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DIFFERENT MAIZE SILAGE CULTIVARS WITH OR WITHOUT UREA AS A FEED FOR RUMINANT: CHEMICAL COMPOSITION AND IN VITRO FERMENTATION AND NUTRIENT DEGRADABILITY

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ABSTRACT

This study compared forage yield and agronomic characteristics of 9 maize cultivars (Pioneer 30N11, Pioneer 4444, Pioneer 30K08, SC-128W, SC-166Y, SC-78Y, TWC-324W, TWC-368Y and TWC-321W) ensiled with or without urea as for ruminants on *in vitro* ruminal fermentation characteristics and nutrient degradability. A factorial randomized complete block design was used. Pioneer 30N11 and Pioneer 4444 showed the greatest silage forage yield, ear weight, and plant height. The chemical composition of maize silage cultivars without urea revealed that Pioneer 30K08 had the greatest (P<0.001) crude protein (CP) content (127 g/kg), while the lowest CP content (89 g/kg) was observed with SC-78Y. CP content ranged from 118 to 156 g/kg when urea was added to the maize silage cultivars. The neutral detergent fiber, acid detergent fiber, and acid detergent lignin contents recorded the highest values (P<0.01) in TWC-324W, SC-128W and SC-78Y. Urea treatment of different cultivars affected (P<0.05) the concentrations of CP, ether extract (EE), non-structural carbohydrates (NSC), and fibers. Urea treatment increased CP and EE in all cultivars and increased or decreased truly degraded dry matter (TDDM), truly degraded organic matter (TDOM), protozoa, ammonia and methane (P<0.05). Overall, Pioneer 30N11, Pioneer 4444 and Pioneer 30K08 showed the greatest forage yield and nutritive value as a feed for ruminants.

Keywords: chemical composition, *in vitro* incubation; maize cultivars; nonprotein nitrogen; urea treatment.

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INTRODUCTION

Feed quality is a very important factor that affect animal productivity and profit (Kırkpınar and Açıkgöz, 2018). Maize (*Zea mays* L.) is a common annual crop in Mediterranean countries such as Egypt (Salama et al., 2021), and is considered as the third-largest cereal crop produced worldwide (FAO, 2022).

In Egypt, maize is important for the rural economy and livelihood, being maize silage one of the main feeds fed to ruminants (Bendary et al., 2022). Whole-plant maize silage is normally used as ruminant feed and the effect of maize cultivars on its nutritive value has been extensively studied in many countries (Opsi et al., 2013; Loučka et al., 2018; Liu et al., 2021). However, there is little information on the nutritional value of different cultivars of maize under local conditions in Egypt, while a considerable number of experiments have evaluated the effect of hybrid corn silage with different varieties to improve nutritive values as feeds for ruminants. The concentrations of different nutrients greatly differ between maize hybrids and affect the density and activity of ruminal microflora in the rumen (Loučka et al., 2018; Ebeid et al., 2020). Loučka et al. (2018) compared the nutritive value of silages made of two maize hybrids and observed that hybrids significantly affected the chemical composition and nutrient digestibility. Opsi et al. (2013) compared two maize hybrid cultivars using the *in vitro* gas production (GP) technique and reported that the yields of fodder and silage differed between cultivars, with no effect on chemical composition and *in vitro* digestibility of maize silage.

Maize grains contain a great content of soluble carbohydrates and could cause digestive problems in ruminants, especially when finely ground because great contents of readily soluble sugars can interfere with rumen function (Jiao et al., 2022). The treatment of feeds containing great contents of readily soluble sugars with urea improve the synchronization between energy and N in the rumen, which results in improved microbial protein synthesis (El-Zaiat et al., 2022).

Ruminants can utilize nonprotein nitrogen (NPN) for microbial protein synthesis in the rumen (Thirumalesh and Krishnamoorthy, 2013). One of the most common NPN is urea, which can be used by ruminal microbes to boost the protein content of feeds (El-Zaiat et al., 2022; Sumadong et al., 2022). The efficiency of urea treatment to improve protein profile of feeds depends on the availability of carbohydrates in the feed (Inácio et al., 2022). Maize contains a low concentration of crude protein (CP) ranging from 7 to 8% (Bendary et al., 2022). Therefore, supplementing maize silage with urea will increase its chemical quality as a feed for ruminants. Moreover, there is evidence that urea treatment can lower ruminal methane production, and thus it may be recognized as a potential methane (CH_4) mitigation agent (Zhang et al., 2018; Saminathan et al., 2022).

The present study aims to compare 9 maize cultivars (Pioneer 30N11, Pioneer 4444, Pioneer 30K08, TWC-324W, SC-128W, SC-166Y, TWC-368Y, TWC-321W, and SC-78Y) in terms of forage yield and agronomic characteristics, and to evaluate the effect of ensiling with or without urea on in vitro ruminal fermentation parameters and nutrient degradability. The hypotheses of this study were: (1) the different concentrations of nutrients in maize cultivars will affect their nutritive values and digestion, and (2) urea treatment during ensiling will compensate the low protein content in maize and will work synchronously with the available carbohydrates in seed to improve the chemical composition of maize silage as a feed for ruminants.

