10.21608/avmj.2025.371043.1643

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EFFECT OF USING GUAVA LEAVES IN RUMINANT RATION ON RUMINAL FERMENTATION CHARACTERISTICS, METHANE EMISSION AND NUTRIENTS DIGESTIBILITY

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Received: 8 May 2025; Accepted: 28 May 2025

ABSTRACT

Guava leaves (GL) are sustainable and locally accessible forage in Egypt, which can serve as a valuable ingredient to ruminants' diets, due to their rich nutritional value and secondary metabolites. The present study was carried out to evaluate the effect of partial/total substitution of corn silage by GL at 0%, 25%, 50%, 75% and 100% on rumen fermentation profile and nutrient degradability, as well as methane emission in vitro using a semi-automatic system of gas production assay. The findings showed that when GL accounted for 25% and 50% of the total mixed diet, volatile fatty acids dramatically decreased. Additionally, there was a very noticeable drop in methane generation as GL increased, particularly at 75% and 100% levels. Furthermore, throughout all recorded fermentation intervals, the diet containing 100% GL produced the least amount of gas. The current study indicated that the partitioning factor as an index of microbial protein synthesis varied significantly between 0% and 100% of GL, ranging from 3.7 to 4.9. Guava leaf inclusion has a negative impact (P<0.05) on the protozoa count, while the rumen pH rises noticeably when GL in the diet increases. The results in the current study concluded that the optimal level for replacing corn silage with GL should not exceed 25% in the TMR of ruminants.

Keywords: Sustainable forage, nutrient degradation, alternative resources, methane emission

INTRODUCTION

There are severe shortages in information in conventional animal feeding. Nearly all the grains are needed for human use. Future aspects of ensuring animal

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feeding will depend on the better use of unconventional feed resources that do not compete with human food, as the demand for livestock products rises due to the world's economies growing quickly and land area decreasing. Perhaps the most pressing challenge facing planners and animal scientists worldwide is the availability of feed supplies and their sensible use for livestock. The issue is a never-ending tale, since it is severe in many developing nations, where recurrent yearly

feed shortages and growing animal populations are prevalent. Therefore, it is necessary to look for less expensive nonconventional feed resources that can improve intake and digestibility of low quality forages. Feedstuffs like fish offal, duckweed, and kitchen leftovers (i.e., potato peel, carrot peel, onion peel, and cabbage leftover), algae/Spirulina, Guava leaf, Leucaena leaf, and cactus are commonly used in India and could be invaluable feed resources for small and medium size holders of livestock (Onte *et al.*, 2021).

Millions of tons of agro-industrial waste are produced in Egypt and many other countries. The accumulation of this trash without treatment has a negative impact on environment. The substantial integration of appropriate agro-industrial by-products into animal diets has been made possible by the growth of the animal feed industry. The potential of various agroindustrial wastes and unconventional feed as animal feed additives has been evaluated (Shi et al., 2014). Given that feed prices can make up more than 70% of the total costs in livestock production, interest in using these alternative feed sources has increased, especially to meet the nutritional gap between animal needs and available feed supplies (Al-shanti et al., 2013).

Research on the use of plant byproducts in animal nutrition is becoming more and more important. Utilizing its co-products in animal feed provides a way for the agrofood industry to recycle nutrients, and ought to be given priority as a way to dispose of by-products. Therefore, it is expected that using agro-industrial wastes will have a good economic impact and lessen the environmental load. Guavas belong to the Myrtaceae family's genus Psidium. Barbalho et al. (2012) state that guavas are native to Mexico and grow throughout tropical and subtropical regions. Guava leaves (GL) (Psidium guajava) contain a variety of flavonoids, such as quercetin, saponins, tannins, and alkaloids. These

