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EVALUATION OF AN AMYLASE-ENABLED CORN SILAGE FED TO LACTATING COWS

A Thesis in

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ABSTRACT

An experiment was conducted to investigate the effects of an amylase-enabled corn silage on the lactation performance, enteric gas emission and rumen fermentation of lactating dairy cows. The amylase-enabled corn hybrid (Enogen; Syngenta Seeds LLC) was harvested, ensiled, and included in the diet at 40% of dry matter (DM) of the cows. The Enogen corn silage (ECS) was compared with silage from a control (CON) isogenic corn hybrid without the amylase trait. Both silages were included at the same inclusion rate of dietary DM and the diet fed, with exception of the silage source, was identical between the treatments. The purpose of the experiment was to investigate the effect of ECS on lactational performance, enteric gas emission, and rumen fermentation of lactating dairy cows.

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. At harvest, the CON hybrid yielded approximated 1.1 t of DM/ha more than the ECS hybrid. Crude protein concentration was 6% lesser in ECS when compared with the CON hybrid. Acid detergent fiber was also 3.9% lesser for ECS. Furthermore, the ECS was greater in starch content, when compared with the CON (9.3%, on average). As expected, the amylase activity in ECS was 13-fold greater when compared with the CON silage. Nevertheless, the differences mentioned in the nutritional content of the silages, did not result in major differences in fermentation end-products between the silages.

Inclusion of ECS at 40% dietary DM did not affect DMI but increased MY (40.8 vs 38.8 kg/d), improved feed efficiency (1.55 vs. 1.47 kg/kg) and tended to improve energy-corrected milk yield (ECM) feed efficiency in lactating cows (1.50 vs. 1.45 kg/kg). Milk lactose was greater (4.92 vs 4.86 %) for cows fed the ECS diet, relative to CON, but milk protein and fat

contents were similar. Methane emission intensity (per unit of milk yield) was decreased by the ECS diet compared with CON; however, treatment did not affect CH_4 emission intensity expressed per kilogram of ECM. Rumen fermentation, apart from a decreased molar proportion of butyrate in rumen fluid of ECS-fed cows, was not affected by treatment. Amylase activity was numerically, but not statistically, greater in rumen fluid of ECS-fed cows, however, the difference could be related both to a greater amylase activity in ECS (as previously described) and greater starch intake with the ECS diet, when compared with CON. As suspected, intake of starch was greater in cows fed the ECS diet relative to CON, and apparent total-tract DM digestibility also tended to be greater in cows fed the ECS. There were no differences in intakes in any of the other nutrients or their apparent total-tract digestibility. Nitrogen intake and utilization, as well as urinary purine derivative excretion, were not affected by the silage treatment. Effects induced by ECS were likely a result of the greater starch content in ECS and greater overall availability of digestible nutrients. Moreover, given that ECS decreased CH_4 emission intensity (per unit of MY, but not of ECM), data would suggest that the carbon footprint of milk production could be reduced by inclusion of ECS,

Inclusion of an amylase-enabled hybrid in dairy rations showed promising effects by improving feed efficiency and ECM feed efficiency in dairy cows. However, the amylase-enabled corn silage used in the current experiment tended to have a greater overall availability of digestible nutrients and was greater in starch content, when compared with its isogenic counterpart. In this regard, effects observed on cow performance can be attributed, at least partially, to differences in silage nutritional composition. Research investigating the effects of feeding amylase-enabled corn silages to dairy cows is limited. This makes it challenging to concretely determine the mode and extent of action of the amylase enzyme in the current study.

Thus, future research on amylase-enabled hybrids should be focused on determining modes and stages of action of the enzyme, potential nutritional and environmental benefits in dairy cows, and possible interactions with other additives or dietary ingredients. The amylase-enabled technology seems to be promising, however, questions remain that must be answered to fully understand the benefits of the inclusion of amylase-enabled corn in dairy cow diets.

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ABBREVIATIONS

ADF	Acid Detergent Fiber
ADG	Average Daily Gain
BMR	Brown Midrib
BW	Body Weight
CON	Control Corn Silage
CP	Crude Protein
CVAS	Cumberland Valley Analytical Services
DIM	Days in Milk
DM	Dry Matter
DMD	Dry Matter Digestibility
DMI	Dry Matter Intake
DU	Dextrinizing Unit
ECM	Energy Corrected Milk
FCM	Fat Corrected Milk
ECS	Enogen Corn Silage
FA	Fatty Acid
MUN	Milk Urea Nitrogen
MY	Milk Yield
NDF	Neutral Detergent Fiber
NDFD	Neutral Detergent Fiber Digestibility
NE_L	Net Energy for Lactation
NFC	Nonfibrous Carbohydrates
OM	Organic Matter
OMDI	Organic Matter Digestibility
RDP	Rumen Undegradable Protein
RFID	Radio-Frequency Identification
RRL	Rock River Laboratory
RUP	Rumen Undegradable Protein
SCC	Somatic Cell Count
SM	Supplementary Material
TDN	Total Digestible Nutrients
TMR	Total Mixed Ration
uNDF	Undigested NDF
UUN	Urinary Urea Nitrogen
VFA	Volatile Fatty Acid

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CHAPTER 1. INTRODUCTION

Cows are organisms with a complex digestive system that enables them to digest forage and feedstuffs due to a mutualistic relationship with microbes that inhabit their rumen. The inclusion of forage in dairy cow diets is a key management strategy implemented by dairy nutritionists to maintain a healthy ruminal environment (Mertens, 1997). In this regard, corn silage has traditionally been a cornerstone forage source in dairy diets (Jordan and Fourdraine, 1993; Wilkinson et al., 2003). Thus, raising and feeding quality corn silage to meet the requirements of lactating dairy cows has been a goal for dairy farmers and corn growers, leading to the production of more than 132 million tons of corn silage in the U.S. in 2019 (USDA, 2019). Furthermore, global population has been exponentially increasing over the past century, with the possibility of reaching 8.5 billion in the next decade according to the United Nations organization. This drives scientists to look for new methods to improve productivity and efficiency of dairy cows with the purpose of meeting the imminent increases in the demand for dairy products. Therefore, the development of new techniques that enhance nutritive value of corn silage is fundamental to optimize forage utilization by the cow and achieve greater levels of productivity.

As mentioned above, corn silage is the predominant forage source in dairy diets, mainly due to its relatively high-energy density and biomass yield when compared with other forages (Wilkinson et al., 2003). Briefly, corn silage is produced by storing the corn plant in anaerobic conditions, which allows microbes to ferment water-soluble carbohydrates, which elicits changes in the plant material's chemical and organoleptic characteristics through fermentation. Therefore, a quality corn silage is determined by how well it is fermented and the changes in nutritional characteristics that ensue (Kung et al., 2018). One of the most important metrics of silage

quality, and forage quality in general, is fiber and starch digestibility of the plant. Therefore, selection and evaluation of corn hybrids has been driven by the potentially digestible fiber and starch in the plant. Development of corn genotypes that are greater in digestible fiber, such as brown midrib (BMR) corn, and increasing duration of ensiling time can increase both fiber and starch digestibility by the cow (Phillippeau and Michalet-Doreau, 1998; Oba and Allen, 2000). Finding the perfect harmony between fiber and starch digestibilities of corn silage is of great benefit for dairy producers by increasing efficiency of cows.

Although increasing fiber and starch digestibility through hybrid selection and management practices is known to increase the productive efficiency of cows, there are other methods that can also be employed. The inclusion of feed additives such as exogenous dietary enzymes and their effects on the productive efficiency of cattle has also been investigated. Some exogenous dietary enzymes have been shown to withstand ruminal degradation, and their supplementation has been shown to aid cattle in the conversion of certain feeds into animal product (Hristov et al., 1998). In some cases, fibrolytic enzmyes have had the effect of increasing fiber digestibility when supplemented in both dairy and feedlot cattle diets (Schingoethe et al., 1999; Kung et al., 2000; Beauchemin et al., 2003). On the other hand, there have been reports that using amylolytic enzymes as feed additives in cattle diets has increased starch digestibility in the rumen and improved feed efficiency (Gencoglu et al., 2010; Nozière et al., 2014). Additionally, utilization of amylolytic enzymes has been inconclusive as there is research reporting only negligible responses to amylase addition in diets of lactating cows (Ferraretto et al., 2011; Weiss et al., 2011). However, considering that degradation and absorption of starch in the small intestine can be limited by insufficient pancreatic alpha-amylase secretion if excessive concentrations of starch escape rumen degradation (Huntington et al., 2006), utilization of

exogenous amylase in dairy diets could be beneficial for dairy cows and further research on this technology should not be discarded. Furthermore, although there is little evidence that supplementation of exogenous enzymes has an effect in reducing absolute CH_4 emission (g CH_4 / d), they could reduce enteric CH_4 emission yield and intensity (g CH_4 emission / kg of DMI or kg of MY) by increasing efficiencies (Hristov et al., 2013). This is of relevance given that the inherent increase in global population would not only increase the demand for dairy products but would also posit environmental challenges. For this, agricultural industries must find methods to increase efficiencies with the purpose of reducing their environmental footprint per unit of production.

Building from what is discussed above, it can be inferred that integrating the dual benefit of a highly digestible forage encoded with an exogenous amylase enzyme in the diet could increase cow performance with promising results for producers. In this context, amylase-enabled corn hybrids are new hybrid lines characterized for showing high levels of amylase activity in the endosperm of their grain. Enogen brand corn hybrids, developed by Syngenta, are the amylase-enabled hybrids currently on the market. These hybrids were originally designed to improve the corn ethanol production process by reducing the need for additional amylase enzymes. These hybrids posit the benefit of offering the cows with more degradable starch when compared to traditional corn hybrids.

More recently, research evaluating the effects of feeding Enogen as grain or silage or both to dairy and feedlot cattle have yielded promising results (Harris et al., 2016; Jolly-Breithaupt et al., 2016; Rebelo et al., 2020), whereas others have reported no relevant production effects on finishing feedlot cattle when silage was fed in the diet at an inclusion rate of 12% dietary DM (Jolly-Breithaupt et al., 2019; Rusche et al., 2020). Evidently, consistent results

cannot be extracted from these experimental trials. Additionally, research elucidating the effects of including Enogen corn hybrids in dairy cattle rations are lacking; thus, specific mode of action of the enzyme in dairy cows and the impact on their productive performance remains a mystery. To the best of our knowledge, Rebelo et al. (2020) along with the scientific research presented in chapter 3 of this thesis are the only studies to date that have investigated the effect of Enogen fed either as grain or as silage in dairy cattle diets. Therefore, the objective of the following thesis is to provide an exploratory evaluation of the inclusion of an amylase-enabled corn silage in the lactational performance, enteric methane emission and rumen fermentation of dairy cows.

References

Beauchemin, K. A., D. Colombatto, D. P. Morgavi, and W. Z. Yang. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81:E27-E47. 10.2527/2003.8114_suppl_2E37x

Ferraretto, L. F., R. D. Shaver, M. Espineira, H. Gencoglu, and S. J. Bertics. 2011. Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. J. Dairy Sci. 94:1490–1499. http://doi.org/10.3168/jds.2010-3736.

Gencoglu, H., R. D. Shaver, W. Steinberg, J. Ensink, L. F. Ferraretto, S. J. Bertics, J. C. Lopes, and M. S. Akins. 2010. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. J. Dairy Sci. 93:723–732. http://doi.org/10.3168/jds.2009-2673.

Hristov, A. N., T. A. McAllister, and K. J. Cheng. 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility. J. Anim. Sci. 76:3146–3156. http://doi.org/10.2527/1998.76123146x.

Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A. T. Adesogan, W. Yang, C. Lee, P. J. Gerber, B. Henderson, and J. M. Tricarico. 2013. Special Topics: Mitigation of methane and nitrous oxide emissions from animal operations: I. A

review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069. https://doi.org/10.2527/jas.2013-6583.

Huntington, G. B., D. L. Harmon, and C. J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84 Suppl:14–24. http://doi.org/10.2527/2006.8413_supplE14x.

Jolly-Breithaupt, M. L., C. J. Bittner, Dirk B. Burken, G. E. Erickson, J. C. MacDonald and M. K. Luebbe.2016. Evaluating Syngenta Enhanced Feed Corn Processed as Dry-Rolled or High-Moisture Corn on Cattle Performance and Carcass Characteristics. Nebraska Beef Cattle Report MP103: 143-145.

Jolly-Breithaupt, M. L., C. J. Bittner, F. H. Hilscher, G. E. Erickson, J. C. MacDonald and M. K. Luebbe. 2018. Impact of Syngenta Enogen Feed Corn on Finishing Cattle Performance and Carcass Characteristics. Nebraska Beef Cattle Report MP105: 92-94.

Jordan, E. R., and R. H. Fourdraine. 1993. Characterization of the management practices of the top milk producing herds in the country. J. Dairy Sci. 76:3247-3256. https://doi.org/10.2134/agronmonogr42.c12

McAllister, T. A., S. J. Oosting, J. D. Popp, Z. Mir, L. J. Yanke, A. N. Hristov, R. J. Treacher, and K.-J. Cheng. 1999. Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. Can. J. Anim. Sci. 79:353–360.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Nozière, P., W. Steinberg, M. Silberberg, and D. P. Morgavi. 2014. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. J. Dairy Sci. 97:2319–2328. http://doi.org/10.3168/jds.2013-7095.

Kung, L., Jr., R. J. Treacher, G. A. Nauman, A. M. Smagala, K. M. Endres, and M. A Cohen. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. J. Dairy Sci. 83:115–122.

Kung, L., R. D. Shaver, R. J. Grant, and R. J. Schmidt. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. J. Dairy Sci. 101:4020–4033. http://doi.org/10.3168/jds.2017-13909.

Oba, M. and M. S. Allen. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 3. Digestibility and microbial efficiency. J. Dairy Sci. 83:1350–1358.

Philippeau, C., C. Martin, and B. Michalet-Doreau. 1999. Influence of grain source on ruminal characteristics and rate, site, and extent of digestion in beef steers. J. Anim. Sci. 77:1587–1596. doi:10.2527/1999.7761587x

Rebelo, L., C. Lee, W. Weiss, and M. Eastridge. 2020. Effects of Enogen Feed corn silage and corn grain on nutrient digestibility, production, and enteric methane emission in lactating cows. J. Dairy Sci. 103 (Suppl. 1): 171 (Abstract).