MATERIALS AND METHODS

Agronomic management

The study was performed at the experimental farm of the Crop Science Department, Alexandria University, during the two successive winter seasons of 2019 and 2020. The meteorological data for both years are shown in Table 1. The soil at the experimental site was a sandy loam, moderately alkaline (pH 8.4), with 1.5% OM content and an electrical conductivity of 1.30 dSm^{-1}.

Table 2 shows a full description of the 9 maize varieties used in this study. The sowing density was 8 plants per m^2. Irrigation was carried out every 10 days.

The plants were removed from each plot randomly during the vegetation period, and morphological traits were recorded (plant height, ear attachment height, leaf area index). Plant height (cm) was measured at harvest time as length of the stem from ground level to the uppermost of tassel. Leaf area index was estimated to main ear as follow: leaf length (cm) × maximum leaf width (cm) × 0.75. The crop was harvested 45 days after flowering with a dry matter (DM) content of about 35%. The fresh mass of the whole plant was measured (ton), while ear weight was measured as the average weight values of 10 ears from each plot (g/plot). The number of ears per plant was also determined. Samples from each plot (10 kg fresh plant) were used for the analysis and *in vitro* ruminal fermentation trial.

Table 1. Average meteorological data for the experimental period (2020 and 2021)[1]

Temperature (° C)	Dew point (° C)	humidity (%)	Wind speed (Kph)	Pressure (Hg)	Precipitation (mm/in)
27-30	19-23	63-74	13-23	29.65-29.80	0

[1]Source: https://weatherandclimate.com/egypt/alexandria

Table 2. Description, maturity, dry matter and seeding rate of maize cultivars used in the study.

Varieties	Description	Maturity (days)	Dry matter (%)	Seeding rate (kg/ha)
Pioneer 30N11	Single Cross-Yellow seeds	90-95	30-35	28.57
Pioneer 4444	Single Cross-Yellow seeds	90-95	30-35	28.57
Pioneer 30K08	Single Cross-White seeds	90-95	30-35	28.57
TWC-324W	Three-way Cross-White seeds	90-95	30-35	33.33
SC-128W	Single Cross-White seeds	90-95	30-35	28.57
SC-166Y	Single Cross-Yellow seeds	90-95	30-35	28.57
TWC-368Y	Three-way Cross-Yellow seeds	90-95	30-35	33.33
TWC-321W	Three-way Cross-White seeds	90-95	30-35	33.33
SC-78Y	Single Cross-Yellow seeds	90-95	30-35	28.57

Urea treatment, ensiling and laboratory analysis

Maize cultivars were chopped (sieve 8-16 mm) and individually treated with urea and sugarcane molasse at 1 and 5%, respectively, and ensiled for 45 d using tightly closed plastic silo bags (40 × 70 cm). Urea was dissolved in water and mixed with molasse before spraying on the grounded maize. The silo bags were compressed manually (Kholif et al., 2022b) and were sealed and kept indoors on a dry concrete floor. Before tightening the bags, and at the end of ensiling after 45 d, sub-samples of 300 g fresh matter per plot were dried at 60°C to determine the DM content. To ensure standardized procedure for sampling for all cultivars, proper mixing before and after ensiling was done and samples from different sites in the silo bags were taken for further analysis. The dried sub-samples were ground to a particle size of 1-mm. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as described by Van Soest et al. (1991). The ADL content was corrected after the residual ash content. Ash was determined by combusting samples in a muffle oven at 550°C for 3 h (Lindberg Blue M, Thermo Fisher Scientific, Pittsburgh, PA, USA) according to the AOAC (2005). Organic matter (OM) was calculated by subtracting the weight of the ash after combustion and expressed as a percentage. The N content was analyzed by the Kjeldahl procedure AOAC (2005), and crude protein (CP) was calculated as N multiplied by 6.25. Total carbohydrate content was determined using the phenol-sulfuric acid method as described by DuBois et al. (1956). For evaluation of silage pH, silage samples (50 g fresh weight) were homogenized for 3 min with a laboratory blender after adding 200 mL of distilled water. The content was filtrated through 4 layers of cheesecloth and filtrate was assessed for pH using a digital pH meter (Thermo Scientific, Orion StarTM A121, Beverly, MA, USA).

***In vitro* analysis**

The *in vitro* trial was carried out as described by Bueno et al. (2005). Planting of maize and animal management was approved by the Research Ethics Review Committee of Alexandria University, Egypt (ID: Alex. Agri. 192305332).

