Enogen Corn Hybrids Can Improve Feed Efficiency and Reduce Environmental Impact in Dairy Production

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Abstract/summary

Agricultural production systems have been implicated as significant sources of environmental impacts, including increased global warming potential due to methane and other greenhouse gases generated by livestock, particularly dairy and beef cattle. Global dairy production, estimated at 852 metric tons in 2019, is projected to grow by 1.6% per year through 2029, as demand for high quality protein increases in developing regions. In parallel, the imperative to manage climate change has risen to worldwide attention and incited international calls to action, with a strong focus on reducing methane emissions. Development and adoption of sustainable intensification technologies is critical to our ability to balance these conflicting societal demands. Enogen® corn hybrids (Syngenta Seeds, LLC, Downers Grove, IL) are genetically modified to express a robust alpha-amylase enzyme in the starchy endosperm tissue of corn kernels. Research trials at leading universities have documented improved feed efficiency and reduced nutrient waste when Enogen corn is fed to cattle, as the amylase renders the starch more readily digestible. Life cycle assessments **(LCA)** based on these controlled studies have been completed for Enogen corn in both beef and dairy production systems. Each LCA was conducted in conformance with the ISO 14040:2016, ISO 14044:2006, and ISO/TS 14071:2006 LCAstandards, including an independent review. In a dairy study comparing the impact of diets containing Enogen silage vs conventional silage (40% of dry matter), feed efficiency was improved by 5.4% ($P<0.001$) and enteric methane intensity was reduced by 7% ($P<0.01$). The LCA based on this study quantified environmental impact reductions of 4.6, 6.3, 4.2, and 4.3% for global warming potential, fossil fuel depletion, land use, and water consumption, respectively, with significance confirmed via Monte Carlo uncertainty analysis (n=1000, CI=95%). Similar environmental impact reductions were seen for Enogen in a second dairy LCA and a beef LCA, confirming that use of Enogen corn in cattle diets can yield significant benefits in the sustainability metrics of primary interest, and can assist the livestock sector in meeting sustainability targets.

Introduction

High-producing dairy cows require relatively high-energy diets to meet their energy needs for maintenance, milk yield and components, and body condition maintenance in early lactation or gain in mid/late lactation (NASEM, 2021). Carbohydrates are the primary source of energy in dairy cattle diets, usually comprising about 70% of the diet dry matter **(DM)**. Since starch often comprises 40% of the

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total carbohydrate fraction, starch is a highly important nutrient in the formulation of dairy cattle diets for energy. The NASEM-2021 provides (DM basis) maximum dietary starch guidelines increasing from 22 to 30% as minimum forage NDF guidelines are increased from 15 to 19%. Others (Grant, 2019; Ishler and Becker, 2021) have previously suggested that starch in diets for lactating dairy cows should range between 23 to 30% of DM. Ruminal, intestinal, and total-tract digestibilities of dietary starch in dairy cattle are variable (Ferraretto et al., 2013; NASEM-2021) with total-tract starch digestibility typically ranging from about 80% to nearly 100% (Ferraretto et al., 2013; Fredin et al., 2014; NASEM-2021). Therefore, dietary starch digestibility, as well as the concentration of starch, in the diet impacts energy utilization by dairy cows.

Corn silage is the predominant forage source for dairy and beef cattle around the world, primarily because of its potential for high energy density coupled with high per acre DM yield potential relative to other forage crops. Starch is a highly important nutrient contributing to the energy value of corn silage, with a normal range in starch concentration of 25 to 39% (DM basis; Shaver, 2017); over half of the energy value of corn silage for dairy cows is attributed to starch (Goeser, 2020). Corn silage starch utilization by dairy cows is influenced by its digestibility, which varies depending on many factors, with the most important silage-based ones being stage of maturity/moisture content at harvest, degree of kernel processing, and length of time in storage prior to feeding (Ferraretto and Shaver, 2012; Kung et al., 2018).

Corn grain is the predominant feed energy source for beef and dairy cattle in the USA, and with 70% starch (DM basis) on average, about 3/4ths of the corn grain energy value is derived from starch (Ferraretto et al., 2013). Corn grain starch utilization by dairy cows is influenced by its digestibility which varies depending on many factors, with the grain-based ones being harvest and storage method (dry vs. high moisture corn), stage of maturity/moisture content at harvest, particle size, and length of time in storage prior to feeding for high-moisture corn, dry corn processing (dry rolled vs. steam flaked vs. fineness of grind), and kernel hardness/vitreousness especially in dry corn (Ferraretto et al., 2013).

