GLOBAL JOURNAL OF ANIMAL SCIENTIFIC RESEARCH

G.J.A.S.R

Journal homepage: www.gjasr.com

Print ISSN:2345-4377 Online ISSN:2345-4385

Original Article

Composition and *in Vitro* Gas Production Evaluation of Corn Silage Cultivated at Twenty and Thirty Thousand Plants per Feddan

Hamed Mohamed Gaafar and R.A. Mesbah

Animal Production Research Institute (APRI), Agricultural Research Center, Dokki, Giza, Egypt

ABSTRACT

Corn hybrid single cross 10 (S.C. 10) was cultivated at two planting density rates of low density was 20 thousand plants per feddan (20 TPF) or high density was 30 thousand plants per feddan (30 TPF) with a split-plot design with randomized complete blocks arrangement in two plant density. Whole corn plants were harvested at the dough stage of maturity (92 days), chopped and ensiled in double plastic bags in three replicates for each density, pressed by hand to exclude the air from the bags and ensiled for 35 days. Results showed that the contents of NFE and NFC were higher, but CF and fiber fractions of corn silage were lower significantly ($P<0.05$) for low plant density compared to high plant density. Gas production and its fractions of soluble (a) and insoluble (*b)* as well as gas production rate (*c*) values were significantly ($P<0.05$) higher for low plant density. However, methane production was significantly ($P<0.05$) higher for high plant density. Gas production from soluble fraction (GPSF), insoluble fraction (GPNSF), short chain fatty acids (SCFA), predicted dry matter intake (DMI), organic matter digestibility (OMD), in vitro dry matter degradability (IVDMD) and microbial protein (MP) were significantly higher ($P<0.05$) for low plant density compared to high plant density. While, predicted metabolizable energy (ME) and net energy (NE) were nearly similar for both low and high plant density.

Keywords: Corn hybrids silage, gas production, energy content, microbial protein.

Corresponding Author: Hamed Mohamed Gaafar < hamedgaafar@gmail.com >

Cite this Article: Gaafar, H.M.A. and Mesbah, R.A. (2020). Composition and in Vitro Gas Production Evaluation of Corn Silage Cultivated at Twenty and Thirty Thousand Plants per Feddan. *Global Journal of Animal Scientific Research*, 8(3), 14-31.
Retrieved from http://www.gjasr.com/index.php/GJASR/article/view/51

Article History: Received: 2020-08-17 Accepted: 2020-11-01

INTRODUCTION

Corn silage is utilized broadly in diets of dairy and beef cattle in most parts of the world. Corn silage is regularly high energy forage with high dry matter yield relative to the other forage crops (Coors, 1996). Whole-plant corn silage (CS) is normally utilized in rations of dairy cows in many parts of the world. It has a excessive content of starch and normally good ensiling traits (Khan *et al*., 2015). Silage preservation is based on anaerobic conditions converting the soluble carbohydrates in organic acids, particularly lactic acid by lactic acid bacteria (McDonald *et al*., 1991).

Corn plant density did no longer have an effect on the quality of whole plant corn destined for silage when precipitation was abundant. Therefore, larger yields of silage can be bought through increasing corn plant density. Crop rotation and administration have to be considered when planning forage management strategies to gain much quantities of good quality forage for dairy farming structures (Ferreira *et al*., 2014). Contents of ADF and NDF a probably indicator of forage quality have been reported that their relations with plant densities were controversial and NDF was affected by using plant densities (Iptas and Acar, 2006).

The technique of in vitro gas production (Menke and Steingass, 1988), or the adjustments by Theodorou *et al*., (1994) in simulating the digestive processes generated from microbial production (Getachew, 1998), lets us to recognize the fermentation and degradation of feeds according to the nutritional quality and availability of nutrients for ruminal bacteria.

There are a variety of factors that affect fermentation of feeds in vitro and should cause intra- or inter-laboratory differences. These are often related with the nature of rumen fluid inoculum, even though breed of animal, its physiological condition, diet, time of feeding, time of collection of rumen fluid relative to feeding time, method of rumen fluid collection (i.e. liquid or stable phase) (Cecava *et al*., 1990), and time elapsed between rumen fluid sampling and inoculation (Robinson *et al*., 1999) are all factors that have been shown to influence microbial activity in vitro.

Since high correlation existed between digestibility measured in vivo and predicted from an in vitro rumen gas production technique in combination with chemical composition, a considerable number of researchers has used in vitro gas techniques to learn about associative effects of various types of feedstuffs, and examine influences on rumen fermentation (Liu *et al*., 2002). Assessment of in vitro gas production (GP) is mostly used to evaluate the nutritive value of ruminant feeds with the aid of incubating substrate in buffered rumen fluid (Getachew *et al*., 1998).

The intention of the present study used to investigate the impact of plant density on *in vitro* gas production, energy value, organic matter digestibility, dry matter intake and microbial protein production of corn silage.