Rumen contents were collected from three Egyptian buffalo steers at the slaughterhouse of the Faculty of Agriculture, Alexandria University. Rumen contents were collected and kept separately in pre-warmed thermos containers (39 ºC) under anaerobic conditions. For the inoculum preparation, rumen contents from each animal were blended for 10 s, squeezed through three layers of cheesecloth, and maintained in a water bath (39 °C) under CO_2 until fermentation.

For each treatment, six replicates (bottles) were used; three for the fermentation parameters (GP, NH_3-N, total and individual volatile fatty acids (VFA) and CH_4) and protozoal count, and

the other three were used for the determination of rumen degradability (truly degraded DM (TDDM) and truly degraded OM (TDOM). Three blanks (rumen fluid and buffer solution) and internal standard bottles (containing rumen inoculum, buffer solution and clover hay) were prepared. Ground samples (0.5 g) of feed were placed into numbered serum bottles (120 mL) and incubated with 45 ml of diluted rumen fluid (15 mL mixed rumen fluid + 30 mL of Menkes buffered medium) for 48 h.

Once filled, bottles were sealed immediately with 20 mm butyl septum stoppers, manually mixed, and incubated in a forced-air oven at 39 °C for 48 h. The bottles were shaken manually after the recording of the gas headspace pressure at 3, 6, 12, 24, 36 and 48 h of incubation and the assay was repeated in different 3 weeks (3 runs). The amount of the GP in all bottles at each measuring time was estimated according to the regression equation predicated between gas volume versus pressure relationship $V = 4.974 \times p + 0.171$ ($n = 500$; $R^2 = 0.98$; where: V is a gas volume (ml); p is the measured pressured (psi).

Rumen fermentation and degradability

After 24 h, bottles were placed in cold water (4 ºC) and determination of TDDM and TDOM was done by calculated from the difference between the amounts of the incubated DM and OM and those remaining non-degraded. The partitioning factor ($PF_{24;}$ mg DMD: mL gas) was estimated as the ratio of TDOM (mg) and gas volume (mL) was calculated according to Blümmel et al. (1997).

After incubation, rumen pH was determined, and 2 ml of rumen fluid was mixed with 2 ml of methyl green-formalin-saline solution and stored in a glass bottle at room temperature for microscopically determination of protozoal count and differentiation as described by Dehority (2018).

Short-chain fatty acids (SCFA) were determined according to Palmquist and Conrad (1971) by gas chromatography (Thermo fisher scientific, Inc., TRACE1300, Rodano, Milan, Italy) fitted with an AS3800 autosampler and equipped with a capillary column HP-FFAP (19091F-112; 0.320 mm o.d., 0.50 µm i.d., and 25 m length; J&W Agilent Technologies Inc., Palo Alto, CA). Hydrogen at 1.35 mL/min was used as a carrier gas. Air, hydrogen, and nitrogen fluxes (make up gas) were kept at 450, 40, and 35 mL/min, respectively. A 0.1 µL aliquot was injected in the splitless mode for the entire run with 31.35 mL/min of H2 flux (63.432 Pa). Injector and flame ionization detector (FID) temperatures were held isothermally at 250°C. The oven heating slope was 80°C (1 min), 120 °C (20 °C/min for 3 min), and 205°C (10 °C/min for 2 min), with 9 min overall analytical time. A mixture of known concentrations of SCFAs was used as an external standard (Sigma Chemie GmbH, Steinheim, Germany) to calibrate the integrator. Ruminal NH_3-N concentration was measured calorimetrically by spectrophotometer (Alpha-1101 model; Labnics Equipment, California). Ruminal NH_3-N concentration was measured calorimetrically using a spectrophotometer (Alpha-1101 model; Labnics Equipment, California).

Statistical analysis

Data of forage yields were analyzed in a randomized complete block design with four blocks. For the seasons, an analysis of variance was performed, and means were compared using Least Significant Difference (LSD). Reported values of the measured parameters are the mean values from the two cultivation seasons.

Chemical composition, *in vitro* ruminal GP and fermentation parameters data were analyzed as a randomized design using the PROC MIXED procedure of SAS (Online Version, SAS® OnDemand for Academics, SAS Inst., Inc., Cary, NC). For calculating the means of chemical composition, samples of the same cultivar from different sites in the same silo bag were averaged and considered as the experimental unit. For the *in vitro* measurements, mean values of each individual run (3 run) were used as the experimental unit. The statistical model was: $Y_{ijk} = \mu + S_i + T_j + S_i \times T_j + \varepsilon_{ij}$; where: Y_{ijk} represents every observation of the *i*th maize cultivars with each treatment, T_j expressed the effect of treatment, $S_i \times T_j$ expressed the interaction between the different cultivars and urea treatment, and e_{ijk} expressed the experimental error. When the treatment *F*-test was significant at $p < 0.05$, means were then compared by applying the probability of difference option of the least squares means statement. Significance was declared at a level of $p < 0.05$.

