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THE EFFECTS OF DIFFERENT NITROGEN DOSES AND IRRIGATION LEVELS ON YIELD, NUTRITIVE VALUE, FERMENTATION AND GAS PRODUCTION OF CORN SILAGE

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ABSTRACT

The study was conducted to investigate the effects of different irrigation levels and nitrogen doses on yield, yield parameters, silage characteristics, digestibility, gas and methane production of corn silage. Three different irrigation levels (50%, 75% and 100% of depleted water) and 3 different nitrogen doses (100, 200 and 300 kg ha^{-1} N) were applied to corn silage. Experiments were implemented in split-split plots design with three replications during the growing seasons of 2013-2014. Plants were harvested at milk-dough stage and yield and morphologic characteristics were determined. Then, harvested plants were silaged and chemical characteristics were investigated. Irrigation level x nitrogen dose interaction was not found to be significant. Increasing nitrogen doses increased plant height, plant diameter, green herbage yield, crude protein, metabolic energy, gas production and organic matter digestibility and decreased pH levels, ADF and NDF ratios. Increased irrigation levels positively affected green herbage yield, plant height, plant diameter and increased ADF and NDF ratios. Gas production, metabolic energy and organic matter digestibility decreased with increasing irrigation levels. Increasing irrigation levels improved corn yields, but reduced the quality. On the other hand, increasing nitrogen doses had positive contributions both to yield and quality characteristics.

Key words: Chemical composition, corn silage, gas production irrigation, nitrogen fertilization

INTRODUCTION

Silage is commonly used in animal feeding of the countries with developed livestock industry since the silage has a high nutritive value and moisture content. It is also easy to store and preserve the silage, it has long-lasting life, it is easily digested due to fermentation, the high-quality feed can be preserved for long durations, it allows the rations with high dry matter and moisture content (Tukel and Hatipoglu, 1997), the protein supplied by silage is cheaper than the protein supplied by fabricated mixed feeds and the silage has high pro-vitamin A content (Tumer, 2001).

Maize can yield quite high green herbage and easily be harvested. It is consumed by livestock with a good appetite (Neylon and Kung, 2003; Kaplan, 2005), suitable for silage and has quite high nutritive values. Thus, maize is the most significant silage crop both worldwide and in Turkey (McDonald et al., 1991; Meeske et al., 1993). Irrigation and fertilizations are the greatest cost items in crop culture and they are also among the most significant factors effecting the yield and quality in pasture and forage crops (Islam et al., 2012; Khelil et al., 2013). Water and nitrogen are the critical issue in maize culture, especially for yield and quality of maize. Therefore, these two parameters should be optimized in maize culture. Current global warming and climate change (World Water Assessment Program, 2009) restrict the water resources and thus enforce the growers to perform such optimizations. Increased water and nitrogen treatments not only increase the production costs, but also result in serious environmental pollution over the agricultural fields (Ferrer et al., 1997) and negative influenceson soil structure (Khelil et al., 2013). Nitrogenous fertilization and synergic effect of water improve nutrient uptake and yields (Kim et al., 2008).

Recently *in vitro* gas production technique with chemical composition have been widely used to evaluate the potential nutritive value of previously uninvestigated forages since *in vitro* gas production technique is quick, cheap, less time consuming (Kamalak and Canbolat, 2010). In addition, *in vitro* gas production technique was used to screen the feedstuffs in terms of their methane reduction potential. Methane production during rumen fermentation is one of important contributors to global warming (Lassey, 2007).

There are many studies regarding the water usage and nitrogen application in silage corn (Istanbulluoglu et al., 2002; Kiziloglu et al., 2009). But in the present studies we have principally focused on the yield, morphological characteristics and chemical composition parameters. There are insufficient knowledge and information about optimum water and nitrogen doses and their effects on yield and silage quality, volatile fatty acids, gas and methane production of maize. Thus, the objectives of the present study were set as assess the effects of irrigation x nitrogen interaction (i) on yield and yield parameters, (ii) silage quality, (iii) metabolic energy (ME), organic matter digestibility (OMD), gas and methane production-like digestibility parameters.