Unfortunately, undigested starch will pass through cattle and be excreted as fecal starch, representing lost opportunity for improved performance, feed efficiency, and producer profitability. Numerous studies have been conducted to assess the value of adding exogenous amylase to cattle diets to improve starch digestion and feed efficiency, with mixed results. Meale et al. (2014) reviewed experiments on the feeding of exogenous enzymes to ruminants for effects on DM intake **(DMI)**, milk yield and components, and total tract nutrient digestibilities in lactating dairy cows. Eight of the trials reviewed included amylase in the treatment regimen with DMI and milk yield each increased in only two studies and milk fat percentage increased in only one study by feeding exogenous amylase. Digestibility of one or more nutrients was increased in 5 of the 8 studies by feeding exogenous amylase. Meale et al. (2014) also reviewed experiments on the feeding of exogenous enzymes to ruminants for effects on DMI, average daily gain, feed conversion, and total tract nutrient digestibilities in beef cattle. Only two of the trials reviewed included amylase in the treatment regimen with only average daily gain affected in only one study by feeding exogenous amylase. Subsequently, Jolly-Breithaupt et al. (2019) published a study showing improved average daily gain and feed conversion with exogenous amylase in finishing cattle diets.

The last dairy cattle trial reviewed by Meale et al. (2014) was published in 2011. Dairy cattle trials involving the feeding of exogenous amylase published since then and a 2010 paper not in their review were reviewed for this paper and included Gencoglu et al. (2010), Weiss et al. (2011), McCarthy et al. (2013), Noziere et al. (2014), Vargas-Rodriquez et al. (2014), Andreazzi et al. (2018), Silva et al. (2018), Gencoglu et al. (2019a, b), and Zillo et al. (2019). Of these 9 trials (10 reports), feed efficiency was increased in 3 trials (1 related to decreased DMI and 2 related to increased milk yield), milk protein percentage was increased in 1 trial, and DM digestibility was increased in 1 trial by the feeding of exogenous amylase. Otherwise, parameters measured were unaffected by treatment across the trials.

Overall, across this review and the review by Meale et al. (2014), the benefits to the feeding of exogenous amylase were infrequent. Furthermore, the added cost of supplementing exogenous amylase coupled with the results of these reviews precludes confident use recommendations.

Among nutrient excretion and other concerns regarding animal agriculture's impact on the environment, its contribution to global greenhouse gas emissions, estimated at 8 to 10% of total annual anthropogenic emissions (McGeough et al., 2012), has come under increasing scrutiny in recent years, with particular emphasis on the contributions of beef and dairy production systems. A recent University of California-Davis Clear Center white paper (Place and Mitloehner, 2021) presents scenarios showing the magnitude of changes the US beef and dairy industries would need to implement in order to reach "zero warming potential" status by 2050, based primarily on the methane generated by these operations. To be successful, these scenarios estimate that direct methane emissions from enteric sources and manure management practices would need to be gradually reduced by 18 to 32% over the next few decades, along with significant reductions in indirect emissions resulting from feed production and other inputs. These scenarios assume essentially static numbers of dairy and beef cattle through 2050, along with significant productivity improvements in that time frame as follows: 47 lb increase in milk/cow/year coupled with a 52% reduction in indirect greenhouse gas **(GHG)** emissions per unit of milk; and a 16% increase in beef yield per head coupled with a 38% reduction in indirect GHG emissions per unit of carcass weight, respectively. Broad ranging innovation will be needed to address the full array of improvements required to achieve this goal, including reduction of direct emissions, improved crop production processes, and methods for increasing livestock feed efficiency and performance.

From a livestock nutrition standpoint, increasing feed efficiency or reducing DMI per unit milk production in dairy cows or gain in beef cattle is of major importance in reducing enteric methane emissions (Hristov et al., 2017). Other important nutritional interventions with regard to reducing enteric methane emissions include increasing dietary concentrate:forage and starch: fiber ratios and increasing the proportion of corn silage in the dietary forage (Uddin et al., 2020). Anti-methanogenic effects of monensin added to dairy and beef cattle diets have been reviewed through a meta-analysis of published trials with stronger effects detected in beef steers than dairy cows but with dose and diet composition modifications potentially improving the response in dairy cows (Ranga Niroshan Appuhamy et al., 2013). Dietary essential oils added to dairy cow diets may also offer potential to reduce enteric methane emissions (Belanche et al., 2020). Supplemental fat and some specific fatty acids, including myristic, linoleic and linolenic acids, added to ruminant diets can reduce enteric methane production (Rasmussen and Harrison, 2011), but potential adverse effects on certain animal performance parameters, i.e., milk fat production in dairy cows (NASEM, 2021), limits field application.

