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Impact of planting density on chemical composition, *in vitro* gas production evaluation and yield of Corn Silage

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Abstract: In a split plot design, a single cross hybrid maize (SC 10) was utilized with randomized complete block creation at low and high planting density of 20 and 30 thousand plants/fed, respectively. When the maize plants reached dough maturity (92 days), they were cut into whole pieces and put into three double-layered plastic bags for every density. The bags were kept for 35 days after being thoroughly compressed to eliminate any last traces of air. The findings showed that corn silage with lower plant density had lower CF and fiber fractions and greater NFE and NFC levels than silage with higher density ($P < 0.05$). As plant density decreased, there was a considerable rise in gas output (C), the soluble to insoluble matter ratio (A), and gas production. As planting density grew, production of methane rose dramatically ($p < 0.05$). Low density planting was associated with higher ($p < 0.05$) levels of microbial protein (MP), soluble fraction (GPSF), insoluble fraction (GPNSF), intake of DM, digestibility of OM, degradability of DM in vitro (IVDMD), and short-chain fatty acids. In the meantime, metabolizable and net energy were almost the same for high and low planting density. However, high plant density corn silage achieved higher yield of dry crop, protein, ME and NE.

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Keywords: Planting density; maize silage; composition; production of gas; yield

1. Introduction

In most world regions, corn silage is a common component in the dairy and beef cattle rations. In comparison to other fodder crops, maize silage typically has a high dry matter yield and high calorie content (Coors, 1996). Throughout the world, dairy cows fed rations that typically include whole-plant corn silage (CS). According to Khan et al. (2015), it is characterized by good silage properties due to its high starch content. Johnson et al. (2003) and Ivan et al. (2005) stated that increasing requirement of animal feed combined to the limited amount of arable land has led to the search for new hybrid varieties of maize that give a high green yield. This suggests that in order to increase the nutritional content of grain and fodder, novel heterosis-based substitutes are needed. Lactic acid bacteria serve as the basis for the silage preservation process by anaerobically converting the soluble carbohydrates into organic acids, primarily lactic acid (McDonald et al., 1991). Among the most significant storage factors that have been examined in recent decades are genetics (Schwarz et al., 1996; Argillier and Barriere, 1996; Johnson et al., 2003), breeding programs (Barriere et al., 1997; Bavec and Bavec, 2002), Date of harvesting (Mayombo et al., 1993; Hartmann et al., 2000), harvesting plants DM content (Yahaya et al., 2002), and storage material's physical attributes (Stockdale and Beavis, 1994 and Johnson et al., 2003).

Quality of whole corn forage stored as fodder silage was not affected by maize plant density during periods of heavy rainfall. Therefore, increasing the density of corn plants can result in higher silage yields. To get large amounts of high-quality fodder for dairy farming systems, forage management strategies should take crop rotation and management into account (Ferreira et al., 2014). ADF and NDF contents, which are reliable markers of fodder quality, were found to have conflicting relationships with plant densities. Plant densities had an impact on NDF (Iptas and Acar, 2006).

Simulation of microbial digestion processes in the rumen (Getachew, 1998) utilizing the process of in vitro gas production (Menke and Steingass, 1988) and changes made by Theodorou and colleagues (1994) enables us to understand the fermentation and decomposition processes of food in the rumen according to the quality of the food and the availability of nutrients necessary for the growth of microorganisms in the rumen. Many variables can influence feed fermentation in the laboratory and lead to variations within or across laboratories. These variables are most often related to the type of rumen fluid inoculated, although other elements that have been demonstrated to affect microbial activity in the laboratory include animal strain, physiological state, diet, feeding schedule, timing of rumen fluid collection and its relation to interval between rumen fluid sampling and incubation, feeding time, and collection method of rumen liquor (solid and liquid state) (Robinson et al., 1999).

Due to the strong correlation between in vivo measured digestibility and predicted in vitro rumen gas production technology in conjunction with chemical composition, many researchers have employed in vitro techniques of gas production to examine the associative effects of various feed types and to take into account the effects on rumen fermentation (Liu et al., 2002; Getachew et al., 2003). The main method for assessing the nutritional value of beef feeds is in vitro gas production (GP) assessment, in this process, the raw ingredients are incubated in rumen fluid for temporary storage (Cone et al., 1996; Getachew et al., 1998 and Dijkstra et al., 2005).