MATERIALS AND METHODS

Field Experiments

Experiments were conducted in Kayseri Province of Turkey (38°43'N; 35°29'E) during the growing seasons of 2013-2014 for two years. Simon hybrid maize cultivar was used as the plant material of the study. Seeds were sown over 6 x 4.2 m plots with 70 cm row spacing and 16 cm on-row plant spacing (Kusvuran et al., 2015). Experiments were conducted in split-split plots experimental design with three replications. Three different irrigation levels based on depleted water from field capacity (50, 75 and 100%) were placed over main plots and 3 nitrogen doses (N1=100, N2=200 and N3=300 kg ha^{-1} N) were placed over sub-plots. Soil moisture content was measured with a neutron probe and the amount of irrigation water to be applied was determined and applied through a drip irrigation method. Based on soil analysis, half of nitrogen was applied at sowing and the other half was applied when the plants had a height of 50 cm. Besides nitrogen fertilization, 100 kg ha^{-1} P_2O_5 was also applied at sowing (Gul et al., 2008). Hoeing and chemical weed control was practiced throughout the growing season. Morphologic observations were measured at milk-dough stage of the plants and then plants were harvested. Side effects were omitted and resultant yields were calculated.

Soil and climate characteristics of research site

Seeds were sown on 23rd of April in the first year and 28th of April in the second year. Experimental years generally had higher temperatures to long-term averages. Precipitations were lower than the long-term averages in the first year and higher than the long-term averages in the second year. Relative humidity levels of the experimental years were generally lower than the long-term averages. Not specified in Table 1, but unexpected short-term low temperatures were also experienced in experimental years.

Table 1. Precipitation, temperature and relative humidity data of the experimental site

	Temperature (°C)			Precipitation (mm)			Relative Humidity (%)		
Months	2013	2014	Long Term*	2013	2014	Long Term*	2013	2014	Long Term*
April	12.1	14.1	10.7	43.6	2.9	54.8	56.2	44.3	62.6
May	18.1	16.7	15.1	31.3	39.7	52.0	44.7	50.4	60.8
June	21.1	19.7	19.1	12.6	52.9	39.1	38.7	46.8	55.3
July	22.5	25.2	22.6	3.4	0.0	10.3	36.9	33.7	49.5
August	22.5	25.1	22.0	0.8	47.4	5.3	36.0	37.4	49.8
September	17.0	18.8	17.1	10.3	85.4	13.3	44.1	54.2	54.4
October	9.2	11.7	11.5	52.5	54.4	30.5	58.9	68.1	64.0
Mean	17.5	18.7	16.8	-	-	-	45.0	47.8	56.6
Total	-	-	-	154.5	282.7	205.3	-	-	-

*from 1970 to 2013

Experimental soils at 0-30 and 30-60 cm were classified as sandy-loam in both years. Soils had low lime and salt content, were rich in potassium and phosphorus, poor in organic matter and were slightly alkaline.

Preparation and analysis of silage samples

The maize grown under three different irrigation levels and nitrogen doses were harvested at milk-dough stage. The plants were chopped in 2.5-3 cm pieces, filled and sealed in 2 kg deflated vacuum bags. Then the samples were preserved in dark (24±2°C) for 60 days. Sample bags were opened and 30 g sample was mixed with 270 ml water to measure the pH of samples. Again 250 g fresh silage sample was dried in an oven at 70 °C for 48 hours to find dry matter ratio. Dried samples were then ground in a mill with 1 mm sieve and made ready for chemical analyses. Dry matter, crude protein and crude ash analyses were performed in accordance with the methods specified in AOAC (1990). NDF and ADF analyses were carried out in accordance with the methods specified in Van Soest and Wine (1967) and Van Soest (1963), respectively by using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp. Fairport, NY, USA). Acetic, propionic and butyric acid contents were determined by using a gas chromatography device (Shimadzu GC-2010 Kyoto, Japan with column characteristics of: 30 m × 0.25 mm × 0.25 µm, Restek, temperature range of 45–230°C) and lactic acid analysis was performed by using spectrophotometric method (Barker and Summerson, 1941).