RESULTS

Field experiment

Reported values of the measured parameters are the mean values from the two cultivation seasons. Years affected the MS of silage forage yield, ear weight, plant height and leaf area index (Table 3). Varieties (cultivars) affected (P<0.01) the MS of silage forage yield (131.0) and leaf area index (47613). Significant varieties × years interactions were observed for the MS of silage forage yield and leaf area index.

The greatest (P<0.05) silage forage yield was observed with the cultivars Pioneer 30N11 (32.6 ton) and Pioneer 4444 (32.3 ton) (Table 4). The

Table 3. Mean squares (MS) of silage forage yield, number of ears, ear weight, plant height and leaf area index of different maize cultivars.

Source of Variance	d.f	MS silage forage yield	MS number of Ears	MS ear weight	MS plant height	MS leaf area index
Replicates (R)	3	41.5$^{n.s}$	0.167$^{n.s}$	2395**	580$^{n.s}$	1856$^{n.s}$
Years (Y)	1	954.1**	0.000001$^{n.s}$	171348**	165281**	242316**
Y × R	3	42.8$^{n.s}$	0.037$^{n.s}$	2956**	4563**	89357**
Varieties (V)	8	131.0**	0.125$^{n.s}$	1621$^{n.s}$	2243$^{n.s}$	47613**
Y × V	48	69.5**	0.062$^{n.s}$	917$^{n.s}$	2503$^{n.s}$	16352**
Error	71	27.6	0.071	738.8	1472	6221

*p<0.05, **p<0.01, n.s = not significant.

Table 4. Means of silage forage yield, number of ears, ear weight, plant height and leaf area index of different maize cultivars.

Varieties	Silage forage yield (ton)	Number of ears	Ears weight (g)	Plant height (cm)	Leaf area index
Pioneer 30N11	32.6a	1.38a	121.6a	287.9a	600.6ef
Pioneer 4444	32.3a	1.00b	108.6ab	248.0bc	710.8bcd
Pioneer 30K08	29.3ab	1.13 ab	115.3a	281.6ab	635.5def
TWC-324W	25.9bc	1.00 b	86.5bc	275.0abc	770.1ab
SC-128W	26.2bc	1.13ab	109.6ab	253.5abc	754.6abc
SC-166Y	23.1c	1.00b	108.6ab	260.0abc	705.2bcd
TWC-368Y	23.6c	1.13ab	105.7ab	246.8bc	804.0a
TWC-321W	23.8c	1.00b	103.6a	241.7c	679.6cde
SC-78Y	21.6c	1.00b	75.8c	251.0abc	578.8ef
L.S.D 0.05	5.280	0.27	27.3	38.57	79.3
SEM	1.91	0.096	10.86	14.12	36.40
P value	0.003	0.012	0.015	0.025	0.003

Means in the same row with different letters differ, p<0.05.

cultivars Pioneer 30N11 and Pioneer 30K08 had the greatest ear weight (121.6 g) and plant height (287.9 cm), while the greatest leaf area index was observed with the cultivars TWC-324W (770.1) and TWC-368Y (804.0).

Chemical composition and fermentation

Effect of maize cultivars and urea treatment on chemical composition and ruminal fermentation

Significant cultivars × treatment interactions (P<0.01) were observed with EE, NSC, NDF, ADF, hemicellulose, cellulose, ADL, and the pH of silage extract (Table 5). The concentrations of CP, EE, NSC, NDF, hemicellulose, cellulose and ADL differed between cultivars (P<0.01).

All cultivars contained similar levels of OM concentration, with values ranging from 87.6 to 90.9%. However, the cultivars Pioneer 30K08 (12.7%), SC-128W (11.4%), TWC-368Y (11.4%), and TWC-321W (10.9%) contained greater CP compared to other cultivars. The highest concentration of EE was recorded in the cultivar TWC-324W. The Pioneer 30K08 had the lowest concentrations of NDF (57.3%), ADF (32.0%) and ADL (5.9%). The greatest NDF, ADF and hemicellulose concentrations were observed with SC-166Y and SC-78Y. However, the cultivars Pioneer 4444, TWC-324W, SC-128W, TWC-321W and SC-78Y had the greatest ADL concentrations. The lowest cellulose concentrations were observed with the cultivars Pioneer 4444, Pioneer 30K08 and SC-128W. Of all the cultivars, Pioneer 30N1 (6.6 ± 0.4) had the highest pH of silage extract.

Significant cultivars × treatment interactions (P<0.05) were observed with GP, TDDM, TDOM, total VFA, acetic, propionic, CH_4 and CH_4: VFA ratio (Table 6). Values of GP, TDDM, TDOM, pH,

Table 5. Chemical composition of different maize cultivars silage with/without urea.