The current study set out to examine the effects of plant density on silage composition, in vitro gas production, the formation of microbial protein in corn silage, organic matter digestibility, predicted dry matter intake, energy values and yield of dry crop, protein and energy.

2. Material and Methods

Corn cultivation management

In a split plot of randomized complete block design, single cross hybrid maize (SC 10) was used at planting density of 20 and 30 thousand plants/fed. for low and high densities, respectively. The two-plant density was used in the main plots. Three replicates were given sub-plots. Four ridges, each measuring 4 m in length and 0.6 m in breadth, made up each sub-plot. Before plowing, suppling of 20–30 cubic meters of organic fertilizer, potassium sulphate (50 kgfed.) and super phosphate (150 kg/fed.). Afterwards, the number of maize plants was reduced to one per hill. Before the first and second irrigation, manual weeding and pesticide spraying are completed as needed. The fertilizer is applied in two stages to maximize productivity: the first stage comes post lizard and prior larvae; 2nd stage comes prior larvae and after weeding. Fertilization is accomplished by applying 120 nitrogen units each fed, which is equivalent to six bags of urea or eight bags of nitrate every fed. The fertilizer is applied beneath and beneath the plants. After 21 days of planting, the first irrigation is done, and then irrigation is stopped approximately two weeks before harvest, and irrigation is done every two or three weeks.

Silage making

After 92 days of planting, whole corn plants were cut with a Holland Chopper machine to a length of 1-1.5 cm when they reached the dough stage of development. Three duplicates of each density of chopped corn silage were kept in double plastic bags for 35 days. The bags were manually compressed to keep the air out.

Chemical analysis

AOAC (1990) methodologies were used to analyze representative silage samples. Van Soest and Markus (1964) discovered NDF (neutral detergent fiber) as one of fiber components. Van Soest's (1963) approach used to determination of ADF (acid detergent fiber) and ADL (acid detergent lignin).

In vitro study

Procedure shown by Menke and Steingass (1988) used to produce *in vitro* gas. A 50 ml glass syringe fitted with pistons was filled with 100 mg of precisely weighed air-dried feed samples. The gas, known as MB9, was produced in the laboratory using a buffer solution (Onodera and Handerson, 1980). To make a buffer solution, dissolve 2.8 gram of sodium chlorid (NaCl), 0.1 gram of calcium chlorid ($CaCl2$), 0.1 gram magnesium Magnesium sulfate ($MgSO4.7H2O$), 2.0 gram of Potassium phosphate ($KH2PO4$), and 6.0 gram of sodium phosphate ($Na2HPO4$) in one litter of distilled water. Carbon dioxide was flushed for 15 minutes after the pH was adjusted to 6.8. Three lambs were removed from the rumen and given a combination of commercial concentrates and rice straw at will. According to Bueno et al. (2005), solid and liquid rumen contents (50:50) were collected from lambs before morning feeding. Solids and liquids contents were delivered air-free to the laboratory in insulated flasks pre-warmed to 39°C. Rumen contents were strained through double layers of cheesecloth and keep it in a water bath at 39°C with saturated carbon dioxide prior to inoculation. In a water bath with saturated carbon dioxide, the buffer and inoculant were combined 2:1 v/v (Salam, 2005; Soliva et al., 2005 and Nasser et al., 2006). Each syringe containing the feed samples was filled with 15 ml of the preserved rumen fluid, and the syringes were then submerged right away in a water bath that was heated to 39°C. Three rounds of each experiment were conducted. One syringe, which served as the raw sample and was incubated, had solely the stored rumen fluid. Each round contained two syringes. The incubation was halted after 96 hours of measuring the gas volume, and the syringes were gently shaking every two hours. Following incubation periods of 3, 6, 9, 12, 24, 48, 72, and 96 hours, gas emissions were measured. Once the total gas values for the raw sample incubation have been adjusted, the reported gas values are given per 200 mg of dry matter. The following is how Ørskov and McDonald (1979) characterized fermentation kinetics:

$Y = a + b (1 – e\text{-}ct)$

Where Y is the gas production (ml/g DM) at time t, c is the gas production rate constant for fraction b, b is the gas production from the insoluble fraction, and a is the gas production from the directly soluble fraction.

