Colegio de Postgraduados

Methane in Dairy Farms in Aguascalientes: Corn Silage

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ABSTRACT

Objective: To evaluate the potential methane gas production from corn silages (CS) intended for Holstein cattle in dairy farms in the state of Aguascalientes (Ags), Mexico.
Design/Methodology/Approach: Methane (CH_4) is one of the greenhouse gases, and worldwide plans and actions are being developed to monitor, control, and reduce their environmental impact. In Mexico, methane emissions from livestock are equivalent to 10.1% of CO_2 equivalent are recorded. CS samples were collected from six municipalities in Ags, representing a total of 18 dairy farms. The *in vitro* gas production technique was used to determine methane gas production, employing a nested mixed model to compare variables between municipalities using residual maximum likelihood method.
Results: The average methane production in CS was 29.3 mL/gDM. The Ags municipality showed significantly higher methane production (35.9 mL/gDM, $p<0.05$), while San Francisco de los Romo (SFR) displayed the lowest production (21.5 mL/gDM, $p<0.05$). In the state of Aguascalientes, CS-derived CH_4 production was projected at approximately 2,884 metric tons (MT) annually.
Study Limitations/Implications: There were no identified limitations in the study.
Findings/Conclusions: The potential CH_4 gas production derived from CS projected in the study represented 0.103% of what was reported by INEGyCEI in 2019.

Keywords: Environmental impact, greenhouse gas, forages.

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INTRODUCTION

Mexico is one of the 125 countries that signed the Global Methane Pledge (CH_4) to reduce methane emissions by 30% from 2020 levels by 2030, which could decrease global temperatures by 0.2 °C by 2050 (www.globalmethanepledge.org). According to the National Institute of Ecology and Climate Change (INECC in Spanish) through the National Inventory of Greenhouse Gases and Compounds Emissions (INEGyCEI in Spanish) of 2018, methane (CH_4) accounted for 20.3% of the total gas emissions, of which 50.7% is attributed to livestock, of this, 75.72% is due to enteric fermentations and 24.27% to manure management.

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In the digestive process of the rumen, there is a microbial ecosystem that facilitates fermentative digestion, within this ecosystem, there are groups of bacteria that can be defined or classified according to the substrate they ferment (Klein, 2014).

There are methanogenic bacteria that can vary depending on the diet and the geographical location of the host (Hook *et al.*, 2010). In the ruminal environment, three groups can be mentioned based on the pathway through which methanogens produce CH_4: the hydrogenotrophic, methylotrophic, and acetoclastic pathways (Extension Circular, 1996; Lambie *et al.*, 2015). In a general stoichiometry of volatile fatty acids (VFAs), acetic acid has a higher proportion relative to propionic acid, as seen in animals that receive a forage-rich diet (Madigan *et al.*, 2015). This would suggest that acetic acid is one of the main pathways for CH_4 production; however, methanogenesis is the primary way to remove or stabilize ruminal H_2. Therefore, as the amount of H_2 increases, methanogenesis would be further triggered (Yuan *et al.*, 2019).

Forages with better fiber quality can eventually reduce CH_4 emissions (Hassanat *et al.*, 2017), or when plants are in early vegetative stages (Brask *et al.*, 2013). Cabezas-García *et al.* (2017) found an increase in CH_4 production when using forages with lower starch content. Hatew *et al.* (2016) found a direct reduction of 7.9% in total CH_4 production when using corn silage (*Zea mays* L.) that reached 40% DM maturity.

Due to the wide range of results presented in various studies using different strategies to mitigate methane production, an alternative for determining potential CH_4 production levels of different ingredients is the *in vitro* gas production technique (Macome *et al.*, 2017; Miranda-Romero *et al.*, 2020). This technique has been used to evaluate and monitor changes in the quality of ingredients and diets used for ruminants, detecting changes derived from nutrients ranging from macro to microelements such as minerals (Sandoval-González *et al.*, 2016). Based on the above, the potential methane gas production of corn silages (CS) intended for Holstein cattle in dairy farms in the state of Aguascalientes, Mexico, was evaluated.