Rusche, W. C., J. A. Walker, and Z. K. Smith. 2020. Effect of inclusion rate of silage with or without alpha-amylase trait on finishing steer growth performance, carcass characteristics, and agronomic efficiency measures. Transl. Anim. Sci. 4:942–949. http://doi.org/10.1093/tas/txaa056.

Schingoethe, D. J., G. A. Stegeman, and R. J. Treacher. 1999. Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. J. Dairy Sci. 82:996–1003.

United States Department of Agriculture National Statistics Service. 2019. Crop Production 2019 Summary 8–102.

Weiss, W. P., W. Steinberg, and M. A. Engstrom. 2011. Milk production and nutrient digestibility by dairy cows when fed exogenous amylase with coarsely ground dry corn. J. Dairy Sci. 94:2492–2499. http://doi.org/10.3168/jds.2010-3766.

Wilkinson, J. M., K. K. Bolsen, and C. J. Lin. 2003. History of silage. In: D. R. Buxton, R. E. Muck, and J. H. Harrison, editors, Silage Science and Technology. Agronomy Monograph Vol. 42 ASA, CSSA, SSSA, Madison, WI. pp. 1-30.

CHAPTER 2. LITERATURE REVIEW

Corn (*Zea mays* L.) is a cereal grain the most widely used feedstuff in animal nutrition. Corn grain is usually processed and fed as an energy source due to its starch content and nutrient availability. Furthermore, given the ability of ruminant animals to convert forage into animal product, utilization of the whole corn plant as silage has become a common practice among cattle nutritionists. In this regard, corn silage is one of the predominant forage sources in dairy cattle nutrition. Corn silage not only provides enough nutritional value to maintain and sometimes improve cow performance but also allows for farmers to have stored forage inventories for extended periods of time. Although the inclusion of corn silage in dairy diets has been a common practice over the last century, understanding the nutritional benefits of ensiling corn and integrating it with novel nutritional techniques could be a viable strategy to increase productivity of dairy farms.

Corn silage in dairy diets

Feeding whole corn plants in the form of silage is a common practice among dairy farmers given that it is a feed ingredient that can be stored for extended periods of time while minimizing nutrient loss (Mahanna and Chase, 2003). When compared with other forages, corn silage also has the advantage of being relatively high in biomass yields and a source of rapidly digestible energy due to its concentration of nonstructural polysaccharides, mainly in the form of digestible starch. Furthermore, corn silage can provide the forage and effective fiber required by dairy cows to support healthy rumen function (Mertens, 1997). Recently, it has been well documented that corn silage commonly makes up more of 50% of the forage in dairy rations and can be included up to 40 to 45% of dietary dry matter (DM; Shaver and Kaiser, 2011; Hristov et al., 2015). Thus, having a measure of the nutrient composition and physical properties of corn

silage is imperative when formulating rations and assessing silage quality. There is a plethora of factors that affect silage fermentation, and consequently, silage quality. In this regard, nutritional quality of silage and cow performance will be directly linked with its content of fiber, starch, and protein along with the fermentation end-products from the ensilement process (Kung and Shaver, 2001; Grant and Ferraretto, 2018). At harvest, whole corn plants are chopped and packed into silos, bags, or piles to limit oxygen exposure and promote anaerobic fermentation. Thus, silage harvest maturity, hybrid selection and the overall ensilement practices can also impact the nutritional quality of corn silage (Johnson et al., 2002; 2003). Therefore, understanding the process of silage fermentation is imperative since differences in the aforementioned characteristics will accrue and ultimately reflect in the fermentation dynamics and end-products that turn the chopped corn plant into silage.

Corn silage fermentation

Comprehensively, the process of ensilement consists of subjecting the corn plant through an anaerobic fermentation process that converts carbohydrates to organic acids, mainly lactic acid (Wilkinson et al., 2003). Microbes in the silage, mainly lactic acid bacteria inhabiting the corn plant at harvest, metabolize sugars into organic acids and which elicits a drop in silage pH (Der Bedrosian et al., 2012; Pahlow et al., 2003). The nutritional changes as a consequence of fermentation will occur within the first few weeks after the plant has been harvested, chopped, packed and subjected to anoxic conditions. The major changes in nutritional characteristics of corn silage can be subcategorized into four different phases.

The initial phase of silage production starts after the corn plant has been harvested and packed and can last up to 24 hrs. Essentially, after the plant is chopped and connection with the

soil is interrupted, there is initiation of enzymatic degradation of the corn plant. Despite the plant being hermetically sealed into a bunker, bag or silo, there is still some oxygen inside which allows for plant respiration during the initial hours after ensilement. Aerobic microorganisms in the plant material at harvest, namely molds and yeasts, utilize the oxygen remaining in the sealed bunker, bag or silo and proliferate while increasing the temperature of the silage (Kung et al., 2018). Additionally, increases in fermentable substrate such as soluble carbohydrates and amino acids, are reported in this phase as a consequence of the degradation of proteins, fiber and starch (Pahlow et al., 2003).

The main fermentation phase begins once all the oxygen has been depleted. Lactic acid producing bacteria, which are anaerobic silage microorganisms, utilize water soluble carbohydrates in the silage and produce lactic acid. This phase is characterized by a steady drop in silage pH as a consequence of an increasing lactate concentration (Kung et al., 2018). Other anaerobic microorganisms present, namely yeasts, bacteria and clostridia will compete for the utilization of nutrients. However, during the fermentation of silage increases in lactate concentration and the drop in pH mostly benefit lactic acid producing bacteria to the point where they become the dominant microbial community and the rest enter a lethargic state of proliferation (Pahlow, 2003). In synthesis, changes in microbial community will cause a reduction in water-soluble, fermentable sugars in the silage with a concomitant increase in lactic acid. An ideal corn silage fermentation will most likely be under pH of 4.0 and will be characterized by lactic acid concentrations of 3 to 6% silage DM, with a lactic to acetic acid ratio of approximately 3 to 1 (Kung et al., 2018). Silage microbial inoculants are popularly used to ensure quality fermentation. Inoculation of the silage with homolactic bacteria such as *Lactobacillus plantarum* can aid in the preservation of the plant material by aiding in the

production of lactic acid and ensuring fast and steady drops in pH. Furthermore, use of different microbial inoculants can shift silage fermentation from homolactic to heterolactic fermentation. *Lactobacillus buchneri* is the most popular heterolactic bacteria that is inoculated to increase the concentration of not only lactic acid, but also acetic acid in the silage (Kung et al., 2018). Acetic acid has antimicrobial properties and is beneficial for silage aerobic stability (Danner et al., 2003). Corn silage can greatly benefit from heterolactic inoculants given that aerobic spoilage is a source of DM loss during the feed-out stage if this stage is not managed properly. It has also been discussed that even though concentration of acetic acid in silage can be indicative of good aerobic stability, greater concentrations of acetic acid could lead to decreases in DMI by the cow (Danner et al., 2003). Ammonia is another indicator of silage quality, given that as degradation of protein occurs, ammonia will increase but should not exceed 8% of total N. The fermentation phase can last up to 4 weeks after the initial aerobic phase.

Once the main fermentation phase is finished, and the metabolic processes in the silage stop, the stable phase begins. Unlike the fermentation phase, the stable phase is characterized by minimal but steady changes in the organoleptic and nutritional characteristics of the corn plant. Most notably, enzymatic degradation of hemicellulose and zein proteins in the protein-matrix of cornstarch granules of the silage occur, thus potentially increasing corn silage fiber and starch digestibility by the cow (Pahlow et al., 2003; Der Bedrosian et al., 2012). Collectively, however, reports on the effect of ensiling time on corn silage neutral-detergent fiber (NDF) digestibility (NDFD) have been inconsistent. In a more recent study, authors reported that in situ degradability of silage NDF linearly decreased from 0 to 150 d of ensiling time as a result of decreases in concentrations of hemicellulose and NDF-bound protein (Hristov et al. 2020). The stable phase can be carried out indeterminately provided that pH and anoxic conditions are

maintained throughout the process. Changes in pH and temperature during this phase would be indicative of unwanted fermentation by microbes and possible aerobic exposure. Thus, feeders must ensure that this stage will not be disrupted until the silage is opened in the final stage.

The final phase of ensilement is the feed-out stage. During this phase the silage is opened and aerobically exposed. During aerobic exposure, bacteria, mold, and yeasts are reinvigorated, leave their quiescent state, and start proliferating causing increases in temperature and spoilage in silage face temperature (Kung et al., 2018). Extended aerobic exposure of silage during this phase will also increase pH. In some cases, increases in corn silage temperature over ambient temperature has been correlated with a decrease in intake (Gerlach et al. 2013). This is likely because opportunistic and unwanted aerobic microbes will increase their toxin secretion and decrease silage digestibility (Pahlow et al., 2003). Overall, temperature will rise as a consequence of aerobic exposure during this phase and management practices such as establishing adequate feed-out rates should be devised with the goal of minimizing DM losses.

Ensiling corn silage can be challenging. It has been reported by several studies that management during corn harvest, ensilement and feed-out will have a large impact on the nutritional value of the forage when fed to cattle. In this context, corn hybrid type, maturity, length of storage, use of inoculants, processing, and packing density are some of the management factors that affect silage nutritive value during feed out (Johnson et al., 1999; 2002). Although understanding the effect of these factors is of immense relevance, this review will focus on the changes and digestibility of fiber and starch in corn silage.

Digestibility of Fiber

Characteristics of fiber

Fiber is a complex array of dietary nutrients composed of protein, lignin, and structural polysaccharides in the form of cellulose, hemicellulose, and pectin. From these, the three major structural components of fiber in plants are cellulose, hemicellulose, and lignin (Van Soest et al., 1991; Moore and Jung, 2001). Cellulose is water-insoluble and consists of d-glucopyranose residues linked by a β-(1, 4) bond (Hilscher, 2018). Unlike cellulose, hemicellulose contains a diversity of polymers in a linear and branched β-(1, 4) bond combination. Despite hemicellulose sharing a hydrogen bond with cellulose, it is also closely associated to lignin through ester and ether bond connections (Van Soest, 1994). Another structural polysaccharide in plants is pectin, which is characterized for having α-(1, 4) linkages. The chemical linkage in pectin closely resembles that of starch and it is soluble in neutral detergent, for which it is considered to be soluble fiber (Van Soest, 1994). However, unlike starch, pectin must be fermented by fiber-degrading rumen microorganisms because it cannot be degraded by amylase enzymes.

Lignin is a term used to describe polymers of indigestible matter that provide structure and rigidity to plants. Briefly, lignin polymerizes between cellulose, hemicellulose, and pectin to provide structure as the plant grows; a process during which lignin binds with hemicellulose. Once lignification occurs, it acts as a physical barrier limiting the access of microbes to the cell wall and is therefore considered the major factor impacting fiber digestibility (Moore and Jung, 2001).

From a ruminant nutritionist standpoint and according to the NRC (2001), fiber in feeds can be subcategorized into acid detergent fiber (ADF) and NDF. Neutral detergent fiber is a measure of the three major structural components (i.e. cellulose, hemicellulose and lignin) of plants, whereas ADF does not include hemicellulose. As mentioned above, the proportion of

lignin in NDF is considered the first limiting factor of fiber digestion in ruminants (Van Soest, 1994). Furthermore, although all the fiber subcategories are highly correlated, the NDF fraction is the one that most completely describes all the compounds that make up dietary fiber, making it a good discerner between structural and nonstructural carbohydrates in plants (NRC, 2001). Thus, NDF has been adopted as the most common measurement of fiber in forages.

The extent and rate of fiber degradation will not only be affected by the characteristics and concentrations of its compounds, but the rumen environment and microorganisms will also play a major role in fiber digestion. In this regard, it is critical to understand that the composition of chemical compounds in fiber and population densities of microbes in the rumen ecosystem have a collaborative role in fiber digestion and a beneficial relationship must be established between them to reach maximal fiber degradation.

Fiber digestion

Inclusion of forages in dairy diets is necessary forages maintain healthy rumen functions and stimulate rumination and formation of a rumen mat (Mertens, 1997; Harper, 2018). Healthy rumen function can be defined as adequate ruminal pH that allow for correct degradation of dietary nutrients (Yang and Beauchemin, 2009). Furthermore, enough particles must be present in the rumen which will allow for a healthy rumen function to ensure adequate absorption of volatile fatty acids (VFA) and also reduce the risk of metabolic diseases such as ruminal acidosis (Zebeli et al., 2012). As mentioned earlier, the extent to which a forage can be digested by rumen microorganisms is determined by both the structural carbohydrates forming the plant cell wall and the accessibility of the rumen microorganisms to those carbohydrates.

The fiber degradation process begins when microorganisms attach themselves to the feed particles that enter the rumen. Once the microbes have adhered, enzymes are secreted and the complex molecules are degraded into nutrients (Varga and Kolver, 1997). For optimal fiber degradation to occur, microbes in the ruminal ecosystem must be provided with the correct environment and adequate amounts of substrate for their proliferation. Ruminal environments that can sustain fiber degrading microbes are characterized for being anoxic and having a pH of between 5.5 and 6.5. It is well known that the concentration of NDF in the diet will be directly related with ruminal pH because of its slower fermentation rate and digestibility when compared with nonstructural polysaccharides. Dietary NDF also promotes chewing and saliva production, which acts as a buffer (NRC, 2001). Furthermore, microorganisms inhabiting the rumen also need a N source to turn fiber into VFA, which they usually find in the ammonia pool inside the rumen. Volatile fatty acids are then absorbed and used for energy.

The predominant communities of fiber-degrading rumen microorganisms are *Fibrobacter*, *Ruminococcus*, and *Butyrivibrio* (Cheng et al., 1991). Apart from bacteria, fungi and protozoa inhabiting the rumen can also have an effect on fiber digestion. Fungi, which account for approximately 8% of the ruminal microbiome, can create lesions in the plant structure, breaking the lignification between cellulose and hemicellulose (Varga and Kolver, 1997). This allows for more bacterial and enzymatic access to the cell wall compounds, consequently increasing the extent of digestible fiber. On the other hand, in a similar fashion to bacteria, protozoa can produce fibrolytic enzymes which are utilized to degrade structural carbohydrates within the rumen. In vitro studies have reported that protozoa can make up to 28% of total cellulase activity and that defaunation would reduce fiber digestion (Varga and Kolver, 1997).