As a new technique for assessing feeds based on these factors, the gas produced at three hours of incubation (GP3) used to compute the gas output from the soluble fraction fermentation (GPSF). Van Gelder et al. (2005) state that the gas production from the insoluble fraction fermentation (GPNSF) can be estimated using the gas production between 3 hours (GP3) and 24 hours (GP24) of incubation.

$\text{GPSF} = \text{GP 3hr} * 0.99 - 3$

$\text{GPNSF} = 1.02*(\text{GP 24hr} - \text{GP 3hr}) + 2$

The formation of gas from the soluble fraction (ml/g dry matter) is known as GPSF, the insoluble fraction (ml/g dry matter) is known as GPNSF, and production of gas for three hours (ml/200 mg dry matter) is known as GP 3hr.

Together with extra measurements of crude protein, ash, and crude fat, values of energy were determined by measuring the produced gas after a 24-hour incubation period. Based on a thorough incubation of feed in the lab, the technique was created by the research team in Hohenheim, Germany (Menke et al., 1979; Menke and Steingass, 1988).

$\text{ME (Mcal/kg DM)} = (2.2 + 0.136*\text{GP 24hr} + 0.057*\text{CP} + 0.0029*\text{CF}^{2})/4.186$

$\text{NE (Mcal/kg DM)} = (2.2 + 0.136*\text{GP 24hr} + 0.057*\text{CP} + 0.0029*\text{CF}^{2} + 0.149*\text{EE}) *2.2/ 14.64$

In this case, ME and NE represents metabolizable and net energy (Mcal/kg DM), GP represents production of gas over a 24-hour period (ml/200 mg DM), EE represents extract (as a percentage of DM), and CP represents crude protein (as % of DM).

$\text{OMD (\%)} = 14.88 + 0.889*\text{GP 24hr} + 0.45*\text{CP} + 0.0651*\text{Ash}$

Where, OMD is the organic matter digestibility (%), CP is the crude protein (% of DM), Ash is the percentage of dry matter, and GP is the 24-hour net gas production (ml/200 mg DM).

According to Getachew et al. (2005), short-chain fatty acids (SCFA) were calculated using the following formula:

$\text{SCFA (mM)} = (- 0.00425 + 0.0222*\text{GP 24hr}) *100$

Where, GP is production of gas (ml) of soluble fraction over a 24-hour period.

Blummel and Ørskove (1993) said that the following formula was used to determine dry matter intake (DMI):

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

Where, c is production rate of gas (ml/h), b is produced gas of insoluble fraction (ml), and a is produced gas of soluble fraction (ml).

According to Czerkawski (1986) calculations, microbial protein yield (MP) was 19.3 g MP/kg OMD.

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

Estimation of yield

The yield of dry crop, protein and energy were estimated and calculated per feddan.

Analysis of statistics

Data was statistically analyzed by independent samples T-test for two treatments User's Guide using IBM SPSS Statistics (2014). To evaluate for statistical significance, a p-value of 0.05 was employed.

3. Results

Chemical composition

Chemical composition and fiber fractions of corn silages with different planting density are displayed in Table 1. Low planting density corn silage had higher levels of DM, OM, EE, NFE and NFC ($P<0.05$). However, high planting density corn silage had higher levels of CP, CF, ash, NDF, ADF, and ADL, cellulose and hemicellulose ($P<0.05$).