antioxidants have been shown to improve immunity and animal performance (Kuo et al., 2023 and Hossain et al., 2024). They also mentioned the antimicrobial qualities of these flavonoids. Methane (CH4) is the second most significant greenhouse gases and absorbs more energy than CO2 (Sarthak et al., 2022). Furthermore, the global warming potential (GWP) of CH4 is 28 times greater than CO2. Cattle and dairy cows were the biggest producers of CH4 emissions, making up 72% of overall sector emissions (Al-Sagheer et al. Akinbode et al. (2024) concluded that guava leaves may be included up to 30% in the diet of West African dwarf goats for effective CH4 reduction with minimal effect on dry matter degradation. Thus, a worldwide effort is required to reduce ruminant emissions. To get the best combination of guava leaves in the total mixed ration (TMR), it was hypothesized that knowledge of the kinetics of gas production and nutrient degradability is crucial. This would encourage more balanced diets and lessen the detrimental effects of CH4 production using an in-vitro gas production system. This study was designed to assess the effects of substituting varying amounts of GL instead of corn silage on CH4 emission, organic matter (OM), the actual breakdown of dry matter (DM) and microbial efficiency.

MATERIALS AND METHODS

The Experiment: *in-vitro* gas production: The Advanced Laboratory of Animal

Nutrition and Agricultural Experimental Station (31°20′N, 30°E), Faculty of Agriculture, Alexandria University, Egypt, is where the current study was conducted.

An in-vitro trial was established to study the effect of partial/or total substitution of corn silage by Guava leaves (GL) on rumen fermentation and nutrient degradability, fermentation parameters and total protozoa count using a semi-automatic system of gas production (GP) and characterization of

rumen populations. Theodorou *et al.* (1994) described the *in-vitro* GP assay, but Bueno *et al.* (2005) modified it to use a semi-automatic system that measured gas production in 120 ml serum bottles incubated at 39°C for 24 hours using a pressure transducer and data logger (GN200, Sao Pualo, Brazil).

Experimental diets and treatments:

Five experimental total mixed rations (TMR) were prepared, which were used as the incubated substrate for the GP assay, the replacement is as follows:

1. GL zero % + CS₁₀₀ % (zero % Guava leaves + 100 % corn silage)

- 2. **GL** 25 % + **CS** 75 % (25 % Guava leaves + 75 % corn silage)
- 3. **GL** 75 % + **CS** 25 % (75 % Guava leaves + 25 % corn silage)
- 4. **GL** 50 % + **CS** 50 % (50 % Guava leaves + 50 % corn silage)
- 5. **GL** 100 % + **CS** zero % (100 % Guava leaves + zero % corn silage)

This diet was formulated according to NRC (2007) nutrient requirements recommended for dairy cattle. The ingredients of the concentrate mixture of the basal diet ration in table (1).

Table 1: Partial or total substitution of corn silage (CS) by Guava leaves (GL) in the experimental total mixed ration (TMR).

Components, %	GL Zero % +	GL 25 % +	GL 50 % + CS	GL 75 % +	GL 100 % + CS
	CS _{100 %}	CS 75 %	50 %	CS 25 %	zero %
GL	0.0	12.5	25.0	37.5	50.0
Corn Silage	50.0	37.5	25.0	12.5	0.0
Yellow Corn	16.0	15.0	15.0	15.0	15.0
Wheat Bran	16.7	16.7	15.7	15.7	15.7
Soybean meal	8.0	9.0	10.0	10.0	10.0
NaCl	1.0	1.0	1.0	1.0	1.0
Limestone	2.0	2.0	2.0	2.0	2.0
Trace minerals	0.3	0.3	0.3	0.3	0.3
Urea	1.0	1.0	1.0	1.0	1.0
Molasses	5.0	5.0	5.0	5.0	5.0

Inoculum donor and preparations:

Fresh ruminal content from three Holstein cows slaughtered at the Faculty of Agriculture slaughterhouse. After being squeezed through four layers of cheesecloth, the combined rumen contents (50:50 v/v) were stored in a water bath at 39°C and saturated with CO2 until the inoculation process.