In the context of silage, it is a common practice to select for hybrids with increased NDF digestibility (NDFD) as this will also increase the amount of energy and nutrients available to the cow. Slow rates of NDF digestion coupled with slow passage rates can limit DMI due to rumen fill (Allen, 1996). In this regard, Oba and Allen (1999a) reported that enhanced forage NDFD increased DMI and MY of dairy cows in a statistical analysis across a wide range of forages and found that 1 unit of enhanced NDFD digestibility was associated with a 0.17 kg increase in DMI and a 0.25 kg increase in 4% fat-corrected milk. Thus, it is worth noting that there are natural mutations of corn that increase NDFD, like in the case of brown midrib (BMR) corn. Research has shown that BMR corn hybrids have lower lignin concentrations which translate to greater NDFD. When comparing between corn silages with low and high NDFD in dairy cow diets, milk and milk fat yield increased for diets with corn silage higher in NDFD (Ivan et al., 2005). Furthermore, research has shown that feeding forages with higher NDFD to cows that are in peak lactation help reduce rumen fill leading to more productivity (Oba and Allen, 1999b).

Management factors such as population densities, fertility, planting dates, plant maturity, processing, as well as environmental factors, will play a big role in the fiber content and digestibility of silage hybrids (Varga and Kolver, 1997; Allen et al., 2003). Research has shown that increasing maturity in silage is concomitant with a decrease in its NDFD and overall NDF concentration (Johnson et al. 1999; Owens, 2008). From these experiments, authors not only inferred that NDF and ADF digestibility decrease as maturity increases due to an increase in lignin accretion in plant material, but also that the increase in starch concentrations, characteristical of more mature corn silage, could have an effect in the rumen ecosystem making it less optimal for fiber digestion (Kung et al. 2018).

It should also be considered that NDF is generally negatively correlated with the overall energy concentration of feeds and diets and that fiber must be broken down and processed by ruminal microorganisms because mammals are not equipped with the enzymes to degrade them. This implies that, to optimize animal performance, a harmonious interaction between the contents and digestibilities of fiber and starch in dietary silage must be found.

Digestibility of Starch

Characteristics of cereal grains

Adoption of cereals in nutritional practices for cattle has been increasing due to their energy density when compared to forage sources. This energy density is correlated to the starch concentration of the grain. Different grains will have different starch concentrations; oats and barley (57 to 58% DM starch) being in the low; corn and sorghum (72% DM) in the middle; and wheat (77% DM starch) on the high end of the starch-content spectrum (Nocek and Tamminga, 1991; Huntington, 1997). Additionally, differences inherent to grain structure, other than total starch content, will influence its utilization by ruminants.

Corn grains are made of three essential parts: pericarp, germ and endosperm. The germ can make up to 2 to 12 % of the grain and is characterized for elevated oils and protein concentrations. The endosperm can make up 60 to 90 % of a kernel and is mainly composed of starch granules (McAllister et al., 1993). Starch is composed of two major molecules: amylose and amylopectin. Amylose is a polymer made of linear α 1-4 glycosidic linkages, whereas amylopectin is also a polymer made of linear α 1-4 glycosidic linkages with an α 1-6 glycosidic

branching point (Tester et al., 2004). The ratio of these two polysaccharides can vary according to grain source and variety and ultimately influence its rate and extent of degradation. Amorphous regions in starch granules represent the amylopectin branching points, whereas the more compact structure of starch can be associated to linear glycosidic linkages of amylose and the more crystalline region of amylopectin (McAllister and Cheng, 1996). Amylopectin, being less compacted and crystalline, is more readily degraded by microbes and enzymes (Tester, 2004). Moreover, endosperm composition within the corn grain can be distinct, being subdivided into vitreous (or horny) or floury endosperm. Proportion of vitreous and floury endosperm will vary depending on corn hybrid genetics, growth environment and maturity at harvest. In the vitreous endosperm area, starch granules are deeply embedded in a protein matrix, whereas the floury endosperm has starch loosely attached to the protein matrix. Therefore, the floury endosperm is more digestible due to easier access from microbes and enzymes. (Tester et al., 2004). Grain processing can increase the availability of starch in floury endosperm to a greater extent than starch in vitreous endosperms (Huntington, 1997). Lastly, the pericarp (3-8% kernel) is made of lignin and wax esters and, along with the protein matrix, is one of the major physical barriers of starch digestion. On this basis, subjecting the grain to different processing methods will result in disruptions of these barriers, allowing for a greater extent and rate of starch degradation (McAllister et al., 1993).

Starch digestion

Anaerobic fermentation of feedstuffs, such as starch, begins once the substrate reaches the rumen and is exposed to microbial attachment. In this context, whole grains with intact pericarps are largely or entirely resistant to digestion in the rumen and are processed to allow

access of microbes to the endosperm of the grain (Huntington et al., 2006). Once the digestion barriers have been broken, starch is degraded by amylolytic bacteria. These bacteria release amylases that hydrolyze amylose and amylopectin linkages, breaking starch down into oligosaccharides. Furthermore, other bacteria in the rumen can produce other types of enzymes that allow for a more extensive digestion of the grain, by helping degrade fiber, lipids and proteins (Tester et al., 2004). Additionally, protozoa are also capable of degrading starch in the rumen by engulfing starch granules. Rhizoids in ruminal fungi can create lesions in the protein matrix of the grain enabling a more complete rumen digestion (McAllister and Cheng, 1996).

Simple sugars obtained from starch degradation in the rumen will be used by the rumen microbes as energy sources to generate VFA and synthesize microbial protein (Huntington 2006). Starch fermentation will drive propionate production, which is the most energy dense VFA. Propionate will be absorbed by the rumen epithelium into the bloodstream to be later removed by the liver, where it will be synthesized into glucose.

Ruminal digestion of starch ranges from 55%-80% of starch intake depending on dietary characteristics, grain source and processing. Starch that escapes rumen fermentation will potentially be digested and utilized in the intestine (Harmon et al., 2004). Ruminant diets with a high grain content and starch availability can result in digestive dysfunctions such as laminitis or acidosis due to excessive rumen fermentation and a consequent drop in pH. Thus, shifting the site of starch digestion to the small intestine could help prevent some metabolic disorders while also being more energetically efficient due to the absorption of energy directly in the form of glucose. Additionally, there is no energy loss in the form of gas and heat in the small intestine as opposed to ruminal fermentation of feedstuffs (Huntington, 1997).

Digestion of starch in the small intestine is driven by luminal enzymatic secretions. Once starch enters the small intestine, the pancreas secretes α-amylase which begins hydrolyzing the glycosidic bonds binding amylose and amylopectin, resulting in limit dextrins and linear oligosaccharides. Further degradation of these compounds into monosaccharides is completed by carbohydrases located on the brush border membrane of the intestine. Once degraded into glucose, sugars are absorbed into the bloodstream through the intestinal lumen through different mechanisms (Huntington, 1997). Paracellular diffusion is one of the mechanisms of glucose transport by absorption through intracellular spaces; however, it is only a major channel of absorption when glucose presence in the intestine is substantially high, which may not occur under normal physiological circumstances (Harmon, 2009). Active transport via SGLT1 (Na+-dependent glucose transporter) is the predominant channels of glucose transport in the intestine. Briefly, there is a concentration gradient between cells that ultimately allows glucose and two sodium ions to be transported into the bloodstream. The density of SGLT1 is higher in the proximal region, with less density in the middle, and the least density in the most distal area of the small intestine (Huntington, 1997). Harmon (2009) also reported that GLUT2 is another mechanism allowing passive transport for glucose and is regulated by insulin and luminal glucose concentrations. When glucose concentrations in the lumen are high, GLUT2 is moved to the brush border membrane allowing absorption without any energy expense.

Huntington (1997) stated that, on average, 5 to 20% of starch intake is digested post-ruminally. Even though digestion in the small intestine is energetically more efficient, it has been argued by several studies (Richards et al., 2003; Harmon et al., 2004; Huntington et al., 2006) that a lack of α-amylase activity is the primary limitation of starch digestion in the intestine and the reason why shifting high quantities of starch to intestinal degradation may be suboptimal.

Starch that escapes digestion in the small intestine will be fermented in the large intestine. However, digestion of starch in the large intestine should be avoided because of its low efficiency of nutrient use. The large intestine will have a system similar to that of the rumen, considering that there will be VFA production and absorption, however, at this point of the gastrointestinal tract the organism will not take advantage of the microbial cell and protein synthesis. Furthermore, starch that is not digested in the intestines will be excreted in feces.

Starch content in corn has been reported to increase as the corn plant matures (Johnson et al., 1999). Furthermore, several studies conducted with lactating dairy cows show that starch digestibility and total-tract starch digestibility decrease when harvesting of corn silage is delayed and when crops are harvested at a greater dry matter (Ferraretto and Shaver, 2012). Results from the studies summarized in the meta-analysis by Ferraretto and Shaver (2012) indicates that MY decreases as starch digestibility decreases. Johnson et al. (1999) and Bal et al. (2000) reported that optimal milk production and intake in dairy cattle were reached when corn silage was harvested between 33 and 36% dry matter. On this basis, it should be noted that production effects of corn silage starch digestibility will be directly affected by the concentration of other dietary ingredients and total starch concentration in the diet. Conveniently, management practices such as kernel processing can be utilized to increase starch digestibility of corn silage.

Whole-plant corn silage can be constituted of up to 50% corn grain thus approximating 35 to 45% starch on a DM basis (NRC, 2001; Ferraretto et al., 2015). When the corn plant is ensiled, the corn grain is fermented along with the plant material, provided anaerobic conditions are met. Harvesting corn at an adequate moisture level (approximating 65%) and storing it in an oxygen-limiting environment will yield a more fermentable grain. Furthermore, younger silages have a lesser energy value when compared with silages that have been ensiled for longer periods

of time. The main reason for this is that starch digestibility continuously increases with ensiling time (Hoffman et al., 2011). Hoffman et al. (2011) reported that the protein-matrix surrounding the starch granules gets hydrolyzed allowing for more loosely attached starch granules to get attacked by rumen microbes or enzymes. Even though digestibility of starch continuous to increase with ensiling time, the rate of increase in starch digestibility will decrease as ensiling time increases. Long ensiling periods may also mask inherent differences in starch digestibility between corn silage hybrids considering that only starch more resistant to degradation could be present after long-term storage and starch digestibility will be high relative to younger silages. Nevertheless, correct fermentation of corn silage over longer ensiling times will increase its nutritive value. Overall, solubility and digestibility of starch in corn silage has been reported to increase as the ensiling period length increases (Phillippeau and Michalet-Doreau, 1998; Owens, 2008; De Bedrosian et al., 2012). These results would hint at increasing ensiling duration as a suggested practice to increase starch digestibility.

Grain processing is characterized by exposing the grain to heat, moisture or mechanical pressure to damage the kernel and reduce particle size; with the main goal of breaking down the endosperm and pericarp structures, rendering the starch more available to gelatinization and degradation. Gelatinization happens when starch granules absorb water, swell and release amylose and amylopectin from their arrangement, increasing their susceptibility to enzymatic hydrolysis (Huntington, 1997; Huntington et al., 2006). Starch granules in barley, oats and wheat are loosely attached to the protein matrix and therefore, the effects of processing are less evident compared to corn. Research done in kernel processing of corn silage reported that there is a reduction in DM losses during ensiling compared with unprocessed silage (Johnson et al., 1999). According to Johnson et al. (1999) processing reduced the number of whole kernels from

20% to 5% while also reducing the amount of undigested grain that would be excreted and lost in the feces. Owens (2008) reported that differences in starch digestibility resulting from different harvest dates or hybrid types can be overridden by processing the kernel, allowing farmers to manage the relationship between silage yield and starch digestibility without significantly altering fiber digestion.

Overall, management factors, plant maturity, kernel processing and ensiling time can all have an effect on the starch protein matrix, affecting the total starch content and digestibility in corn silage (Der Bedrosian et al., 2012; Ferraretto and Shaver, 2012). Additionally, utilization of some exogenous dietary enzymes is yet another proposed strategy to increase starch digestibility by cows.

Exogenous Enzymes in Cattle Diets

Exogenous enzymes

Enzymes are specialized proteins produced by living organisms that act as biocatalysts of biochemical reactions. Ruminants not only benefit from enzyme secretion originating from their organism, but also from enzymatic secretion by the microbes inhabiting their rumen. In ruminant nutrition, exogenous enzymes are enzymes that are not produced by the organism or by the ruminal microbes, but enzymes that are included as a dietary supplement. Dietary supplementation of exogenous enzymes in cattle diets is a practice that can be traced back to the early 1960s (Burroughs et al., 1960). Multiple researchers have investigated the effects of including enzyme mixtures with promising results (McAllister et al., 1999; Beauchemin et al., 2003). The premise of this technology is to aid the animal by increasing digestibility and nutrient availability of feeds and improving animal performance.

Research on supplementing exogenous enzymes has mostly focused on the utilization of fibrolytic enzymes. Fibrolytic enzymes, as the name suggests, are fiber-degrading and improve animal performance by enhancing ruminal fiber digestion. As discussed in the previous chapter, enhancing forage NDFD could increase DMI and milk yield of high-producing dairy cows (Oba and Allen, 1999). Thus, use of fibrolytic enzymes in the cattle industry has been extensively researched with several studies showing that adding exogenous fibrolytic enzymes to ruminant diets have increased milk production (Schingoethe et al., 1999; Kung et al., 2000; Beauchemin et al., 2003) and average daily gain (McAllister et al., 1999).

Amylases

Although digestion of starch in the rumen is extensive, and generally not limiting, some studies have suggested that addition of amylase enzyme as a dietary supplement could improve animal performance (Tricarico et al., 2005; Klingermann et al., 2009; Nozière et al., 2014). Amylase enzymes are able to hydrolyze starch into simple sugars. More specifically, amylases are endo-specific enzymes with the ability to hydrolyze alpha 1,4-linkages in the amylose and amylopectin found in starch (Monteiro de Souza et al., 2010). Use of alpha-amylase is not limited to the animal and livestock industry but can also be used for other industrial processes, such as production of bioethanol, detergents and the food industry which rely on alpha-amylase to obtain their final product (Melnichuk et al., 2020). In the livestock industry, amylase enzymes were originally researched for inclusion in swine and poultry diets. However, as mentioned above, Hristov et al. (1998) and Beauchemin et al. (1995) reported that some exogenous enzymes are resistant to ruminal degradation, which makes supplementing alpha amylase in cattle diets viable. In vitro and in situ studies have reported greater DM and starch digestibility of grains treated with amylase products (Rojo-Rubio et al., 2001). Effect of in vivo supplementation

of amylolytic enzymes on starch and carbohydrate degradation has been more inconsistent with multiple studies showing different responses when included in beef and dairy diets (Klingermann et al, 2009; DiLorenzo et al., 2011; Ferraretto et al., 2011). The response to supplementation of amylolytic enzymes make it an attractive strategy to be adopted by ruminant nutritionists to increase starch digestion albeit by increasing ruminal degradation of starch, or in the small intestine if the enzyme supplemented can withstand ruminal and abomasal degradation. Furthermore, there have been no negative consequences on cow health from inclusion of dietary exogenous amylase. Thus, considering that starch digestion in ruminants is limited by post-ruminal pancreatic α-amylase secretion, and that ruminal starch digestion is a fundamental determinant of animal performance (Huntington, 1997), further research in amylase supplementation should not be discarded.

