geq 0.38$ for both variables. Samples for digestibility measurements were collected during experimental wk 4.

Table 5. Effect of an amylase-enabled (Enogen, Syngenta Seeds LLC) corn silage on enteric gas emissions in dairy cows

Item	Treatment[1]		SEM[2]	*P*-value[3]
	CON	ECS		
CH_4				
CH_4, g/d	420	411	8.2	0.32
CH_4 per DMI, g/kg	15.9	15.7	0.23	0.63
CH_4 per milk yield, g/kg	11.1	10.3	0.22	0.007
CH_4 per ECM[4] yield, g/kg	11.1	10.7	0.22	0.20
CO_2, g/d	13,730	13,835	158.0	0.62
H_2, g/d	1.25	1.17	0.053	0.30

[1]Treatments were control (CON) and Enogen (ECS) corn silages, both at a 40% inclusion rate on DM basis. Means are covariate-adjusted LSM.

[2]Largest SEM published in table; n = 288 (n represents number of observations used in the statistical analysis).

[3]Main effect of treatment.

[4]Energy-corrected milk (kg/d) = kg of milk × [(38.3 × % fat × 10 +24.2 × % true protein × 10 + 16.54 × % lactose × 10 + 20.7) ÷ 3,140]; Sjaunja et al. (1990).

cows (Table 4), and no treatment × week interaction ($P \geq 0.12$) was detectable for any of the milk composition variables or BW.

Enteric Gas Emission

Daily enteric emissions of CH_4, H_2, and CO_2 did not differ between cows fed CON and ECS diets (Table 5). Methane emission yield, expressed in relation to DMI, was also similar between treatments. Methane emission intensity (per kilogram of MY) was decreased ($P < 0.01$) by the ECS diet compared with CON; CH_4 emission intensity expressed per kilogram of ECM, however, was not affected by treatment. Little evidence exists of direct effects of exogenous enzymes on enteric CH_4 production (Hristov et al., 2013). Clearly, the 7% reduction in CH_4 emission intensity (per kilogram of MY) by ECS-fed cows in the current experiment was a result of the increased MY. However, previous research has reported linear increases in in vitro gas production with increasing proportions of Enogen corn in a dry-rolled corn blend (Horton and Drouillard, 2018). Moreover, Rebelo et al. (2020) reported a decrease in CH_4 yield when ECS was added in the diet at a rate of 48% dietary DM compared with an isogenic hybrid, an effect that was likely a result of increased DMI by cows fed ECS. In vivo studies investigating the effect of amylase supplementation on enteric CH_4 emission in dairy cattle are lacking, and this is the first full-length report, along with that of Rebelo et al. (2020), documenting the effects of ECS on enteric CH_4 emission in vivo. Inclusion of exogenous enzymes could reduce CH_4 emission yield and CH_4 emission intensity through the increase of feed efficiency in dairy cows (Holtshausen et al., 2011). Considering that DMI is a major driver of enteric CH_4 emission in ruminants (Hristov et al., 2018), similarities in DMI between treatments in the current experiment also help explain similarities in daily enteric CH_4 emission.

Rumen Fermentation

Effects of ECS on rumen fermentation variables are presented in Table 6. As indicated earlier, samples for these analyses were collected from a limited number of cows, using the ororuminal sampling technique, and only once during the experiment; therefore, it is noted that variability in the data was large, and results should be interpreted with caution. We found no differences between CON- and ECS-fed cows in total or individual VFA concentrations, except for a decrease ($P = 0.04$) in the molar proportion of butyrate for cows fed ECS. Hu et al. (2010) observed no difference in total VFA production with supplementation of an amylase-enabled corn hybrid (CA3272; Syngenta Biotechnology Inc.) when incubated in vitro. Hristov et al. (2008) also reported no change in total or individual VFA concentrations in dairy cows after supplementation of the diet with an exogenous amylase product. Nozière et al. (2014) reported a reduction in the molar proportion of butyrate in the rumen fluid of Holstein cows when exogenous amylase was supplemented via the TMR. This was associated with an increase in the molar proportion of propionate. In that experiment, the authors suggested that the reduction in the molar proportion of butyrate was because glucose released from starch hydrolysis by the exogenous amylase was predominantly fermented via the odd-chain VFA pathways. Furthermore, microbial sequencing in the current experiment revealed some changes in bacteria and protozoa in rumen fluid from cows fed ECS (Supplemental Table S2, https://doi.org/10.26208/am92-yn24; Cueva et al., 2021). In

this context, the ororuminal sampling technique does not permit sampling from different sites within the rumen. Furthermore, Lage et al. (2020) reported that using the ororuminal sampling technique could affect the observed distribution of microbial population in the rumen. However, this would apply equally to both diets in the current study, and potential shortcomings of the sampling technique would not significantly affect the comparative nature of the data. In the current experiment, we detected no difference in the acetate-to-propionate ratios and NH_3 concentrations between treatments. Amylase activity in ruminal contents (Table 6), although numerically greater for cows fed ECS, was not statistically affected by treatment. Whereas previous experiments have reported higher amylase activities in ruminal contents of beef heifers or dairy cows fed exogenous amylase products (Hristov et al., 1998; Nozière et al., 2014), the numerically higher amylase activity in the rumen of ECS-fed cows in the current experiment could be related to both greater amylase activity in ECS (as previously described) and greater starch intake with the ECS diet, compared with CON.