In-vitro experiment:

The in-vitro gas production assay by Theodorou *et al.* (1994) was modified to a semi-automatic system by Bueno *et al.* (2005), which includes a data logger (GN200, Sao Pualo, Brazil) and a pressure transducer. Samples of the incubated diet (500 mg as-fed) were precisely weighed into 120 ml glass bottles, incubated with 30 ml of buffer solution (MB9; Onodera and

Handerson, 1980), and then 15 ml of inoculum was added to the bottles, leaving a 75 ml head space. 2.8 g NaCl, 0.1 g CaCl2, 0.1 g MgSO4.7H2O, 2.0 g KH2PO4, and 6.0 g Na2HPO4 were dissolved in distilled water to create a 1 L buffer. After 15 minutes of CO2 flushing, the pH was manually fixed and immediately sealed with 20 mm butyl septum stoppers (Bellco Glass Inc., Vineland, NJ, USA). The bottles were incubated for 24 hours at 39°C in a forced air oven. After 3, 6, 12, and 24 hours, the head space gas pressure was measured. $V = 4.974 \times p + 0.171$ (n = 500; r2 = 0.98; data not reported) is the formula used to compute gas production. V stands for gas volume (ml), and p for observed pressure (psi). In order to determine the concentration of CH4, gas is extracted from the bottles using a 2 ml syringe after 3, 6, 12, and 24 hours of incubation. The gas is then collected in vacutainer tubes and infused into a Gas Pro detector (Gas Analyzer CROWCON Model Tetra3). Following the correction of the total gas GP and incubated or genuinely degraded organic matter readings for the corresponding blank, both GP and CH4 expressed as ml/g DM and ml/g TDOM.

Rumen degradability and fermentation characteristics:

According to Van Soest et al. (1991), the amount of genuinely degraded dry matter (TDDM) and organic matter (TDOM) was measured when the incubation period ended at 24 hours. The ratio of the gas volume (ml) to the milligrams of TDOM at 24 hours of incubation is the partitioning factor (PF) (Blümmel et al., 1997). Rumen pH was determined with a portable pH meter (GLP 21 model; CRISON, Barcelona, Spain) within two to three minutes after sampling. A spectrophotometer (Alpha-1101 model; Labnics Equipment, California, USA) uses a commercial lab test that Konitzer and Voigt (1963) describe to quantify the ruminal NH3-N concentration calorimetrically.

Gas chromatography (GC) was used to determine each VFA's concentration. Quickly, after thawing, a 1.6 mL aliquot was made with 0.4 mL of a 4:1 ratio of 25% metaphosphoric acid, and it was centrifuged at $15,000 \times g$ for 20 minutes at 4 °C (K1015 Micro Prime; Centurion Scientific Ltd, Stoughton, Chichester, UK). Using a GC Fisher Scientific, (Thermo TRACE1300, Rodano, Milan, Italy) with an AS3800 auto sampler and a capillary column HP-FFAP (19091F-112; 0.320 mm o.d., 0.50 µm i.d., and 25 m length; J and W Agilent Technologies Inc., Palo Alto, CA), the supernatant was used to measure the concentration of VFA. The carrier gas is hydrogen at a rate of 1.35 mL/min. The make-up gas fluxes of nitrogen, hydrogen, and air were maintained at 35, 40, and 450 mL/min, respectively.

For the duration of the run, a 0.1 µl aliquot was injected in splitless mode using 31.35 mL/min of H2 flow (63.432 Pa). The temperatures of the injector and the flame ionization detector (FID) were maintained at 250°C isothermally. With a total analysis time of nine minutes, the oven heating slope was 80°C for one minute, 120°C for three minutes (20°C/min), and 205°C for two minutes (10°C/min). For a subsequent total protozoa count, two milliliters of rumen fluid sample were combined with two milliliters of methyl green-formalin-saline (MFS) solution and kept at room temperature in a glass bottle. Using an Olympus biological microscope (model CX31RBSFA, Philippines), count the total number of protozoa using the method outlined by Dehority et al. (1983).