Amylase in beef diets

Experiments investigating the effect of feeding amylase as an exogenous additive in beef cattle rations can be traced back as far as the year 1960 when a blend of amylolytic and proteolytic enzymes were fed to beef steers (Burroughs et al., 1960). More recently, studies evaluating alpha amylase supplementation have investigated feedlot cattle performance and carcass characteristics (Tricarico et al., 2007). More specifically, Tricarico et al. (2007) ran 3 different experiments evaluating the effects of varying concentrations of amylase supplementation with different roughage sources and corn processing methods on feedlot cattle performance. In the first experiment, Tricarico et al. (2007) included the commercial amylase product at 0 and 950 dextrinizing units (DU)/kg of feed. A DU is the amount of enzyme needed to solubilize starch at 1g/h at 30°C and pH of 4.8. Results from that experiment suggested no

interaction between roughage source and amylase inclusion and no effect of the enzyme on any of the feedlot performance and carcass characteristics. In their second experiment, yearling heifers were used to evaluate the effect of dry-rolled corn and high moisture corn with amylase inclusion of 0, 580 and 1,160 DU/kg. Again, no interaction between corn processing method and amylase inclusion was observed for feedlot performance and carcass characteristics. Nevertheless, there was a quadratic effect on DMI and ADG with increasing amylase inclusion, with the 580 DU/kg dose having the greatest ADG and DMI. Ultimately, in their third experiment, beef steers were used, and supplementation of amylase was conducted at the 0 and 930 DU/kg level, however, no differences were reported for feedlot performance in the beef steers when the amylase was supplemented. Furthermore, amylase supplementation increased up to 16% live-weight gain in beef cattle and sheep in other research trials (Crosby et al., 2006; Salem et al., 2013). Conversely, DiLorenzo et al. (2011) did not observe any effects of amylase supplementation in feedlot beef steer diets. Inconsistent production response to amylase supplementation in beef makes establishing the benefits of this feeding strategy difficult. Previous studies have indicated that type of animal, diet composition, side activities and modes and levels of application are all factors that could affect enzymological response (McAllister et al., 2001; Hristov et al., 2008).

Amylase in dairy diets

The premise of including exogenous amylase in dairy diets is that they will improve starch digestibility and subsequently increase milk production. In this regard, Tricarico et al. (2005) reported a quadratic increase in milk production with no effect on DMI when exogenous amylase (Amaize, Alltech Inc., Nicholasville, KY) was mixed with the TMR of lactating Holstein cows. In a larger investigation, supplementation with the same additive tended to

increase milk production without reducing milk fat and protein yield in commercial dairy herds across the United States and Canada (Harrison and Tricarico, 2007). Andreazzi et al. (2018) reported no effects of exogenous amylase on energy-corrected milk (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 effect on MY or ECM when applying liquid amylase onto the feed but reported greater feed efficiency due to reduced DMI in Holstein cows. However, Vargas-Rodriguez et al. (2014) did not observe an effect of enzyme supplementation, whereas Ferraretto et al. (2011) observed only a trend for increased feed conversion in response to exogenous dietary amylase in reduced starch diets (starch, 21 to 22% DM). Improved feed efficiency has been observed in response to dietary supplementation of exogenous amylases (Gencoglu et al., 2010; Andreazzi et al., 2018) but overall, supplementation of amylase in dairy diets has had inconsistent effects on lactational performance of dairy cows. Evaluation of the effects of amylase supplementation has not been limited to productive variables, studies have also focused on changes in milk composition.

Similar to what has been observed in the productive performance of dairy cows fed exogenous amylases, changes in milk composition have not been consistent. In this regard, Klingerman et al. (2009) observed an increase in milk protein yield as a result of increased MY. McCarthy et al. (2013) and Gencoglu et al. (2010), respectively, observed an increased and a tendency for increased milk protein concentration, but observed no effect on milk protein yield. Earlier studies, however, suggest that exogenous amylase supplementation increased milk fat through microbial cross-feeding mechanisms (Tricarico et al., 2005, 2008; Klingerman et al., 2009).

Clearly, the response in both dairy and beef cattle to exogenous amylase supplementation has been inconsistent. As mentioned above, it has been previously discussed that the erratic enzymatic response may have, at least partially, originated from factors such as level of supplementation, method of enzyme application, diet composition, and type and stage of animal growth (Beauchemin et al., 2002; Hristov et al., 2008) Furthermore, due to the intricate interrelationship between the rumen ecosystem and ruminal microbes, specific mode of action of the amylase enzyme in the rumen remains unclear. However, it has been suggested that changes in site of starch digestion, microbial cross-feeding mechanisms, increased ruminal starch digestion and changes in VFA profile could all be possible mechanisms of action of amylase supplementation (Tricarico et al., 2007; Nozière et al., 2014). However, in spite of a high variability in productive responses and the unclear mode of action, positive responses to amylase supplementation with no deleterious effects reported by the aforementioned experiments may be interpreted as an indication that this technology warrants further investigation. Building from this, a new technology that integrates the benefits from corn silage fermentation with the potential positive effects of exogenous amylase supplementation has been developed. These are the amylase-enabled corn hybrids and have the purpose of further enhancing nutrient utilization in cattle.

Amylase-enabled corn hybrids

Amylase-enabled corn hybrids are genotypes characterized for having a bacterial transgene coding for the expression of alpha-amylase in the starchy endosperm of the grain during crop growth. This technology consists in an endo-amylase that can degrade the glycosidic bonds within the starch molecules, in random-attack patterns, resulting in the breakdown of complex starch molecules into an array of linear and branched oligosaccharides

(Atichokudomchai et al., 2006). Traditionally, in the ethanol industry a form of exogenous amylase must be added to degrade starch into glucose; but use of amylase-enabled corn hybrids eliminates the need of exogenous enzyme addition in the process. In this same context, Enogen brand corn hybrids (Syngenta Seeds LLC, Downers Grove, IL) are amylase-enabled genotypes that were originally developed to improve production efficiency in the ethanol milling industry. Enogen hybrids are characterized for having the AMY797E gene encoded into their DNA. The enzyme AMY797E is a genetically engineered amylase, thermostable, pH stable and linked to the maize gamma-zein protein promoter which causes the protein to be produced and stored in the starchy endosperm of Enogen grain, without altering other nutritional constituents of the corn plant (APHIS, 2011).

The thermostability and pH tolerance of the amylase in Enogen corn allows it to withstand the rumen environment, which could increase the potentially degradable nutrients of the plant and consequently offer a greater availability of digestible nutrients to the cow. In vitro results have reported that 6-h starch disappearance increased from 1.60 to 1.99% of DM when at a 40°C incubation and from 0.85% to 10.56% of DM at a 60°C incubation when compared with an isogenic line without the amylase trait (Hu et al., 2010). Although in vitro effects are not always similar to in vivo responses, results from that experiment along with previous studies done with exogenous amylase addition in cattle diets suggest that including Enogen corn in diets could potentially benefit cattle by increasing rumen amylase activity and starch digestibility when compared to hybrids without the endo-amylase trait. Consequently, a series of in vivo experiments evaluating the effects of feeding Enogen corn to both beef and dairy cattle were reviewed.

Enogen Corn in beef diets

Feeding Enogen corn has yielded positive effects when fed as dry-rolled corn by improving feed conversion of feedlot cattle when compared with cattle fed dry-rolled corn not containing the amylase trait (Harris et al., 2016; Jolly-Breithaupt et al., 2016). Conversely, negligible responses were reported in an earlier study when Enogen was fed as ground corn to feedlot steers (Schoonmaker et al., 2014). Differences in production performance were of little significance when Enogen was fed as dry-rolled (Jolly-Breithaupt et al., 2019), or high-moisture (Jolly-Breithaupt et al., 2016; Brinton et al., 2020b) corn to finishing cattle. Horton et al. (2018) investigated the effect of feeding steam-flaked Enogen corn grain on finishing beef steers. In that experiment, a 5% increase in feed efficiency was reported for steers fed Enogen corn when compared with steers fed a conventional hybrid. In terms of Enogen corn fed as silage, 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 tended to increase DMI. The production responses from the latter experiment are in line with research with beef steers where Enogen was fed in the diet either as grain, 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, little to no production effects were reported when Enogen corn silage (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).

Results, from these studies suggest that feeding Enogen corn as dry-rolled corn to growing or finishing feedlot cattle may improve feed efficiency. Nevertheless, feeding Enogen as

high-moisture corn did not have significant effects on cattle performance when compared to an isogenic line without the amylase trait. Concrete results cannot be gathered from Enogen included as silage in beef diets. However, results from the aforementioned experiments suggest that improvements in feed efficiency might be observed when ECS is included at approximately 40% of dietary DM in growing steers.

Enogen Corn in dairy diets

Studies investigating the effect of feeding Enogen corn in the dairy industry are scarce. Rebelo et al. (2020) investigated the effect of feeding Enogen corn to lactating cows either as grain or silage or a combination of both. In that experiment, the authors reported that inclusion of Enogen corn in the diet increased DMI and MY, but not energy-corrected milk (ECM), when Enogen corn was included as silage at a rate of 48% of dietary DM. An increase in milk true protein yield was also reported by Rebelo et al. (2020). Discrepancies in data from the feedlot 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. Evidently, more data are needed to clearly conclude on the effect of including Enogen corn in dairy diets. In this regard, differences in basal composition between dairy and beef diets, animal type and growth stage and certain differences within the ruminal ecosystem could crucially change enzymatic responses for dairy cattle when compared with beef. Therefore, the benefits of feeding Enogen corn cannot be accurately established based on the currently available data.

However, results from feedlot experiments feeding ECS and that of Rebelo et al. (2020) indicate that inclusion of ECS in cattle diets could have potential benefits in the productive performance of dairy cows. Previous research reports increased in situ starch degradability of

ECS when compared to silages without the amylase trait (Shaver, 2019). Shaver (2019) also reported increases in small particle starch and increased NDFD for Enogen corn varieties. The increases in small particle starch could offer the cows with more immediately available energy. Furthermore, as mentioned above, the possibility of microbial cross-feeding mechanisms as a consequence of amylase supplementation (Tricarico et al., 2007; Nozière et al., 2014) could help explain the increases in NDFD reported by Shaver (2019). These data would suggest that ensiling Enogen corn could offer both the inherent benefits of an anaerobic in-silo fermentation and a potential improvement in nutrient utilization in dairy cattle in response to addition of an exogenous amylase.

Furthermore, the projected increase in global population also posits environmental challenges. For this, agricultural industries must find methods to increase production efficiencies with the purpose of reducing their environmental footprint per unit of production. Colombini et al. (2015) investigated absolute CH_4 production when feeding corn and sorghum silage to lactating cows. Diets were balanced for both starch and NDF in the diets. In that experiment, absolute enteric CH_4 emission was not different between dietary treatments, but CH_4 yield was the lowest for the cows fed the diet formulated with corn silage. The authors also reported that DMI and milk components yields were the same between treatments and attributed the difference in CH_4 yield to NDFD and passage rates of fiber. It is unlikely that amylase-enabled corn hybrids will have an effect in CH_4 synthesis in the rumen given that there is little evidence of direct effects of exogenous enzymes on enteric CH_4 production (Hristov et al., 2013). Nevertheless, 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). 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 when compared to an isogenic hybrid, an effect that was likely a result of increased DMI by the cows fed ECS. In vivo studies investigating the effect of amylase supplementation on enteric CH_4 emission in dairy cattle are lacking and the study presented in chapter 3 of the current thesis is the first full-length published report documenting the effects of ECS on enteric CH_4 emission in vivo.

Building from this literature review, there is promise in feeding ECS to lactating cows considering that it could optimize nutrient digestion and improve production efficiencies. Results from utilization of exogenous amylase enzymes as feed additives in the dairy industry have been inconsistent. Thus, more research is needed to clarify the potential benefits of amylase-enabled hybrids in ruminant nutrition. Additionally, studies investigating the effect feeding amylase-corn hybrids specifically as silage and in the dairy industry are lacking; use of amylase-enabled corn silage could offer the benefits of both an in-silo fermentation and exogenous-alpha amylase supplementation. Therefore, inclusion of ECS in dairy diets should be investigated to completely characterize the effects of amylase-enabled corn hybrids on animal performance and shed more light on this novel technology.

References

APHIS. 2011. Final Environmental Assessment Alpha-Amylase Maize Event 3272 https://www.aphis.usda.gov/brs/aphisdocs/05_28001p_ea.pdf (accessed Sep. 22, 2021).

Atichokudomchai, N., J. Jane, and G. Hazlewood. 2006. Reaction pattern of a novel thermostable α-amylase. Carbohydrate Polymers 64:582-588.

Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74:3063-3075.

Allen, M. S., J. G. Coors., G. Roth. 2003. Corn Silage. In: D. R. Buxton, R. E. Muck, and J. H. Harrison, editors, Silage Science and Technology. Agronomy Monograph Vol. 42 ASA, CSSA, SSSA, Madison, WI. Pp. 547-607.

Andreazzi, A. S. R., M. N. Pereira, R. B. Reis, R. A. N. Pereira, N. N. Morais Júnior, T. S. Acedo, R. G. Hermes, and C. S. Cortinhas. 2018. Effect of exogenous amylase on lactation performance of dairy cows fed a high-starch diet. J. Dairy Sci. 101:7199–7207. https://doi.org/10.3168/jds.2017-14331.

Bal, M. A., R. D. Shaver, A. G. Jirovec, K. J. Shinners, and J. G. Coors. 2000. Crop processing and chop length of corn silage: Effects on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 83:1264-1273.