Apparent Total-Tract Digestibility

Nutrient intake (from the digestibility sample collection week) and total-tract, apparent digestibility data are shown in Table 7. Treatment did not affect DMI during fecal and urine collection week, and thus nutrient intakes, except for a greater ($P = 0.04$) starch intake by cows fed ECS, were similar between diets. There was a trend for greater ($P = 0.08$) DM digestibility (**DMD**) with the ECS diet compared with CON. If the difference in DMD during collection week is extrapolated to the entire experiment, cows fed the ECS diet would have been consuming 0.18 kg/d more digestible DM than cows fed the CON. Research in steers reported linear increases in in situ DM degradability with increasing proportions of Enogen corn in a dry-rolled corn blend (Horton and Drouillard, 2018). These observations agree with a previous report in which Enogen corn grain was fed to beef cattle (Johnson et al., 2020). As discussed earlier, ECS had a greater estimated OMDI (resulting from greater starch content and 30-h NDF digestibility and lower ADF and lignin compared with CON), which is in line with the observed trend for greater DMD of the ECS diet. Collectively, these differences and trends are likely the explanation for the improvement in milk production of ECS-fed cows relative to CON. However, apart from the greater DMD, no other statistical differences in nutrient digestibility were detectable between the 2 diets fed in the current experiment. Hu et al. (2010) performed a 6-h in vitro incubation on an amylase-enabled cultivar and observed marginal differences in in vitro starch digestibility, speculating that incubation temperature could affect enzyme activity expression. In the current experiment, total-tract starch digestibility was, as expected, high, which may have masked potential differences in ruminal starch digestibility due to the amylase enzyme in ECS. It is possible that the friction and associated heat of silage chopping and kernel processing, followed by conditions during the initial stages of silage fermentation, could elicit enzymatic activation to produce

Table 6. Effect of an amylase-enabled (Enogen, Syngenta Seeds LLC) corn silage on rumen fermentation in dairy cows

	Treatment[1]			
Item	CON	ECS	SEM[2]	*P*-value[3]
pH	6.77	6.52	0.193	0.25
Total VFA, m*M*	86.2	105.1	11.44	0.17
VFA (mol %)				
Acetate	58.7	59.0	1.99	0.91
Propionate	22.8	26.4	2.94	0.29
Butyrate	14.6	11.3	1.06	0.04
Isobutyrate	0.71	0.58	0.068	0.13
Valerate	1.87	1.62	0.14	0.15
Isovalerate	1.37	1.15	0.24	0.24
Acetate:propionate	2.59	2.41	0.38	0.67
NH_3, m*M*	4.45	4.02	0.97	0.68
Total protozoa,[4] $\times 10^4$/mL	11.9	13.7	0.43	0.75
Amylase activity[5]	69.0	76.5	8.46	0.48

[1]Treatments were control (CON) and Enogen (ECS) corn silages, both at a 40% inclusion rate on DM basis.

[2]Largest SEM published in table; n = 10 (data are from 10 cows, 5 per treatment).

[3]Main effect of treatment.

[4]Statistical analysis was performed on log-transformed data.

[5]Expressed as nanomoles of reducing sugars as glucose released per milliliter of ruminal fluid per minute.

differences in silage digestibility. Additionally, physiological rumen temperatures could also enhance enzyme activation, thus increasing starch degraded in the rumen. However, we speculate that increasing ensiling duration can cause a disruption in the protein matrix cross-linked to starch granules, allowing for microbial attachment and enzymatic hydrolysis, thereby increasing starch digestibility (Hoffman et al., 2011). In this context, the long ensiling period of our silages (over 220 d at the beginning of the experiment) could have affected treatment responses, as we suspect that only starch more resistant to degradation could be present after long-term storage, thus hindering potential differences in starch digestibility between ECS and CON at earlier stages of in-silo fermentation. Furthermore, specific mode of action of the enzyme during ensilement of Enogen corn and when feeding Enogen corn to cattle has not been clearly demonstrated in published research to date, and discrepancies in in vitro and in vivo responses indicate that further investigation of this technology is needed. No effect of the ECS diet on total-tract fiber digestibility was observed. From these data, it could be concluded that the production effects of ECS observed in the current experiment result from both greater starch intake and a trend for higher DMD compared with CON.