MATERIALS AND METHODS

Area of Study

The study was conducted in six municipalities of Aguascalientes State, Mexico, in the year 2021. A total of 18 dairy farms were included in the study, with a total of 11,904 animals. Of these, 51.47% (6,128) were lactating cows, 8.97% (1,068) were dry cows, and 39.55% were replacement animals from newborns to pre-first-calving. The projected volume of corn silage required annually for consumption in the sampled dairy farms was 77.8 thousand metric tons (MT).

Corn Silage Sampling

Samples of corn silage (CS) were collected from 18 dairy farms across the following municipalities: Aguascalientes (AGS), El Llano (ELL), Pabellón de Arteaga (PAR), Rincón de Romos (RR), San Francisco de los Romo (SFR), and Tepezalá (TPZ). From each dairy farm, 0.5 kg of CS was taken from 10 different points on the silo face, and then mixed, and a final 1.0 kg sample was obtained for each ranch. Subsequently, 200 g of each sample was taken and dried in a forced-air oven at 65 °C for 48 hours to determine partial dry matter. The samples were ground using a Wiley mill with a 2 mm sieve. Four subsamples of 0.5 g each were weighed and placed into individually identified amber glass bottles with a capacity of 120 mL each.

Nutritional Content Analysis of Corn Silage

Bromatological analyses of CS were conducted, dry matter, protein (Prot), ether extract (EE), and ashes (Ash) using the AOAC (1990) method. Neutral detergent fiber (NDF) was determined by the technique of Van Soest *et al.* (1991). These records were documented in an Excel® spreadsheet database.

In Vitro Gas Production Technique

Gas determination was conducted at the Food and Forage Analysis Laboratory of the Technological Institute El Llano, Aguascalientes. The *in vitro* gas production technique cited by Miranda-Romero *et al.* (2020) was employed for this purpose.

Extraction of CH_4, CO_2, and Global Warming Potential Index (GWPI)

For the estimation of CH_4, CO_2, and other minor gas production, values were expressed as mL/g DM. The GWPI was calculated using the formula cited by Martínez-Hernández *et al.* (2019) with values for CH_4 and CO_2, expressed as mL CO_2 eq gDM.

$$GWPI\left(mL\ \mathrm{CO_2}\ eq\ \ gDM\right)=\mathrm{CO_2}\left(mL\,/\,g\right)+\mathrm{CH_4}\left(mL\,/\,g\right)*23$$

The readings were taken every 6 hours over a total period of 24 hours, and these records were entered into an Excel® spreadsheet database.

Production of Gas Fractions

For the quantification of different carbohydrate fractions, the technique of production of gas fractions was used (Miranda-Romero *et al.*, 2020). In this technique, GP8 represents gas production in the first 8 hours and corresponds to simple sugars, GP24 encompasses gas production accumulated between 8 and 24 hours, representing starches, and GP72 is the gas production accumulated between 24 and 72 hours, representing cellulose content, values were expressed in mL/g DM (Jiménez-Santiago *et al.*, 2019).

Degradation at 24 and 72 hours

For the *in vitro* degradation at 24 and 72 h, the technique adapted from Theodorou *et al.* (1994) was used. The samples used for CH_4 gas production and gas fractions were dried in a forced-air oven at 65 °C for 48 h after the process, to determine degradation at 24 and 72 h.

Statistical Analysis

A nested mixed-effect model was employed with "n" ranches nested within "r" localities and the variable "t" representing time within "n" ranches, to compare study variables across municipalities.

Statistical Model

$$Y_{ijkl} = \mu + Ra\left(L_j\right)_i + A_k + L_l + (A \times L)_{kj} + \varepsilon_{ijkl}$$

Y_{ijkl} represents the response variables such as Methane, Prot (Protein), NDF (Neutral Detergent Fiber), EE (Ether Extract), Ash (Ash), NFC (Non-fiber carbohydrates), *In vitro* Degradability at 24 and 72 hrs, GP8, GP24, GP72. μ=is the overall mean of the experiment. $Ra\left(L_j\right)_i$ = the effect of the *i*-th ranch within the *j*-th locality. A_k=the effect of the *k*-th time. L_l=is the effect of the *l*-th locality. $(A \times L)_{kj}$ = is the interaction effect of the *k*-th year and the *l*-th locality. ε_{ijkl}=represents the experimental error effect.