Some promising technologies for reduction of enteric methane are emerging. Yu et al (2021) summarized the studies published to date for the methane inhibitor, 3-nitrooxypropanol **(3NOP)**, including 27 *in vivo* studies in beef and dairy cattle. The authors concluded that these studies suggest an average 30% reduction in enteric methane emissions, with higher doses needed for similar efficacy in beef cattle fed high-concentrate diets than for dairy cows on high forage diets, but many questions remain unanswered. Studies to date are mostly of short duration in confined feeding settings, therefore do not address the potential for long-term efficacy, use in grazing animals, interaction with other methane reduction strategies, or long-term effects on rumen microbiome composition. With some variability regarding effects on milk fat and protein, the product does not currently appear to have negative effects on milk yield in dairy cows or weight gain in beef cattle, but it also does not appear to increase gain in beef cattle or milk yield in dairy cattle. Another area of active research involves the addition of seaweed-based ingredients to cattle feed to reduce methane emissions. Vijn et al. (2020) summarized the existing knowledge for this approach and presented an exhaustive analysis of the significant research that will be needed to bring these products to the market at scale. Areas for additional research range from "foundational research" needed to identify algal species suited for ocean or tank cultivation, to develop processing methods, and to validate product performance in animal trials, to research into "industrial-scale application" in order to establish robust and reliable supply and utilization by commercial producers, and research into ecological implications of this industrial-scale production. Regulatory issues will need to be resolved as well.

As is the case for exogenous amylase products, these methane inhibitors will come at a cost to the industry, which may not be offset by improved performance. Additional innovations will be needed that offer a return on investment, and Enogen hybrids may be one such innovation to consider.

Enogen hybrids are genetically modified to express high levels of a specific alpha-amylase enzyme in corn grain. Originally developed for the ethanol industry, this alpha-amylase protein is produced by a chimeric gene constructed from gene fragments isolated from three different thermophilic bacteria and reassembled into a new, unique gene (Richardson et al, 2002). This new alpha-amylase gene is linked to a zein promoter which causes the protein it produces to be expressed selectively in the starchy endosperm tissue of the grain as the kernels mature (Tuttle et al, 2006). As a result of laboratory selection processes, the Enogen alpha-amylase exhibits a broad range of temperature and pH tolerance and a low requirement for calcium ion cofactor for efficacy. This biotech event has been authorized in Canada since 2008 and fully deregulated in the US since 2011. Enogen hybrids are currently sold for use in ethanol and feed production across the US, and more recently in Canada. Enogen hybrids express very high levels of alpha-amylase protein, which is embedded within the starch/protein matrix in the kernels. This is expected to improve enzyme content and distribution within a silage pile or in mixed feed rations, to place the enzyme in close proximity to the kernel starch, and to offer some protection for the enzyme until activation occurs. The enzyme is not expected to be denatured by any conditions experienced during crop production, harvesting, or processing, and its wide range of pH and temperature tolerance allows activity under conditions which would be limiting for most exogenous amylase products. These differences in enzyme level, distribution, and robustness form the basis for the performance of Enogen in animal studies, as summarized below.

Enogen Performance and Digestion Studies in Beef Cattle

Previous research has shown that Enogen corn can improve feed efficiency in beef cattle when included in balanced rations as silage or as whole-shelled, dry-rolled or steam-flaked grain. A series of trials conducted at the University of Nebraska-Lincoln showed feed efficiency gains ranging from 1 to 9% when Enogen was included as dry-rolled corn **(DRC)** in the total mixed ration **(TMR)** fed to finishing beef cattle (Jolly-Breithaupt et al., 2019; Brinton, 2019). Variation in feed efficiency was noted based on factors such as the other components of the TMR, especially the type and amount of other corn byproducts, such as distillers' grains or corn gluten meal in the rations. Feed efficiency gains of approximately 5% have also been documented in finishing beef cattle when Enogen corn was fed as either steam-flaked corn **(SFC)** (Horton et al. 2020) or as whole plant corn silage, but similar feed efficiency gains were not observed when both Enogen silage and Enogen SFC were fed simultaneously to finishing cattle in this study (Baker et al., 2019). Enogen hybrids also showed potential to improve feed efficiency in backgrounding cattle when fed as whole-shelled corn **(WSC)** or DRC, or as whole plant corn silage, with feed efficiency gains of 5.5% and 4.4%, respectively, observed for WSC/DRC and silage (Johnson et al., 2018, Johnson et al., 2019). However, rations containing both Enogen DRC and Enogen corn silage did not show an additional feed efficiency gain over the group receiving only Enogen silage in this study (Johnson et al., 2019). These results suggest that there is more to learn about optimizing the use of Enogen hybrids in feed for beef cattle. Where significant feed efficiency improvements were observed, greater average daily gain **(ADG)** tended to be the key driver of efficiency, with reduced DMI playing a secondary role, or no role, in most cases.