Nitrogen Utilization

We detected no difference in N intake between treatments during the urine sampling week (Table 8). One of our hypotheses for this experiment was that increased degradation of starch in the rumen would stimulate microbial protein synthesis and outflow from the rumen and decrease urinary N excretion due to improved ruminal NH_3 utilization. In spite of the lower MUN concentration observed for ECS-fed cows, we found no statistical differences in urinary N excretion between treatments. Unaccounted N (as percent of intake), despite being numerically higher for ECS-fed cows, was not statistically different between treatments. Excretion of urinary purine derivatives, allantoin, and uric acid, which are commonly used as indirect indicators of ruminal microbial protein synthesis and outflow, were not affected by treatment. In the current experiment, as mentioned previously, urine volume was estimated using creatinine concentrations from urine spot collections. Thus, it is possible that urine outputs were underestimated, as it has been reported that changes in diurnal urine volume may affect urinary creatinine concentrations (Lee et al., 2019). However, Lee et al. (2019) also concluded that, despite underestimations of urine output, using urinary creatinine from urine spot sampling can still be useful when determining dietary effects on urine outputs. Therefore, results from the current experiment are in accord with previous research, indicating no response in urinary N excretion when dairy cow diets were supplemented with exogenous amylases (Hristov et al., 2008; Nozière et al., 2014).

CONCLUSIONS

Inclusion of ECS at 40% dietary DM did not affect DMI but increased MY, improved feed efficiency, and

Table 7. Effect of an amylase-enabled (Enogen, Syngenta Seeds LLC) corn silage on nutrient intake and apparent total-tract digestibility in dairy cows

	Treatment[1]			
Item	CON	ECS	SEM[2]	*P*-value[3]
Intake,[4] kg/d				
DM	25.7	26.5	0.77	0.39
OM	24.7	25.5	0.74	0.41
NDF	8.57	8.42	0.252	0.62
ADF	5.07	4.99	0.150	0.66
CP	4.28	4.37	0.138	0.59
Starch	6.16	6.68	0.188	0.04
Apparent total-tract digestibility, %				
DM	71.3	72.5	0.60	0.08
OM	74.1	75.2	0.59	0.12
NDF	52.8	53.4	1.09	0.68
ADF	49.5	50.3	1.03	0.52
CP	76.1	76.6	0.80	0.65
Starch	97.7	97.6	0.14	0.43

[1]Treatments were control (CON) and Enogen (ECS) corn silages, both at a 40% inclusion rate on DM basis.
[2]Largest SEM published in table; n = 48 (n represents number of observations used in the statistical analysis).
[3]Main effect of treatment.
[4]DM intake reported is during the fecal collection period (experimental wk 4) for the digestibility analysis.

Table 8. Effect of an amylase-enabled (Enogen, Syngenta Seeds LLC) corn silage on nitrogen utilization and urinary purine derivative excretion in dairy cows

Item	Treatment[1]		SEM[2]	*P*-value[3]
	CON	ECS		
N intake, g/d	685.5	699.6	20.18	0.59
N excretion or secretion, g/d				
Urine N	262.7	249.6	13.24	0.42
UUN[4]	197.2	175.6	19.56	0.43
Fecal N	158.8	155.2	7.96	0.70
Total excreta N	421.5	404.8	18.34	0.43
Milk N	193.1	191.2	6.42	0.79
As % of N intake				
Urine N	38.2	35.7	1.43	0.23
UUN	28.5	25.0	2.59	0.34
Fecal N	23.4	22.3	1.08	0.40
Total excreta N	61.5	58.0	2.00	0.18
Milk N	28.6	27.5	0.99	0.43
Unaccounted N	9.86	14.4	2.66	0.18
Urine output[5], kg/d	23.4	21.7	1.03	0.20
Urinary PD[6] excretion, mmol/d				
Allantoin	678.6	745.0	49.01	0.28
Uric acid	60.3	56.3	4.21	0.44
Total PD	738.9	801.3	49.62	0.33

[1]Treatments were control (CON) and Enogen (ECS) corn silages, both at a 40% inclusion rate on DM basis.
[2]Largest SEM published in table; n = 48 (n represents number of observations used in the statistical analysis).
[3]Main effect of treatment.
[4]UUN = urinary urea N.
[5]For details, see Materials and Methods.
[6]PD = purine derivatives.

tended to improve ECM feed efficiency in dairy cows, compared with its isogenic counterpart. The diet with ECS decreased CH_4 emission intensity (per unit of milk, but not ECM), which would have a positive effect on the carbon footprint of milk production. Effects induced by ECS in this study were likely a result of both greater silage starch intake and overall availability of digestible nutrients, as suggested by a trend for increased total-tract DM digestibility.

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