MATERIALS AND METHODS

Corn silage making:

Corn hybrid single cross 10 (S.C. 10) was cultivated at two planting densities of low density was 20 thousand plants per feddan (20 TPF) or high density was 30 thousand plants per feddan (30 TPF). Whole corn plants were harvested after 92 days of planting at the dough stage of maturity and chopping using Holland Chopper machine to 1-1.5 cm of length. Chopped corn forage was ensiled in double plastic bags in three replicates for each density, pressed via hand to exclude the air from the bags and ensiled for 35 days.

Chemical analysis

Representative samples of silages were analyzed according to the techniques of AOAC (1990). Fiber components including neutral detergent fiber (NDF) was determined according to Van Soest and Marcus (1964). Acid detergent fiber (ADF) and acid detergent lignin (ADL) were decided in accordance to Van Soest (1963).

In vitro study

In vitro gas production was undertaken according to the procedure described by Menke and Steingass (1988). Buffer and mineral solution were prepared and placed in a water bath at 39ºC under continuous flushing with CO_2. Both solid and liquid rumen fractions (50 % solid: 50 % liquid) were collected before the morning feeding from three rumen cannulated sheep fed on berseem hay and commercial concentrate mixture diet. Rumen fractions were collected into pre-warmed insulted bottles, combined among sheep, homogenized in a laboratory blender, filtered through three layers of cheesecloth and purged with CO_2. The well mixed and CO_2 flushed rumen fluid was added to the buffered rumen fluid solution (1:2 v/v), which was maintained in a water bath at 39ºC, and mixed. Samples (200 mg) of air-dry feedstuffs were accurately weighted into syringe fitted with plungers. Buffered rumen fluid (30 ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39ºC. Three syringes with only buffered rumen fluid were incubated and considered as the blanks (Blümmel and Ørskov, 1993). The syringes were gently shaken every 2 hr, and the incubation terminated after recording the 96 h gas volume. The gas production was recorded after 3, 6, 9, 12, 24, 48, 72, and 96 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM. Cumulative gas production was fitted iteratively to the exponential model proposed by France *et al.*, (1993):

Y = a + b(1 – e-ct)

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

As a new approach to evaluate feeds from those parameters, gas production brought on by way of fermentation of the soluble fraction (GPSF) used to be estimated with the aid of gas produced after 3hr (GP3) of incubation. Gas production brought on by means of fermentation of the insoluble fraction (GPNSF) may want to be estimated from the gas production between 3hr (GP3hr) and 24hr (GP24hr) of incubation according to Van Gelder *et al.*, (2005) as follows:

GPSF (ml/g DM) = GP 3hr * 0.99 - 3

GPNSF (ml/g DM) = 1.02*(GP 24hr – GP 3hr) + 2

Where: GP 3hr is 3hr net gas production (ml/200 mg DM), GP 24hr is 24hr net gas production (ml/200 mg DM).

The energy values had been calculated from the quantity of gas produced at 24hr of incubation with supplementary analyses of crude protein, ash and crude fat. This method was once developed via the research group in Hohenheim (Germany) and is based upon big in vitro incubation of feedstuffs (Menke and Steingass, 1988).

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

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

Where: ME is the metabolizable energy (Mcal/kg DM), GP is 24hr net gas production (ml/200 mg DM), CP is crude protein (% of DM) and EE is either extract (% of DM).

The procedure of Van Soest *et al.*, (1991) was used to determine in vitro truly degraded DM. The ivTDDM coefficient was calculated as follows:

(Feed DM incubated - residue DM recovered in the crucibles)*100/feed DM incubated.

OMD (%) of legume hays was calculated using equation of Menke and Steingass (1988) as follows:

OMD (%) = 14.88 + 0.889*GP 24hr + 0.45*CP + 0.0651*Ash

Where: OMD is organic matter digestibility (%), GP is 24hr net gas production (ml/200mg DM), CP is crude protein (% of DM), Ash (% of DM).

Short chain fatty acids (SCFA) have been calculated in accordance to the Getachew *et al.*, (2005) as follow:

SCFA (mmol/l) = (- 0.00425 + 0.0222*GP 24hr)*100

Where: GP is 24hr net gas production from the soluble fraction (ml).

Dry matter intake (DMI) was calculated according to Blümmel and Ørskove (1993) as follow:

DMI = 1.66 + 0.49 * (a) + 0.0297 * (b) – 4 * (c)

Where: a = the gas production from the soluble fraction (ml), b = the gas production from the insoluble fraction (ml), c = the gas production rate (ml/hr).

Microbial protein (MP) production was calculated as 19.3 g microbial nitrogen per kg OMD according to Czerkawski (1986).

MP (g/kg DM) = OMD * 19.3 * 6.25/100

Statistical analysis

The data had been subjected to statistical analysis using general linear model procedure adapted by IBM SPSS Statistics (2014) for user's guide with one-way ANOVA. Duncan test within program SPSS was done to decide the degree of significance between the means (Duncan, 1955).