Two digestion studies were conducted in parallel to several of the previously described feeding studies - one examining digestion of Enogen as DRC in finishing beef cattle diets (Jolly-Breithaupt et al., 2016) and another examining digestion of Enogen as DRC or WSC in backgrounding cattle diets (Johnson et al., 2020), compared with similarly processed conventional corn. No differences in DMI were observed in either study, and there were no differences in ruminal pH or production of volatile fatty acids **(VFA)** between the Enogen diets and conventional corn diets. Total tract digestibility of starch was increased by 4% in each of these studies (P=0.01 and P=0.16 in finishing and backgrounding studies, respectively), while fecal starch output was reduced by 38% (P=0.01) and 49% ($P \leq 0.01$), respectively, in the finishing and backgrounding study with Enogen DRC diets, and by 37% ($P \leq 0.01$) for the Enogen WSC diet in backgrounding cattle compared to conventional diets. In addition, total tract digestibility of DM and organic matter **(OM)** were increased by 5 to 9% in each study for cattle fed Enogen corn, while fecal output of OM was reduced by 21% in finishing cattle (P=0.05) and fecal DM output was reduced by 10 to 11% in backgrounding cattle ($P \leq 0.01$) fed Enogen diets when compared with conventional corn diets. This suggests that the increased starch digestibility related to the feeding of Enogen corn may have some effect on other components of the diet as well.

Enogen Performance Studies in Dairy Cows

Research results in dairy cows are more limited; however, two recent studies evaluating Enogen corn silage in diets of lactating cows compared to diets containing corn silage from a genetically similar hybrid without the amylase-expression trait (an “Isoline” hybrid) demonstrated positive performance outcomes. Each study is summarized below.

Study #1

A 10-week feeding study was conducted using 48 mid-lactation cows in a randomized complete block design with two treatments (Cueva et al., 2021). Simple replacement of Isoline silage with Enogen silage, each at 40% of TMR DM in an otherwise identical diet, resulted in a 4.4 lb/cow/day (5.2%) increase in milk yield (P<0.001) and a 5.4% increase in feed efficiency (P<0.001). There was also a numerical increase of 3.1 lb/day in energy-corrected milk **(ECM)** yield (P=0.12) and a tendency toward increased ECM efficiency (3.4%, P=0.09) for cows fed Enogen silage. There was no difference in DMI between treatments and, while the Enogen silage had approximately 3%-unit higher starch content than the Isoline silage, this difference translated to 1.2%-unit higher starch in the TMR which was estimated to account for only about 20% of the observed milk yield difference. No significant effects on milk fat or protein percentages were observed, but protein yield, lactose percentage, and lactose yields were increased by 4.2% (P=0,05), 1.2% (P=0.02) and 5.8% (P<0.001), respectively, for the cows fed Enogen silage, while milk urea nitrogen **(MUN)** content was reduced by 9% (P=0.002) for cows fed Enogen silage compared to the cows fed Isoline silage. Single time point ruminal sampling of a subset of the test cattle did not show differences in ruminal fermentation characteristics, except for a reduced molar proportion of butyrate in cows fed Enogen silage (P=0.04). Except for a trend for increased DM digestibility in cows fed Enogen silage (P=0.08), there were no differences in apparent total-tract digestibility of nutrients. Enteric emissions of methane, hydrogen, and CO_2 were measured using two GreenFeed units (C-Lock Inc., Rapid City, SD) available in the free-stall barn. There were no differences in daily emissions per cow for any of the measured gases, but methane per unit of milk (methane intensity) was reduced by 7% for the Enogen-fed cows (P<0.01). Amylase presence and activity were confirmed in Enogen silage samples from this study collected between 220 and 300 days after harvest/ensiling. Amylase activity in the Enogen silage was shown to be 13-fold greater than in the Isoline silage, even after long ensiling times.