Chemical analyses:

Feed samples were grounded to pass a 1mm screen and chemically analyzed for dry matter (DM), organic matter (OM), ether extract (EE) and crude protein (CP) according to the analytical procedures of AOAC (2006). Sequentially, NDF and ADF were determined in filter bags and expressed exclusive of residual ash, as described by Van Soest et al. (1991), using Ankom200 Fiber Analyzer Unit (ANKOM Technology Corporation, Macedon, NY, USA). ADL was determined according to Van Soest and McQueen (1973) by solubilization of ADF with 720 ml/l sulfuric acid.

Statistical Analysis

The SAS software package's least significant difference procedure (LSD) was used to analyze the experiment's data using analysis of variance (ANOVA) (2002). The model that was employed was $Yij = \mu + Ti + eij$, in which eij is the random error, Ti is the treatment, and μ is the overall mean. The LSD test was used to determine the significant differences between individual means.

RESULTS

Among different total mix rations (TMR) found in Table (2) OM% and Cellules decrease with 100% GL supplementation diet compared with other groups. The previous results could be explained as shown in Table (3) that GL had lower OM, Cellules, compared with Corn silage (CS).

Crude protein (CP) and Ether extract (EE) of Guava leaves (GL) were 6.62% and

3.39%, respectively. Crude fiber (CF), as shown in Table (3), had a lower value than that found by Raju, *et al.* (2024) (10.39 and 16.47, respectively). Neutral detergent fiber (NDF) was 40.08, while Ash was 9.02. No big differences were found between corn silage (CS) and GL in OM, Ash, NDF, ADF, and hemicellulose (Table 3). In the same context, GL was twice times in ADL compared with CS, while CS was twice times in Cellulose compared to GL.

Table 2: Chemical analysis of different TMR used in the experiment on DM basis.

Itoma 0/	Experimental TMR						
Items, %	0%GL	25%GL	50%GL	75% GL	100% GL		
OM	91.10	91.07	90.96	91.09	88.53		
CP	15.31	15.65	15.27	15.05	14.59		
EE	1.39	1.03	1.34	2.28	2.47		
NDF	42.60	38.30	37.60	34.20	37.10		
ADF	17.80	17.50	16.90	15.70	15.70		
ADL	0.72	0.67	0.81	0.89	0.60		
Hemicellulose	24.80	20.80	20.70	18.50	21.40		
Cellulose	17.08	16.83	16.09	14.81	15.10		

OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, TMR: Total mix ration, GL: Guava leaves

Table 3: Chemical composition of Guava leaves and corn silage on DM basis

Components, %	Guava leaves (GL)	Corn silage (CS)
OM	90.98	91.52
Ash	9.02	8.58
CP	6.62	8.48
EE	3.39	1.18
CF	10.39	23.35
NDF	40.08	48.50
ADF	22.63	31.90
ADL	8.82	4.15
Hemicellulose	17.45	16.60
Cellulose	13.81	27.75

Om: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin

Data in Table (4) also showed a highly significant effect of treatments on gas production (GP). Also, 100% of GL in the diet had the lowest GP in all measured fermentation intervals, starting from 3 hours to 24 hours. While percentages of GL (25, 50 and 75) had lower GP compared with 0% GL, with no noticeable difference among them (Figure 1).

Moreover, *In-vitro* gas production reduced drastically when guava leaves incubated alone. Data in Table (4) showed a highly significant (P<0.05) decrease in CH4

production with increasing GL, especially with 75% and 100% levels. Methane production decreased with about 34% when GL reached 100% in the experimental diet.

Highly significant effects (0.001) were found among treatments on TDDM and TDOM (Table 4) with remarkable decreasing in both TDDM and TDOM between zero and 100% GL. The partitioning factor (PF) ranged from 3.7 and 4.9 in the present study, with significant differences between 0% and 100% of GL.

