

Impact of molasses and ground corn grains as a source of energy supplementation on composition, quality, nutritional values and in vitro gas production parameters of corn stover silage

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ABSTRACT: As soon as the ears were harvested, green corn stover was collected and cut to a length of 1.5 to 2.0 cm with a harvester chopper machine. Chopping corn stover was unsupplemented in T1 or supplemented with 3% molasses in T2 or 3% ground yellow corn grains in T3 and stored for 45 days in plastic bags that can hold roughly 0.5 kg of weight. Then, samples were taken for determinations of chemical analysis, silage quality and in vitro gas production. Supplementing corn stover silage with either molasses (T2) or ground corn grains (T3) results in significant increases in dry matter (DM), crude protein (CP) and nitrogen-free extract (NFE) contents, while reducing crude fiber (CF) and fiber fractions include neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, and hemicellulose contents, compared to the control (T1). However, organic matter (OM), ether extract (EE), and ash contents remain relatively stable and unaffected by the treatments. Supplementation of corn stover silage with molasses and ground corn grain results in a noticeable decrease in pH and butyric acid concentration, but increase total volatile fatty acids (TVFAs), lactic, acetic and propionic acids concentrations, while ammonia-N levels remain largely unaffected. Cumulative gas production and the parameters of rapidly degradable fraction (a), potentially degradable fraction (b), the rate of gas production (c), and the total amount of gas produced from both the soluble (GPSF) and insoluble (GPNSF) were significantly increased, while methane production components decreased when molasses and ground corn grains were added to corn stover silage (T2 and T3). In the first 12 hours, gas production was fast; in the next 12 hours, it was moderate; and in the final 24 to 96 hours, it was slower. Short-chain fatty acid (SCFA) concentration, microbial protein production (MP), dry matter intake (DMI), effective dry matter degradability (EDMD), organic matter digestibility (OMD), total digestible nutrients (TDN), gross energy (GE), digestible energy (DE), metabolizable energy (ME), and net energy (NE) were all significantly ($P < 0.05$) improved when corn stover silage was supplemented with molasses and ground corn grains (T2 and T3). In conclusion, supplementing corn stover silage with molasses or ground corn grains improves its chemical composition, quality, nutritional value and leads to a more efficient in vitro gas production.

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INTRODUCTION

In Egypt, three million feddans were planted in the corn crop overall (Agriculture Economics and Statistics Institute, 2024). Green corn stalks represent about 55 to 65% of the whole corn plant at harvest (Bendary et al., 2001a). The average productivity of green corn stalks per acre is about 15 tons per feddan (Bandari et al., 2001b). Making high-quality silage from fresh corn stover, either with or without additions, is crucial for summertime animal feeding (Ahmed et al., 2003). Corn stover silage is a method of preserving corn stalks, leaves, and husks (stover) after grain harvest for animal feed, particularly for ruminants like

cows. It involves fermenting the chopped stover in an anaerobic (oxygen-free) environment, similar to how other types of silage are made. This process preserves the stover's nutritional value for later use (El-Garhy, 2007).

Adding molasses and ground corn grains as energy additives to corn stover silage can improve its nutritional value and fermentation quality. Molasses can increase lactic acid and volatile fatty acid (VFA) production, while decreasing ammonia-N, leading to better silage preservation. Ground corn grains can enhance dry matter (DM) content and increase the nitrogen-free extract (NFE) content (El-Garhy, 2007). Lactic acid bacteria (LAB) use the easily fermentable

carbohydrates found in molasses to create lactic acid, which lowers pH. By preventing the growth of unwanted microorganisms, this lower pH improves silage preservation (Oduguwa et al., 2007). The optimal molasses input at 35 days of fermentation was found to be 3%. As the amounts of additions and fermentation times grew, the pH dropped and the lactic acid rose. Crude protein and dry matter contents somewhat increased (Bilal, 2009).

Adding molasses and corn grains to corn stover silage can improve its nutritional value and fermentation characteristics. Molasses, a readily available sugar source, enhances lactic acid production, lowers pH, and improves digestibility. Corn grains, as a source of starch, increase dry matter (DM) and potentially crude protein (CP) content, while also contributing to improved fermentation (Bautista-Trujillo et al., 2009; Bostami et al., 2008). Molasses addition significantly increases lactic acid production in corn stover silage, contributing to a more stable and nutritious silage. Also, molasses can help reduce ammonia-N (a byproduct of protein breakdown) in the silage, indicating improved protein preservation (Wang et al., 2022).

Ground corn grains, being a source of dry matter and readily available carbohydrates, can boost the overall DM content of the silage. The NFE content, which includes readily available carbohydrates, also increases, further improving the silages nutritional value. The changes in nutrient composition and digestibility due to corn grain additives can result in improved feeding values for livestock (Bilal, 2009). By carefully selecting and using energy additives, farmers can improve the nutritional value and preservation of corn stover silage, making it a more effective and efficient feed for livestock (Gaafar et al., 2023). Incorporating corn grains into corn stover silage can be a valuable strategy for enhancing its nutritional profile and fermentation quality, ultimately leading to better livestock performance (Li et al., 2024).

Corn stover silage's nutritional content and fermentation properties can be enhanced by adding molasses and corn grains, which will affect the kinetics of in vitro gas production. Molasses, as readily available energy source, can promote lactic acid fermentation, leading to lower pH and improved digestibility. Corn grains, with their starch content, can also contribute to fermentation and potentially increase gas production. The specific effects depend on the type and amounts of additives used, as well as how long the fermenting process takes (El-Garhy, 2007). Adding energy additives like molasses or bacterial inoculants to corn stover silage can significantly affect in vitro gas production, influence

fermentation and potentially enhancing its nutritional value. In the end, these additions may affect gas production patterns by encouraging the growth of lactic acid bacteria, which may result in more lactic acid output and better overall fermentation quality (Mejia-Urbe et al., 2013).