Study #2

A second study to evaluate the effects of Enogen corn silage on lactating cows (Rebelo et al., 2020) reported similar findings in most respects. This Latin square design employed 15 cows (including 6 cannulated cows) and three treatments, with each period consisting of 14 days of diet acclimation followed by 14 days of sample collection. Two of the treatments directly compared Enogen silage with Isoline silage, each at 48% of TMR DM in an otherwise nearly identical diet, and only these two treatments are discussed here. Key differences between this study and Study #1 include a 6.4% increase in DMI for the cows fed Enogen silage (P<0.01) vs the Isoline group, and equivalence in starch content between the two silages tested. As in the previously described study, this trial also found significant milk yield increases of 7.5 lb/cow/day (10.3%, P=0.01) and feed efficiency was numerically increased by 3.8% (P=0.24) for cows fed Enogen silage vs cows fed Isoline silage. ECM yield was numerically increased by 4.2 lb/cow/day (5.5%, P=0.32), but ECM feed efficiency did not differ between treatments. There were no differences in milk fat, protein, or lactose percentages, but there were significant increases in protein yield (11.4%, $P<0.01$) and lactose yield (9.4%, $P=0.01$) for the cows fed Enogen silage vs Isoline silage. Analysis of samples from the rumen cannulated cows did not show differences in fermentation characteristics between cows fed the two silages. Enteric emissions of methane, hydrogen and CO_2 were measured using a GreenFeed unit (C-Lock Inc., Rapid City, SD) in the tie-stall barn. There were no differences in daily emissions per cow for any of the measured gases, but methane per unit of milk (methane intensity) was reduced by 26% for the Enogen-fed cows ($P=0.04$) and methane per unit DMI was reduced by 15% ($P<0.01$). Amylase presence and activity were confirmed in Enogen silage samples from this study as well, with amylase activity in the Enogen silage shown to be 7-fold greater than in the Isoline silage, even after long ensiling times (Syngenta, personal communication).

Surprisingly, standard laboratory analytical methods, including *in vitro* and *in situ* rumen starch digestibility assays which often show clear differences between silage produced from Enogen hybrids and other silages (Shaver, 2019), did not show such differences between the Enogen and Isoline silages used in either of these two studies, whereas appreciable amylase activity was verified in the Enogen silages from both locations. Animal performance also showed significant differences in both studies, especially in the longer, larger feeding study conducted by Cueva et al. (2021), suggesting that direct activity of the enzyme during digestion may be playing a critical role in improved performance. Additional research will be needed to clarify and validate these observations.

Dairy Life Cycle Assessment

LCA is a rigorous study of the inputs and outputs of a particular product or product system which provides a scientific basis for evaluating the environmental impacts through each phase of the life cycle and provides an alternative to the single-criterion decision-making that currently guides many environmental choices. As animal agriculture's contribution to global anthropogenic GHG emissions, estimated at 8 to 10% of total annual emissions (McGeough et al. 2012) has come under increasing scrutiny, the use of LCA is growing rapidly in the agricultural and dairy industries to measure, understand, and combat these significant emissions. The dairy LCA study reported here (Sustainable Solutions Corp, Royersford, PA, 2021) was conducted in collaboration with Syngenta, utilizing the data generated in the two dairy studies reviewed above (Cueva et al., 2021; Rebelo et al. 2020) as the main sources of primary data. Primary data related to seed corn production for both Enogen and Isoline hybrids were provided by Syngenta.

The LCA documents the methodology, data, details, and results of the analysis of the environmental impacts associated with one kilogram of energy-corrected milk **(ECM)** produced by animals on Enogen diets compared with the impacts of one kilogram of ECM produced by animals on Isoline diets. The assessments were conducted separately for each of the two primary data sources and in conformance with the ISO 14040 series standards and processes (Klöpffer, 2005).

LCA goal and scope definition

This LCA study is characterized as a "cradle-to-gate" study, examining the milk production from animals fed a diet containing either Enogen corn silage or Isoline corn silage, extending from raw material extraction through milk production. The processing of raw milk and all downstream stages that follow were excluded as they would be identical for Enogen and conventional milk.

Functional unit

The functional unit utilized for this study is one kilogram (kg) of ECM. ECM values were taken directly from the study comparisons in each animal study to avoid altering primary data.

System boundaries

This study considers the life cycle activities from seed production to milk production (milk at the farm gate). The study system boundary (Figure 1) includes the transportation of major inputs to (and within) each activity stage.

Key assumptions

- Identical seed production, processing and packaging for Enogen and Isoline hybrids;
- Transportation distances were identical for Enogen and Isoline hybrid seed and for crops grown from the seed;
- Planting rates and other inputs, such as fertilizers and pesticides, were identical for Enogen and Isoline hybrids at each silage crop production location;
- Annual composition for a herd with 1000 lactating cows was as follows: 850 cows in milk, 150 dry cows, 850 replacement heifers, 369 culled adults, 80 culled heifers, 31 dead adults and 70 dead heifers;
- Dry cow and heifer diets were identical for Enogen and Isoline at each study location; and
- Manure management was accomplished by lagooning at each location.