Cultivars	Treatment	OM	CP	EE	NSC	NDF	ADF	HC	Cel	ADL	pH silage extract
Pioneer 30N11	Untreated	903	104	20.8e	153bc	626bcde	350cde	276cdef	302abcd	48e	6.6a
	Urea	921	133	37.3b	152bc	599def	342edf	257 efg	269ef	73c	3.8h
Pioneer 4444	Untreated	907	104	12.9h	173ab	617cdef	345def	272def	261ef	84ab	3.9efg
	Urea	899	132	47.7a	135cd	585ef	341edf	243g	269ef	72c	4.1ef
Pioneer 30K08	Untreated	909	127	16.7fg	192a	573ef	320efg	254fg	261ef	59d	4.3ef
	Urea	898	156	16.6fg	115def	611cdef	310fg	300abc	279cde	32g	3.9efg
TWC-324W	Untreated	901	108	28.7d	99dfg	666abcd	357bcd	309ab	316a	41f	3.8h
	Urea	882	134	31.8c	25i ±	691a	409a	282cde	321a	88a	5.2c
SC-128W	Untreated	894	114	18.5f	130def	632abcd	347cde	285bcde	283bcde	64d	4.2ef
	Urea	902	142	33.6c	70h	657abcd	381abc	276cdef	299abcd	82b	4.5de
SC-166Y	Untreated	897	104	21.5e	85dfg	687ab	366bcd	320a	325a	42f	4.2ef
	Urea	907	133	32.9c	185a	556f	298g	258efg	247f	50e	3.8h
TWC-368Y	Untreated	890	114	17.2fg	107	651abcd	334edf	317a	307ab	27g	4.1efg
	Urea	897	141	26.9d	103dfg	626bcd	367bcd	259efg	306abc	61d	4.0fgh
TWC-321W	Untreated	902	109	10.9h	133cd	649abcd	352cde	296abcd	282bcde	70c	4.3ef
	Urea	912	135	28.5d	134cd	614cdef	346cde	268efg	275def	72c	3.8h
SC-78Y	Untreated	876	89	15.3g	103dfg	669abc	405a	263efg	324a	82b	5.6b
	Urea	876	118	16.7fg	80fgh	661abc	387ab	274cdef	313a	74c	4.9cd
SEM		23.60	3.67	0.80	10.34	18.38	10.69	8.30	8.76	2.00	0.14
P value											
Cultivars		0.908	<0.001	<0.001	<0.001	0.005	<0.001	0.001	<0.001	<0.001	<0.001
Treatment		0.872	<0.001	<0.001	0.003	0.038	0.887	<0.001	0.035	<0.001	<0.001
Cultivars × Treatment		0.997	0.999	<0.001	<0.001	0.003	0.001	<0.001	<0.001	<0.001	<0.001

Means in the same column with different letters differ at $P<0.05$. *P*-value is the observed significance level of the *F*-test for cultivars × urea. ADF, acid detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; NDF, neutral detergent fiber; NSC, non-structural carbohydrates; OM, organic matter; HC, hemicellulose; Cel, cellulose; SEM, standard error of the mean.

NH_3-N, total VFA, acetic, propionic, butyric, CH_4 and CH_4: VFA ratio differed between cultivars (P<0.01). The cultivars Pioneer 30K08, SC-166Y, TWC-368Y and TWC-321W had the greatest (P<0.001) GP, while SC-78Y showed the lowest GP. Pioneer 30K08 showed the greatest TDDM and TDOM (P<0.001), while the cultivars SC-166Y and TWC-321W showed the lowest values. The lowest concentrations (P<0.001) of ruminal NH_3-N were observed with the cultivars Pioneer 4444, and TWC-321W. Each of Pioneer 30K08, TWC-324W, SC-128W and SC-166Y showed the greatest concentrations of total VFA, while the greatest concentrations of acetate were observed

Table 6. ***In vitro*** **fermentation characteristics of different maize cultivars silage with/ without urea.**