RESULTS

Chemical composition

Results of chemical composition and fiber fractions of low and high plant density corn silage are presented in Table (1). Low corn plant density revealed significantly higher contents of DM, OM and NFE ($P<0.05$) and lower contents of CP, CF and ash ($P<0.05$) in contrast to high corn plant density. Also, high plant density confirmed greater contents of NDF, ADF, ADL, hemicellulose and cellulose and decrease content of NFC ($P<0.05$) in contrast to low corn plant density.

Cumulative gas production

Cumulative gas production for low and high plant density corn silage is shown in Fig. (1). Gas production was significantly ($P<0.05$) greater for low plant density compared to high plant density corn silage at the different incubation time. The greater gas production for low plant density than that of high plant density may be attributed to higher NFE and NFC contents and lower CF and fiber fractions for low plant density compared to high plant density (Table 1). The difference in gas production between low and high plant densities of corn silage extended linearly with the development of incubation time. Gas production increased markedly until 24 hrs of incubation and then increased slightly up to 96 hrs of incubation.

Table 1: Chemical composition and fiber fractions of low and high plant density corn silage

Item	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	94.04^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

a, b: means with different superscripts differ significantly at $P < 0.05$.

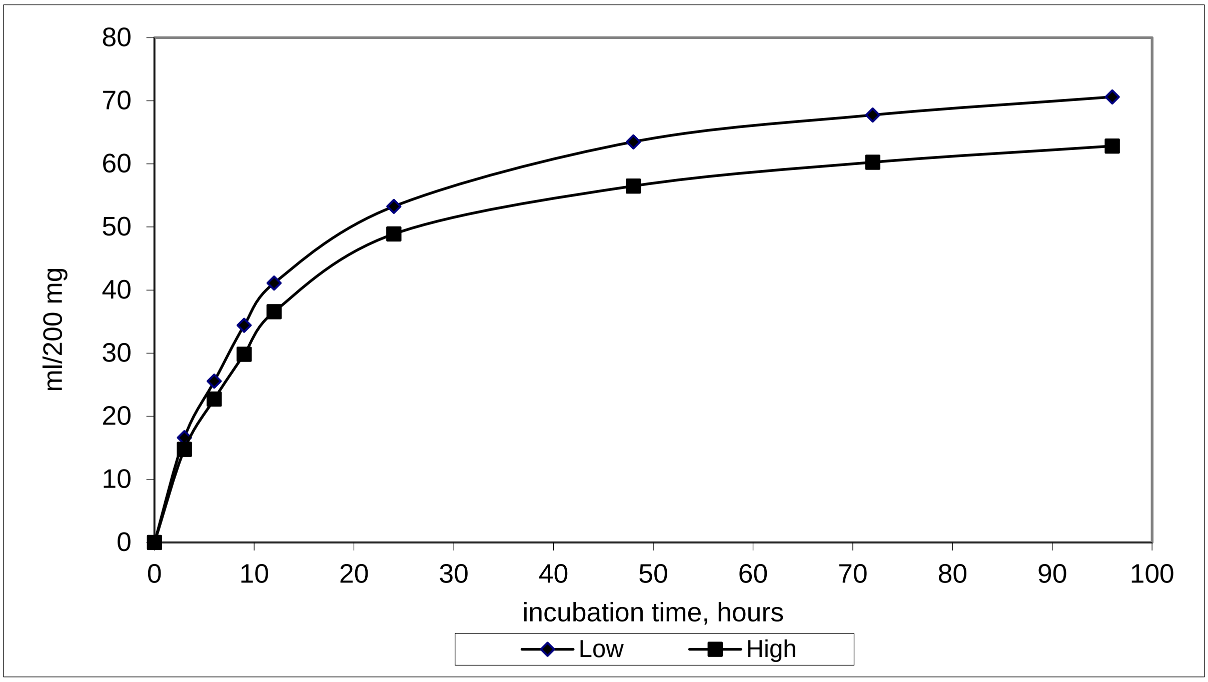

Fig. 1: Cumulative gas production of low and high plant density corn silage

Gas production fractions:

Fractions and rate of gas production of different plant densities corn silage are shown in Table (2). The gas production from the immediately soluble fraction (*a*), insoluble fraction (*b*) and soluble and insoluble fractions (*a* + *b*) as well as the gas production rate constant for the insoluble fraction (*c*) values had been considerably (P<0.05) greater for low plant density compared to high plant density corn silage.

Table 2: Gas production fractions for different plant densities corn silage

Item	Low density	High density	SEM	*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

a, b: means with different superscripts differ significantly at P < 0.05.

Methane production (CH_4):

Methane production of low and high plant densities corn silage is presented in Fig. (2). Concentration of CH_4 was once notably (P<0.05) greater for high plant density compared to low plant density corn silage. Methane production decreased with increasing NFE and NFC contents of corn silage as shown in Table (1). The concentration of CH_4 increased rapidly up to 24 hours after incubation, and then increased slightly with the advancement incubation period up to 96 hours.