*Life Cycle Inventory **(LCI)**, modeling tools and databases*

As previously noted, the LCA utilized the data generated in the two dairy studies reviewed above (Study #1, Cueva et al., 2021; and Study #2, Rebelo et al., 2020) as the main sources of primary data. Where primary data were unavailable, critically reviewed secondary studies and secondary data, selected for consistency, precision, and reproducibility, were utilized to fill in data gaps.

This LCA was conducted using SimaPro software with the ecoinvent v3.5 and Agri-footprint v5 databases serving as the primary sources of LCI data for raw materials and processes not directly collected from Syngenta. Where data were not available in the ecoinvent and Agri-footprint databases, data from the Agribalyse database and published reports were used. Data from European databases were adapted using US electricity impacts.

*Life Cycle Impact Assessment **(LCIA)***

The four key impact categories evaluated in this LCA included global warming potential (also referred to as climate change potential), fossil fuel depletion, land use, and water consumption. Eight additional impact categories included ozone depletion, smog, acidification, eutrophication, human health: carcinogens and non-carcinogens, respiratory effects, and ecotoxicity.

Established impact methodologies were used for calculating environmental impacts as follows:

- International Panel on Climate Change (IPCC, 2013) methodology used to calculate global warming potential impact values;

- ReCiPe (H) Midpoint methodology (2016), developed through a cooperative effort among RIVM (National Institute for Public Health and the Environment, Bilthoven, Netherlands); Radboud University, Nijmegen, Netherlands; Leiden University, Leiden, Netherlands; and Pré Sustainability, Amersfoort, Netherlands, used for land use and water consumption impacts; and

- US EPA Tools for Reduction and Assessment of Chemical and other environmental Impacts (TRACI version 2.1) used to calculate all remaining environmental impacts.

Magnitude of environmental impacts in each of the selected impact categories was estimated separately for the feed production phase and for the milk production phase for each hybrid at each study location, and an overall impact for the Enogen diets and the Isoline diets at each study location was determined. These were compared between the two hybrids to determine benefits of the Enogen diet versus the Isoline diet at each study location.

Sensitivity testing and validation

The accuracy and validity of LCA studies are highly reliant on the data used to model the system; therefore, to account for the high amount of variability in agricultural practices, a range of sensitivity studies were conducted to confirm the validity of the results and their dependence on the assumptions made throughout the LCA.

- US average - Replacing state specific cultivation processes for corn grain and corn silage

- Regional – Irrigated vs. non-irrigated systems in key dairy producing states/regions

- Manure management practices – Lagooning vs. dry lot manure management

- Enteric emission differences in dry cows and heifers (held constant in baseline model)

LCA results and interpretation

<u>Study #1</u> compared the impacts of diets containing Enogen silage vs Isoline silage (40% of DM). Enogen increased milk yield by 5.2% (P<0.001), improved feed efficiency by 5.4% (P<0.001) and reduced enteric methane intensity by 7% (P<0.01), with no difference in DMI.

The LCA quantified overall environmental impact reductions per kg ECM of 2 to 6% for the Enogen diet vs. the Isoline diet across all impact categories (Table 1), with reductions for the four key impact categories as follows:

- Global Warming Potential – 4.57%
- Fossil Fuel Depletion – 6.33%
- Land Use – 4.17%
- Water Consumption – 4.26%

Impact reductions are directly related to increased milk production, reduced enteric methane emissions, and increased feed efficiency. Monte Carlo analysis results were less than ±5% different and less than one standard deviation away from the median value through 1000 iterations, confirming significance of these results. Equivalencies in more real-world terms for reductions in the four key impact categories are shown in Figure 2a.

<u>Study #2</u> compared the impacts of diets containing Enogen silage vs Isoline silage (48% of DM). Enogen increased milk yield by 10.3% (P<0.01), improved feed efficiency by 3.8%

(P=0.24) and reduced enteric methane intensity by 26% (P=0.04). DMI was increased by 6.4% for the Enogen diet (P<0.01), which reduced feed efficiency, despite significantly higher milk production for the Enogen diet as compared with Study #1.

The LCA quantified overall environmental impact reductions per kg ECM of 1 to 5% for the Enogen diet vs. the Isoline diet across all impact categories (Table 2), with reductions for the four key impact categories as follows:

- Global Warming Potential – 5.26%
- Fossil Fuel Depletion – 2.21%
- Land Use – 2.10%
- Water Consumption – 1.15%

Impact reductions are directly related to increased milk production and reduced enteric methane emissions. Differences in reductions vs Study #1 are likely due to increased DMI for the Enogen diet. Monte Carlo analysis results were less than ±5% different and less than one standard deviation away from the median value through 1000 iterations (excluding ozone depletion, carcinogenics, non-carcinogenics, and water consumption), confirming significance of these results.