		Parameters[1]											
Cultivars	**Treatment**	**GP**	**TDDM**	**TDOM**	**PF[2]**	**Protozoa[3]**	**pH**	**NH_3-N**	**VFA**	**C_2**	**C_3**	**C_4**	**CH_4**
Pioneer 30N11	Untreated	118cdef	523def	500cdef	3.93	3.67ab	5.87	13.1abc	55.8cdef	33.1bcde	12.0de	10.7	28.4cd
	Urea	122abcde	531de	526cd	4.08	2.37bc	5.78	15.2a	56.7bcde	31.9edf	13.4cde	11.5	26.9gh
Pioneer 4444	Untreated	126abc	533cd	502cdef	3.90	3.03abc	5.73	10.4c	54.3cdef	31.0ef	12.6cde	10.7	27.2gh
	Urea	120bcdef	513def	522cde	4.00	2.27bc	5.76	10.2c	65.4abcde	39.9abcd	14.6cd	10.9	28.0def
Pioneer 30K08	Untreated	129a	540bcd	530bcd	3.90	3.30abc	5.75	13.6abc	78.5a	48.5a	16.1abc	14.0	29.2bc
	Urea	128a	572ab	571ab	4.12	3.03abc	5.85	15.0a	63.3abcde	38.7abcd	14.0cd	10.7	28.2ed
TWC-324W	Untreated	120bcdef	484fgh	478ef	3.72	3.43abc	5.81	13.2abc	74.1a	46.7a	14.9bcd	12.6	29.6ab
	Urea	100g	407i	392h	3.45	2.83abc	5.97	15.0a	64.6abcd	40.5abcd	12.3cde	11.8	30.3a
SC-128W	Untreated	113f	508def	490def	4.11	2.07c	5.86	13.6abc	68.2abcd	44.0a	13.4cde	10.8	30.0ab
	Urea	114ef	509def	489def	3.99	2.83abc	5.85	15.3a	50.4ef	32.6cdef	10.0e	7.8	29.8ab
SC-166Y	Untreated	118cdef	448h	435g	3.40	2.87abc	5.87	12.7abc	69.9abc	43.1a	14.3cd	12.6	29.4abc
	Urea	123abcd	589a	579a	4.30	2.07c	5.74	13.5abc	74.4a	42.5ab	18.9a	13.0	25.7i
TWC-368Y	Untreated	116def	499defg	487def	3.74	2.80abc	5.86	13.2abc	68.5abcd	41.2abcd	15.6abc	11.7	27.6defg
	Urea	121bcdef	504defg	509cdef	3.76	3.33abc	5.79	14.6ab	72.5ab	41.8abc	18.1ab	12.6	26.0gh
TWC-321W	Untreated	122bcdef	525ed	487def	3.64	4.03a	5.78	10.0c	67.6abcd	39.6abcd	15.6abc	12.4	27.3efg
	Urea	119bcdef	569abc	542abc	7.12	2.93abc	5.74	11.2abc	55.1cdef	31.1ef	14.1cd	9.9	25.5i
SC-78Y	Untreated	104g	490defg	468fg	4.18	3.30abc	5.90	15.2a	45.4f	27.1f	10.0e	8.4	28.2ed
	Urea	105g	466gh	430gh	3.69	2.87abc	5.95	15.8a	53.1cdef	30.8ef	12.7de	9.6	26.7gh
SEM		2.6	12.6	14.2	0.694	0.435	0.051	1.10	4.8	2.92	1.1	0.9	0.3
P value													
Cultivars		<0.001	<0.001	<0.001	0.035	0.282	0.109	<0.001	0.001	<0.001	<0.001	0.005	<0.001
Treatment		0.278	0.038	0.003	0.180	0.039	0.921	0.026	0.192	0.058	0.427	0.102	<0.001
Cultivars × Treatment		<0.001	<0.001	<0.001	0.168	0.287	0.130	0.988	0.026	0.021	0.004	0.061	<0.001

Means in the same column with different letters differ at $P<0.05$. *P*-value is the observed significance level of the *F*-test for cultivars × urea; SEM, standard error of the mean. GP, gas production (mL/g DM); TDDM, true degradable DM (g/kg DM); TDOM, true degradable OM (g/kg DM), NH_3-N, ammonia-N (mg/g DM); VFA, volatile fatty acids (mmol/g DM); C_2, Acetic; C_3, propionic, C_4, butyric; CH_4, methane (mL/g DM). [2]PF, partitioning factor (mg DMD: mL gas). [3]Protozoa ($\times 10^5$/mL).

with the cultivars Pioneer 30K08, TWC-324W, SC-128W, SC-166Y and TWC-368Y. SC-78Y had the lowest total VFA, propionate acetate and butyrate (P<0.001). Pioneer 30K08, TWC-368Y and TWC-321W had the greatest concentration of propionate. Increased CH_4 production was observed with Pioneer 30K08, TWC-324W, SC-128W and SC-166Y, while the lowest values were obtained with TWC-368Y (P<0.001).

The concentrations of CP, EE, NSC, NDF, hemicellulose, cellulose and ADL were affected by urea treatment (P<0.05) (Table 5). Urea treatment increased the concentrations of CP by 22.8 to 32.6% with Pioneer 30K08 and SC-78Y, respectively, while EE increased by 10.8 to 270% with TWC-324W and Pioneer 4444, respectively (P<0.05). Increased NDF concentrations were observed after the ensiling of the cultivars Pioneer 30K08 (by 6.6%), TWC-324W (by 3.8%) and SC-128W (by 4%) with urea; however, decreased NDF concentrations were observed in the cultivars Pioneer 30N11 (by 4.3%), Pioneer 4444 (by 5.2%), SC-166Y (by 19.1%) and TWC-368Y (by 90.5%). Urea treatment decreased the concentration of hemicellulose in all maize cultivars (by 3.2 to 19.4%), except for Pioneer 30K08 that recorded an increase of 18.1%. With respect to cellulose concentrations, Pioneer 4444, Pioneer 30K08, TWC-324W, and SC-128W recorded increases of 3.1%, 5.7%, 1.6%, and 5.7%, respectively; while decreases of 24% and 9.5% were observed with SC-166Y and TWC-321W, respectively. Regarding pH of silage extract, Pioneer 4444 recorded the highest increase (5.1%), followed by Pioneer 30K08 (36.8%), and SC-128W (7.1%), while decreases were observed in Pioneer 30N11 (42.4%), Pioneer 30K08 (9.3%), SC-166Y (9.5%), TWC-368Y (2.4%), TWC-321W (11.6%) and SC-78Y (12.5%) cultivars treated with urea compared to those untreated.