Gas production from soluble and insoluble fractions

Gas production from the fermentation of soluble fraction (GPSF) and insoluble fraction (GPNSF) of low and high plant density corn silage are presented in Table (3). The concentrations of GPSF and GPNSF were notably (P<0.05) higher for low plant density compared to high plant density corn silage. The gas production of corn silage from the insoluble fraction used to be about three folds higher than the gas production from the soluble fraction.

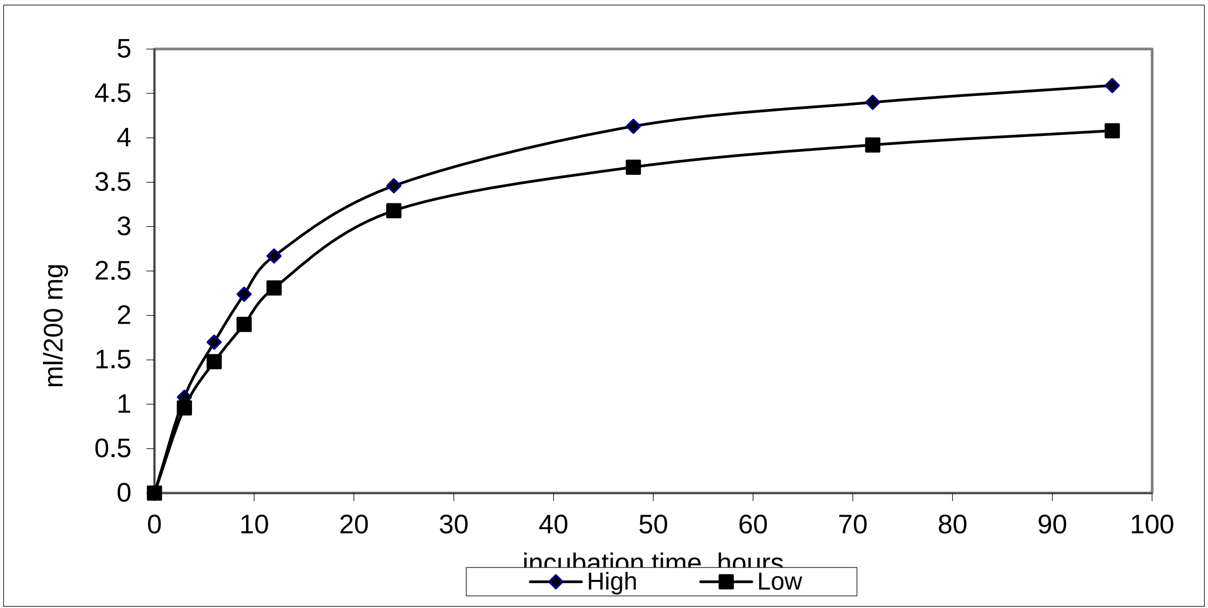

Fig. 2: Concentration of CH_4

Short chain fatty acids (SCFA):

Concentration of short chain fatty acids (SCFA) of *in vitro* fermented low and high corn silage is shown in Table (3). The concentration of SCFA was significantly (P<0.05) higher for low plant density compared to high plant density (122.36 vs. 108.13 mmol/l).

Table 3: Fermentation of the soluble (GPSF) and insoluble (GPNSF) fractions and short chain fatty acids (SCFA) of different corn hybrids silage

Item	Low density	High density	SEM	*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

a, b: means with different superscripts differ significantly at P < 0.05.

Dry matter intake (DMI):

From the results in Table (4), we believe that in vitro gas production of corn silage are valuable predictors of the voluntary intake potential when fed alone or in mixed rations. The predicted DMI of corn silage was greater significantly (P<0.05) for low plant density compared to high plant density corn silage. The predicted DMI for low and high plant density corn silage have been 6.71 and 6.14 kg/day or 63.87 and 58.52 g/kg $LBW^{0.75}$ and these values had been higher compared with the other forages reflecting high palatability of corn silage.

Organic matter digestibility (OMD):

Based on the strong relationship between measured digestibility and that predicted from gas production, regression equations have been developed and the technique has been standardized. As introduced in Table (4) the OMD of low plant density was once greater substantially ($P<0.05$) than that of high plant density corn silage (67.94 vs. 62.43%).

***In vitro* dry matter degradability (IVDMD:**

In vitro DMD of low and high plant density corn silage is presented in Table (4). *In vitro* DMD used to be considerably ($P<0.05$) higher for low plant density than that of high plant density corn silage.

Table 4: Dry matter intake (DMI), organic matter digestibility (OMD) and in vitro dry matter degradability (IVDMD) of different corn hybrids silage

Item	Low density	High density	SEM	*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^{c}	1.41	0.031

a, b: means with different superscripts differ significantly at $P < 0.05$.

Metabolizable energy (ME) and net energy (NE):

The predicted metabolizable energy (ME, Mcal/kg DM) and net energy (NE, Mcal/kg DM) from gas production for corn hybrids silage are presented in Table (5). The estimated ME and NE contents had been nearly comparable for low and high plant densities corn silage without large variations ($P>0.05$). These effects may additionally be due to the reality that fiber is covered in the representative of ME and NE.