Independent validation of results

After extensive review by an independent panel of experts, the LCA was determined to be in conformance with the applicable ISO standards and the plausibility, quality, and accuracy of the LCA-based data and supporting information were confirmed.

Beef Life Cycle Assessment

A similar LCA was conducted for Enogen in beef cattle (Matlock et al., 2021) based on published studies in backgrounding and finishing cattle with Enogen as DRC replacing conventional corn grain as DRC in the TMR. This LCA showed similar environmental benefits as were observed in dairy cows, with reductions for the four key impact categories as follows:

- Climate change potential – 5.49%
- Fossil Fuel Depletion – 5.72%
- Land Use – 5.78%
- Water Consumption – 5.10%

Equivalencies in more real-world terms for reductions in the four key impact categories are shown in Figure 2b.

Conclusions

Feeding studies conducted with Enogen corn hybrids have demonstrated the potential for feed efficiency improvements of about 5% when Enogen is included as silage or as whole shelled, dry-rolled or steam-flaked grain in beef cattle diets or when included as silage in diets of lactating dairy cows. The Enogen trait technology is fully approved in the US and Canada and can be adopted without increased seed cost compared with other elite corn hybrids and without the need for any other changes to crop production processes or operations at the feedlot or dairy. It is being utilized at commercial scale today. Direct measurements have shown reductions in methane emissions per unit of milk produced by dairy cows in controlled studies. LCA for Enogen in both beef and dairy demonstrate that these feed efficiency gains can also result in significant reductions to environmental impacts in key impact categories, including global warming potential, fossil fuel depletion, land use and water use. While additional technologies are being developed to reduce the environmental impact of animal agriculture, adoption of Enogen hybrids can make immediate contributions to improved efficiency and reduced environmental effects in beef and dairy production.

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Table 1. Dairy Study #1 - Overall life cycle assessment results for cows fed Enogen silage vs cows fed Isoline silage, per kg energy-corrected milk (ECM) yield, by environmental impact category.

		Environmental Impact*		Enogen Impact Reduction*	
Impact Category	Unit	Enogen	Isoline	Absolute	%
Global warming	kg CO_2 eq	2.08	2.18	-0.10	-4.59
Fossil fuel depletion	MJ surplus	0.813	0.868	-0.055	-6.34
Land use	m^2a crop eq	1.47	1.54	-0.07	-4.55
Water consumption	m^3	0.0804	0.0840	-0.0036	-4.29
Ozone depletion	kg CFC-11 eq	3.73E-08	3.81E-08	-8.00E-10	-2.10
Smog	kg O_3 eq	0.205	0.215	-0.010	-4.65
Acidification	kg SO_2 eq	0.0235	0.0245	-0.0010	-4.08
Eutrophication	kg N eq	0.0159	0.0166	-0.0007	-4.22
Carcinogenics	CTUh	6.59E-08	6.87E-08	-2.80E-09	-4.08
Non carcinogenics	CTUh	4.89E-06	5.11E-06	-2.20E-07	-4.31
Respiratory effects	kg PM2.5 eq	0.00112	0.00116	-0.00004	-3.45
Ecotoxicity	CTUe	15.9	16.60	-0.70	-4.22

*per kg ECM yield

Table 2. Dairy Study #2 – Overall life cycle assessment results for cows fed Enogen silage vs cows fed Isoline silage, per kg energy-corrected milk (ECM) yield, by environmental impact category.

		Environmental Impact*		Enogen Impact Reduction*	
Impact category	Unit	Enogen	Isoline	Absolute	%
Global warming	kg CO_2 eq	2.07	2.18	-0.11	-5.26
Fossil fuel depletion	MJ surplus	0.801	0.819	-0.018	-2.21
Land use	m^2a crop eq	1.55	1.59	-0.04	-2.10
Water consumption	m^3	0.0146	0.0148	-0.0002	-1.15
Ozone depletion	kg CFC-11 eq	4.20E-08	4.24E-08	-4.00E-10	-0.87
Smog	kg O_3 eq	0.210	0.219	-0.009	-4.07
Acidification	kg SO_2 eq	0.0194	0.0202	-0.0008	-3.71
Eutrophication	kg N eq	0.0108	0.0110	-0.0002	-2.33
Carcinogenics	CTUh	6.16E-08	6.32E-08	-1.60E-09	-2.51
Non carcinogenics	CTUh	1.73E-06	1.77E-06	-4.00E-08	-2.21
Respiratory effects	kg PM2.5 eq	0.00102	0.00105	-0.00003	-3.01
Ecotoxicity	CTUe	11.7	12.1	-0.40	-2.62