Objectives of the study

The objective of this study is to examine how the addition of molasses and ground corn grains affects the nutritional values, qualitative attributes, chemical composition, and parameters for in vitro gas production of corn stover silage.

MATERIALS AND METHODS

Ethical Approval

The following study was declared by the Local Experimental Animals Care and welfare Committee's Ethics Committee and done according to the rules of Kafrelsheikh University, Egypt. (No. 4/2016 EC).

Silage Making

As soon as the ears were harvested, the corn stover was collected and cut to a length of 1.5 to 2.0 cm using a harvester chopper machine. In T1, chopped corn stover was left alone; in T2, it was supplemented with 3% molasses; and in T3, it was supplemented with 3% crushed yellow maize grains. After that, they were manually compressed to keep the air out of the silos and encased in plastic bags that could hold roughly 0.5 kg of weight. Three of each treatment were administered in duplicate, and they were ensiled for forty-five days. Representative samples were collected following the ensiling period in order to assess the nutritional values, proximate composition, silage quality, and in vitro gas production.

Silage Quality

To determine the quality of the silage, 20 grams of wet silage and 100 milliliters of distilled water were blended together and the extracts had been filtered through Whatman No. 40 filter sheets. The pH level of silage was measured in the filtrate solution using a Bechman pH meter. Warner (1964) determined the concentration of total volatile fatty acids (TVFAs), AOAC (2005) determined the concentration of ammonia-N, and the Analytical Chemistry of Foods (1995) methods used the following equation to estimate the concentration of lactic acid through titration with 0.1 sodium hydroxide solution using 2-3 drops of phenolphthalein indicator: Liquid NaOH x 0.09/sample weight equals lactic acid percentage of DM.

Proximate Analysis

The dry matter content and dry crop yield were estimated by weighing the fresh crop, taking samples, and drying them completely in an air oven set at 60 degrees Celsius. In accordance with AOAC (2005), representative samples were ground and subjected to chemical analysis in order to identify the different components. NDF, ADF, and ADL fiber fractions were calculated using Van Soest (1991) methodology. Cellulose = ADF – ADL Hemicellulose = NDF - ADF

Nutritional Values

The calculation of total digestible nutrients (TDN) followed AAFCO (1997) guidelines:

$$\text{TDN} \quad \% \quad = \quad 40.2625 + 0.1969 * \text{CP} + 0.4228 * \text{NFE} + 1.1903 * \text{EE} - 0.1379 * \text{CF}$$

Digestible crude protein (DCP) was calculated according to Villamide & Fraga (1998), DCP % = 0.803 * CP - 1

where NFE stands for nitrogen-free extract, EE for ether extract, CP for crude protein, and CF for crude fiber.

MAFF (1975) was used to compute digestible energy (DE) and gross energy (GE):

$$\text{GE} \quad (\text{Mcal/kg} \quad \text{DM}) \quad = \quad (0.226 * \text{CP} + 0.177 * \text{CF} + 0.407 * \text{EE} + 0.192 * \text{NFE}) / 4.184$$

$$\text{DE} = (\text{GE} * (87.9 - (.88 * \text{CF}))) / 100$$

In vitro gas production

The assessment of in vitro gas production was conducted using the methodology described by Menke & Steingass (1988). In conclusion, a 50 ml glass syringe that was calibrated and fitted with plungers for the creation of in vitro gas using the buffer solution was filled with 100 mg of the air-dry feed materials, which were carefully weighed (Onodera & Henderson, 1980). One liter of distilled water was used to dissolve 2.8 g of NaCl, 0.1 g of CaCl₂, 0.1 g of MgSO₄ 4.7 H₂O, 2.0 g of KH₂PO₄, and 6.0 g of Na₂HPO₄ to create the buffer. The CO₂ flushed for 15 minutes and pH was adjusted to 6.8. Bueno et al. (2005) report that three rumen-cannulated lambs fed ad libitum a mixture of commercial concentrate and rice straw had their rumen contents (50 percent liquid and 50 percent solid) collected. The contents of the animals' rumen were collected before they were fed in the morning. Before being brought to the lab in anaerobic circumstances, samples of both liquids and solids were put in insulated flasks that had been heated to 39 °C. The contents of the rumen were squeezed through four layers of cheesecloth and then kept at 39 °C in a water bath with CO₂ saturation until inoculation. The buffer and inoculant (2:1 v/v) were mixed in a water bath

maintained at 39 °C with CO₂ saturation (Nasser et al., 2006; Sallam, 2005; Soliva et al., 2005). The syringes are immediately inserted in the water bath, which is set at 39 °C, after 15 ml of buffered rumen fluid containing the feed samples has been pipetted into each one. Three iterations of each experiment were conducted. Two syringes containing buffered rumen fluid were used as the blank in each run after incubation. Every two hours, the syringes are gently shaken, and after 96 hours, the incubation is terminated when the gas volume is noted. Following incubation for 3, 6, 9, 12, 24, 48, 72, and 96 hours, the gas production was measured using the procedure outlined by Theodorou et al. (1994). The headspace gas pressure against gas volume was evaluated using linear regression. According to Pellikaan et al. (2011), 10 µl of the headspace gas was taken out of the bottles at different incubation times (3, 6, 9, 12, 24, 48, 72, and 96 hours) and directly injected into a GC to determine the methane (CH₄) content. Once the total gas measurements have been corrected for the blank incubation, the reported gas and methane readings are provided per 200 mg of DM. The following is how Ørskov & McDonald (1979) explained fermentation kinetics:

$$Y = a + b * (1 - e^{-ct})$$

where a, b, and c are the gas production rate constants for fraction b, the gas production from the instantly soluble fraction, the insoluble fraction, and the insoluble fraction, respectively. The gas production (ml/g OM) at time (t) is denoted by Y.