Table 5: Metabolizable energy (ME), net energy (NE) and microbial protein (MP) of different corn hybrids silage.

Item	Low density	High density	SEM	*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 DM)	81.96[a]	75.31[b]	1.72	0.026

a, b: means with different superscripts differ significantly at P < 0.05.

Microbial protein

Improving the yield of rumen microbial protein (MCP) has significant importance in the promotion of animal performance and the reduction of protein feed waste. Amount of energy supplied to rumen microorganisms is an vital factor affecting the amount of protein nitrogen incorporated into rumen. Rich energy diet induces a significant increase in rumen MCP yield, whereas rich protein diet has no extensive influences on it **(**Lu *et al*., 2019**).** Results in Table (5) confirmed that microbial protein yield (MP) was higher significantly (P<0.05) for low plant density compared to high plant density corn silage. Microbial protein yield ranged from 75.31 to 81.96 g/kg DMI.

DISCUSSION

The results of chemical composition of corn silage agreed with those obtained by Pinter *et al*., (1994) who determined that CP and fiber fractions increased, whilst starch and soluble carbohydrates diminished with increasing plant density. Wang *et al*., (2005) observed that crude protein, ether extract, crude fiber and nitrogen free extract increased with plant density. The contents of DM, OM and NFE of corn silage decreased significantly (P<0.05), while the contents of CP, CF, EE and ash accelerated significantly (P<0.05) with increasing plant density (Gaafar, 2009). Contents of ADF and NDF a suitable indicator of forage quality have been reported that their relations with plant densities (Iptas and Acar, 2006). Marsalis *et al*., (2009) demonstrated that none of the quality parameters of corn (e.g., NDF, ADF) was affected by plant density. Valdez *et al*., (1989) determined that NDF and ADF concentrations increased by increasing corn plant density.

Incubation of feedstuff with buffered rumen fluid *in vitro*, the carbohydrates are fermented to short chain fatty acids (SCFA), gases mainly CO and CH and microbial cells. Gas production is essentially the end result of fermentation of carbohydrates to acetate, propionate and butyrate. Gas production from protein fermentation is relatively small as in contrast to carbohydrate fermentation while, contribution of fat to gas production is negligible (Blümmel and Ørskov, 1993). Fiber quality is extremely important for the complete action of cellulolytic bacteria, responsible for high rates of microbial colonization to the substrate, and resulting in efficient energetic use of the evaluated feeds (Sun *et al.*, 2007). Silva and Ørskov (1988) observed that the presence of a supply of readily digestible cellulose and hemicellulose increased the numbers of ruminal fibrolytic microorganisms and, consequently, may additionally enhance digestibility of other much less degradable fiber sources. Gas production is an indirect measure of substrate degradation and it is not continually positively related to microbial mass production (Liu *et al.*, 2002). The method of in vitro gas production (Menke and Steingass, 1988), or the modifications by using Theodorou *et al.*, (1994) in simulating the digestive approaches generated from microbial production (Getachew, 1998), allows us to comprehend the fermentation and degradation of feeds in accordable to the nutritional excellent and availability of nutrients for ruminal bacteria. Haddi *et al.*, (2003) reported that there had been significant negative correlation between NDF and ADF, and the rate and extent of GP.

Garcıa-Rodriguez *et al.*, (2005) suggested that differences in parameters of *b* and *c* between silages indicate one of a kind fermentation patterns. The aforementioned traits of whole plant corn are in line with the observations that each the asymptotic GP of the soluble fraction (*a*) and the related most rate of gas production (*c*) diminished with increasing maturity, whereas the half time of maximum GP of the soluble fraction (*a*) increased with increasing maturity. Furthermore, the maximum rate of gas production of the insoluble fraction (*b*) improved with increasing maturity, thereby reflecting the increased digestibility of starch versus NDF (Macome *et al.*, 2017).

The principal factors influencing CH_4 emissions from ruminants are: i) level of feed intake, ii) kind of carbohydrate fed, and iii) the ruminal microflora (Lascano and Cardenas, 2010). Greater starch content of corn hybrids corresponded to decrease CH_4 emissions through its have an impact on propionate (Aboagye *et al.*, 2017). Amount of CH_4 produced is influenced with aid of type and content of dietary carbohydrates (Ellis *et al*., 2007) and lipids (Grainger and Beauchemin, 2011).

The ruminant forages are currently described through three extraordinary features; the soluble fraction, insoluble fraction and the rate of degradation (Ørskov, 1991). The soluble fraction, often named washing loss, represents the water soluble components of the organic matter or the dry matter. It includes the soluble sugars and soluble compounds as polyphenolics liberated for the duration of the fermentation process (Ly *et al*., 1997). Besides, these parameters are used for assessing the nutritive value of feeds (Ørskov, 1991; Ly and Preston, 1997).