In the mixed model, the Type IIIA model as described by Sanni and Ukaegbu (2012) was used, with the locality variable considered as fixed and the ranch and time variables as random. Two types of analyses were conducted to compare the results: ANOVA and Maximum Likelihood Residual methods. Pairwise mean comparisons were performed using the Student's T-test ($p<0.05$). The analyses were conducted using R-Studio® version 2022.02.3., with the libraries readxl, agricolae, ggplot2, tidyverse, tseries, nlme, sjPlot, nortest, quadprog, emmeans, MuMIn, and sjstats (www.R-project.org).

RESULTS AND DISCUSSION

Nutritional Content of Corn Silage

The protein (Prot) content was significantly higher in El Llano (ELL) compared to the municipalities of PAR, RR, and SFR (+1.9, +1.8, +1.8; $p<0.05$) respectively. The silages from PAR, SFR, and TPZ had the highest content of NFC (40.5%, 38.1%, and 37.6% respectively). The municipality of ELL had a higher ash content than PAR (+2.07%; $p<0.05$). The neutral detergent fiber (NDF) content was statistically higher in the municipalities of ELL and RR (48.5% and 48.4% respectively) compared to PAR (44.5%, $p<0.05$). There were no significant differences in the contents of ether extract (EE), acid detergent fiber (ADF), and lignin (Lig) among the municipalities (Table 1).

CH_4, CO_2 and GWPI production and of Corn Silages

Methane (CH_4) production in corn silage was significantly higher in AGS compared to SFR, ELL, TPZ, and PAR (+14.4, +11.1, +9.9, and +8.2, $p<0.05$) respectively, and RR was higher than SFR (+7.3, $p<0.05$). CO_2 production did not differ significantly among municipalities. The Global Warming Potential (GWPI) production was higher in AGS compared to SFR (+327, $p<0.05$) (Table 2).

The approximate potential volume of CH_4 and CO_2 derived from the total volume of corn silage required annually by the dairy farms was 664,230 thousand liters of CH_4 per MT DM Tot and 2,993,788 thousand liters of CO_2 per MT DM Tot (Table 3).

Production of gas fractions PG8, PG24, and PG72

The AGS municipality had significantly lower GP-8 production of gas fractions (mL/gDM) than ELL and RR (−25.0, $p<0.05$; −23.1, $p<0.05$) respectively, although

Table 1. Average nutritional values (%) per municipality of corn silages sampled in 2021.

Municipality	Corn silage (%) 2021							
	PDM	Prot	NDF	NFC	EE	Ash	ADF	Lig
Aguascalientes	26.4	8.7 ^{ab}	47.1^{ab}	36.4 ^{ns}	1.80^{ns}	7.10 ^{ab}	26.7^{ns}	5.4^{ns}
El Llano	28.9	9.8 ^{a}	48.5^{a}	33.2 ^{ns}	1.66^{ns}	8.08 ^{a}	27.0^{ns}	4.9^{ns}
Pabellón de Arteaga	31.6	7.9 ^{b}	44.5^{b}	40.5 ^{ns}	2.03^{ns}	6.01 ^{b}	25.3^{ns}	4.8^{ns}
Rincón de Romos	29.6	8.0 ^{b}	48.4^{a}	35.4 ^{ns}	1.58^{ns}	7.47 ^{ab}	27.6^{ns}	5.6^{ns}
San Francisco de los Romo	26.8	8.0 ^{b}	45.4^{ab}	38.1 ^{ns}	1.62^{ns}	7.74 ^{ab}	26.3^{ns}	5.5^{ns}
Tepezalá	27.4	8.5 ^{ab}	46.0^{ab}	37.6 ^{ns}	1.88^{ns}	7.07 ^{ab}	25.6^{ns}	5.5^{ns}
Average	28.4	8.5	46.7	36.9	1.76	7.20	26.4	5.3
Coefficient of Variation (CV)		0.16	0.12	0.17	0.23	0.15	0.13	0.16
R^2		0.26	0.09	0.16	0.17	0.35	0.07	0.19