Table 2. Physical and chemical characteristics of soils of the experimental site

Properties	2013		2014	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm
Clay (%)	13.10	8.94	12.58	9.18
Silt (%)	4.16	10.40	5.11	9.55
Sand (%)	82.74	80.66	82.31	81.27
Class	Sandy-Loam	Sandy-Loam	Sandy-Loam	Sandy-Loam
pH	7.94	7.75	7.48	7.60
Organic Matter (%)	1.05	1.27	1.09	1.14
$CaCO_3$ (%)	0.28	0.27	0.24	0.29
K_2O (kg ha^{-1})	1092.20	755.14	1184.20	842.34
P_2O_5 (kg ha^{-1})	89.63	11.56	110.41	12.58
EC (mmhos cm^{-1})	0.96	0.23	0.83	0.27

In vitro Hohenheim gas production technique

Rumen fluid, which required for *in vitro* gas production technique, was obtained from a Simmental steer beef (at 12 months of age and in 600 kg live weight). Animal care procedures for the experiment were conducted under a research protocol approved by the Local Ethics Committee for Animal Experiments of Erciyes University, Kayseri-Turkey.

Rumen fluid was collected into a glass bottle (Isolab, Germany). The bottle was transported to the laboratory in a sealed thermos container at 39±1°C and filtered through four layers of cheesecloth under CO_2 gas. *In vitro* gas production was analyzed in twenty seven different silage samples. The samples were incubated in rumen fluid and buffer mixture in 100 ml glass syringes (Model Fortuna, HaberleLabortechnik, Germany) following the procedures of Menke and Steingass, (1988). About 200±10 mg dry samples were weighed in triplicate into glass syringes. The syringes were prewarmed at 39°C in a thermostatically controlled cabinet (Lovibond, Austria), before 10 ml of rumen fluid and 20 ml of prewarmed buffer mixture were dispensed anaerobically in each syringe using an automatic bottletop dispenser (Isolab, Germany). The syringes were closed using one position polypropylene clamps and incubated at 39±0.5°C for 24h. In addition, three blank syringes (no template; rumen fluid + buffer mixture) were used to calculate the total gas production.

Determination of total gas and methane productions

After 24 h of incubation, the total gas volume was recorded from the calibrated scale on the syringe. After measuring the total gas volume, the tubing of the plastic syringe outlet was inserted into the inlet of the methane analyzer (Sensor, Europe GmbH, Erkrath, Germany) and the piston was pushed to insert the accumulated gas into the analyzer. The methane as percent of the gas was displayed on a PC (Goel et al., 2008).

Determination of metabolic energy (ME) and organic matter digestibility (OMD)

The ME and OMD levels of silage samples were calculated using the equations of Menke *et al.*(1979) and Blümmel *et al.* (1997) as follows:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136 \times GP + 0.057 \times CP$$

$$OMD\ (g/kg\ DM) = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times A$$

GP = 24 h net gas production (ml/200 mg).

CP = Crude protein

A = Ash content

Statistical analysis

The two-year experimental data were subjected to variance analysis with SAS (SAS Inst. 1999) software in accordance with split-split plots experimental design. LSD test was used to assess the significance of differences in treatment means.

RESULTS

Morphological characteristics of corn silage grown under different irrigation levels and nitrogen doses are provided in Table 3. Irrigation levels and nitrogen doses had highly significant effects on green herbage yield, plant height, cob and leaf ratios ($P<0.01$), irrigation levels had significant effects on plant diameter ($P<0.05$), and nitrogen doses had significant effects on plant diameter ($P<0.01$). The effects of irrigation x nitrogen interaction were not significant ($P>0.05$). Significant increases were observed in green herbage yields with increasing irrigation levels and nitrogen doses and the highest yield 75.2 t ha^{-1} was obtained from 100% water levels and 75.0 t ha^{-1} was obtained from N3 nitrogen doses. Plant height had also similar responds to water and nitrogen treatments and increased with increasing water levels and nitrogen doses plant height of maize samples varied between 184.13 and 207.56 cm in irrigation levels and 188.28-203.41cm in nitrogen doses.