Incubation of feedstuff with buffered rumen fluid in vitro, the carbohydrates are fermented to short chain fatty acids (SCFA) and gases basically CO and CH (Blümmel and Ørskov, 1993). The degradability measurement accounts for feed conversion into all products of microbial degradation and synthesis, essentially microbial biomass, short chain fatty acids (SCFA) and gases, whereas the gas volume measurement reflects feed conversion into SCFA and gases (Grings *et al*., 2005). The complicated interactions within a mixed rumen microbial population lead to the conversion of plant aspects to gas and SCFA (Van Soest, 1994). Getachew *et al.*, (2002) reported the close association between SCFA and the in vitro GP, and used the relationship between SCFA and GP to estimate the SCFA production from gas values, which is an indicator of energy availability to the animals. The gas production of different classes of feed incubated in vitro in buffered rumen fluid was carefully related to the production of SCFA, s which was based on carbohydrates fermentation (Kanak *et al.*, 2012). Concentration of SCFA obtained in the existing study ranged from 110.35 to 119.97 mmol/l, which are within the ordinary range from 70 to 150 mmol/l (McDonald *et al.*, 2002).

Forage intake is commonly confined through low digestibility, where the content of the cell wall constituents has the greatest influence on digestibility (Mould, 2003). Several authors observed high correlations between in vitro GP studies and DMI of forages (Hetta *et al.*, 2007).

Using the in vitro gas measurement and chemical composition in multiple regression equation, Van Soest (1994) found a high precision in prediction of in vivo OMD. The effective rumen degradability of OM as well as the total-tract digestibility of OM decreased with advanced maturity of whole plant corn at harvest (Hatew *et al*., 2016).

The degradation of soluble carbohydrates, starch and part of the fiber fraction, since soluble carbohydrates are definitely degraded in the rumen and the starch is of high ruminal digestion (Cammell *et al*., 2000), it is evident that the fibrous portion of the stover fractions (stalks, leaves, husks and cobs) would possibly have been poorly degraded. Chemical analysis should supply a beast prediction towards degradable traits of feedstuffs in ruminants (Dua *et al*., 2016).

There was once a wonderful correlation between metabolizable energy calculated from 24 hours in vitro gas production together with protein and fiber contents with metabolizable energy value of traditional feeds measured in vivo (Menke and Steingass, 1988). The in vitro gas production technique has additionally been widely used to evaluate the energy value of several classes of feeds (Getachew *et al*., 1998), particularly straws (Makkar *et al*., 1999).

The source and quantity of fed carbohydrate are the principle factors affecting the energy available for rumen microbial growth (synthesis of MCP in particular), and that of fed protein affects the production of microbial dry matter (DM) per unit of carbohydrate fermented (Hoover and Stokes, 1991). In the dairy cow, 12–13% protein content is needed to maximize the ruminal synthesis of MCP (Satter and Roffler, 1975). More protein N is incorporated into rumen MCP only if greater non-fiber carbohydrate (NFC), known to be the major energy substrate for ruminal microorganisms, are fed to the animals (Schwab *et al*., 2005). This microbial protein supplies 60 to 85% of amino acids (AA) reaching the animal's small intestine. In the small intestine, more than 80% of rumen MCP is digested, accounting for 50–80% of the total absorbable protein contained there (Storm *et al*., 1983).

CONCLUSION

From this study it could be concluded that low plant density corn silage confirmed greater NFE and NFC contents, higher gas production, fractions of gas production, gas production from soluble and insoluble fractions, short chain of fatty acids, dry matter intake, organic matter digestibility, in vitro dry matter degradability and microbial protein production and lower contents of CF and fiber fractions and methane production compared to high plant density. Whereas, ME and NE contents had been almost comparable for low and high plant density corn silage.

REFERENCES

Aboagye, I.A.; M. Oba; V. Baron and J. Goyader (2017). In vitro degradation and methane production of short-season corn hybrids harvested before or after a light frost. *Canadian Journal of Animal Science,* 99(4): 741-753.

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis. 15th Ed,, Washington DC.

Blümmel, M. and E.R. Ørskove (1993). Comparison of in vitro gas production and nylon bag degradability of roughages in prediction of feed intake in cattle. Animal Feed Science and Technology, 40: 109-119.

Cammell, S.B.; J.D. Sutton; D.E. Beever; D.J. Humphries and R.H. Phipps (2000). The effect of crop maturity on the nutritional value of maize silage for lactating dairy cows. 1. Energy and nitrogen utilization. Anim. Sci., 71: 381-390.

Cecava, M.J.; N.R. Merchen; L.L. Berger and D.R. Nelson (1990). Effect of energy level and feeding frequency on site of digestion and postruminal nutrient flows in steers. J. Dairy Sci., 73: 2470–2479.

Coors, J.G. (1996). Findings of the Wisconsin Corn Silage Consortium. p. 20-28. Proc. of the 58th Cornell Nutrition Conference for Feed Manufacturers. October 22-24, 1997. Rochester, NY. Cornell University Press, Ithaca, NY.

Czerkawski, J.W. (1986). An Introduction to Rumen Studies. Oxford, New York: Pergamon Press.

Dua, S.; M. Xub and J. Yaoa (2016). Relationship between fibre degradation kinetics and chemical composition of forages and by-products in ruminants. Journal of Applied Animal Research, 44(1): 189–193.