Baker, A., V. de A. Veloso, and L. Barros. 2019. Feedlot performance and carcass characteristics of steers fed diets containing steam-flaked grain and corn silage from Enogen Feed Corn. J. Anim. Sci. 97 (Suppl. 2):137. https://doi.org/10.1093/jas/skz122.243.

Brinton, M., B. M. Boyd, F. H. Hilscher, L. J. Mcphillips, J. MacDonald, and G. Erickson. 2020a. University of Nebraska - Lincoln Evaluating Syngenta Enogen Feed Corn Silage or Grain on Growing Beef Cattle Performance.

Brinton, M., B.M. Boyd, F.H. Hilscher, L.J. Mcphillips, J. MacDonald, and G. Erickson. 2020b. University of Nebraska - Lincoln Impact of Feeding Syngenta Enhanced Feed Corn as Dry-Rolled Corn, High- Moisture Corn, or a Blend to Finishing Yearlings.

Beauchemin, K. and L. Holtshausen. 2010. Developments in enzyme usage in ruminants. Enzymes in farm animal nutrition:206-230.

Beauchemin, K. A., D. Colombatto, D. P. Morgavi, and W. Z. Yang. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81:E27-E47. 10.2527/2003.8114_suppl_2E37x.

Cheng, K. J., C. W. Forsberg, H. Minato and , J. W. Costerton (1991) Microbial ecology and physiology of feed degradation within the rumen. Proceedings of the Seventh International Symposium on Ruminant Physiology. Academic Press Toronto, ON.

Colombini, S., M. Zucali, L. Rapetti, G. M. Crovetto, A. Sandrucci, and L. Bava. 2015. Substitution of corn silage with sorghum silages in lactating cow diets: In vivo methane emission and global warming potential of milk production. Agric. Syst. 136:106–113.

Crosby, M., G. Mendoza, L. Melgoza, R. Bárcena, F. Plata, and E. Aranda. 2006. Effects of Bacillus licheniformis amylase on starch digestibility and sheep performance. Journal of Applied Animal Research 30(2):133-136.

Danner, H., M. Holzer, E. Mayrhuber, and R. Braun. 2003. Acetic acid increases stability of silage under aerobic conditions. Appl. Environ. Microbiol. 69:562–567. http://doi.org/10.1128/AEM.69.1.562-567.2003.

Der Bedrosian, M. C., K. E. Nestor, and L. Kung. 2012. The effects of hybrid, maturity, and length of storage on the composition and nutritive value of corn silage. J. Dairy Sci. 95:5115-5126.

DiLorenzo, N., D. Smith, M. Quinn, M. May, C. Ponce, W. Steinberg, M. Engstrom, and M. Galyean. 2011. Effects of grain processing and supplementation with exogenous amylase on nutrient digestibility in feedlot diets. Livestock Science 137(1-3):178-184.

Ferraretto, L. F., A. C. Fonseca, C. J. Sniffen, A. Formigoni, and R. D. Shaver. 2015. Effect of corn silage hybrids differing in starch and neutral fiber digestibility on lactation performance and total-tract nutrient digestibility by dairy cows. J. Dairy Sci. 98 :395–405.

Ferraretto, L. F., and R. D. Shaver. 2012. Meta-analysis: Impact of corn silage harvest practices on intake, digestion and milk production by dairy cows. Prof. Anim. Sci. 28:141–149.

Ferraretto, L. F., R. D. Shaver, M. Espineira, H. Gencoglu, and S. J. Bertics. 2011. Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. J. Dairy Sci. 94:1490–1499. http://doi.org/10.3168/jds.2010-3736.

Gencoglu, H., R. D. Shaver, W. Steinberg, J. Ensink, L. F. Ferraretto, S. J. Bertics, J. C. Lopes, and M. S. Akins. 2010. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. J. Dairy Sci. 93:723–732. http://doi.org/10.3168/jds.2009-2673.

Gerlach, K., F. Ros, K. Weiss, W. Buscher, and K. H. Sudekum. 2013. Changes in maize silage fermentation products during aerobic deterioration and effects on dry matter intake by goats. Agric. Food Sci. 22:168–181.

Grant, R. J. and L. F. Ferraretto. 2018. Silage review: Silage feeding management: Silage characteristics and dairy cow feeding behavior. J. Dairy Sci. 101:4111–4121. https://doi.org/10.3168/jds.2017-13729

Harmon, D.L. 2009. Understanding starch utilization in the small intestine of cattle. Asian-Australasian J. Anim. Sci. 22:915–922. doi:10.5713/ajas.2009.r.08.

Harmon, D.L., R.M. Yamka, and N.A. Elam. 2004. Factors affecting intestinal starch digestion in ruminants: A review. Can. J. Anim. Sci. 84:309–318. doi:10.4141/A03-077.

Harper, M. T. The use of sorghum, oat, wheat, triticale and pearl millet silages in lactating dairy cow diets. PhD Dissertation. The Pennsylvania State Univ., University Park.

Harris, M. E., M. L. Jolly-Breithaupt, B. L. Nuttelman, D. B. Burken, G. E. Erickson, I. G. Rush and M. K. Luebbe. 2016. Evaluating Syngenta Enhanced Feed Corn on Finishing Cattle Performance and Carcass Characteristics. Nebraska Beef Cattle Report MP103: 135-138.2

Harrison, G. A. and J. M. Tricarico. 2007. *Case Study* : Effects of an *Aspergillus oryzae e*xtract containing *α*-amylase activity on lactational performance in commercial dairy herds. The Professional Animal Scientist 23 (2007):291–294.

Hilscher, F. H. 2018. Effect of corn silage harvest, hybrid, and concentration on performance in growing and finishing beef cattle. PhD Dissertation. University of Nebraska-Lincoln. http://digitalcommons.unl.edu/animalscidiss/164.

Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465–2474. http://doi.org/10.3168/jds.2010-3562.

Holtshausen, L., Y. H. Chung, H. Gerardo-Cuervo, M. Oba, and K. A. Beauchemin. 2011. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. J. Dairy Sci. 94:899–907. http://doi.org/10.3168/jds.2010-3573.

Horton, L., and Drouillard, J. 2018. Effects of particle size and heat treatment on in situ and in vitro digestion of dry-rolled Enogen® Feed Corn. Journal of Animal Science, Volume 96, Issue suppl_3, December 2018, Page 391, https://doi.org/10.1093/jas/sky404.857.

Hristov, A. N., C. E. Basel, A. Melgar, A. E. Foley, J. K. Ropp, C. W. Hunt, and J. M. Tricarico. 2008. Effect of exogenous polysaccharide-degrading enzyme preparations on ruminal fermentation and digestibility of nutrients in dairy cows. Anim. Feed Sci. Technol. 145:182–193. http://doi.org/10.1016/j.anifeedsci.2007.05.051.

Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A. T. Adesogan, W. Yang, C. Lee, P. J. Gerber, B. Henderson, and J. M. Tricarico. 2013. Special Topics: Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069. https://doi.org/10.2527/jas.2013-6583.

Hristov, A. N., K. Heyler, E. Schurman, K. Griswold, P. Topper, M. Hile, V. Ishler, E. Wheeler, and S. Dinh. 2015. Reducing dietary protein decreased the ammonia emitting potential of manure from commercial dairy farms. Prof. Anim. Sci. 31:68-79.

Hristov, A. N., M. T. Harper, G. Roth, C. Canale, P. Huhtanen, T. L. Richard, and K. DiMarco. 2020. Effects of ensiling time on corn silage neutral detergent fiber degradability and relationship between laboratory fiber analyses and in vivo digestibility. J. Dairy Sci. 103. https://doi.org/10.3168/jds.2019-16917

Hristov, A. N., T. A. McAllister, and K. J. Cheng. 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility. J. Anim. Sci. 76:3146–3156. http://doi.org/10.2527/1998.76123146x.

Hu, W., M. E. Persia, and L. Kung. 2010. Short communication: In vitro ruminal fermentability of a modified corn cultivar expressing a thermotolerant α-amylase. J. Dairy Sci. 93:4846–4849. http://doi.org/10.3168/jds.2009-2321.

Huntington, G.B. 1997. Starch Utilization by Ruminants: From Basics to the Bunk. J. Anim. Sci. 75:852–867. doi:10.2527/1997.753852x.

Huntington, G.B., D.L. Harmon, and C.J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84 Suppl:14–24. doi:10.2527/2006.8413_supplE14x.

Ivan, S. K., R. J. Grant, D. Weakley, and J. Beck. 2005. Comparison of a corn silage hybrid with high cell-wall content and digestibility with a hybrid of lower cell-wall content on performance of Holstein cows. J. Dairy Sci. 88:244–254.

Johnson, M. A., T. Spore, S. P. Montgomery, W. R. Hollenbeck, R. N. Wahl, E. D. Watson, and D. A. Blasi. 2019. Syngenta Enogen Feed Corn silage containing an alpha amylase expression trait improves feed efficiency in growing calf diets. Kansas Agricultural Experiment Station Research Reports 5. http://doi.org/10.4148/2378-5977.7719.

Johnson, M. A., T. J. Spore, S. P. Montgomery, C. S. Kubick, J. S. Garzón, W. R. Hollenbeck, R. N. Wahl, C. I. Vahl, E. D. Watson, and D. A. Blasi. 2020. Syngenta Enogen Feed Corn containing an alpha amylase expression trait improves digestibility in growing calf diets. Kansas Agricultural Experiment Station Research Reports 6. http://doi.org/10.4148/2378-5977.7890.

Johnson, L., J. H., Harrison, C. Hunt, K. Shinners, C. G. Doggett, and D. Sapienza. 1999. Nutritive value of corn silage as affected by maturity and mechanical processing: A contemporary review. J. Dairy Sci. 82:2813-2825. Invited Review.

Johnson, L. M., J. H. Harrison, D. Davidson, M. Swift, W. C. Mahanna, and K. Shinners. 2002. Corn silage management II: Effects of hybrid, maturity, and mechanical processing on digestion and energy content. J. Dairy Sci. 85:2913-2927.

Johnson, L. M., J. H., Harrison, D. Davidson, W. C. Mahanna, and K. Shinners. 2003. Corn silage management: Effects of hybrid, chop length, and mechanical processing on digestion and energy content. J. Dairy Sci. 86:208-231.

Jolly-Breithaupt, M. L., C. J. Bittner, Dirk B. Burken, G. E. Erickson, J. C. MacDonald and M. K. Luebbe. 2016. Evaluating Syngenta Enhanced Feed Corn Processed as Dry-Rolled or High-

Moisture Corn on Cattle Performance and Carcass Characteristics. Nebraska Beef Cattle Report MP103: 143-145.

Jolly-Breithaupt, M. L., C. J. Bittner, F. H. Hilscher, G. E. Erickson, J. C. MacDonald and M. K. Luebbe. 2018. Impact of Syngenta Enogen Feed Corn on Finishing Cattle Performance and Carcass Characteristics. Nebraska Beef Cattle Report MP105: 92-94.

Klingerman, C. M., W. Hu, E. E. McDonell, M. C. DerBedrosian, and L. Kung. 2009. An evaluation of exogenous enzymes with amylolytic activity for dairy cows. J. Dairy Sci. 92:1050–1059. http://doi.org/10.3168/jds.2008-1339.

Kung, L., R. D. Shaver, R. J. Grant, and R. J. Schmidt. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. J. Dairy Sci. 101:4020–4033. http://doi.org/10.3168/jds.2017-13909.

Kung, L., Jr., and R. Shaver. 2001. Interpretation and use of silage fermentation analysis reports. Focus on forage. vol. 3, no. 13. Accessed Sep. 20, 2021. http:// fyi .uwex .edu/ forage/ interpretation-and -use -of -silage -fermentation -analysis -reports.

Kung, L., Jr., R. J. Treacher, G. A. Nauman, A. M. Smagala, K. M. Endres, and M. A Cohen. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. J. Dairy Sci. 83:115–122.

Mahanna, W., and L. E. Chase. 2003. Practical applications and solutions to silage problems. Pages 855–895 in Silage Science and Technology. Agronomy Monograph No. 42. ASA-CSSA-SSA, Madison, WI.

McAllister, T. A., A. N. Hristov, K. A. Beauchemin, L. M. Rode, and K. J. Cheng. 2001. Enzymes in ruminant diets. Enzymes in farm animal nutrition 273–298. http://doi.org/10.1079/9780851993935.0273.

McAllister, T.A., and K. J. Cheng. 1996. Microbial strategies in the ruminal digestion of cereal grains. Anim. Feed Sci. Technol. 62:29–36. doi:10.1016/S0377-8401(96)01003-6.

McAllister, T.A., R.C. Phillippe, L.M. Rode, and K.J. Cheng. 1993. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. J. Anim. Sci. 71:205–212. doi:10.2527/1993.711205x.

McAllister, T. A., S. J. Oosting, J. D. Popp, Z. Mir, L. J. Yanke, A. N. Hristov, R. J. Treacher, and K.J. Cheng. 1999. Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. Can. J. Anim. Sci. 79:353–360.

McCarthy, M. M., M. A. Engstrom, E. Azem, and T. F. Gressley. 2013. The effect of an exogenous amylase on performance and total-tract digestibility in lactating dairy cows fed a high-byproduct diet. J. Dairy Sci. 96:3075–3084. http://doi.org/10.3168/jds.2012-6045.

Melnichuk, N., M. J. Braia., P. A. Anselmi., M-R Meini., and D. Romanini. 2020. Valorization of two agroindustrial wastes to produce alpha-amylase enzyme from Aspergillus oryzae by solid-state fermentation. Waste Management 106 (2020) 155–161. https://doi.org/10.1016/j.wasman.2020.03.025

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Moore, K. J. and H-J. G. Jung. 2001. Lignin and fiber digestion. J. Range Manage. 54:420-430.

Monteiro De Souza, P., De Oliveira e Megalhaes, P., 2010. Application of microbial -amylase in industry – a Review Brazilian. J. Microbiol. 41, 850–861. https://doi.org/10.1590/S1517-83822010000400004.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press. Washington, DC.

Nocek, J.E., and S. Tamminga. 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74:3598–3629. doi:10.3168/jds.S0022-0302(91)78552-4.

Nozière, P., W. Steinberg, M. Silberberg, and D. P. Morgavi. 2014. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. J. Dairy Sci. 97:2319–2328. http://doi.org/10.3168/jds.2013-7095.