^{abc} Values with different letters in the same column are different ($p<0.05$). ns=not significant, CV=Coefficient of Variation, R^2=Coefficient of Determination, PDM=Partial Dry Matter, Prot=Protein, NDF=Neutral Detergent Fiber, NFC=Non-Fiber Carbohydrates, EE=Ether Extract, Ash=Ashes, ADF=Acid Detergent Fiber, Lig=Lignin.

Table 2. Methane and carbon dioxide production (mL/gDM) and Global Warming Potential Index expressed in CO_2 eqgDM of corn silages sampled by municipality in 2021.

Municipality	CH_4 mL/gDM	CO_2 mL/gDM	GWPI mL CO_2 eq g^1MS
Aguascalientes	35.9 ^{a}	119.7 ^{ns}	945 ^{a}
El Llano	24.8 ^{bc}	125.9 ^{ns}	696 ^{ab}
Pabellón de Arteaga	27.7 ^{bc}	127.5 ^{ns}	765 ^{ab}
Rincón de Romos	28.8 ^{ab}	120.5 ^{ns}	782 ^{ab}
San Francisco de los Romo	21.5 ^{c}	124.0 ^{ns}	618 ^{b}
Tepezalá	26.4 ^{bc}	144.7 ^{ns}	751 ^{ab}
Average	27.6	127.0	759.5
CV	0.147	0.045	0.123
R^2C	0.247	0.112	0.26
*LogLiK	−121.49	162.42	−223.14
**REML p=	0.052	0.919	0.041

^{abc} Values with different letters in the same column are different ($p<0.05$). ns=not significant, CV=Coefficient of Variation, R^2C=Conditional Coefficient of Determination, *LogLik=Likelihood coefficient. **REML p=p-value of the Residual Maximum Likelihood method. CH_4=Methane, CO_2=Carbon dioxide, GWPI=Global Warming Potential Index. gDM=grams dry matter, eq=equivalent, mL=milliliter.

production of gas fractions in PAR and SFR was lower than ELL (−15.0 and −16.3) respectively, it was not significant. Regarding GP24 gas fraction production (mL/gDM), AGS had lower content than ELL and RR (−27.3 and −22.6 $p<0.05$) respectively. GP72 gas production did not show a statistically significant difference between municipalities (Table 4).

Degradability at 24 and 72 hours

The 24 h degradability (DG24) averaged 45.1% across all municipalities, with AGS being significantly lower than ELL (−5.1, $p<0.05$) and PAR (−5.63, $p<0.05$). The 72 h

Table 3. Total DM tons required per year by sampled dairy farms and their potential methane and carbon dioxide production per municipality derived from corn silage.

Municipality	MT Tot DM	Lt CH_4/ MT Tot DM (K)	Lt CO_2/ MT Tot DM (K)
Aguascalientes	4,146	151,261	485,468
El Llano	2,281	55,218	296,670
Pabellón de Arteaga	5,496	150,813	723,053
Rincón de Romos	1,943	54,029	248,673
San Francisco de los Romo	4,256	101,263	511,690
Tepezalá	4,543	151,646	728,236
Total	22,665	664,230	2,993,788

TM DM Tot=Total dry matter tons, Lt CH_4/TM DM Tot (K)=Thousand liters of methane per ton of total dry matter, Lt CO_2/TM DM Tot (K)=Thousand liters of carbon dioxide per ton of total dry matter.

Table 4. Average of gas fractions production at 8, 24, and 72 hours from sampled corn silage in 2021.