Table (5) showed an increase in ammonia and pH with increasing GL, while Protozoa decreased.

Table 4: Impact of substituting corn silage with GL in TMR on cumulative GP, CH₄, truly degraded in dry and organic matter and PF *in vitro*.

RT 3 hr	GP (n	nl/g DM)		CH ₄	TDDM	TDOM	DE	
	3 hr	6 hr	12 hr	24 hr	(ml/g DM)	(g/kg DM)	(g/kg DM)	PF
0%GL	55.10 ^a	91.95 a	147.35 a	202.58 a	32.80 a	802.66a	796.52 a	3.73°
25%GL	45.83 b	75.92 b	118.70 b	168.98 b	32.05 ab	721.17^{cd}	710.23 °	4.38^{ab}
50%GL	47.18 b	77.51 b	120.45 b	173.16 ^b	29.86 ^b	$752.36^{\text{ cb}}$	746.77 ^ь	4.06^{cb}
75%GL	46.46 b	75.86 b	119.33 b	170.51 b	24.48 °	762.23 ^b	754.20 b	$4.07^{ m cb}$
100%GL	39.87°	62.54°	93.71 °	131.91°	21.72 ^d	695.71 ^d	685.72 °	4.91 a
SEM	1.3004	3.0816	5.7456	3.0502	0.8787	11.0218	11.8084	0.189
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0025

GP: gas production, CH₄: methane, TDDM: truly degraded dry matter, TDOM: truly degraded organic matter, PF: partitioning factor (gas volume (ml mg⁻¹) of truly degraded OM).SEM: standard error of mean. Values with different superscript letters within the same column differ significantly.

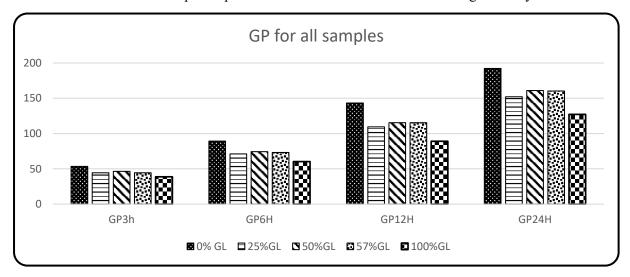


Figure 1: Differences of gas production (GP) among measured intervals.

Table 5: Impact of substituting corn silage by GL in TMR on ammonia-N concentration, protozoa count and rumen pH *in vitro*.

TRT	NH ₃ -N (mg/ dl)	Protozoa (10 ⁵ / ml)	Rumen pH
0%GL	9.73°	2.09 a	5.73°
25%GL	11.69 ^b	1.50°	5.77 ^d
50%GL	13.73 a	1.51 °	5.87 °
75%GL	11.69 b	1.84 ^b	5.95 b
100%GL	14.33 a	1.92 b	6.02 a
SEM	0.3236	0.027	0.007
P-Value	0.0001	0.0001	0.0001

NH₃-N: Ammonia, SEM: standard error of mean, values with different superscripts letters within the same column differ significantly.

Table (6) showed that VFA decreased significantly when GL represented 25% and 50% of the TMR. Treatment had a significant effect on all volatile fatty acids (VFA) like acetic, propionic, isobutyric,

butyric, valeric and isovaleric. Acetic is the highest value among all VFA, followed by Propionic and Butyric, while the lowest value found in Valeric.

Table 6: Impact of substituting corn silage by GL in TMR on *in vitro* total and individual volatile fatty acids concentrations (mM).