Table 1: impact of planting density on chemical composition and fiber fractions of corn silages.

items	Low density	High density	MSE	P-value
DM %	32.86^{a}	30.65^{b}	0.61	0.036
Composition of DM %				
OM	95.16^{a}	90.04^{b}	0.31	0.037
CP	7.95^{b}	8.21^{a}	0.07	0.026
CF	22.12^{b}	25.45^{a}	0.84	0.017
EE	2.89^{b}	2.95^{a}	0.03	0.027
NFE	62.20^{a}	57.43^{b}	1.22	0.019
Ash	4.84^{b}	5.96^{a}	0.48	0.018
Fiber fractions %				
NDF	43.78^{b}	47.87^{a}	0.97	0.015
ADF	25.73^{b}	28.31^{a}	0.61	0.014
ADL	5.13^{b}	5.43^{a}	0.07	0.016
Hemicellulose	18.05^{b}	19.56^{a}	0.36	0.018
Cellulose	20.60^{b}	22.88^{a}	0.53	0.014
NFC	40.44^{a}	34.81^{b}	1.29	0.013

At the 0.05 level, the means with different superscripts (a, b) differ considerably.

Accumulative production of gas

Figure (1) displays the total amount of gas produced from corn silage at both low and high planting density. At different incubation times, low plant density corn silage produced more gas than high plant density corn silage ($P < 0.05$). The lower levels of CF, NDF, ADF, ADL, cellulose, and hemicellulose, as well as the greater levels of NFE and NFC, and the larger percentage of CF, may be the cause of the higher production of gas of corn silage at low compared to high planting density (Table 1). As incubation period extended, the variations in production of gas of corn silage grew linearly for low and high plant demisting. Up to 24 hours of incubation, gas production rose significantly; after that, it climbed progressively for the next 96 hours.

Gas production fractions

The gas generation rates and ratios of corn silage at varying plant densities are displayed in Table (2). In low planting density corn silages, there was a substantial increase ($P<0.05$) in the percentage that is instantly soluble (a), The portion that is insoluble (b), the portions that are soluble and insoluble (a + b) and the insoluble fraction's rate constant for gas production (c).

Table 2: Impact of planting density on gas production fractions of corn silage.

items	Low density	High density	MSE	P-value
a (ml/g DM)	6.98^a	6.20^b	0.08	0.018
b (ml/g DM)	63.23^a	56.25^b	0.72	0.012
a+*b* (ml/g DM)	70.21^a	62.45^b	0.80	0.014
c (ml/hour)	0.063^a	0.056^b	0.001	0.015

At the 0.05 level, the means with different superscripts (a, b) differ considerably.

Production of methane (CH4)

Fig. (2) shows the methane production of corn silage with low and high plant densities. When comparing high and low plant density corn silages, the former CH4 concentration was significantly higher ($P<0.05$). As indicated in Table (1), methane generation declined as the NFE and NFC levels in corn silage increased.

Production of gas from soluble and insoluble portions

Amount of gas production from ferment of soluble fractions (GPSF) and insoluble fractions (GPNSF) of corn silages with varying planting densities is shown in Table (3). GPSF and GNPSF levels in different planting density corn silage were substantially higher ($P<0.05$).

Concentration of short-chain fatty acids

Table (3) displays amount of short-chain fatty acids (SCFA) in the different planting density corn silages that were fermented in vitro. Low planting density corn silages had a considerably ($P<0.05$) greater SCFA concentration than high planting density corn silages.

Table 3: Impact of planting density on the fermentation of corn silage's short chain fatty acids (SCFA) and soluble (GPSF) and insoluble (GPNSF) fractions.

items	Low density	High density	MSE	P-value
GPSF (ml/g DM)	13.44^a	11.62^b	0.43	0.014
GPNSF (ml/g DM)	41.47^a	36.81^b	1.13	0.018
SCFA (mM/L)	122.36^a	108.13^b	3.42	0.017

At the 0.05 level, the means with different superscripts (a, b) differ considerably.

Dry matter intake prediction (DMI)

When fed either alone or in mixed diets, the in vitro gas generation of corn silage is a useful indicator of the voluntary feed intake, according to the results in Table (4). When comparing low and high plant densities of corn silages, the estimated DMI of the former was significantly higher ($P<0.05$). It was predicted indicated the DMI would be 6.71 and 6.14 kg/day, or 63.87 and 58.52 g/kg $LBW^{0.75}$, for corn silage with low and high planting densities, respectively.

Digestibility of organic matter

Given the high connection between the method was standardized and regression models were created to compare the measured digestibility with that anticipated by gas generation. The OMD of low planting density corn silages was substantially greater ($P<0.05$) than that of high planting density silages, as shown in Table (4).