Oba, M., and M. S. Allen. 1999a. Evaluation of the importance of the digestibility of neutral detergent fiber from forge: Effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82:589–596.

Oba, M. and M. S. Allen. 1999b. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 3. Digestibility and microbial efficiency. J. Dairy Sci. 83:1350–1358.

Owens, F. 2008. Corn silage – facts, fantasies, and the future. 2008. In: Proc. Florida Ruminant Nutrition Symposium. Univ. of Flordia, Gainesville.

Pahlow, G., R. E. Muck, F. Driehuis, S. J. W. H. O. Elferink, and S. F. Spoelstra. 2003. Microbiology of Ensiling. Silage Science and Technology. P. 31-93.

Philippeau, C., C. Martin, and B. Michalet-Doreau. 1999. Influence of grain source on ruminal characteristics and rate, site, and extent of digestion in beef steers. J. Anim. Sci. 77:1587–1596. doi:10.2527/1999.7761587x.

Richards, C.J., K.C. Swanson, S.J. Paton, D.L. Harmon, and G.B. Huntington. 2003. Pancreatic exocrine secretion in steers infused postruminally with casein and cornstarch. J. Anim. Sci. 81:1051–1056. doi:10.2527/2003.8141051x.

Rebelo, L., C. Lee, W. Weiss, and M. Eastridge. 2020. Effects of Enogen Feed corn silage and corn grain on nutrient digestibility, production, and enteric methane emission in lactating cows. J. Dairy Sci. 103 (Suppl. 1): 171 (Abstract).

Rojo-Rubio, R., G. Mendoza-Martinez, and M. Crosby-Galvan. 2001. Use of thermostable amylase from Bacillus licheniformis on in vitro starch digestion of sorghum and corn. Agrociencia 35:423-427.

Rusche, W. C., J. A. Walker, and Z. K. Smith. 2020. Effect of inclusion rate of silage with or without alpha-amylase trait on finishing steer growth performance, carcass characteristics, and agronomic efficiency measures. Transl. Anim. Sci. 4:942–949. http://doi.org/10.1093/tas/txaa056.

Salem, A., H. Gado, D. Colombatto, and M. Elghandour. 2013. Effects of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. Livestock Science 154(1-3):69-73.

Schingoethe, D. J., G. A. Stegeman, and R. J. Treacher. 1999. Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. J. Dairy Sci. 82:996–1003.

Schoonmaker, J. P., M. E. Persia, and D. C. Beitz. 2014. Effect of feeding corn modified to contain a unique amylase on performance and carcass characteristics of feedlot steers. Prof. Anim. Sci. 30:561–565. http://doi.org/10.15232/pas.2014-01322.

Shaver, R.D. 2019. Enogen Corn silage research summary. Proceedings of the Four-State Dairy Nutrition Conference. Page 159-161. http://fourstatedairy.org/proceedings/2019/19_4state_proceedings.pdf.

Shaver, R., and R. Kaiser. 2011. Top producing dairy herds in Wisconsin feed more forage than you may think. Accessed Sep. 17, 2021. http://www.uwex.edu/ces/dairynutrition/documents/mfaforage focusnov-2011shaver.pdf.

Tester, R.F., J. Karkalas, and X. Qi. 2004. Starch - Composition, fine structure and architecture. J. Cereal Sci. 39:151–165. doi:10.1016/j.jcs.2003.12.001.

Tricarico, J. M., J. D. Johnston, and K. A. Dawson. 2008. Dietary supplementation of ruminant diets with an Aspergillus oryzae α-amylase. Anim. Feed Sci. and Tech. 145:136–150. http://doi.org/10.1016/j.anifeedsci.2007.04.017.

Tricarico, J. M., J. D. Johnston, K. A. Dawson, K. C. Hanson, K. R. McLeod, and D. L. Harmon. 2005. The effects of an Aspergillus oryzae extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating Holstein cows. Anim. Sci. J. 81:365–374. http://doi.org/10.1079/ASC50410365.

Tricarico, J. M., M. D. Abney, M. L. Galyean, J. D. Rivera, K. C. Hanson, K. R. McLeod, and D. L. Harmon. 2007. Effect of dietary *Aspergillus oryzae* extract containing α-amylase activity on performance and carcass characteristics of finishing beef cattle. J. Anim. Sci. 85:802-811.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2 nd ed. Cornell Univ. Press, Ithaca, NY.

Varga, G. A. and E. S. Kolver. 1997. Microbial and animal limitations to fiber digestion and utilization. J. Nutr. 127:819S–823S.

Vargas-Rodriguez, C. F., M. Engstrom, E. Azem, and B. J. Bradford. 2014. Effects of dietary amylase and sucrose on productivity of cows fed low-starch diets. J. Dairy Sci. 97:4464–4470. http://doi.org/10.3168/jds.2013-7845.

Yang, W. Z., and K. A. Beauchemin. 2009. Increasing physically effective fiber content of dairy cow diets through forage proportion versus forage chop length: Chewing and ruminal pH. J. Dairy Sci. 92:1603-1615.

Wilkinson, J. M., K. K. Bolsen, and C. J. Lin. 2003. History of silage. In: D. R. Buxton, R. E. Muck, and J. H. Harrison, editors, Silage Science and Technology. Agronomy Monograph Vol. 42 ASA, CSSA, SSSA, Madison, WI. pp. 1-30.

Zebeli, Q., J. R. Aschenbach, M. Tafaj, J. Boguhn, B. N. Ametaj, and W. Drochner 2012. *Invited review:* Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J. Dairy Sci. 95:1041-1056.

CHAPTER 3.

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 effect 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 harvested as silage (ECS) containing a bacterial transgene expressing α-amylase in the endosperm of the grain. The ECS and CON silages were included at 40% of the dietary dry matter (DM) and contained 43.3 and 41.8% DM and (%, DM): neutral-detergent fiber, 36.7 and 37.5, starch, 36.1 vs. 33.1 (on average), 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, Rapid City, SD). Dry matter intake 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, by ECS-diet. There was no effect of treatment on energy-corrected MY (ECM), but there was a trend 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 by ECS-diet (11.1 vs. 10.3 g/kg 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 milk yield with ECS led to a decrease in enteric CH_4 emission intensity, compared with the control silage.

Keywords: 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, 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, 2020). Dairy farmers and corn growers are striving 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 bacteria in the rumen in converting nutrients into animal product (Hristov et al., 1998). Extensive research has been done with feeding exogenous fibrolytic 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., 2013b). 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, Downers Grove, IL) were originally developed by Syngenta to improve corn ethanol production efficiency. These hybrids are characterized by containing a bacterial transgene expressing high levels of thermotolerant α-amylase 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, along with Rebelo et al. (2020), are the only studies that 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 post-ruminal 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 in 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 (Downers Grove, IL). Both hybrids were planted on June 1, 2018, grown in Centre County, Pennsylvania, and were 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 (https://www.ncdc.noaa.gov/climate-monitoring; accessed Apr. 8, 2021). Both crops were planted with a John Deere 1755 no-till planter (John Deere Co. Moline, Illinois) into 2.02 and 1.01 ha fields. Fields utilized 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 of 79,040 plants/ha. Corn harvest was conducted using a John Deere 6750 self-propelled forage harvester with a kernel processor (John Deere Co.). 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; Fulton, IL) through an applicator on the harvester and

ensiled in 3.0-m diameter plastic silage bags (Up North Plastics, Cottage Grove, MN). 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 d and MY 44.3 ± 10.6 kg/d; at the beginning of the covariate period. Cows were housed at The Pennsylvania State University's Dairy Teaching and Research Center free-stall barn, equipped with a Calan Broadbent Feeding System (American Calan Inc., Northwood, NH) for individual cow monitoring of DMI. Two GreenFeed units (C-Lock Inc., Rapid City, SD) 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 ± 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. For the

blocking process, matching parity was the main criteria followed by reducing the coefficient of variation between the other variables. Cows had free access to drinking water and diets were fed from a Rissler model 1050 TMR mixer (I.H. Rissler Mfg. LLC, Mohnton, PA). Feeding was once daily at around 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 nearly genetically 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 milk containing 3.50% fat and 3.15% true protein at 26 kg/d 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 combined 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; Swedesboro, NJ) 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 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 done by wet chemistry at RRL, whereas lignin, undigested NDF at 240-h (uNDF) and water-soluble carbohydrates were done 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 composition of the silages obtained from RRL was 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 commercially available qualitative test kit (QuickStix for Enogen, EnviroLogix Inc., Portland, ME), 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., Cambridge, MA; incubated at 37°C for 24h). 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 an approximate depth of 2m (San Diego, CA). 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) by using a DS1922L-F5# Thermochron iButton 8K probe (OnSolution Pty Ltd, Baulkham Hills, Australia). 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 composition of the diets (i.e., CP, NDF, ADF, ether extract, starch, ash, Ca, and P) was 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 milk urea nitrogen (MUN) using infrared spectroscopy (MilkoScan 4000; Foss Electric, Hillerød, Denmark). Milk

composition data were weighed for the corresponding weekly 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 wks 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 supplemental material [(SM); https://doi.org/10.26208/am92-yn24)].

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

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. (2020). A pelletized bait feed (Stocker Grower 14, Purina Animal Nutrition LLC, Shoreview, MN) 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 (RFID) 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 done 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 DMI) and intensity (i.e., g/kg milk or ECM yield).

Ruminal contents. Rumen samples were collected from 10 cows (5 CON- and 5 ECS-fed cows) on day 3 of wk 3 of the experimental period (i.e., following 2 wks of adaptation to treatment diets), at 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, Dieterich, IL) 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 (pH meter 59000–60 pH Tester, Cole-Parmer, Instrument Company, Vernon Hills, IL) 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 SM (https://doi.org/10.26208/am92-yn24). 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, Lake Forest, IL), centrifuged (Sorvall RC-5C Plus, Marshall Scientific, Hampton, NH) 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 feed delivery 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- (**UUN**), and total-N. Allantoin was analyzed following the procedure by Chen et al. (1992). Stanbio Laboratory (Boerne, TX) kits were used to analyze uric acid (Uric Acid Kit 1045), creatinine (Creatinine Kit 420), and UUN (Urea Nitrogen Kit 580). Total N was analyzed in freeze-dried, diluted 1:10 and acidified urine samples using a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA). 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, UUN, and purine derivatives excretions were calculated using the estimated urine output.

Fecal samples were oven-dried at 65°C, ground through 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, Haan, Germany) 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., Macedon, NY) 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 25-μm pore size Ankom filter bags (F57; Ankom Technology, Macedon, NY) 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., Cary, NC). Descriptive statistics of the nutrient composition of the silages were done 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 Shapiro-Wilk test was less than 0.05 (i.e., SCC, protozoal counts, and rumen microbial diversity data). The statistical model for the production (DMI, MY, milk composition, BW, feed efficiency and ECM feed efficiency) and CH_4 emission data included the fixed effect 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 kg of DMI) and intensity (per kg 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 indicated otherwise, data are presented as LSM.

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 profile of the silages are presented in Table 1 and Table 2, respectively. Silage DM was high, most likely a consequence of delayed harvest. Overall, with exception of the high DM, nutrient composition of the silages fell within the ranges of silages used by Ferraretto and Shaver (2015). Silage DM at feed out 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 greater 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 greater for ECS than CON silage (Table 1). 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, when 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, there were some differences in their nutritional composition. This emphasizes the importance of investigating the effect of environmental conditions on ECS nutrient composition.

Particle size distribution of the samples (as % 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. There were also no differences in fermentation acids between silages, except ECS had a 13.8% lower acetic acid concentration when 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 also 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. There were 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 et al. (2008) and Kung et al. (2018). Average OMDI of ECS was 73% while 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 concentration of the diets was 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 when 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 that 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 where Enogen was fed in the diet either as grain, 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, little 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 the above 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 effect on MY or ECM when applying liquid amylase onto the 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 when 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 adding liquid amylase to a TMR formulated with coarsely ground corn and 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 increased in dietary starch concentration. Assuming 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 to a difference in MY of 0.1 kg/d. Other data, however, suggest a much greater response in MY to differences in dietary starch concentration. In a study by Agle et al. (2010), the MY of Holstein cows was 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 by 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, there was 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 milk yield, relative to CON. Increased milk protein yield in response to ECS inclusion in the diet

was also reported by Rebelo et al. (2020). Similar effect 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 increased (respectively) 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. There were only minor changes in milk FA concentrations due to treatment and details on this analysis are presented as Supplemental Table S4 (https://doi.org/10.26208/am92-yn24). 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 discussion below). 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 Rebelo et al. (2020; L. Rebelo, The Ohio State University, Wooster, OH, personal communication) study. 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 (discussed below). In the current experiment, treatment had no effect on BW or BW change of the cows (Table 4) and there was no treatment × week interaction ($P \geq 0.12$) 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 kg of MY) was decreased ($P < 0.01$) by the ECS-diet, when compared with CON; CH_4 emission intensity expressed per kg of ECM, however, was not affected by treatment. There is little evidence of direct effects of exogenous enzymes on enteric CH_4 production (Hristov et al., 2013b). Clearly, the 7% reduction in CH_4 emission intensity (per kg 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 when compared to 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 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. There were 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., Research Triangle Park, NC) 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). 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 equally apply 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, there was no difference in the acetate to propionate ratio and NH_3 concentration 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 (see above) and greater starch intake with the ECS-diet, when 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 a greater ($P = 0.08$) DM digestibility (DMD) by ECS-diet when 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, which along with the greater starch intake showed by cows fed the ECS diet could partially explain the differences in MY. Research with 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 when Enogen corn grain was fed to beef cattle (Johnson et al., 2020). As discussed above, ECS had a greater estimated OMDI (resulting from greater starch content and 30-h NDF digestibility and lower ADF and lignin, when 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, there were no other statistical differences in nutrient digestibility 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 any 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 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 discrepancy in in vitro and in vivo responses indicate that further investigation of this technology is needed. There was also no effect of the ECS-diet on total-tract fiber digestibility. 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

There was 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, there were 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 above, 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 in 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 line 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 tended to improve ECM feed efficiency in dairy cows, when 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 impact 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.

References

Agle, M., A. N. Hristov, S. Zaman, C. Schneider, P. Ndegwa, and V. K. Vaddella. 2010. Effect of dietary concentrate on rumen fermentation, digestibility, and nitrogen losses in dairy cows. J. Dairy Sci. 93:4211–4222. https://doi.org/10.3168/jds.2009-2977

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334. https://doi.org/10.2527/jas.2009-1779.