Plant diameter increased with increasing nitrogen doses, but the difference between N2 (21.32 mm) and N3 (22.39 mm) treatments was not significant. Plant diameters also increased with irrigation levels, but the difference was observed only at 100% irrigation level (21.90 mm). While irrigation levels and nitrogen doses increased cob ratios, a decrease was observed in leaf ratio. Cob ratio of the samples varied between 51.64-54.35% in irrigation levels and 51.98-54.59% in nitrogen doses. Leaf ratio of the samples varied between 20.60-17.05% in irrigation levels and 20.53-16.54% in nitrogen doses. Stem ratio increased with nitrogen doses, but the difference was observed in N3 treatment. The greatest stem ratio (28.88%) was obtained from N3 treatment.

Table 3. The effects of irrigation levels and nitrogen doses on morphological characteristics and herbage yield of corn

Irrigation Level	Green Herbage Yield ($t\ ha^{-1}$)				Irrigation Level	Plant Height (cm)			
	Fertilizer Doses			Means		Fertilizer Doses			Means
	N1	N2	N3			N1	N2	N3	
50	48.9	63.6	70.5	61.0 b	50	174.72	185.95	191.72	184.13 c
75	54.9	68.0	73.6	65.5 b	75	189.56	204.33	206.89	200.26 b
100	64.4	80.3	80.9	75.2 a	100	200.56	210.50	211.61	207.56 a
Means	56.1 c	70.6 b	75.0 a		Means	188.28 c	200.26 b	203.41 a	

Irrigation Level	Plant Diameter (mm)				Irrigation Level	Cob Ratio (% DM)			
	Fertilizer Doses			Means		Fertilizer Doses			Means
	N1	N2	N3			N1	N2	N3	
50	18.86	20.39	21.94	20.40 b	50	50.29	51.28	53.37	51.64 b
75	18.60	20.81	22.20	20.54 b	75	52.17	53.77	55.08	53.67 a
100	19.92	22.74	23.03	21.90 a	100	53.47	54.27	55.32	54.35 a
Means	19.13 b	21.32 a	22.39 a		Means	51.98 c	53.11 b	54.59 a	

Irrigation Level	Leaf Ratio (% DM)				Irrigation Level	Stem Ratio (% DM)			
	Fertilizer Doses			Means		Fertilizer Doses			Means
	N1	N2	N3			N1	N2	N3	
50	23.24	21.05	17.51	20.60 a	50	26.47	27.68	29.11	27.75
75	19.95	18.18	16.56	18.23 b	75	27.87	28.05	28.38	28.10
100	18.38	17.23	15.54	17.05 c	100	28.15	28.50	29.15	28.60
Means	20.53 a	18.82 b	16.54 c		Means	27.50 b	28.08 b	28.88 a	

N1:100, N2:200 and N3:300 $kg\ ha^{-1}$ N

Nitrogen treatments had highly significant effects on pH (P<0.05), ADF (P<0.01), NDF (P<0.01), dry matter (P<0.01), crude protein (P<0.01) and crude ash (P<0.01). Irrigation levels had also highly significant effects on ADF, NDF and dry matter (P<0.01), significant effects on crude ash (P<0.05), but insignificant effects on pH and crude protein. The effects of irrigation x nitrogen interaction on chemical composition were not significant (P>0.05). Increasing nitrogen doses reduced dry matter contents. Dry matter contents of silage samples varied between 28.80-32.30%. The pH of silage samples decreased with increasing nitrogen doses. With regard to pH values, N1 and N2 treatments were placed in the same group, but the decrease accelerated in N3 treatment. The pH values of the study varied 3.92 – 4.02 which were within the ideal value for corn silage. ADF and NDF values increased with increasing irrigation levels, but decreased with increasing nitrogen doses. The lowest ADF and NDF values were respectively observed as 22.15% and 46.08% and the greatest values were respectively observed as 28.55% and 49.85%. Crude protein contents of silage samples increased with increasing nitrogen doses and varied between 6.41-7.70%. Crude ash content of silage samples increased with increasing levels and decreased with increasing nitrogen doses. Crude ash content of silage samples increased with increasing irrigation levels and decreased with increasing nitrogen doses.