Urea treatment affected TDDM, TDOM, protozoa, NH_3-N and CH_4 (P<0.05) (Table 6). Regarding TDDM and TDOM, urea treatment resulted in increases of 5.9 and 7.7% in Pioneer 30K08; 31.5 and 33.1% in SC-166Y; and 8.4 and 11.3% in TWC-321W, respectively. Conversely, decreases of 15.9 and 18% were observed in TWC-324W, and of 4.9 and 8.1% in SC-78Y, respectively (P<0.05). Urea treatment lowered (P<0.05) the number of ruminal protozoa in the cultivars Pioneer 30N11(35.4%), Pioneer 4444 (25.1%), Pioneer 30K08 (8.2%), TWC-324W (17.5%), SC-166Y (27.9%), TWC-321W (27.3%) and SC-78Y (13%), but increases were observed in SC-128W (36.7%) and TWC-368Y (18.9%). Urea treatment increased (P<0.05) the concentrations of ruminal NH_3-N in all cultivars from 3.9 to 16%, except for Pioneer 4444 as it recorded a decrease of 1.9%. Moreover, urea treatment decreased (P<0.05) CH_4 production in Pioneer 30N11 (5.3%), SC-166Y (12.6%), TWC-321W (6.6%), Pioneer 30K08 (3.4%) and SC-78Y (5.3%), but it increased it in Pioneer 4444 (2.9%), andTWC-324W (2.4%).

Correlations between nutrient concentration and measured parameters

Positive correlations were observed between the concentration of NSC and GP, true degradable DM and true degradable OM (Table 7). However, negative correlations were observed between fibers (NDF, ADF and cellulose) and GP, true degradable DM and true degradable OM.

Table 7. Pearson correlation between in vitro fermentation products and nutrient contents (mean from all maize cultivars).

	OM	CP	EE	NSC	NDF	ADF	HC	Cel
Gas production	0.18	0.21	-0.02	0.58**	-0.50**	-0.64**	-0.07	-0.54**
True degradable DM	0.25	0.30*	-0.01	0.72**	-0.62**	-0.66**	-0.27	-0.67**
True degradable OM	0.21	0.39**	0.15	0.66**	-0.66**	-0.68**	-0.30*	-0.64**
Partitioning factor	-0.12	0.08	0.07	0.12	-0.26	-0.19	-0.23*	-0.26
Protozoa	0.01	-0.22	-0.34*	-0.01	0.17	0.15	0.11	0.21
pH	-0.21	-0.10	-0.09	-0.47**	0.36**	0.36**	0.17	0.42*
Ammonia-N	-0.04	0.18	0.02	-0.28*	0.16	0.22	0.01	0.26
Total volatile fatty acids	0.13	0.14	0.05	0.21	-0.17	-0.35**	0.16	-0.13
Acetic	0.10	0.12	0.05	0.11	-0.09	-0.29*	0.22	-0.05
Propionic	0.15	0.23	0.07	0.37**	-0.34*	-0.46**	-0.01	-0.29**
Butyric	0.17	0.04	-0.02	0.29*	-0.16	-0.31*	0.12	-0.15
CH_4	-0.09	-0.16	-0.03	-0.38**	0.37**	0.28*	0.31*	0.35**

Notes: * p<0.05, **P<0.001. ADF, acid detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; NDF, neutral detergent fiber; NSC, non-structural carbohydrates; OM, organic matter; HC, hemicellulose; Cel, cellulose; CH_4, methane.

DISCUSSION

Yield and maize plant characteristics

The year of seeding affected the yield of maize, which may be related to the heat stress resistance of varieties, differences in climate, and the length of the growing season (Bruns and Abbas, 2006). Moreover, timely sowing is critical for maximizing yield for both grain and biomass in maize (Maresma et al., 2019). Djaman et al. (2022) stated that planting date can affect grain yield, crop height and leaf area index in maize.

Obtaining great forage yield with Pioneer 30N11 and Pioneer 4444, stability of produced quantity along with good quality represents the major importance might not be the choice of forage grower (Bacchi et al., 2021). The greatest green fodder yield may be related to plant height, ears weight, stem girth and number of leaves per plant (Brar et al., 2021), and this is an important issue regarding the availability of green fodder to fed animals.