Duncan, D.B. (1955). Multiple range and Multiple F-Tests. Biometrics, 11: 1-42.

Ellis, J.L.; E. Kebreab; N.E. Odongo; B.W. McBride; E.K. Okine and J. France (2007). Prediction of methane production from dairy and beef cattle. J Dairy Sci., 90(7): 3456–466.

Ferreira, G.; M. Alfonso; S. Depino and E. Alessandri (2014). Effect of planting density on nutritional quality of green-chopped corn for silage. J. Dairy Sci., 97: 5918–5921.

France, J.; M.S. Dhanoa; M.K. Theodorou; S.J. Lister; D.R. Davis and D. Isac (1993). A model to interpret gas accumulation profiles associated with in vitro degradation of ruminant feeds. J. Theor. Biol., 163: 99.

Gaafar, H.M.A. (2009). Some factors affecting on nutritive value of whole plant corn silage. XVth International Silage Conference Proceedings, July 27-29, 2009 – Madison, Wisconsin, USA. P 383-391.

Garcıa-Rodriguez, L.J.; R. Valle; A. Durán and C. Roncero (2005). Cell integrity signaling activation in response to hyperosmotic shock in yeast. *FEBS Lett,* 579(27): 6186-90.

Getachew, G.; E. De Peters; P. Robinson and J. Fadel (2005). Use of an in vitro rumen gas production techniques to evaluate microbial fermentation of ruminant feeds and its impact on fermentation products. Anim. Feed Sci. Technol., 124: 547-559.

Getachew, G.; H.P.S. Makkar and K. Becker (2002). Tropical browses: content of phenolic compounds, in vitro gas production and stoichiometric relationship between short chain fatty acids and in vitro gas production. J. Agric. Sci., 139: 341.

Getachew, G.; M. Blummel; H.P.S. Makkar and K. Becker (1998). In vitro gas measuring techniques for assessment of nutritional quality of feeds: A review. Anim. Feed Sci. Technol., 72: 261-281.

Grainger, C. and K.A. Beauchemin (2011). Can enteric methane emissions from ruminants be lowered without lowering their production? Anim Feed Sci Technol., 166–167: 308–320.

Grings, E.E.; M.Blummelb and K.H. Sudekumc (2005). Methodological considerations in using gas production techniques for estimating ruminal microbial efficiencies for silage-based diets. Animal Feed Science and Technology, 123–124: 527–545.

Haddi, M.L.; S. Filacorda; K. Meniai; F.P. Rollin and P. Susmel (2003). In vitro fermentation kinetics of some halophyte shrubs sampled at three stages of maturity. Anim. Feed Sci. Technol., 104: 215-225.

Hatew, B.; A.Bannink; H.van Laar; L.H. de Jonge and J. Dijkstra (2016). Increasing harvest maturity of whole-plant corn silage reduces methane emission of lactating dairy cows. J. Dairy Sci., 99(1): 354-368.

Hetta, M.; G. Bernes; J.W. Cone and A.M. Gustavsson (2007). Voluntary intake of silages in dairy cows depending on chemical composition and in vitro gas production characteristics. Livestock Science, 106(1): 47-56.

Hoover, W.H. and S.R. Stokes (1991). Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci., 74: 3630.

IBM SPSS Statistics (2014). Statistical package for the social sciences, Release 22, SPSS INC, Chicago, USA.

Iptas, S. and A. A. Acar (2006). Effects of Hybrid and Row Spacing on Maize Forage Yield and Quality. Plant Soil Environ., 52(11): 515-522.

Kanak, A.R.; M.J. Khan; M.R. Debi; M.K. Pikar and M. Aktar (2012). Nutritive value of three fodder species at different stages of maturity. Bangladesh J. Anim. Sci., 41 (2): 90-95.

Khan, N.A.; P.Q. Yu; M. Ali; J.W. Cone and W.H. Hendriks (2015). Nutritive value of maize silage in relation to dairy cow performance and milk quality. J. Sci. Food Agric., 95: 238-252

Lascano, C.E. and E. Cardenas (2010). Alternatives for methane emission mitigation in livestock systems. Rev. Bras. Zootecn., 39:175-182.

Liu, J.X.; A. Susenbeth and K.H. Südekum (2002). In vitro gas production measurements to evaluate interactions between untreated and chemically treated rice straws, grass hay and mulberry leaves. J. Anim. Sci., 80: 517-524.

Lu, Z.; Z. Xu; Z. Shen; Y. Tian and H. Shen (2019). Dietary energy level promotes rumen microbial protein synthesis by improving the energy productivity of the ruminal microbiome. Front Microbiol., 17: 10:847.

Ly, J. and T.R. Preston (1997). An approach to the estimation of washing losses in leaves of tropical trees. Livestock Research for Rural Development, 9(3): 931.

Ly, J.; V.L. Nguyen and T.R. Preston (1997). A study of washing losses and *in vitro* gas production characteristics of nine leaves from tropical trees and shrubs for ruminants. Livestock Research for Rural Development, 9(3): 932.