Mean values for acid composition (lactic, acetic, propionic and butyric acid) of maize silage are provided in Table 5. The effects of nitrogen doses, irrigation levels, nitrogen x irrigation interaction on lactic, acetic, propionic and butyric acid contents were not found to be significant. Lactic acid contents of silage samples varied between 4.39-4.62% DM, acetic acid contents varied between 1.22-1.25% DM, butyric acid contents varied between 0.000-0.008% DM and propionic acid contents varied between 0.003-0.008% DM (Table 5).

The effects of irrigation levels and nitrogen doses on gas production, metabolic energy and organic matter digestibility were found to be highly significant (P<0.01) (Table 6). The effects of irrigation and nitrogen treatments on methane production were not found to be significant. Similarly, the effects of irrigation x nitrogen interaction on gas and methane production were not found to be significant (P>0.05). Increasing nitrogen doses increased gas production, metabolic energy and organic matter digestibility. Gas production values of silage samples varied between 40.51-45.78 mL, metabolic energy varied between 8.10-8.82 MJ/kg DM and organic matter digestibility varied between 54.82-59.58%.

Table 4. The effects of irrigation levels and nitrogen doses on chemical composition of corn silage

Irrigation Level	Dry Matter (%) Fertilizer Doses N1	N2	N3	Means	Irrigation Level	pH Fertilizer Doses N1	N2	N3	Means
50	33.47	31.11	29.27	31.28 a	50	4.02	4.00	4.00	4.01
75	32.29	30.63	28.59	30.50 b	75	4.02	3.99	3.86	3.97
100	31.13	29.74	28.54	29.81 c	100	4.02	3.99	3.91	3.96
Means	32.30 a	30.50 b	28.80 c		Means	4.02 a	3.99 a	3.92 b	
Irrigation Level	**ADF (%) Fertilizer Doses** N1	N2	N3	**Means**	**Irrigation Level**	**NDF (%) Fertilizer Doses** N1	N2	N3	**Means**
50	26.89	23.89	22.10	24.29 b	50	48.99	47.13	45.69	47.27 b
75	29.00	26.21	21.85	25.68 ab	75	49.29	47.56	45.43	47.43 ab
100	29.75	27.28	22.51	26.51 a	100	51.27	47.79	47.14	48.73 a
Means	28.55 a	25.79 b	22.15 c		Means	49.85 a	47.49 b	46.08 c	
Irrigation Level	**Crude Protein (%) Fertilizer Doses** N1	N2	N3	**Means**	**Irrigation Level**	**Crude Ash (%) Fertilizer Doses** N1	N2	N3	**Means**
50	6.64	7.36	7.73	7.24	50	6.66	5.94	5.69	6.10 b
75	6.31	7.11	7.69	7.04	75	6.66	6.43	5.81	6.30 ab
100	6.27	7.03	7.67	6.99	100	7.12	6.54	6.23	6.63 a
Means	6.41 c	7.17 b	7.70 a		Means	6.81 a	6.30 b	5.91 c	

N1:100, N2:200 and N3:300 kg ha^{-1} N

Table 5. The effects of irrigation levels and nitrogen doses on silage acids of corn silage

Irrigation Level	Lactic Acid (% DM) Fertilizer Doses N1	N2	N3	Means	Irrigation Level	Acetic Acid (% DM) Fertilizer Doses N1	N2	N3	Means
50	4.39	4.40	4.60	4.46	50	1.24	1.25	1.24	1.24
75	4.51	4.53	4.62	4.55	75	1.22	1.22	1.23	1.22
100	4.51	4.55	4.62	4.56	100	1.24	1.24	1.25	1.24
Means	4.47	4.49	4.62		Means	1.23	1.24	1.24	
Irrigation Level	**Butyric Acid (% DM) Fertilizer Doses** N1	N2	N3	**Means**	**Irrigation Level**	**Propionic Acid (% DM) Fertilizer Doses** N1	N2	N3	**Means**
50	0.000	0.000	0.000	0.000	50	0.005	0.003	0.003	0.004
75	0.000	0.000	0.000	0.000	75	0.008	0.008	0.003	0.007
100	0.008	0.000	0.000	0.003	100	0.007	0.007	0.008	0.007
Means	0.003	0.000	0.000		Means	0.007	0.006	0.005	

N1:100, N2:200 and N3:300 kg ha^{-1} N

Table 6. The effects of irrigation levels and nitrogen doses on gas production, methane, metabolic energy and organic matter digestibility of corn silage