Dry matter degradability in vitro (IVDMD):

The DMD in vitro of maize silage with different planting densities is displayed in Table (4). Low planting density corn silages had a considerably ($P<0.05$) greater in vitro DMD than high plant density corn silages.

Table 4: Impact of planting density of maize silage on dry matter intake (DMI), organic matter digestibility (OMD), and in vitro dry matter degradability (IVDMD).

items	Low density	High density	MSE	P-value
DMI (kg/day)	6.71^a	6.14^b	0.14	0.023
DMI (g/kg $LBW^{0.75}$)	63.87	58.52	1.37	0.028
OMD (%)	67.94^a	62.43^b	1.42	0.026
IVDMD %	69.65^a	64.28^b	1.41	0.031

At the 0.05 level, the means with different superscripts (a, b) differ considerably.

Contents of metabolizable and net energy

The estimated metabolizable and net energy (ME and NE) from gas generation for corn silage are displayed in Table (5). For the various plant densities of corn silage, the expected ME and NE contents were almost identical, with negligible variations ($P>0.05$). ME and NE had respective values of 2.77 and 1.81 and 2.67 and 1.75 Mcal/kg DM. Compared to high plant density, low plant density tended to enhance the ME and NE contents of corn silage.

Production of microbial protein

To improve animal performance and reduce waste from high-protein diets, it is essential to increase the production of rumen microbial protein (MCP). The energy given to rumen microorganisms has a great impact on the amount of protein nitrogen that rumen MCP absorbs. A food high in energy greatly increases the formation of rumen MCP, whereas a diet high in protein has no discernible effect on this process (Lu et al., 2019). In comparison to high planting density silages, the microbial protein yield (MP) for low planting density corn silages was considerably greater ($P<0.05$), according to the results in Table (5). Both low and high plant densities produced microbial protein in the range of 81.96 to 75.31 g/kg DMI. However, the yields of dry crop, protein, ME, and NE were considerably ($P<0.05$) greater when corn silage was planted at a high density.

Table 5: impact of plant density on the dry crop yield, protein, metabolizable energy (ME), net energy (NE), and microbial protein (MP) as well as the concentrations of these nutrients in corn silage.

items	Low density	High density	MSE	P-value
ME (Mcal/kg DM)	2.77	2.67	0.04	0.228
NE (Mcal/kg DM)	1.81	1.75	0.02	0.251
MP (g/kg OM)	81.96^{a}	75.31^{b}	1.72	0.026
Dry crop yield (ton/fed)	6.86^{b}	8.31^{a}	0.35	0.010
Protein yield (ton/fed)	0.55^{b}	0.68^{a}	0.03	0.006
ME yield (Mcal/fed)	19002^{b}	22188^{a}	952	0.018
NE yield (Mcal/fed)	12417^{b}	14543^{a}	632	0.017

At the 0.05 level, the means with different superscripts (a, b) differ considerably.