Feeds were evaluated based on those factors using a new technique for measuring gas production produced by fermentation of the soluble fraction (GPSF), or gas produced after three hours (GP3) of incubation. According to Van Gelder et al. (2005), the amount of gas produced as a result of the fermentation of the insoluble fraction (GPNSF) may be determined using the gas produced during the GP3–GP24 incubation hours in the following manner:

$$\text{GPSF} = (\text{GP3hr} * 0.99) - 3$$

$$\text{GPNSF} = 1.02 * (\text{GP24hr} - \text{GP3hr}) + 2$$

where GPSF is the gas production from a soluble fraction (ml/g DM), GPNSF is the gas production from a non-soluble fraction (ml/200 mg DM), and GP 3hr is the net gas production (ml/200 mg DM) over the course of three hours.

Together with extra measurements of crude protein, ash, and crude fat, the energy values were calculated based on the amount of gas produced following a 24-hour incubation period. Based on a thorough in vitro incubation of feedstuffs, this method was created by a research team in Hohenheim, Germany (Menke et al., 1979; Menke & Steingass, 1988).

$$\text{ME (Mcal/kg DM)} = (2.2 + 0.136 \cdot \text{GP} + 0.057 \cdot \text{CP}) / 4.184$$

$$\text{NE (Mcal/kg DM)} = (2.2 + 0.136 \cdot \text{GP} + 0.057 \cdot \text{CP} + 0.149 \cdot \text{EE}) \cdot 2.2 / 14.64$$

where CP is crude protein (a percentage of DM), EE is extract (a percentage of DM), GP is 24-hour net gas production (ml/200 mg DM), ME is metabolizable energy (Mcal/kg DM), and NE is net energy (Mcal/kg DM).

Organic matter digestibility (OMD) was calculated as established by Getachew et al. (1998):

$$\text{OMD (\%)} = 14.88 + 0.889 \cdot \text{GP} + 0.45 \cdot \text{CP} + 0.0651 \cdot \text{A}$$

where A is ash (percent of DM), CP is crude protein (percent of DM), GP is 24-hour net gas production (ml/200 mg DM), and OMD is organic matter digestibility (percent).

Effective Dry Matter Degradability (EDMD) from in vitro gas production data can be calculated using the equation developed by Ørskov & McDonald (1979):

$$\text{EDMD} = (a+b) + ((a \cdot b) \cdot c) / (c+k)$$

'a' represents the gas production of the soluble fraction, 'b' represents the projected gas production of the insoluble fraction, 'c' represents the gas production rate constant, and 'k' represents the rumen outflow rate, which is at the maintenance level and means 2% per hour.

Getachew et al. (2005) state that the following formula was used to determine short chain fatty acids (SCFA):

$$\text{SCFA (Mm)} = (-0.00425 + 0.0222 \cdot \text{GP}) \cdot 100$$

where GP is the net gas production (ml) of the soluble fraction over a 24-hour period.

In compliance with Blümmel & Ørskove (1993), the following formula was used to calculate the predicted dry matter intake (DMI) of silage:

$$\text{DMI} = 1.66 + 0.49 \cdot a + 0.0297 \cdot b - 4 \cdot c$$

where a is the amount of gas produced from the soluble fraction (ml), b is the amount of gas produced from the insoluble fraction (ml), and c is the gas production rate (ml/hr).

Czerkawski (1986) calculated that the production of microbial protein (MP) was 19.3 g of microbial nitrogen per kilogram of OMD.

$$\text{MCP (g/kg DM)} = \text{OMD} \cdot 19.3 \cdot 6.25 / 100$$

Statistical Analysis

The data was statistically evaluated using the general linear models (GLM) technique, which IBM SPSS Statistics (2020) tailored for the user guide using one-way ANOVA. Within the SPSS program, Duncan's tests were used to assess significant differences in mean values among dietary regimens at the level of significance $P < 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSIONS

Proximate Composition

Molasses and ground corn grain supplementation significantly affects the proximate composition of corn stover silage are presented in Table 1. Supplementing corn stover silage with either molasses (T2) or ground corn grains (T3) results in significant increases in DM and NFE contents, while reducing CF content, compared to the control (T1). Additionally, molasses supplementation (T2) leads to significant increase in CP content compared to the control, with no significant difference observed when compared to ground corn grains (T3). However, OM, EE and ash contents remain relatively stable and unaffected by the treatments. Molasses addition can also lead to a slight increase in DM and CP contents (El-Garhy, 2007). Molasses can help in the breakdown of cell wall components (Bilal, 2009). Ground corn grains increase the dry matter content, elevates the nitrogen-free extract (NFE) content of the silage and dilute the ash content (Hartutik et al., 2021). The DM content of corn stover silage significantly ($P < 0.05$) increased when ground corn grains were added. Additionally, adding molasses and ground corn grains to corn stover silage resulted in a substantial increase ($P < 0.05$) in its NFE concentration but a significant decrease ($P < 0.05$) in its ash content (Gaafar et al., 2023).