*per kg ECM yield

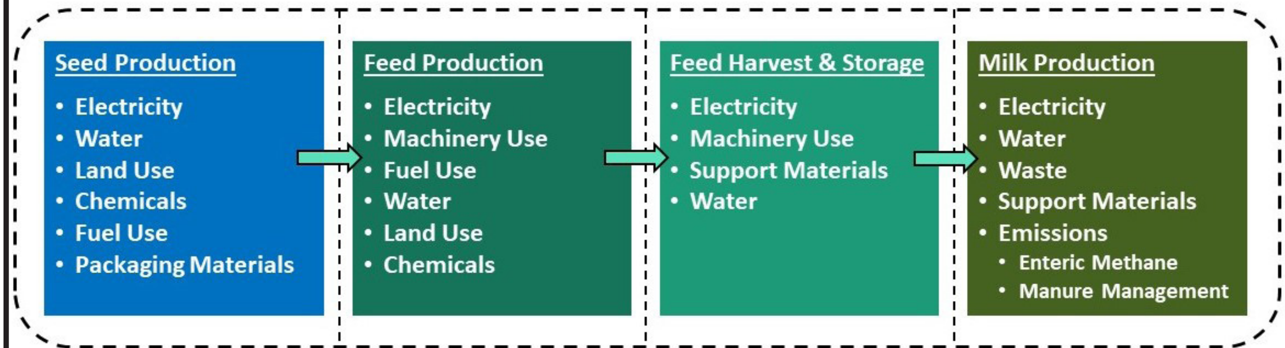

Figure 1. LCA system boundary diagram.

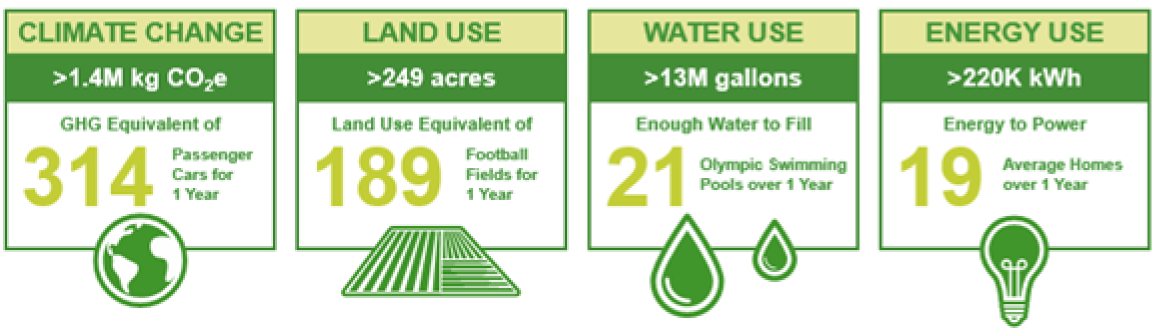

Figure 2a. Results of LCA for Dairy Study #1; per herd with 1,000 lactating cows.

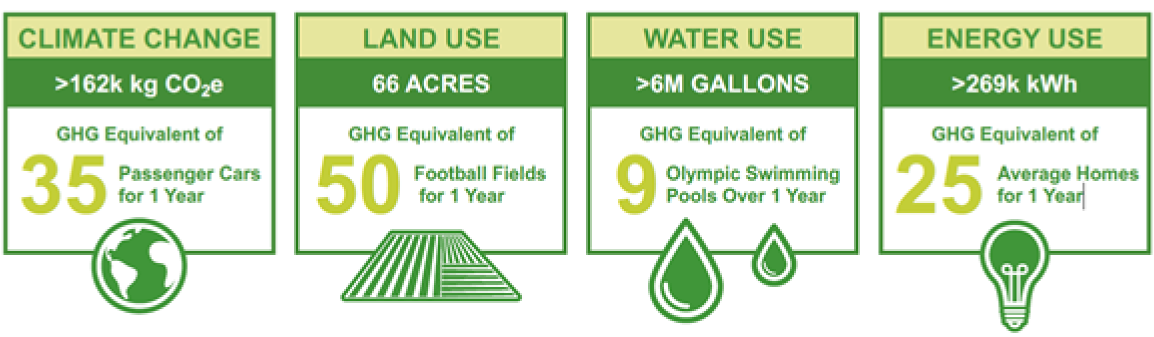

Figure 2b. Results of LCA for beef cattle; per herd of 1,000 cattle.

Figure 2. Life Cycle Assessment (LCA) results, expressed as real-world equivalents[1]

[1]Equivalencies estimated using these calculators: https://www.epa.gov/energy/greenhouse-gas-equivalencies-calculator; https://www.eia.gov/energyexplained/units-and-calculators/energy-conversion-calculators.php