Municipality	GP-8	GP-24	GP-72
	mLg^{-1} DM^{-1}		
Aguascalientes	83^{b}	159^{b}	157^{ns}
El Llano	108^{a}	186^{a}	148^{ns}
Pabellón de Arteaga	93^{ab}	169^{ab}	144^{ns}
Rincón de Romos	106^{a}	182^{a}	150^{ns}
San Francisco de los Romo	92^{ab}	183^{a}	142^{ns}
Tepezalá	100^{ab}	183^{a}	148^{ns}
Coefficient of variation (CV)	0.075	0.085	0.108
R^2C	0.308	0.221	0.221
*LogLiK	−149.65	−149.5	−154.11
**REML p=	0.176	0.086	0.867

abc Values with different letters in the same column are different ($p<0.05$). ns=not significant. CV=Coefficient of variation, R^2C=Conditional determination coefficient, *LogLik=likelihood coefficient. **REML p=p-value of residual maximum likelihood method, GP-8=gas fraction production at 8 hrs, GP24=gas fraction production at 24 hrs, GP72=gas fraction production at 72 hrs. gDM=grams per dry matter, eq=equivalent, mL=milliliter.

degradability (DG72) averaged 66.3%, with no significant differences found between municipalities (Table 5).

Correlation of variables in the production of CH_4, CO_2, and GWP Index

The production of CH_4 in corn silages was negatively affected by GP24 and DG24 but positively affected by DG72 ($p=0.0000134$, $p=0.000307$, and $p=0.000108$; $R^2=56.01$), respectively (Table 6).

The NDF content (p=0.000228, R2=39.19) had a negative effect on CO2 production (Table 7).

The variables GP24 and DG24 had a negative effect, and DG72 had a highly significant positive effect on GWPI when expressed as mL CO_2eq/gDM ($p=0.0000127$,

Table 5. Means of Degradability at 24 and 72 hours per Municipality of Corn Silage Sampled in 2021.

Municipality	DG24 %	DG72 %
Aguascalientes	41.8^{b}	65.6^{ns}
El Llano	46.9^{a}	68.6^{ns}
Pabellón de Arteaga	47.4^{a}	68.8^{ns}
Rincón de Romos	44.1^{ab}	65.3^{ns}
San Francisco de los Romo	45.2^{ab}	63.5^{ns}
Tepezalá	45.5^{ab}	65.7^{ns}
Coefficient of variation (CV)	0.067	0.065
R^2C	0.234	0.199
*LogLiK	131.67	−99.4
**REML p=	0.217	0.115

[abc] Means with different letters in the same column are significantly different ($p<0.05$). ns=not significant. CV=Coefficient of variation, R^2C=Conditional coefficient of determination, *LogLik=likelihood coefficient. **REML p=p-value from the residual maximum likelihood method. DG24=24-hour degradability, DG72=72-hour degradability.

Table 6. Model by variable selection with the highest correlation to CH_4 production in corn silages.

Model CH_4	R^2 Adjusted
$Y=31.822-0.261GP24+0.092GP72-1.049DG24+1.133DG72+\varepsilon_{ijkl}$	0.56
$Y=31.822-0.261GP24+0.092GP72-1.049DG24$	0.33
$Y=31.822-0.261GP24-1.049DG24$	0.29
$Y=31.822-0.261GP24+1.133DG72$	0.25
$Y=31.822-0.261GP24$	0.20
$Y=31.822+1.133DG72$	0.10

Y=response variable, GP24=gas fraction production between 8 and 24 h, GP72=gas fraction production between 24 and 72 h, DG24=degradation at 24 h, DG72=degradation at 72 h.

Table 7. Model by variable selection with the highest correlation to CO_2 production in corn silages.

Model CO_2	R^2 Adjusted
$Y=140.864-2.10NDF+0.234GP24+0.891DG24+\varepsilon_{ijkl}$	0.39
$Y=140.864-2.10NDF$	0.35
$Y=140.864+0.891DG24$	0.06
$Y=140.864+0.234GP24$	0.01

Y=response variable, NDF=neutral detergent fiber, GP24=gas fraction production between 8 and 24 hours, DG24=degradation at 24 hours.