Fiber Fractions

The addition of molasses and ground corn grains (treatments T2 and T3) to corn stover silage resulted in a significant decrease in all fiber fractions (NDF, ADF, ADL, cellulose, and hemicellulose) compared to the unsupplemented control (T1) as presented in Table 1. The reduction in fiber content, specifically in the digestible fractions (NDF, ADF), implies a possible rise in the energy content is available to the animal consuming the silage. Specifically, the addition of molasses can decrease NDF and ADF contents, which are measures of fiber. Ground corn grains, when added, can also contribute to may affect the overall fiber content (Gao et al., 2019). Molasses can help in the breakdown of cell wall components (Bilal, 2009). Molasses and corn grain supplementation can also lead to a reduction in lignin content, another fiber component (kareem & Saeed, 2021). Molasses and corn grains are beneficial additives for corn stover silage, which decreasing fiber components (Bilal, 2009).

Silage Quality

Results of silage quality characteristics in Table 1, revealed that supplementing corn stover silage with

molasses or ground corn grain significantly ($P < 0.05$) improves silage quality by lowering pH and butyric acid while increasing lactic, acetic, propionic acids, and total volatile fatty acids (TVFAs), with molasses providing a greater positive effect. However, ammonia-N level, was not significantly ($P > 0.05$) affected by these energy supplements. Both molasses and ground corn provide the readily fermentable energy needed to promote beneficial bacteria and achieve superior silage fermentation compared to unsupplemented stover. Molasses acts as a readily

available carbohydrate source, promoting lactic acid fermentation and reducing pH (El-Garhy, 2007). Both molasses and corn grains contribute to a higher quality silage with better preservation (Ahmed et al., 2021). With the addition of molasses and pulverized maize grains, the silage's pH value dramatically dropped ($P < 0.05$), even when the lactic acid concentration increased ($P < 0.05$) according to Gaafar et al. (2023). Additionally, the quality of the silage was greatly enhanced by adding 2 and 4% molasses to new corn stover (Zhang et al., 2024).

Table 1. Effect of energy additives on proximate composition, fiber fractions and silage quality of corn stover silage.

Item	T1	T2	T3	±SE	P-value
Proximate Composition %					
DM	34.47b	36.66a	37.05a	0.45	0.010
OM	90.85	91.27	91.32	0.10	0.101
CP	7.29b	7.51a	7.45ab	0.04	0.046
CF	29.45a	27.59b	27.65b	0.35	0.011
EE	2.38	2.42	2.44	0.02	0.376
NFE	51.73b	53.75a	53.78a	0.43	0.039
Ash	9.15	8.73	8.68	0.10	0.101
Fiber Fractions %					
NDF	69.45a	64.59b	65.06b	0.86	0.018
ADF	39.75a	36.97b	37.24b	0.49	0.020
ADL	5.35a	4.98b	5.01b	0.07	0.015
Cellulose	34.40a	31.99b	32.23b	0.43	0.017
Hemicellulose	29.70a	27.62b	27.82b	0.37	0.022
Silage Quality					
pH value	4.37a	4.18b	4.20b	0.04	0.043
Ammonia -N (% of total-N)	2.98	3.02	3.07	0.02	0.265
TVFA's (% of DM)	2.55b	2.74a	2.68a	0.06	0.011
Lactic acid (% of DM)	4.68b	5.14a	5.01a	0.07	0.013
Acetic acid (% of DM)	2.03b	2.23a	2.17a	0.03	0.025
Propionic acid (% of DM)	0.30b	0.35a	0.34a	0.01	0.042
Butyric acid (% of DM)	0.17a	0.12b	0.13b	0.002	0.034

^{a, b}: Means in the same row with different superscripts differ significantly at $P < 0.05$.

Accumulative Gas Production

Fig. 1 shows the cumulative gas production of incubated corn stover silage with energy additions over the course of the 96-hour incubation period. In comparison to the control (T1), adding molasses and ground corn grains to corn stover silage (T2 and T3) resulted in a significant ($P < 0.05$) increase in cumulative gas production throughout the course of the incubation period, with a minor decrease when molasses was added instead of corn grains. The observed gas production pattern, with the rapid increase in the first 12 hours, followed by moderate increases in the next 12 hours, and then a slower increase from 24 to 96 hours, is likely due to the readily available carbohydrates in the added molasses and corn grains (Fig. 1). The carbohydrates are rapidly fermented by microorganisms, leading to a quick initial surge in gas production. As these readily available sugars are consumed, the rate of fermentation and gas production slows down, resulting in the moderate and then slower increases observed over the longer time frame. Corn grains and molasses have a major impact on the in vitro cumulative gas production of corn stover silage, affecting the rate and degree of fermentation. Gas production is often increased by the addition of molasses, possibly as a result of its easily accessible carbohydrates and enhanced fermentation. Corn grains, as a source of starch, can also impact gas production, with variations depending on the specific grain type and processing (Daoud et al., 2024). Molasses addition can enhance the fermentation process by providing a readily available substrate for microbial growth, leading to increased volatile fatty acid (VFA) production and other fermentation products (Xu et al., 2013). Molasses can improve gas production by raising the amount of water-soluble carbohydrates (WSC) and crude protein (CP) in silage

(Xia et al., 2018). Starch, which is abundant in corn grains, can be fermented by rumen microorganisms to create gas and VFA. As the starch is gradually broken down, adding maize grains to silage can raise the total amount of gas produced, especially in the later stages of fermentation (Campos et al., 2020).