Macome, F.M.; W.F. Pellikaan; W.H. Hendriks; J. Dijkstra; B. Hatew; J.T. Schonewille and J.W. Cone (2017). In vitro gas and methane production of silages from whole-plant corn harvested at 4 different stages of maturity and a comparison with in vivo methane production. J. Dairy Sci., 100: 8895–8905.

Makkar, H.P.S.; E.M. Aregheore and K. Becker (1999). Effect of saponins and plant extracts containing saponins on the binding efficiency of ammonia during urea-ammoniation of wheat straw and fermentation kinetics of the treated straw. J. Agric. Sci. (Camb.), 132: 313-321.

Marsalis, M.A.; S. Angadi and F.E. Contreras-Govea (2009). Effect of seeding and nitrogen rates on limited irrigated corn and forage sorghum yield and nutritive value. In Abstracts of Annual Meeting, Western Society of Crop Science, June 22–24, 2009, Ft. Collins, CO.

Mcdonald, P.; A.R. Henderson and S.J.E. Heren (1991). Water, In: The biochemistry of silage (Eds.). Chalcombe Publ., Marlow, United Kingdom, pp. 167-183.

McDonald, P.; R.A. Edwards and J.F.D. Greenhalgh (2002). Animal Nutrition. 6th Edition. Longman, London and New York.

Menke, K.H. and H. Steingass (1988). Estimation of the energetic feed value obtained by chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev., 28: 7-55.

Mould, F.L. (2003). Predicting feed quality – chemical analyses and in vitro evaluation. Field Crops Research, 84:31-44.

Ørskov, E.R. (1991). Manipulation of fiber digestion in the rumen. Proceedings of the Nutrition Society, 50: 187-196.

Pinter, L.; Z. Alfoldi; Z. Burucs and E. Paldi (1994). Feed value of forage maize hybrids varying in tolerance to plant density. Agron. J., 86: 799.

Robinson, J.J.; K.D. Sinclair and T.G. McEvoy (1999). Nutritional effects on foetal growth. Anim. Sci., 68: 315–331.

Satter L.D. and R.E. Roffler (1975). Nitrogen requirement and utilization in dairy cattle. *J. Dairy Sci.*, 58: 1219–1237.

Schwab, M.A.; S. Kolker; L.P. Van Den Heuvel; S. Sauer; N.I. Wolf; D. Rating; G.F. Hoffmann; J.A. Smeitink and J.G. Okun (2005). Optimized spectrophotometric assay for the completely activated pyruvate dehydrogenase complex in fibroblasts. *Clin. Chem.*, 51 151–160.

Silva, A.T. and E.R. Ørskov (1988). Fibre degradation in the rumen of animals receiving hay, untreated or ammonia-treated straw. Anim. Feed Sci. Tech., 19, 277-287.

Storm, E.; D.S. Brown and E.R. Orskov (1983). The nutritive value of rumen micro-organisms in ruminants. 3. The digestion of microbial amino and nucleic acids in, and losses of endogenous nitrogen from, the small intestine of sheep. *Br. J. Nutr.*, 50 479–485.

Sun, P.F.; Y.M. Wu and J.X. Liu (2007). In vitro gas production technique to evaluate associative effects among lucerne hay, rice straw and maize silage. Journal of Animal and Feed Sciences, 16(Suppl. 2): 272–277.

Theodorou, M.K.; B.A. Williams; M.S. Dhanoa; A.B. McAllan and J. France (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Tech., 48: 185-197.

Valdez, F.R.; J.H. Harrison and S.C. Fransen (1989). Effect of feeding silage of early and late maturing corn planted at two population densities to lactating dairy cows. J. Dairy Sci., 72: 2081-2086.

Van Gelder, M.H.; M.A.M. Rodrigues; J.L. De Boever; H. Den Hartigh; C. Rymer; M. Van Oostrum; R. Van Kaahthoven and J.W. Cone (2005). Ranking of in vitro fermentability of 20 feedstuffs with an automated gas production technique: Results of a ring test. Anim. Feed Sci. Technol., 123-124: 243-253.

Van Soest, P.J. (1963). The use of detergents in the analysis of fibrous feeds: II. A rapid method for the determination of fiber and lignin. Official Agriculture Chemistry, 46: 829.

Van Soest, P.J. (1994). Nutritional Ecology of the Ruminant. Ithaca, NY: Cornell University Press.

Van Soest, P.J. and W.C. Marcus (1964). Methods for the determination of cell wall constituents in forages using detergents and the relationship between this fraction and voluntary intake and digestibility. J. Dairy Sci., 47: 704-705.

Van Soest, P.J.; J.B.Robertson and B.A.Lewis (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74(10): 3583-3593.

Wang, Y.; G.H. Mi; F.J. Chen; J.H. Zhang and F.S. Zhang (2005). Response of root morphology to nitrite supply and its contribution to nitrogen accumulation in maize. J. Plant Nutr., 27:2189–2202.