$p=0.000492$, and $p=0.0000872$; $R^2=56.34$), respectively. GP72 showed a positive trend ($p=0.06$) (Table 8).

Nutritional Content of Corn Silage

The content of protein (Prot), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in corn silages fall within the ranges typical for corn silage reported by Dairy One

Table 8. Model by variable selection with the highest correlation to GWPI production in corn silage.

Model GWPI	R^2 Ajusted
$Y=759.789-5.722GP24+2.178GP72-22.007DG24+25.215DG72+\varepsilon_{ijkl}$	0.56
$Y=759.789-5.722GP24-22.007DG24+25.215DG72$	0.48
$Y=759.789-5.722GP24+25.215DG72$	0.28
$Y=759.789-22.007DG24+25.215DG72$	0.28
$Y=759.789-5.722GP24$	0.21
$Y=759.789+25.215DG72$	0.11

Y=response variable, GP24=gas fraction production between 8 and 24 h, GP72=gas fraction production between 24 and 72 h, DG24=degradation at 24 h, DG72=degradation at 72 h.

(revised in September 2023), except in the municipality of ELL with 9.8% protein. It has been observed that the use of nitrogen fertilizers in corn silage leads to increased protein content (Soto *et al.*, 2004). Conversely, when manure is used as fertilizer, which is a common practice in Aguascalientes to minimize the use of chemical fertilizers, there has been an increase in protein yield per hectare. However, this response is not linear, and there is no difference in the protein:DM ratio with varying levels of manure inclusion per hectare (Ortíz-Díaz *et al.*, 2022).

The average content of non-fiber carbohydrates (NFC) found in the municipalities is within the limits reported by Dairy One (2023), albeit it is just above the lower range. However, the content of ether extract (EE) in the different municipalities is 46% lower than that reported by Dairy One (2023). This discrepancy may be related to the lower limits of NFC content, as NFC largely represents the carbohydrate content of corn.

CH_4, CO_2 and GWPI production of Corn Silages

Estimating the weighted average volume of CH_4 production derived from the fermentation of CS among the total active animals in the sampled dairy farms, an average annual production of 55.8 m^3 CH_4 per animal was observed. Based on the census reported by SIAP (2021) for the state of Aguascalientes, this equates to 2,884 metric tons (MT) of CH_4 annually.

The GP24 fraction, identified by Miranda-Romero *et al.* (2020) as starches, had a negative effect on the CH_4 production of corn silage, consistent with findings by Hatew *et al.* (2016). Increasing starch content in silage with higher maturity or rapidly fermenting starches (Hatew *et al.*, 2014) lowers ruminal pH due to starch fermentation, which has been found to decrease protozoa population over time, thereby reducing CH_4 production.

Starch fermentation favors bacteria that produce propionate (Hook *et al.*, 2011; Benchaar *et al.*, 2014). However, increasing starch concentration within the first days can alter the rumen bacterial population (Neubauer *et al.*, 2018).

GP72, as indicated by Miranda-Romero *et al.* (2020), representing cellulose within slowly fermenting carbohydrates, was positively associated with CH_4 production, as mentioned by Hatew *et al.* (2014). Cellulose fermentation pathway leads to volatile fatty acids (VFA), CO_2, and CH_4 production (Madigan *et al.*, 2015), resulting in an expected scenario (Danielsson *et al.*, 2017). Although the NDF content in the current study was lower (46.7% *vs.* 51.91%, respectively) compared to findings by Kara (2015), the average CH_4 production was similar (27.6 *vs.* 25.9 mL/g DM, respectively). This contrast highlights the results sometimes associated with the 24-hour incubation period, where despite insufficient time for NDF fermentation, greater amounts of CH_4 can be produced (Pirondini *et al.*, 2012).

CONCLUSIONS

Under the conditions of the present study, the effect found in the production of gas fractions representing cellulose, and the very marked negative effect that starch content had on methane production derived from corn silages, it is important to consider determining the carbohydrate content using the *in vitro* fermentation technique, this would provide greater precision in estimating the potential production of methane and CO_2 gases from corn silages.

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