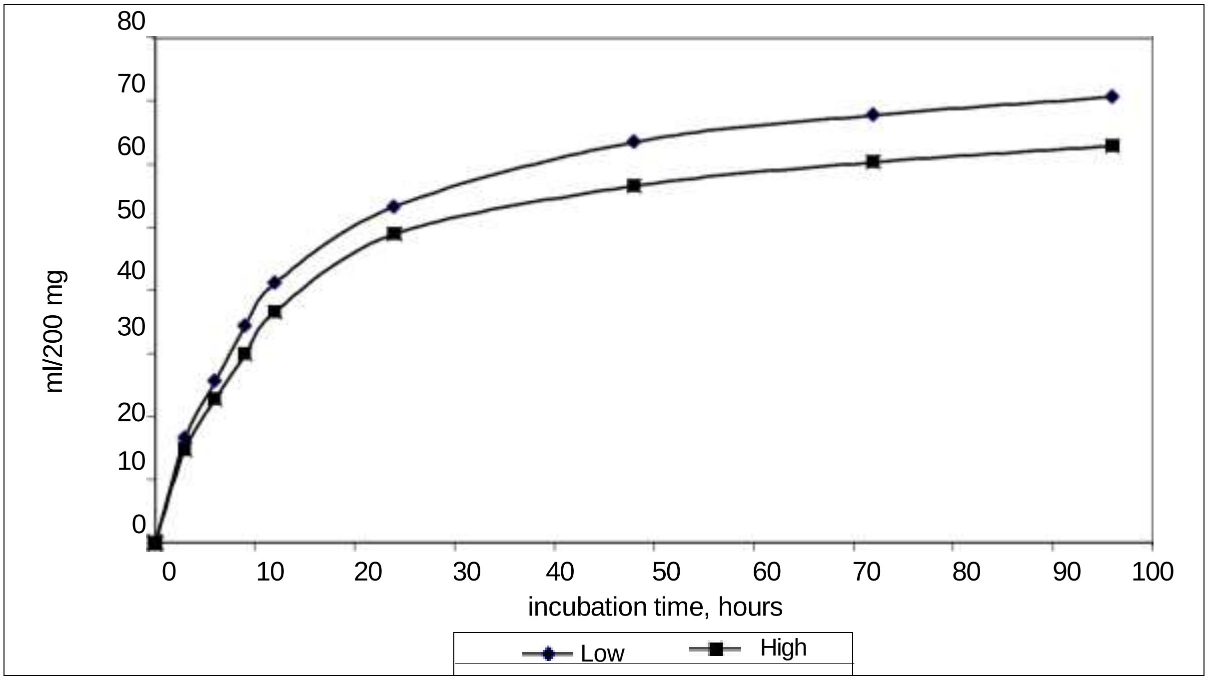

Fig. 1: Cumulative gas production of low and high plant density corn silage.

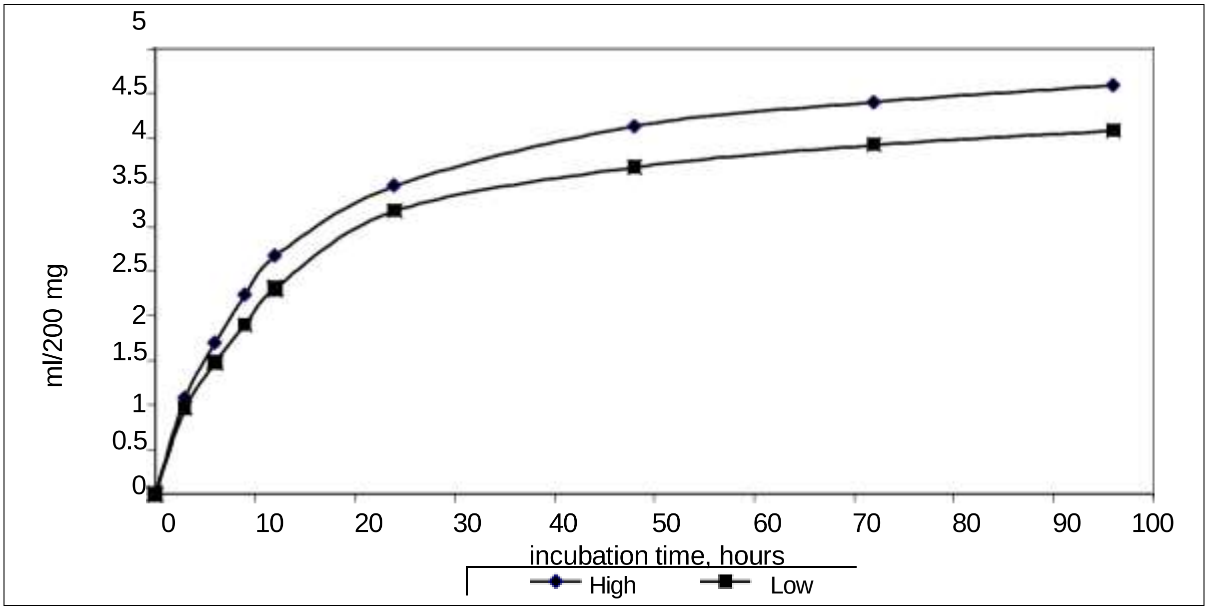

Fig. 2: Methane production of low and high plant density corn silage.