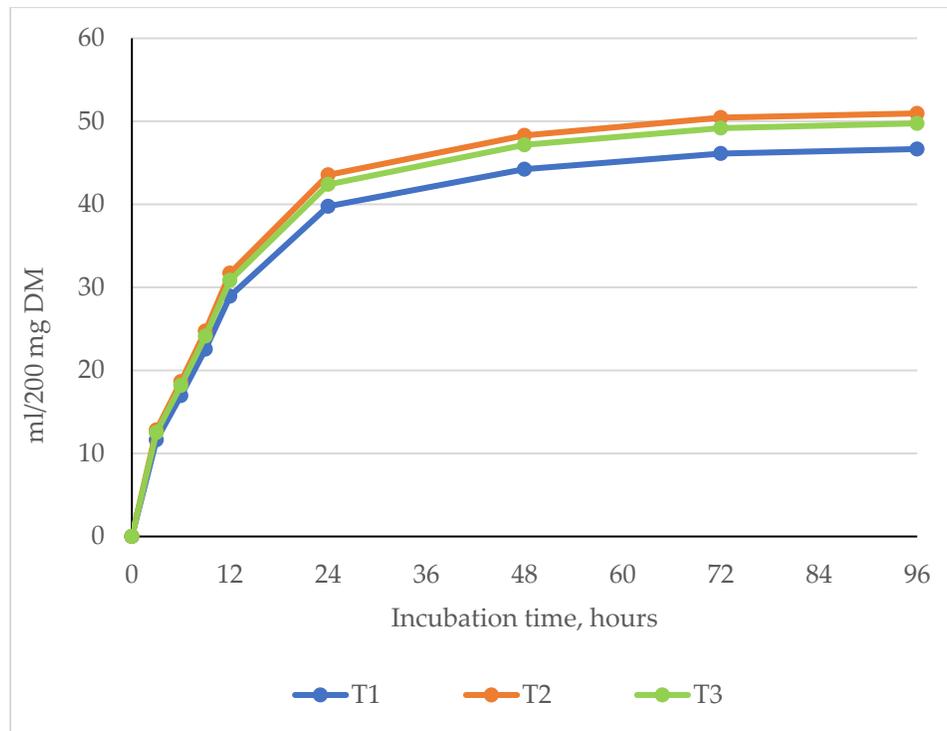


Figure 1. Effect of energy additives on accumulative gas production of corn stover silage.

Kinetics Parameters

Energy additions have an impact on the in vitro gas production kinetics of maize stover silage, as shown in Table (2). In particular, the rate of gas production (c), the possibly degradable fraction (b), and the rapidly degradable fraction (a) are all considerably ($P < 0.05$) higher when molasses and ground maize grains (treatments T2 and T3) are added than when the unsupplemented control (T1) is used. Corn stover silage ferments more quickly and thoroughly when energy supplements are added. maize stover silage's in vitro gas production kinetics can be greatly impacted by the addition of molasses and maize grains, which may improve silage usage and influence fermentation characteristics (Xia et al., 2018). Because there are more fermentable substrates available, molasses may raise the total potential for gas production. Additionally, the amounts of volatile fatty acids (VFAs) generated during fermentation can be influenced by corn grains, which may have an effect on animal performance (Pongsub et al., 2024). Molasses offers easily accessible sugars that improve fermentation, accelerating the rate at which gas is produced. It can also decrease the lag period of fermentation, which accelerates the onset of gas production. Additionally, a higher total gas production potential may result from the higher starch content (Yulistiani & Nurhayati, 2018). Molasses has the ability to change the asymptotic gas production (a) and the rate constant (c) of gas production curves (da Silva Zornitta et al., 2021). Because of their high starch content, corn grains can accelerate the formation of gas (DePeters et al., 2003).

Gas Fractions

According to the statement, in an experiment where statistical significance was set at $P < 0.05$, adding molasses and ground corn to corn stover silage (treatments T2 and T3) led to significantly ($P < 0.05$) higher gas production from both the soluble (GPSF) and insoluble (GPNSF) fractions of fermentation in comparison to the control (T1) (Table 2). The breakdown of the feed's more resistant and more accessible components was improved by energy supplements. Molasses and corn grains can significantly affect in vitro gas production from corn stover silage, influencing both the soluble and insoluble fractions. While corn grain can contribute to gas production from its insoluble, potentially degradable part, adding molasses often increases gas production from the soluble fraction (Nayel et al., 2019). The

amount and rate of gas production can be influenced by molasses, which may raise both the total amount of gas produced and the rate constant for the insoluble fraction (Ayandiran et al., 2024).

Table 2. Effect of energy additives on kinetics parameters and fractions of gas production of corn stover silage.

Item	T1	T2	T3	±SE	P-value
Kinetics parameters					
a (ml/200 mg DM)	5.14b	5.53a	5.48a	0.06	0.012
b (ml/200 mg DM)	36.79b	39.56a	39.23a	0.46	0.010
c (ml/hour)	0.054b	0.058a	0.057a	0.001	0.021
Gas fractions					
GPSF (ml/200 mg DM)	8.51b	9.69a	9.39a	0.19	0.017
GPNSF (ml/200 mg DM)	30.71b	32.72a	32.48a	0.34	0.026

a, b: Means in the same row with different superscripts differ significantly at $P < 0.05$.