Andreazzi, A. S. R., M. N. Pereira, R. B. Reis, R. A. N. Pereira, N. N. Morais Júnior, T. S. Acedo, R. G. Hermes, and C. S. Cortinhas. 2018. Effect of exogenous amylase on lactation performance of dairy cows fed a high-starch diet. J. Dairy Sci. 101:7199–7207. https://doi.org/10.3168/jds.2017-14331.

AOAC International. 1997. Yeast and Mold Counts in Foods. J. AOAC Int. 80,806(1997).

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International, Arlington, VA.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International, Arlington, VA.

APHIS. 2011. Final Environmental Assessment Alpha-Amylase Maize Event 3272 https://www.aphis.usda.gov/brs/aphisdocs/05_28001p_ea.pdf (accessed May 14, 2021).

Atichokudomchai, N., J. Jane, and G. Hazlewood. 2006. Reaction pattern of a novel thermostable α-amylase. Carbohydrate Polymers 64:582-588.

Baker, A., V. de A. Veloso, and L. Barros. 2019. Feedlot performance and carcass characteristics of steers fed diets containing steam-flaked grain and corn silage from Enogen Feed Corn. J. Anim. Sci. 97 (Suppl. 2):137. https://doi.org/10.1093/jas/skz122.243

Brinton, M., B. M. Boyd, F. H. Hilscher, L. J. Mcphillips, J. MacDonald, and G. Erickson. 2020a. University of Nebraska - Lincoln Evaluating Syngenta Enogen Feed Corn Silage or Grain on Growing Beef Cattle Performance.

Brinton, M., B. M. Boyd, F. H. Hilscher, L. J. Mcphillips, J. MacDonald, and G. Erickson. 2020b. University of Nebraska - Lincoln Impact of Feeding Syngenta Enhanced Feed Corn as Dry-Rolled Corn, High- Moisture Corn, or a Blend to Finishing Yearlings.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clinical chemistry 8:130–132. http://doi.org/10.1093/clinchem/8.2.130.

Chen, X. B., Y. K. Chen, M. F. Franklin, E. R. Orskov, and W. J. Shand. 1992. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. J. Anim. Sci. 70:1534–1542. http://doi.org/10.2527/1992.7051534x.

Cueva, S. F., H. Stefenoni, A. Melgar, S. E. Räisänen, C. F. A. Lage, D. Wasson, M. E. Fetter, A. M. Pelaez, G. W. Roth and A. N. Hristov. 2021. Lactational performance, rumen fermentation and enteric methane emission of dairy cows fed an amylase-enabled corn silage. Supplemental Material and Tables. https://doi.org/10.26208/am92-yn24.

Danner, H., M. Holzer, E. Mayrhuber, and R. Braun. 2003. Acetic acid increases stability of silage under aerobic conditions. Appl. Environ. Microbiol. 69:562–567. http://doi.org/10.1128/AEM.69.1.562-567.2003.

Ferraretto, L. F., and R. D. Shaver. 2015. Effects of whole-plant corn silage hybrid type on intake, digestion, ruminal fermentation, and lactation performance by dairy cows through a meta-analysis. J. Dairy Sci. 98:2662–2675. http://doi.org/10.3168/jds.2014-9045.

Ferraretto, L. F., R. D. Shaver, M. Espineira, H. Gencoglu, and S. J. Bertics. 2011. Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. J. Dairy Sci. 94:1490–1499. http://doi.org/10.3168/jds.2010-3736.

Ferraretto, L. F., P. M. Crump, and R. D. Shaver. 2013. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. J. Dairy Sci. 96:533–550. doi:10.3168/jds.2012-5932.

Gencoglu, H., R. D. Shaver, W. Steinberg, J. Ensink, L. F. Ferraretto, S. J. Bertics, J. C. Lopes, and M. S. Akins. 2010. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. J. Dairy Sci. 93:723–732. http://doi.org/10.3168/jds.2009-2673.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (Apparatus, reagents, procedures and some applications. Agriculture Handbook No. 379. Agricultural Research Service, USDA, Washington, DC.

Goeser, J. 2016. Rock River Laboratory in situ Rumen Digestion Method Description.

Hall, M. B. 2009. Determination of starch, including maltooligosaccharides, in animal feeds: Comparison of methods and a method recommended for AOAC collaborative study. J. AOAC Int. 92:42–49. http://doi.org/10.1093/jaoac/92.1.42.

Heinrichs, A. J. and P. J. Kononoff. 2002. Evaluating particle size of forages and TMRs using the New Penn State Forage Particle Separator. DAS 02-42. Penn State Coop. Ext., University Park, PA.

Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465–2474. http://doi.org/10.3168/jds.2010-3562.

Holtshausen, L., Y. H. Chung, H. Gerardo-Cuervo, M. Oba, and K. A. Beauchemin. 2011. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. J. Dairy Sci. 94:899–907. http://doi.org/10.3168/jds.2010-3573.

Horton, L., and Drouillard, J. 2018. Effects of particle size and heat treatment on in situ and in vitro digestion of dry-rolled Enogen® Feed Corn. Journal of Animal Science, Volume 96, Issue suppl_3, December 2018, Page 391, https://doi.org/10.1093/jas/sky404.857

Hristov, A. N., C. E. Basel, A. Melgar, A. E. Foley, J. K. Ropp, C. W. Hunt, and J. M. Tricarico. 2008. Effect of exogenous polysaccharide-degrading enzyme preparations on ruminal fermentation and digestibility of nutrients in dairy cows. Anim. Feed Sci. Technol. 145:182–193. http://doi.org/10.1016/j.anifeedsci.2007.05.051.

Hristov, A. N., C. Lee, T. Cassidy, K. Heyler, J. A. Tekippe, G. A. Varga, B. Corl, and R. C. Brandt. 2013a. Effect of Origanum vulgare L. leaves on rumen fermentation, production, and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 96:1189–1202. http://doi.org/10.3168/jds.2012-5975.

Hristov, A. N., C. Lee, T. Cassidy, M. Long, K. Heyler, B. Corl, and R. Forster. 2011. Effects of lauric and myristic acids on ruminal fermentation, production, and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 94:382–395. http://doi.org/10.3168/jds.2010-3508.

Hristov, A. N., E. Kebreab, M. Niu, J. Oh, C. Arndt, A. Bannink, A. R. Bayat, A. F. Brito, D. Casper, L. A. Crompton, J. Dijkstra, P. C. Garnsworthy, N. Haque, A. L. F. Hellwing, P.

Huhtanen, M. Kreuzer, B. Kuhla, P. Lund, J. Madsen, S. C. McClelland, P. Moate, C. Muñoz, N. Peiren, J. M. Powell, C. K. Reynolds, A. Schwarm, K. J. Shingfield, T. M. Storlien, M. R. Weisbjerg, Z. Yu, T.B. Boland, C. Martin, M. Eugène, D. R. Yáñez-Ruiz, S. Muetzel. 2018. Symposium review: Uncertainties in enteric methane inventories, measurement techniques, and prediction models. J. Dairy Sci. 101:6655-6674. doi.org/10.3168/jds.2017-13536.

Hristov, A. N., J. Oh, J. L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H. P. S. Makkar, A. T. Adesogan, W. Yang, C. Lee, P. J. Gerber, B. Henderson, and J. M. Tricarico. 2013b. Mitigation of methane and nitrous oxide emissions from animal operations: A review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069. http://doi.org/10.2527/jas.2013-6583.

Hristov, A. N., K. Heyler, E. Schurman, K. Griswold, P. Topper, M. Hile, V. Ishler, E. Wheeler, and S. Dinh. 2015. Reducing dietary protein decreased the ammonia emitting potential of manure from commercial dairy farms. Prof. Anim. Sci. 31:68-79.

Hristov, A. N., T. A. McAllister, and K. J. Cheng. 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility. J. Anim. Sci. 76:3146–3156. http://doi.org/10.2527/1998.76123146x.

Hu, W., M. E. Persia, and L. Kung. 2010. Short communication: In vitro ruminal fermentability of a modified corn cultivar expressing a thermotolerant α-amylase. J. Dairy Sci. 93:4846–4849. http://doi.org/10.3168/jds.2009-2321.

Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. and Technol. 48:211–227. http://doi.org/10.1016/0377-8401(94)90173-2.

Huntington, G. B., D. L. Harmon, and C. J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84 Suppl:14–24. http://doi.org/10.2527/2006.8413_supplE14x.

Johnson, M. A., T. J. Spore, S. P. Montgomery, W. R. Hollenbeck, R. N. Wahl, E. D. Watson, and D. A. Blasi. 2019. Syngenta Enogen Feed Corn silage containing an alpha amylase expression trait improves feed efficiency in growing calf diets. Kansas Agricultural Experiment Station Research Reports 5. http://doi.org/10.4148/2378-5977.7719.

Johnson, M. A., T. J. Spore, S. P. Montgomery, C. S. Kubick, J. S. Garzón, W. R. Hollenbeck, R. N. Wahl, C. I. Vahl, E. D. Watson, and D. A. Blasi. 2020. Syngenta Enogen Feed Corn containing an alpha amylase expression trait improves digestibility in growing calf diets. Kansas Agricultural Experiment Station Research Reports 6. http://doi.org/10.4148/2378-5977.7890.

Jordan, E. R., and R. H. Fourdraine. 1993. Characterization of the management practices of the top milk producing herds in the country. J. Dairy Sci. 76:3247-3256. https://doi.org/10.2134/agronmonogr42.c12

Klingerman, C. M., W. Hu, E. E. McDonell, M. C. DerBedrosian, and L. Kung. 2009. An evaluation of exogenous enzymes with amylolytic activity for dairy cows. J. Dairy Sci. 92:1050–1059. http://doi.org/10.3168/jds.2008-1339.

Kung, L., A. C. Sheperd, A. M. Smagala, K. M. Endres, C. A. Besset, N. K. Ranjit, and J. L. Glancey. 1998. The effect of preservatives based on propionic acid on the fermentation and aerobic stability of corn silage and a total mixed ration. J. Dairy Sci. 81: 1322-1330. http://doi.org/10.3168/jds.S0022-0302(98)75695-4.

Kung, L., R. D. Shaver, R. J. Grant, and R. J. Schmidt. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. J. Dairy Sci. 101:4020–4033. http://doi.org/10.3168/jds.2017-13909.

Lage, C. F. A., S. E. Räisänen, A. Melgar, K. Nedelkov, X. Chen, J. Oh, M.E. Young, J. Bender, B. Vecchiarelli, N, Indugu, D. Pitta, and A. N. Hristov. 2020. Comparison of two sampling techniques for evaluating ruminal fermentation and microbiota in the ruminal planktonic phase of dairy cows. Frontiers Microbiology. https://doi.org/10.3389/fmicb.2020.618032

Lee, C., A. N. Hristov, T. W. Cassidy, K. S. Heyler, H. Lapierre, G. A. Varga, M. J. de Veth, R. A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. J. Dairy Sci. 95:6042–6056. http://doi.org/10.3168/jds.2012-5581.

Lee, C., D. L. Morris, and P. A. Dieter. 2019. Validating and optimizing spot sampling of urine to estimate urine output with creatinine as a marker in dairy cows. J. Dairy Sci. 102: 1-10. https://doi.org/10.3168/jds.2018-15121

McAllister, T. A., R. Feniuk, Z. Mir, P. Mir, L.B. Selinger, and K. J. Cheng. 1998. Inoculants for alfalfa silage: Effects on aerobic stability, digestibility and the growth performance of feedlot steers. Livest. Prod. Sci. 53:171–181. http://doi.org/10.1016/S0301-6226(97)00150-4.

McCarthy, M. M., M. A. Engstrom, E. Azem, and T. F. Gressley. 2013. The effect of an exogenous amylase on performance and total-tract digestibility in lactating dairy cows fed a high-byproduct diet. J. Dairy Sci. 96:3075–3084. http://doi.org/10.3168/jds.2012-6045.

Melgar, A., C. F. A. Lage, K. Nedelkov, S. E. Räisänen, H. Stefenoni, M. E. Fetter, X. Chen, J. Oh, S. Duval, M. Kindermann, N. D. Walker, and A. N. Hristov. 2020. Enteric methane emission, milk production, and composition of dairy cows fed 3-nitrooxypropanol. J. Dairy Sci. 104 http://doi.org/10.3168/jds.2020-18908.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press. Washington, DC.

NOAA (National Oceanic and Atmospheric Administration). 2021. National centers for environmental information – climate monitoring. Accessed Apr. 8, 2021. https://www.ncdc.noaa.gov/climate-monitoring

Nozière, P., W. Steinberg, M. Silberberg, and D. P. Morgavi. 2014. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. J. Dairy Sci. 97:2319–2328. http://doi.org/10.3168/jds.2013-7095.

Oh, J., A. N. Hristov, C. Lee, T. Cassidy, K. Heyler, G. A. Varga, J. Pate, S. Walusimbi, E. Brzezicka, K. Toyokawa, J. Werner, S. S. Donkin, R. Elias, S. Dowd, and D. Bravo. 2013. Immune and production responses of dairy cows to postruminal supplementation with phytonutrients. J. Dairy Sci. 96:7830–7843. http://doi.org/10.3168/jds.2013-7089.

Penn State Extension. 2020. PA Commercial Grain and Silage Hybrid Corn Tests Report. https://extension.psu.edu/2020-results-pa-commercial-grain-and-silage-hybrid-corn-tests-report. Accessed January 2021

Rebelo, L., C. Lee, W. Weiss, and M. Eastridge. 2020. Effects of Enogen Feed corn silage and corn grain on nutrient digestibility, production, and enteric methane emission in lactating cows. J. Dairy Sci. 103 (Suppl. 1): 171 (Abstract).

Richards, C. J., J. F. Peterson, R. A. Britton, R. A. Stock, and C. R. Krehbiel. 1995. In vitro starch disappearance procedure modifications. Anim. Feed Sci. Technol. 55:35–45.

Rico, D. E., and K. J. Harvatine. 2013. Induction of and recovery from milk fat depression occurs progressively in dairy cows switched between diets that differ in fiber and oil concentration. J. Dairy Sci. 96:6621–6630. https://doi .org/10.3168/ jds.2013-6820.