4. Discussions

Results of chemical composition were consistent with those of Painter et al. (1994), who found that increasing plant density increased the content of CP and CF and decreased soluble carbohydrates and starch in corn silage. Wang et al. (2005) found that increasing plant density significantly increased the content of CP, EE, CF and NFE. Additionally, as planting density grew, the contents of CP, CF, EE, and ash in corn silage increased significantly ($P < 0.05$), whereas DM, OM, and NFE contents fell significantly ($P < 0.05$) in corn silage (Gaafar, 2009). Roth and Hinrich (2001) found that CP level of corn silage varied between 7.2 and 10.0%. According to MacDonald et al. (1998), the amounts of ash, EE and CF were 10.0%, 5.7, and 23.3 percent, respectively. Plant densities and ADF and NDF contents, which are reliable markers of fodder quality, have a contentious connection (Iptas & Acar, 2006). As the density of maize plants grew, so did the concentrations of NDF and ADF (Valdez et al., 1989). According to Roth & Hinrich (2001), corn silage contained 23.6–33.2% acid detergent fiber (ADF) and 41.0–54.1% neutral detergent fiber (NDF).

When feeds are incubated in a laboratory, the fermentation of carbohydrates in the presence of rumen buffer fluid results in the production of microbial cells, short-chain fatty acids and gases, particularly carbon and methane. Gas is basically the result of the fermentation of carbohydrates into acetate, propionate, and butyrate. While fat contributes very little to gas production, protein fermentation produces comparatively less gas than carbohydrate fermentation (Beuvink & Spoelstra, 1992; Blummel & Ørskov, 1993). The full activity of cellulolytic bacteria, which leads to high rates of microbial colonization to the substrate and an energy-efficient usage of the evaluated feeds, depends on the fiber's quality (Sun et al., 2007). Ruminal fibrolytic bacteria were more prevalent in the presence of easily digested cellulose and hemicellulose, which may aid in the digestion of other less degradable fiber sources (Silva & Ørskov, 1988). Gas production is an indirect indicator of substrate breakdown rather than a direct correlate of microbial mass production (Liu et al., 2002). By employing the in vitro gas generation technique, we can understand how food ferments and breaks down based on its nutritional value and ruminal bacteria's access to nutrients (Menke and Steingass, 1988) or the changes made by Theodorou et al. (1994) to model the digestive processes that are produced by microbes (Getachew, 1998). Haddi et al. (2003) found a substantial negative correlation between both NDF and ADF and the rate and severity of GP.

According to Garcia-Rodriguez et al. (2005), silages with variations in parameters b and c show distinct fermentation patterns. The asymptotic GP of the soluble fraction (a) and the associated maximum gas production rate (c) declined with increasing maturity, whereas the half-time of the maximum GP of the soluble fraction (a) increased. These results align with the previously reported characteristics of whole corn. Furthermore, as the insoluble fraction (b) matured, its maximal gas generation rate increased, suggesting that starch is more digestible than NDF (Macome et al., 2017).

As the incubation period grew to 96 hours, the CH4 level slightly dropped after increasing significantly up to 24 hours. i) level of feed intake, i) type of feeding carbhydrate, and iii) rumen microbiota are the main determinants influencing CH4 emissions from ruminants (Johnson & Johnson, 1995; Lascano & Cardenas, 2010). According to Aboagye et al. (2017), a higher starch content in corn silage was associated with decreased CH4 emissions because of its effect on propionate. The kind and content of dietary lipids and carbohydrates affect the amount of CH4 produced (Grainger & Beauchemin, 2011; Ellis et al., 2007).

About three times as much gas was created by the insoluble fraction in corn silage as by the soluble fraction. Ruminant diets are now defined by three factors: degradation rate, soluble fraction, and insoluble fraction (Orskov et al., 1988; Orskov, 1991). The components of the dry matter or organic matter that are soluble in water are represented by the soluble fraction, which is also called loss of washing. According to Ly et al. (1997), it consists of the soluble sugars and soluble compounds, like polyphenolics, that are created during fermentation. Additionally, these characteristics are used to evaluate meals for nutritional value (Orskov, 1991; Ly & Preston, 1997).