Irrigation Level	Gas Production (mL)				Irrigation Level	CH_4 (mL)			
	Fertilizer Doses			Means		Fertilizer Doses			Means
	N1	N2	N3			N1	N2	N3	
50	39.13	43.01	44.49	42.21 b	50	13.55	13.38	12.75	13.23
75	38.14	40.93	44.31	41.13 b	75	13.68	12.93	13.97	13.53
100	44.25	46.52	48.55	46.44 a	100	12.88	13.60	14.07	13.52
Means	40.51 c	43.49 b	45.78 a		Means	13.37	13.31	13.59	

Irrigation Level	Metabolic Energy (MJ/kg DM)				Irrigation Level	Organic Matter Digestibility (%)			
	Fertilizer Doses			Means		Fertilizer Doses			Means
	N1	N2	N3			N1	N2	N3	
50	7.96	8.45	8.65	8.35 b	50	53.92	57.06	58.48	56.49 b
75	7.77	8.20	8.60	8.19 b	75	52.67	55.54	58.11	55.44 b
100	8.58	8.97	9.21	8.92 a	100	57.88	60.58	62.16	60.21 a
Means	8.10 c	8.54 b	8.82 a		Means	54.82 c	57.73 b	59.58 a	

N1:100, N2:200 and N3:300 kg ha^{-1} N

DISCUSSION

This study was conducted to investigate the effects of different irrigation levels and nitrogen doses on yield, morphologic traits, chemical composition, and digestibility characteristics of corn silage. Experiments were conducted during the summer growing season for two years. Unexpected short-term low temperatures during the spring and autumn of the experimental years had significant impacts on yield, morphologic characteristics and chemical composition. Such low temperatures resulted in a constriction in leaves, thickening in cell walls (increasing ADF and NDF ratios), a recess in growth and development (Galdamez-Cabrera et al., 2002; Mahajan and Tuteja 2005) and various other relevant changes in plant cells. All these variations might have resulted in differences between the years.

Increasing irrigation levels and nitrogen doses increased plant height, plant diameter, stem and cob ratios and ultimately increased green herbage yields. Leaf ratio was reduced by irrigation levels and nitrogen doses. Nitrogenous fertilization speeds up the growth and development in meristem cells located at the bottom sections of internodes, thus increases plant heights of maize (Sezer and Yanbeyi, 1997). It was indicated in previous studies that nitrogenous fertilization generally increased plant heights (Islam et al., 2010), but such increases were not continuous and insufficient nitrogen levels resulted in decrease in plant heights, biomass and kernel yield (Subedi and Ma, 2005). Among the plant nutrients like nitrogen, phosphorus and potassium, Ping et al., (1993) indicated nitrogen as the most significant nutrient specifying plant height. Otegui et al. (1995) and Istanbulluoglu et al. (2002) reported decreasing plant heights and Kiziloglu et al. (2009) reported reduced green herbage yield with water deficits. Nitrogen deficiency negatively affects vegetative structure in maize and result in poor plant development (Akar et al., 2014). When the plants are not able to find the required nitrogen, they are forced to bloom and growing season is shortened. In this case, top tassel and cob tassel development is reduced and quite low kernel is formed at the tip of cobs (Kirtok, 1998). When the plants are able to find sufficient nitrogen, they keep normal growth and speed up kernel development in cobs (Akar et al., 2014). In this case, cob ratio increases and leaf and shoot ratio decreases in plants. Yılmaz (2005) reported decreasing leaf ratios with increasing nitrogen doses. Other researchers also indicated decreasing leaf area index and thus leaf ratios (Kang et al., 1986) and increasing green herbage yield (Kumar et al., 2001) with increasing nitrogen doses in maize. Water stress effects leaf and stem elongation (Song et al., 2010) and limits root development (Sharp and Davies 1979). Water stress is commonly resulted from reduced carbohydrate and photosynthesis rates and accelerated stomal closures (Kaiser, 1987; Foyer et al., 1998). Plant stomas close under stress conditions and consequently carbohydrate, proline, glycine and protein metabolites accumulate in leaves (Pelleschi et al., 1997). Such results support the our current findings.