Methane Production (CH₄)

The addition of energy sources and the duration of incubation have an impact on the synthesis of methane from maize stover silage (Fig. 2). During the whole incubation period, methane production in corn stover silage (T2 and T3) supplemented with molasses and ground corn grains was considerably ($P < 0.05$) lower than that of unsupplemented T1. Additionally, molasses was found to somewhat reduce methane output when compared to maize grains. Methane production increases sharply in the first 12 hours of incubation, then steadily increases between 12 and 24 hours, and continues to increase slightly up to 96 hours (Fig. 2). Generally, increasing fiber content in animal feed tends to increase methane production, particularly in ruminants like cows. This is because fiber, especially when poorly digestible, is fermented in the rumen by microbes, producing methane as a byproduct. However, the relationship can be complex and influenced by factors like fiber digestibility, the overall diet composition, and animal type. Adding molasses and corn grains to corn stover silage can affect *in vitro* methane production, but the effects can vary. Molasses can improve fermentation and potentially decrease methane production, while corn grains, especially when included at high levels, can increase methane production due to their higher starch content (Xia et al., 2018). The type of grain can also affect methane emissions. Methane emissions were lower in cows fed a corn-based diet during the finishing phase (Beauchemin & McGinn, 2005).

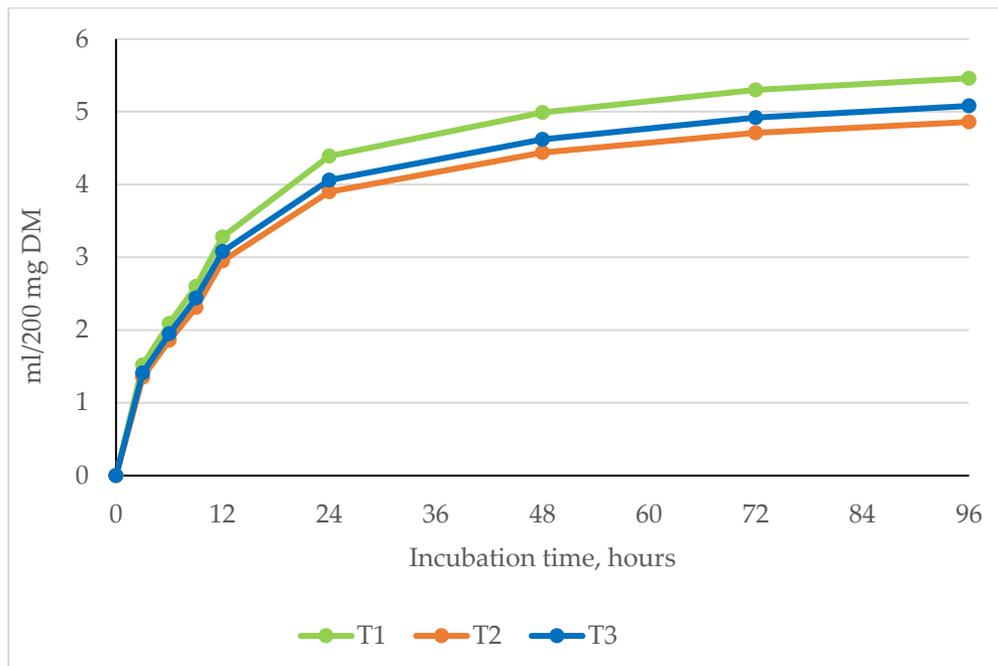


Figure 2. Effect of energy additives on *in vitro* methane production of corn stover silage.

Short Chain Fatty Acids (SCFA)

According to the results shown in Fig. 3, adding molasses and ground corn grains to corn stover silage (treatments T2 and T3) led to noticeably greater concentrations of short-chain fatty acids (SCFAs) than the unsupplemented treatment (T1); this difference was statistically significant ($P < 0.05$). More SCFAs are produced in the silage as a result of the improved fermentation process brought about by the additional molasses and crushed maize grains. Indeed, the primary byproducts of fermentation in ruminants are short-chain fatty acids (SCFAs), especially acetate, propionate, and butyrate, which are essential sources of energy for these creatures. These SCFAs are created when bacteria in the rumen digest carbohydrates. The production of gas and short-chain fatty acids (SCFAs) can be greatly impacted by molasses and corn grains during the *in vitro* fermentation of corn stover silage. Adding molasses can boost gas production, improve digestibility, and perhaps change the ratios of SCFAs. As a source of starch, corn grains can also impact fermentation processes, which in turn can affect the kind and quantity of SCFAs that are generated (Xu et al., 2013). The amounts of SCFAs produced during fermentation can be affected by molasses. For example, it may raise the production of propionate while lowering the ratio of acetate to propionate (Palmonari et al., 2023). The starch content and the particular makeup of the maize grain might affect the kind and quantity of SCFAs generated. For instance, more propionate may be produced when there is a higher starch content (Khan et al., 2012).

Microbial Protein Production

Gas production from corn stover silage with energy additions was employed to measure the rumen's microbial protein synthesis (MCP) (Fig. 3). When compared to unsupplemented silage (T1), molasses and ground corn grains supplementation (T2 and T3) significantly ($P < 0.05$) increased MCP, indicating that rumen bacteria are better able to use the silage when energy sources are given. Although the precise effects can vary, adding molasses and corn grains to corn stover silage can have a good impact on the production of gas and protein by microorganisms *in vitro*. For rumen microorganisms, molasses provides an easily accessible energy source that may increase microbial gas production and protein synthesis. Corn grains, rich in starch, can also enhance fermentation and gas production, but their impact on microbial protein synthesis may depend on the extent of starch degradation and utilization by rumen microbes (Liu et al., 2020). By providing readily available energy, molasses supports microbial growth in the rumen, potentially leading to increased microbial protein production (Xu et al., 2013). Although corn grains can promote microbial development, the balance between rumen microorganisms' use of other nutrients and the breakdown of starch determines how much microbial protein synthesis occurs (DePeters et al., 2003).