TRT	Acetic	Propionic	Isobutyric	Butyric	Valeric	Isovaleric	TVFA
0%GL	68.80^{a}	27.82a	7.45 a	17.68 a	4.04 a	9.12 a	136.17 a
25%GL	57.99^{bc}	23.84^{bc}	5.84 bc	14.48 bc	3.45 a	6.94 ^b	109.39 bc
50%GL	52.65°	23.37°	5.25 °	11.35 °	2.54^{b}	5.37°	96.03°
75%GL	62.55^{ab}	26.49 ab	6.43^{b}	13.27^{bc}	3.47 a	7.39 b	116.56 b
100%GL	64.60^{ab}	25.44 ^{abc}	5.79^{bc}	14.75 ab	3.91 a	6.39 bc	121.15 ab
SEM	2.678	0.9048	0.3383	1.0276	0.2952	0.4028	5.5149
P-Value	0.0030	0.0103	0.0014	0.0039	0.0126	0.0001	0.0005

TVFA: Total volatile fatty acids, SEM: Standard error of mean, Values with different superscript letters within the same column differ significantly

DISCUSSION

Concerning chemical analysis of GL, some studies reported that CP was higher than our study and ranged between 8.60% and 10.09%, and EE ranged from 9.69 to 11.68% (Lira. et al., 2011 and Santos et al., 2009). While Raju, et al. (2024) found that EE for GL was 2.66% which is lower than our study. Also, crude fiber (CF) had a lower value than that found by Raju, et al. (2024) (10.39 and 16.47, respectively). Neutral detergent fiber (NDF) was also lower 40.08 compared with 61.04 which was reported by Raju et al. (2024).

Al-Sagheer et al. (2018) indicated that presence of GL leads to a highly significant decrease in GP (P<0.001), CH4 emission Moreover, (P < 0.05). In-vitro production reduced drastically when guava leaves were incubated alone compared to Barseem (Akinbode et al., 2024). The authors also illustrated that this decrease in gas production may have resulted from differences in some plant metabolites. In the same context, Sarthak et al. (2023) illustrated that the presence of polyphenolic compounds in GL is capable of interfering with specific microbial adhesion to feed particles, resulting in inhibiting ruminal fermentation.

Akinbode *et al.* (2024) found that methane production decreased with about (59%) when GL represented 30% of the diet. Moreover, according to Al-Sagheer *et al.* (2018), GL can considerably lower CH4 emissions (P<0.05). Chatterjee *et al.* (2014) suggested that this decrease could be attributed to ethanol extract of GL, which strongly inhibited the *In-vitro* methanogenesis process. According to Huang *et al.* (2010) and Hassen *et al.* (2016), this decrease may be related to the high levels of TP, condensed tannins, and flavonoids in GL, which may have a significant role in restricting in-vitro fermentation.

In the current study, the decrease in both TDDM and TDOM between zero and 100% GL was observed by Al-Sagheer *et al.* (2018), who discovered that a significant linear (P < 0.05) drop in both TDDM and TDOM values was produced by GL at 37.5 and 50% levels in the diet. Additionally, McSweeney *et al.* (2001) demonstrated that this effect might be linked to GL's condensed tannins (CT)'s ability to disrupt microbial attachment to feed particles, which in turn had a negative effect on the microbial population and partially inhibited ruminal fermentation.

In the current study, the partitioning factor (PF) rose as GL increased. Similarly, Al-Sagheer et al. (2018) found that as dietary GL levels increased, PF values ranged from 2.40 to 4.60. Tannin molecules that formed interactions with proteins may be the reason for the dramatic increase in GL's PF (Makkar et al. 1997). In contrast to Bhatta et al. (2009), who showed that the presence of GL causes a decrease in NH3-N concentration, this study shows an increase in ammonia as GL increases. Additionally, he stated that the decreased proteolysis to produce ammonia may be due to the protein binding activity and the ensured creation of condensed tannins-protein complexes (Pal et al., 2015).

Akinbode et al. (2024) found that the reduction in the degradability as a result of guava leaves may be associated with the effect of phytochemicals on protozoa, for that incubated of guava leaves alone tended to reduce the protozoa population by 50%. Al-Sagheer et al. (2018) reported that the effect of supplementing guava leaves on invitro rumen fermentation is having a detrimental effect on protozoa number, as methanogenic archaea are symbiotically associated to rumen protozoa. Moreover, Rahmat et al. (2021) reported that protozoa are more susceptible to saponins than bacteria, because the membrane walls of protozoa contain cholesterol, whereas bacteria are peptide bonds with glycerol (peptidoglycan).