Rusche, W. C., J. A. Walker, and Z. K. Smith. 2020. Effect of inclusion rate of silage with or without alpha-amylase trait on finishing steer growth performance, carcass characteristics, and

agronomic efficiency measures. Transl. Anim. Sci. 4:942–949. http://doi.org/10.1093/tas/txaa056.

Schneider, B. H., and W. P. Flatt. 1975. The evaluation of feeds through digestibility experiments. University of Georgia Press, Athens, GA.

Schoonmaker, J. P., M. E. Persia, and D. C. Beitz. 2014. Effect of feeding corn modified to contain a unique amylase on performance and carcass characteristics of feedlot steers. Prof. Anim. Sci. 30:561–565. http://doi.org/10.15232/pas.2014-01322.

Shaver, R.D. 2019. Enogen Corn silage research summary. Proceedings of the Four-State Dairy Nutrition Conference. Page 159-161. http://fourstatedairy.org/proceedings/2019/19_4state_proceedings.pdf

Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1990. A Nordic proposal for an energy corrected milk (ECM) formula. Pages 156-157 in 27th Session of the International Commission for Breeding and Productivity of Milk Animals, Paris, France. Wageningen Academic Publishers, Wageningen, the Netherlands.

Tricarico, J. M., J. D. Johnston, K. A. Dawson, K. C. Hanson, K. R. McLeod, and D. L. Harmon. 2005. The effects of an Aspergillus oryzae extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating Holstein cows. Anim. Sci. J. 81:365–374. http://doi.org/10.1079/ASC50410365.

United States Department of Agriculture National Statistics Service. 2020. Crop Production 2019 Summary 8–102.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. http://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Vargas-Rodriguez, C. F., M. Engstrom, E. Azem, and B. J. Bradford. 2014. Effects of dietary amylase and sucrose on productivity of cows fed low-starch diets. J. Dairy Sci. 97:4464–4470. http://doi.org/10.3168/jds.2013-7845.

Ward, R. T. and M. B. de Ondarza. 2008. Fermentation analysis of silage: Use and Interpretation 1–18. Cumberland Valley Analytical Services, Inc. Hagerstown, MD.

Weiss, W. P., W. Steinberg, and M. A. Engstrom. 2011. Milk production and nutrient digestibility by dairy cows when fed exogenous amylase with coarsely ground dry corn. J. Dairy Sci. 94:2492–2499. http://doi.org/10.3168/jds.2010-3766.

Yang, C. M. J., and G. A. Varga. 1989. Effect of three concentrate feeding frequencies on rumen protozoa, rumen digesta kinetics, and milk yield in dairy cows. J. Dairy Sci. 72:950–957. http://doi.org/10.3168/jds.S0022-0302(89)79188-8.

Table 3-1. Nutrient composition mean ± standard error (% of DM or as indicated) of Control (CON) and Enogen (ECS) corn silages

	Corn Silage[1]	
Item	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[4,2]	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., Waynesboro, PA and Rock River Laboratory, Watertown, WI), 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 NIR 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 3-2. Fermentation characteristics and aerobic stability means ± standard error (% of DM or as indicated) of Control (CON) and Enogen (ECS) corn silages

	Corn Silage[1]	
Item	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], $\times 10^5$ cfu/g	45.5 ± 19.13	5.35 ± 5.32
Yeast[7], $\times 10^9$ 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., Waynesboro, PA and Rock River Laboratory, Watertown, WI), 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.

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

	Diet[1]	
Item	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,[9] % 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/day	235	136

[1]Average diet composition for adaptation and experimental periods (i.e wks 2 to 8)
[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, Ames, IA).
[5]Liquid molasses from Westway Feed Products (Tomball, TX).
[6]The mineral and vitamin premix (Cargill Animal Nutrition, Cargill Inc., Roaring Spring, PA) 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., Way nesboro, PA) of individual feed ingredients and their inclusion rate in the diets.
[8]Estimated based on NRC (2001) by Cumberland Valley Analytical Services Inc., Waynesboro, PA.
[9]Estimated based on NRC (2001) using actual DMI, milk yield, milk composition, and BW of the cows throughout the experiment.

Table 3- 4. Effect of an amylase-enabled (Enogen) 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], × 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]Somatic cell count. 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.

Table 3-5. Effect of an amylase-enabled (Enogen) corn silage on enteric gas emissions in dairy cows

	Treatment[1]			
Item	CON	ECS	SEM[2]	*P*-value[3]
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				
CO_2, g/d	13,730	13,835	158.0	0.62
H_2				
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).

Table 3-6. Effect of an amylase-enabled (Enogen) 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.

Table 3-7. Effect of an amylase-enabled (Enogen) 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 3-8. Effect of an amylase-enabled (Enogen) corn silage on nitrogen utilization and urinary purine derivative excretion in dairy cows

	Treatment[1]			
Item	CON	ECS	SEM[2]	*P*-value[3]
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 nitrogen
[5]For details, see Materials and Methods.
[6]PD = purine derivatives.

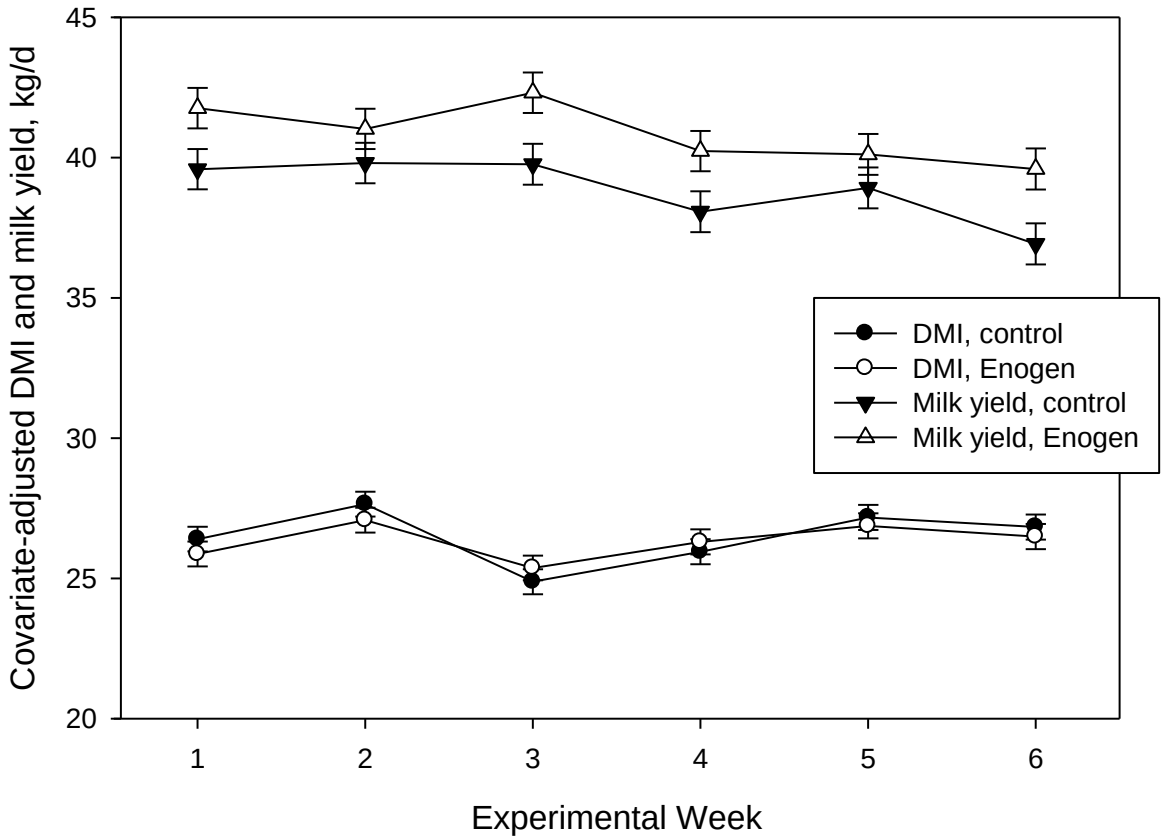

Figure 3-1. Effect of an amylase-enabled (Enogen Feed Corn) corn silage on DMI and milk yield (MY) in dairy cows over the course of the experiment. Treatments were Control (CON) and Enogen (ECS) corn silages, both fed at 40% of DM. Data are covariate-adjusted LSM, and error bars represent SEM. Main effect 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.

CHAPTER 4. CONCLUSIONS AND FUTURE RESEARCH

Conclusions

Utilization of amylase-enabled corn hybrids as a forage for cattle is relatively new. Discrepancies in data from the studies reviewed indicate that corn processing, animal type and growth stage, nutritional characteristics of the plant material, dietary characteristics, and dietary inclusion rate are all factors that can affect animal response to both exogenous amylase additives and amylase-enabled hybrids. Therefore, more research must certainly be done when feeding these hybrids to cattle to concretely elucidate their effects on productive performance.

Enogen corn hybrids, originally developed by Syngenta for ethanol production, are the amylase-enabled hybrids currently on the market that have been used in the cattle industry. Despite this, there is a lack of research done evaluating its effects when fed to dairy cows and results reported for the beef industry have been generally inconsistent. Nevertheless, it should be highlighted that increases in feed efficiency have been observed by various experiments, mostly as a result of improved dry and organic matter digestibility when feeding Enogen corn, suggesting that the potential benefits posited by inclusion of Enogen hybrids in cattle diets should not be discarded. Thus far, it is difficult to infer about the specific mechanisms of action of the amylase enzyme since reports on the production effects of these hybrids have also highlighted the confounding effect that type, processing and amount of dietary starch and other ingredients have on animal response to Enogen hybrids.

In the context of ECS, the friction and heat that characterize the chopping and kernel processing during ensilement could activate the enzyme even before being consumed by the cow. The relatively high temperatures and consequent drop in pH during the initial stages of ensiling could also provide the adequate environment for enzymatic activation and produce differences in

silage fermentation and digestibility. Furthermore, it is known that increasing ensiling duration is concomitant with increases in starch digestibility of the silage; this might preclude the detection of enzymatic effects on starch digestibility of silages that have been ensiled for longer periods of time. Thus, it is possible that the presence of the enzyme in the ensiled material could affect fermentation during the initial phases of ensilement. Additionally, physiological rumen temperatures could also enhance enzyme activation, thus increasing starch degraded in the rumen. This is in line with previous research investigating amylase enzymes that suggest that enzymatic activation in the rumen can not only have effects on milk yield but also on milk components.

The experiment in chapter 3 of the current thesis tested the inclusion of ECS at 40% of dietary DM. Cows fed the ECS diet in that experiment did not show any effects on DMI but increased MY by 2 kg/d, improved feed efficiency and tended to improve ECM feed efficiency, when compared with the isogenic line without the amylase trait. As mentioned above, it is possible that the long ensiling period of the silages (over 220 d at the beginning of the experiment) could have affected treatment responses, as 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. It should be noted that ECS had a greater starch content when compared to the isogenic line. However, it appears, that the difference in starch intake between treatments in the experiment could only partially account for the increased milk production reported for cows fed the ECS diet relative to the CON.

Absolute CH_4 emission was not different between treatments, but the diet with ECS decreased CH_4 emission intensity (per unit of milk, but not ECM). Results from the two research

trials done in dairy cattle to this date suggest that inclusion of ECS in the diet could have a positive impact on the carbon footprint of milk production by increasing productive efficiency of dairy cows (Rebelo et al., 2020; Cueva et al., 2021).

Regarding rumen fermentation, there were no differences between treatments in total VFA concentration or molar proportion of individual VFA, except for a lower molar proportion of butyrate reported for ECS-fed cows. 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 and greater starch intake with the ECS-diet, when compared with CON.

It is likely that effects induced by ECS in the study of the current thesis 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. These results are in line with previous research in cattle showing that Enogen hybrids can in fact offer greater contents of available nutrients for the animal. However, no difference between treatments were observed in N utilization and excretion and, to the best of my knowledge, there are no published reports to date investigating the effect of amylase-enabled hybrids on these variables.

Overall, the 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 discrepancy in vitro and in vivo responses indicate that further investigation of this technology is necessary.

Future Research

Currently, follow-up research is being done at Penn State University feeding ECS to dairy cows in mid-lactation. It is a continuation of the work presented in chapter 3 of this thesis, which branched from the production effects observed in that experiment as a result of feeding

ECS. In the follow-up study, ECS is being fed to cows with the purpose of determining if whether silage (with an expected greater starch digestibility but lower in starch concentration) with or without corn supplementation will be comparable, from a productive standpoint, with a corn silage with greater starch content (used as a control). Results from that experiment, along with what is reported in the current thesis might provide with further knowledge needed to better understand the mechanisms behind the technology and elucidate its potential nutritional benefits.

Moreover, it is clear that agronomic practices and environmental factors can have a significant effect on the nutritional characteristics of the corn plant, making a nutritional parity, even between genetically identical hybrids, challenging. Considering that Enogen corn hybrids have been on the market for the past decade, it would be recommended to do a summary and analysis of the nutritional characteristics of these hybrids across different locations and climates. This would provide a better understanding of the variation in nutritional composition of the plant material.

There has been little research done feeding amylase-enabled corn, specifically in the form of silage. Reviewed literature and the results from the experiment in chapter 3 of this thesis would suggest that the amylase gene encoded in the endosperm of the grain could have an effect on the nutritional characteristics of the corn during ensilement. Thus, future investigation must scrutinize the potential changes in the nutritional composition of the ensiled material accounting for increases in ensiling time. This would require collecting samples across the different stages of silage fermentation and running in situ incubations to try and elucidate the potential differences in ruminal DM, OM, NDF and starch degradabilities between the ECS and a control silage. It would also be advisable to measure amylase activity in the silage across different ensiling times to explore for a possible correlation between amylase activity in the silage and in

the rumen of cows fed ECS. In chapter 3, amylase activity was only measured in samples collected during the feed-out stage of the silage and not during the fermentation phase. Thus, collecting silage samples during the different stages of ensilement is necessary to determine if amylase activity changes with ensiling time.

Ultimately, despite interesting results, no research has been done evaluating potential interactions between other feed additives or dietary ingredients and amylase-enabled corn hybrids in lactating cows. If it can be concretely demonstrated that these hybrids can have a significant effect in rumen or silage fermentation or both, future research should focus on potential interactions between other additives or ingredients and amylase-enabled corn hybrids.

Future research is necessary to completely characterize the mechanisms and modes of action of this technology and more importantly, to determine if the effects of including amylase-enabled hybrids in cattle diets are consistent.