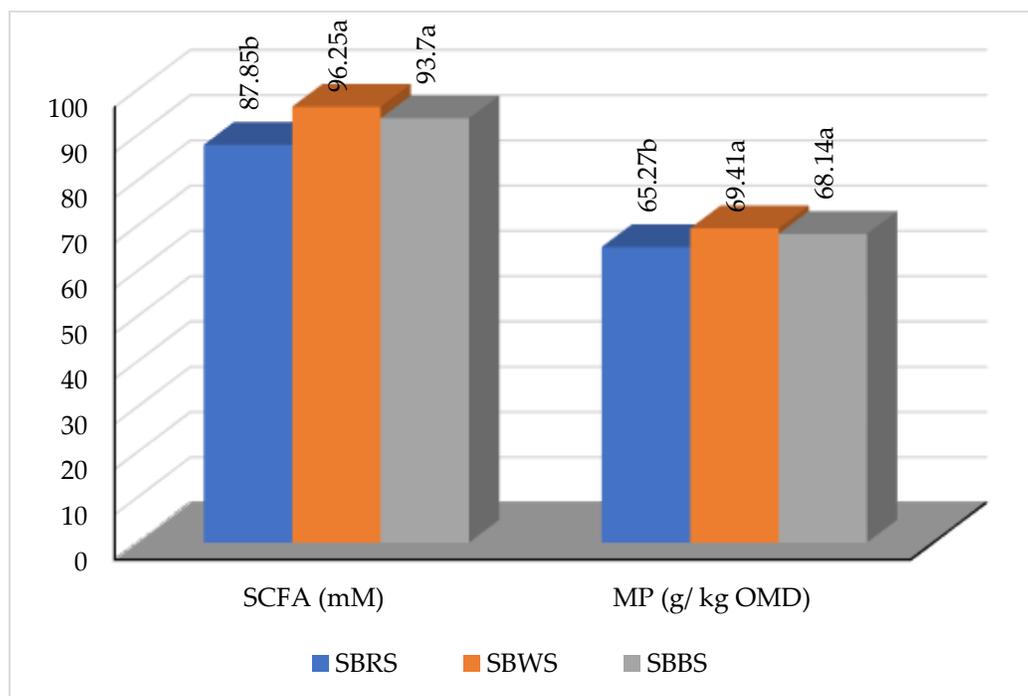


Figure 3. Effect of energy additives on short chain fatty acids (SCFA) and microbial protein production (MCP) of corn stover silage.

Predicting Dry Matter Intake

As indicated in Table (3), adding molasses and ground corn grains to corn stover silage (treatments T2 and T3) results in a significantly ($P < 0.05$) higher dry matter intake (DMI) than an unsupplemented control (T1). When DMI is represented as kg/day or as kg/kg metabolic live body weight (LBW^{0.75}), an increase is seen. Additionally, the text implies that DMI can be predicted using in vitro gas production techniques. Molasses and maize grains added to corn stover silage have the ability to enhance dry matter intake (DMI) and have a beneficial effect on in vitro gas production. Molasses, as a readily fermentable carbohydrate source, enhances fermentation, leading to increased gas production and potentially higher DMI. Corn grains, similarly, contribute to fermentation and digestibility, further boosting gas production and potentially DMI (Dong et al., 2020). Molasses can improve the digestibility of the silage, making more nutrients available for microbial fermentation and potentially increasing DMI (Ayandiran et al., 2024). By improving fermentation and digestibility, corn grains can indirectly contribute to increased dry matter intake (Kareem and Saeed, 2021). Higher gas production in vitro often correlates with higher DMI in vivo, making it a useful indicator of silage quality (Ayandiran et al., 2024).

Effective Dry Matter Degradability and Organic Matter Digestibility

Data in Table 3, highlights that supplementing corn stover silage with molasses and ground corn (treatments T2 and T3) significantly ($P < 0.05$) increases the effective dry matter degradability (EDMD) and organic matter digestibility (OMD) compared to the unsupplemented treatment (T1). This suggests that the additives improve the nutritional value of the silage. Furthermore, the digestibility of the silage's organic matter (OMD) and dry matter (IVDMD) can be estimated using in vitro gas production. In vitro effective dry matter degradability (EDMD) and organic matter digestibility (OMD) can be enhanced by adding molasses and corn grains to corn stover silage. Molasses, as an energy source, can improve EDMD, while corn grains, especially when processed, can increase OMD by providing readily available carbohydrates (Ayandiran et al., 2024). Molasses, with its readily fermentable sugars, can enhance the activity of rumen microbes, leading to increased breakdown of dry matter in the silage. This is reflected in higher EDMD values (Xu et al., 2013). By providing a readily available energy source, molasses can indirectly improve organic matter digestibility in the silage. The increased microbial activity can break down complex carbohydrates and other organic components more efficiently, leading to higher OMD (Ayandiran et al., 2024). Corn grains, particularly when processed (e.g., ground or steam-flaked), can be a good source of starch, which is readily digested in the rumen. This can significantly contribute to the overall OMD of the silage. While the primary impact of corn grains is on OMD, the increased availability of readily digestible carbohydrates can also positively influence EDMD by stimulating microbial activity (Lee et al., 2002).