The carbohydrates in feedstuff undergo in vitro fermentation to create SCFA and gases, primarily CO and CH, when incubated with buffered rumen fluid (Beuvink & Spoelstra, 1992; Blummel & Ørskov, 1993). While feed conversion into SCFA and gases is demonstrated by the gas volume measurement, the degradability assessment takes into account conversion of feed into all byproducts of microbial synthesis and degradation, mostly microbial biomass, SCFA, and gases (Grings et al., 2005). Methane and SCFA are produced from plant components through complex relationships between a diverse community of rumen bacteria (Van Soest, 1994). Getachew et al. (2002) reported a substantial association between the in vitro GP and SCFA. This correlation used to determine the generation of SCFA using gas measurements, which are an indicator of an animal's energy availability. Depending on the fermentation of carbohydrates, the synthesis of SCFA was closely linked to the gas generation of several diets grown in buffered rumen fluid in vitro (Sallam et al., 2007, Kanak et al., 2012; Blummel & Oraskov 1993). The SCFA concentration in this study was between 110.35 and 119.97 mM/L, which is usually between 70 and 150 mM/L (McDonald et al., 2002).

These values were greater than those of the other forages, indicating that corn silage is very palatable. The amount of fodder that can be ingested is limited by low digestibility, which is mostly brought on by the concentration of cell wall components (Blummel & Becker, 1997; Mould, 2003). Several researchers discovered strong relationships between forage DMI and in vitro GP (Blummel & Becker, 1997; Hetta et al., 2007).

Menke et al. (1979) found that they could predict in vivo OMD with high accuracy. Chemical composition and a multiple regression equation used in vitro gas measurements by McLeod & Minson (1971) and Van Soest (1994). Both the overall rumen OM digestibility and the effective ruminal OM degradability decreased when whole maize achieved advanced harvesting maturity (Hatew et al., 2016).

In vitro dry matter disappearances of maize silage are significantly positively correlated with gas generation (Taghizadeh et al., 2006). During the various incubation periods for the diets, Tuah et al. (1996) found a high, positive, and significant association (r = 0.58 to 0.95) between the in vitro gas production values and the in Sacco dry matter degradability. Kamalak et al. (2004) claim that in situ DM disappearance parameters can be predicted using in vitro gas production characteristics.

The ME derived from in vitro gas production at 24 hours showed a favorable correlation with the amount of carbs in conventional diets (Menke & Steingass, 1988). Additionally, the in vitro gas production method has been widely utilized to assess the energy value of various feed classes, especially straws (Makkar et al., 1999; Getachew et al., 1998).

Energy available for rumen microbial development (i.e., the synthesis of MCP) is largely determined by where and how much carbohydrate is supplied, however the amount of fed protein influences microbial dry matter (DM) content produced for every fermented carbohydrate unit (Hoover & Stokes, 1991). Dairy cows must have a protein level of 12–13% in order to maximize ruminal production of MCP (Satter & Roffler, 1975). Given that ruminal bacteria mostly obtain their energy from non-fiber carbohydrates (NFC), additional protein N is only absorbed into rumen MCP when the animals are fed more of them (Schwab et al., 2005). Microbial protein provides 60–85% of the amino acids (AA) that enter the animal's small intestine (Storm et al., 1983). The gut more than 80% of the time breaks down rumen MCP, which accounts for 50–80% of the total absorbable protein in the small intestine (Tas et al., 1981; Storm et al., 1983). The DM crop, protein, and energy yield increased in tandem with the corn silage's plant density (Gaafar, 2009). Compared to low planting density, high corn plant density showed a noticeably higher yield of dry crop, protein, and total digestible nutrients (Sayed & El-Nahrawy, 2021).

Conclusion

In conclusion, low planting density led to higher levels of NFE and NFC, gas production, gas producing fractions, gas production from soluble and insoluble fractions, short chain fatty acids, dry matter intake, organic matter digestibility, in vitro dry matter decomposition capacity, and microbial protein production in corn silage. Nevertheless, larger CF, fiber fractions, and methane production, along with the yield of dry crop, protein, ME, and NE, are achieved by high planting density corn silage. In contrast, the metabolizable and net energy contents of corn silage were nearly identical for both planting densities.

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