Increasing irrigation levels increased ADF and NDF ratios and increasing nitrogen doses reduced ADF and NDF ratios. Yosef et al. (2009) reported increased NDF ratio and *in vitro* digestibility in sorghum with increasing water levels and Islam et al. (2012) reported the similar findings in maize. Increasing ADF and NDF ratios decrease crude protein and WSC ratios (Hargreaves et al. 2009). Increased nitrogen doses increase CP and *in vitro* digestibility (Cox and Charney, 2005). In this study, protein ratios decreased with increasing water levels, but the changes were not found to be significant. Increasing ADF and NDF ratios make the digestion difficult and ultimately result in decreased crude protein, gas production, metabolic energy and digestible organic matter content (Kamalak et al., 2011). Increasing nitrogen doses and irrigation levels may also increase cob ratios and thus kernel ratios and energy values (Islam et al., 2012).

Nitrogen has a critical role in protein and enzyme synthesis in plants. Entire metabolic processes are controlled by enzymes. Moreover, nitrogen constitutes a component in chlorophyll synthesis which is the primary molecule absorbing the energy needed in photosynthesis (Islam et al., 2010). In maize plants, available water and nitrogen increase photosynthesis rates especially in kernel-fill period (Uribelarrea et al., 2004), then sucrose and other sugars are regularly transported into ears like nitrogen (Spielbauer et al., 2006; Swank et al., 1982). Continuous post-flowering nitrogen transport from vegetative organs to ears and leaflets may improve the kernel nitrogen content (Christensen et al., 1981; Swank et al., 1982).

Ball et al. (1996) indicated that dry matter and protein contents of cultivars might vary not only based on plant genetics but also based on leaf, spike and stem ratios, ripening period, temperature and fertilization. Increased cob ratio with increasing nitrogen doses and irrigation levels of the our study contributed to crude protein ratios. Water stress may reduce nutrient transport in plants and thus terminate dry matter accumulation (Kruse et al., 2008; Setter and Parra 2010). In our study, increasing nitrogen doses and irrigation levels reduced the dry matter contents. Besides, current dry matter ratios (28.54-33.47%) were quite close to expected ratios (about 30%) (Keady, 2005).

Low pH levels indicate the existence of acidified atmosphere with fermentation of soluble sugars (Islam et al., 2012). Low pH ranges in silage samples of the our study (3.86-4.02) are the indicator of lactic acid accumulation providing the preservation of silage quality and leading the microbial activities (McDonald et al., 1991). The decrease in pH levels with increasing irrigation levels and nitrogen doses increased lactic acid contents. Water stress has direct impact on cob development and kernel-fill (Andrade, 2002). The dissolved carbohydrates with increasing water levels increase lactic acid production and increased lactic acid then decrease pH levels (Bates 2009). Reduced kernel ratio also decreases energy levels (Mould et al., 1983) and reduces silage quality (Cox et al., 1993).

Lopez et al. (2010) classified the anti-methanogenic potential of feeds based on methane ratio of the gas released through fermentation as low (>%11 and ≤%14), medium (%>6 and <%11) and high (>%0 and <%6). According to this classification, current finding under different irrigation levels and nitrogen doses indicated the anti-methanogenic potential of maize silage as low (12.88-14.07 mL CH_4).

CONCLUSION

Our results revealed that increasing irrigation levels increased plant height and diameter but relatively reduced the quality of corn silage. Increased nitrogen doses increased plant height, diameter, green herbage yield, cob ratio and crude protein ratio, but reduced ADF and NDF ratios. Increasing nitrogen doses also increased metabolic energy and organic matter digestibility, but did not have significant effects on methane production. Nitrogen doses had positive impacts on silage fermentations, slightly increased lactic acid content and reduced pH levels. Irrigation levels x nitrogen doses interaction was not found to be significant, but the effects of years were significant. Ultimately, the effects of different irrigation levels and nitrogen doses on yield, yield parameters, chemical composition, gas and methane production, organic matter digestibility and metabolic energy were elucidated. Although we recommend applying 100 irrigation levels and N3 nitrogen dose, performing similar studies in different climatic and soil conditions will yield better results.

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