Nutritional Values

In a study on corn stover silage (Table 3), adding molasses (T2) and ground corn grains (T3) to the silage increased its level of total digestible nutrients (TDN) significantly ($P < 0.05$) when compared to the unsupplemented control (T1). Although the crude protein (CP) content was raised by both molasses and corn grain supplementation, only molasses (T2) demonstrated a substantial ($P < 0.05$) increase in digestible crude protein (DCP) when compared to the control, whereas the DCP difference from corn grain (T3) supplementation was negligible. Corn stover silage's nutritional value can be raised by adding molasses and corn grains, especially by raising TDN and DCP. Molasses, as a readily available carbohydrate source, can enhance fermentation, improve palatability, and boost energy content. Corn grains, also a good source of energy and nutrients, contribute to a higher TDN value and can also improve the protein content of the silage (El-Garhy, 2007). Molasses also contributes to a higher TDN value of the silage due to its energy content (Bostami et al., 2008). Adding ground corn can increase the TDN value by providing more digestible energy. Furthermore, corn grain can increase the overall protein content of the silage, leading to a higher DCP value (Bilal, 2009).

Table 3. Effect of energy additives on predicting dry matter intake, degradability and digestibility and nutritional values of corn stover silage.

Item	T1	T2	T3	±SE	P-value
predicting DM intake					
DMI (kg/day)	5.05b	5.31a	5.28a	0.04	0.012
DMI (g/kg LBW ^{0.75})	48.14b	50.57a	50.30a	0.41	0.014
Degradability and digestibility					
EDMD %	46.91b	51.27a	50.68a	0.73	0.022
OMD %	54.11b	57.54a	56.49a	0.53	0.018
Nutritive values					
TDN %	62.34b	63.54a	63.56a	0.23	0.015
DCP %	4.85b	5.03a	4.98ab	0.03	0.046

a, b: Means in the same row with different superscripts differ significantly at $P < 0.05$.

Energy Values

The energy value of the corn stover silage is increased by these energy additions, according to the results in Figure 4. In comparison to the unsupplemented silage (T1), the addition of molasses and ground corn grains to corn stover silage (T2 and T3) resulted in a statistically significant increase ($P < 0.05$) in gross energy (GE), digestible energy (DE), metabolizable energy (ME), and net energy (NE). By improving fermentation and digestibility, molasses and corn grain additions to corn stover silage can have a positive impact on in vitro metabolizable and net energy. As an easily accessible source of carbohydrates, molasses encourages the growth of lactic acid bacteria, which enhances silage fermentation and may raise metabolizable energy. Because corn grain is high in nutrients, it can directly increase the silage's energy content. The amounts of each addition and the silage's overall composition determine the precise effects (Bostami et al., 2008). By giving lactic acid bacteria (LAB) an easy-to-access supply of sugars, molasses can enhance the fermentation quality of maize stover silage. The silage's digestibility and metabolizable energy (ME) content may increase as a result of this enhanced fermentation (Bostami et al., 2008). Compared to maize stover, corn grain has more starch and other easily digested carbohydrates, making it a suitable source of energy for ruminants. Corn grain can be added to corn stover silage to boost the feed's net energy content and total energy density (Daoud et al., 2024). Corn stover silage can have its energy content raised by adding corn grains and molasses. An easily accessible energy source, molasses promotes fermentation and raises the nutritious content overall. Similarly, adding crushed corn grains to corn stover silage raises the amount of nitrogen-free extract (NFE) and dry matter (DM) considerably, both of which add to the energy value (El-Garhy, 2007). The energy content of silage is increased by molasses, which is a good source of easily accessible carbohydrates. Additionally, the total energy density is increased when ground corn is added to corn stover silage (Gaafar et al., 2023).

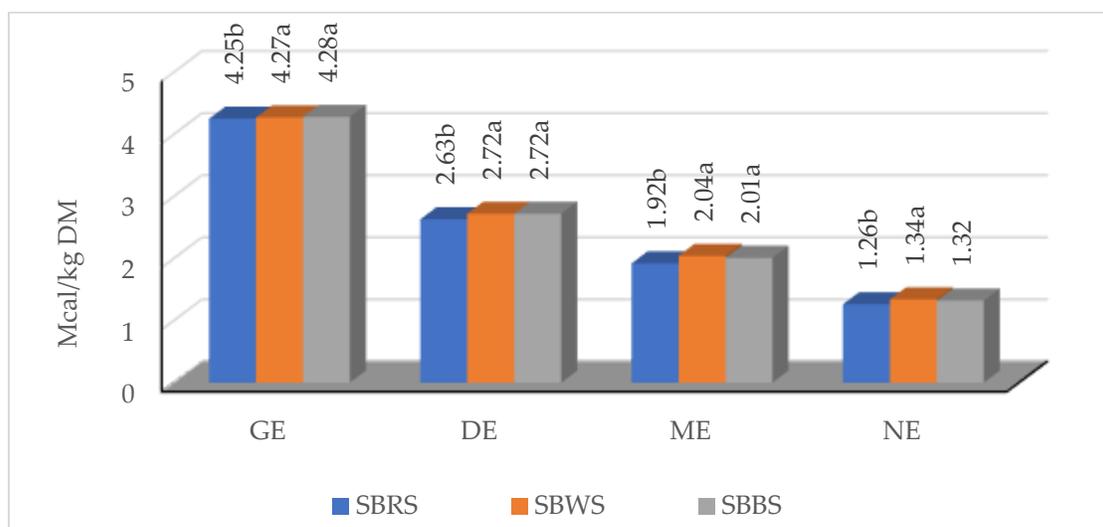


Figure 4. Effect of energy additives on energy contents of corn stover silage.

CONCLUSION

Supplementing corn stover silage with molasses or ground corn grains improves its chemical composition, nutritional value, enhances fermentation characteristics, and leads to a more efficient *in vitro* gas production. These findings suggest that these additives can be valuable tools for improving the quality and utilization of corn stover silage as a feed for ruminants.

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Conflict of Interest

The authors have no conflict of interest regarding the publication of this article.

Authors Contribution

Each author made an equal contribution to the paper.

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