Increasing GL in the diet leads to a notable rise in the rumen pH (Al-Sagheer *et al.*, 2018). The previous result was compatible with our results. Moreover, the levels of GL in diets linearly influenced the ruminal pH (Nobre *et al.*, 2020). Additionally, Meng *et al.* (2016) discovered that GL had pH values that were noticeably higher than Barseem, which may be related to the decrease in GP, TDDM, TDOM, and VFA.

Similar to the findings of our study, Al-Sagheer et al. (2018) found that the

presence of GL alone or at 37.5 and 50% levels in the diet considerably decreased the total VFA content, including the individual fraction of acetate and valerate. Also, Ngamsaeng *et al.* (2020) reported that acetic acid reduced from 122.35 mmol/L to 43.99 mmol/L, when GL represented 30% in the diet, and butyric acid decreased from 9.58 mmol/L to 2.17 mmol/L at the level of 30% GL. The previous authors mentioned that this reduction may be related to the increase of fat in diets.

CONCLUSION

Highly significant effects were found among treatments in GP, CH4, TDDM, TDOM, PF, ammonia-N concentration, protozoa count and rumen pH. Hundred percent of GL in the diet had the lowest GP in all measured fermentation intervals, starting from 3 hours until 24 hours. Highly significant decreased of CH4 production with an increasing GL, especially with 75% and 100% levels. Partitioning factor (PF) Gas production, TDDM and TDOM decreased from 0 to 100% of GL in the diet. Ammonia and pH increased with increasing GL, while Protozoa decreased. significantly decreased when GLrepresented 25% and 50% of the TMR.

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تأثير استخدام أوراق الجوافة في علائق المجترات على خصائص التخمرات في الكرش وانبعاث غاز الميثان وهضم العناصر الغذائية

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تعتبر أوراق الجوافة أعلافًا مستدامة ويمكن الوصول إليها محليًا في مصر ويمكن أن تكون بمثابة عنصر قيم في النظام الغذائي للحيوانات المجترة نظرًا لانها غنية بالعناصر الغذائية والمركبات الثانوية. أجريت هذه الدراسة لتقييم تأثير الاستبدال الجزئي/الكلي لسيلاج الذرة بأوراق الجوافة بنسبة 0.1, و0.1, و0.1, و0.1, و0.1, على خصائص تخمرات الكرش، وقابلية هضم العناصر الغذائية، بالإضافة إلى انبعاث غاز الميثان في المختبر باستخدام نظام شبه آلي لتحليل إنتاج الغاز. أظهرت النتائج أنه عندما شكلت أوراق الجوافة 0.1, و0.1, من إجمالي العلف المختلط، انخفضت الأحماض الدهنية المتطايرة بشكل كبير. بالإضافة إلى ذلك، كان هناك انخفاض ملحوظ في إنتاج الميثان مع زيادة أوراق الجوافة، وخاصةً عند مستويات 0.1, و0.1, علاوة على ذلك، خلال جميع فترات التخمير أنتج النظام الغذائي المحتوي على 0.1, من اوراق الجوافة تأثير سلبي الوراق الجوافة أقل كمية من المغاز. أشارت الدراسة الحالية إلى أن عامل التوزيع كمؤشر لتخليق البروتين الميكروبي تفاوت بشكل ملحوظ بين 0.1, من الجوافة في النظام المغذائي. يمكن الاستنتاج من هذه الدراسة أنه يمكن استبدال اوراق الجوافة في الخلطة العلفية المكتملة للمجترات بما لا يزيد عن 0.1, من سيلاج الذرة لتحسين نمط تخمرات الكرش، والهضم، وانبعاث غاز الميثان.