Increased plant height with Pioneer 30N11 and Pioneer 30K08 and increased ear weight with TWC-324W and TWC-368Y probably due to competition for light between plants. Competition for light is crop responses to avoid competitive interactions to avoid shading. That results in low ratios roots/shoots, thinner stalks and stronger apical dominance with low intensity branching characteristics favoring height growth of stalk, allowing them to rise above the branches of neighboring plants (Sérgio et al., 2014). Such conditions were reflected as increased yield and quality of silages were made from Pioneer 30N11 and Pioneer 30K08 cultivars. Moreover, the low roots/shoots ratios, thinner stalks and low intensity branching characteristics affect the concentrations of nutrient (e.g., structural, and nonstructural carbohydrates) (Bláha, 2019), which will affect nutritive value of silage as a feed for animals.

Chemical composition and fermentation

The density and activity of ruminal microflora depend mainly on nutrient concentrations in diets fed to host animals (Ebeid et al., 2020). Moreover, the significant interactions suggest that the fermentation kinetics from cultivars could be enhanced by addition of urea.

Chemical composition and ruminal fermentation of maize cultivars

The concentrations of basic nutrients (e,g., OM, CP, NSC and fibers) differed between cultivars. As a result, it is expected that the proximate analysis of each cultivar will differ. In the present experiment, the cultivars Pioneer 30K08, SC-128W, TWC-368Y, and TWC-321W contained the greatest CP and low concentration of NDF, ADF and ADL. Great CP and low fiber fractions are indicators of greater nutritive values with most animals. These were reflected on GP, degradability and fermentation parameters making them recognized feeds for ruminants (Kholif et al., 2022a; Morsy et al., 2022). Based on the results of chemical composition and nutrient degradability, it would be suggested that these cultivars could be used effectively in animal feeding.

Great concentrations of plant fiber in SC-166Y and SC-78Y negatively affect rumen microflora activity (Ammar et al., 2022), and reduce their potential intake and degradability when be used as animal feeds.

The chemical composition and degradability of plants depend mainly on genotypic variation and some environmental factors (Bhattarai et al., 2020). Moreover, the ratios of leaf to stem, growth rate and plant resistance to stress could be other factors influencing the chemical composition of plant (Melesse et al., 2012). Ünlü et al. (2022) showed that plant species greatly affected the chemical composition, gas and methane production, ME, and OMD of plants.

All of the measured parameters of GP and degradability differed between cultivars, as a result of the differed nutrient concentrations (Kholif et al., 2017). The activity and growth of ruminal microbes depend mainly on the type of diet fed. The cultivars Pioneer 30K08, SC-166Y, TWC-368Y and TWC-321W had the greatest GP, which may be due to the great concentration of CP and low fiber fractions and indicate great nutritive values of these cultivars for ruminants and that more available carbohydrates in these cultivars increasingly came to the rumen for microbial fermentation, resulting in greater kinetics of gas and total gas. On the other side, the cultivar SC-78Y showed the lowest GP, which may be due to the great fiber fractions concentrations. Gas production may be used as a good indicator of nutrient digestibility, fermentability and microbial protein production (Kholif et al., 2017). It is well documented that the nutrient contents (e.g., OM, CP, fat, NSC, and soluble fraction) of feeds affect their potential production of gas quantity, and the level of gas produced tends to decrease or increase with changing chemical content of feeds (Elghandour et al., 2016). Elghandour et al. (2016) observed a strong relationship between GP and CP and NSC, and a weak relationship between GP and NDF content. Talebi et al. (2022) reported a negative correlation with NDF and ADF and GP and DM degradability.

The greatest TDDM and TDOM with Pioneer 30K08, which may be due to the great concentration of CP and low concentration of fiber fractions and may partially explain the increased total GP. Moreover, the cultivars SC-166Y and TWC-321W showed he lowest TDDM and TDOM, which may be due to the great fiber fractions concentrations. Talebi et al. (2022) observed a positive correlation between DM degradability and NDF and ADF contents.

The low concentrations of ruminal NH_3-N observed with some cultivars (e.g., Pioneer 4444, and TWC-321W) may be due to the great fiber fractions concentrations. Pioneer 30K08, TWC-324W, SC-128W and SC-166Y showed the greatest CH_4 production which may be due to the low fiber fractions concentrations and great CP concentration.

Due to the great fiber fractions concentrations, the cultivars Pioneer 30K08, TWC-324W, SC-128W, SC-166Y and TWC-368Y had the greatest concentrations of acetate. Moreover, the cultivars SC-78Y had the lowest total VFA, propionate acetate and butyrate which may be due to the low fiber fractions concentrations. The cultivars Pioneer 30K08, TWC-368Y and TWC-321W showed the greatest propionate, which may be due to the great fiber fractions concentrations.

Effect of urea on chemical composition and ruminal fermentation from maize silage cultivars

The treatment of feeds with urea is an approach to increase N concentration and improve the nutritive value of ensiled feed (Sumadong et al., 2022). The increased CP concentrations with urea treatment is attributed to the conversion of ammoniated maize silage to NH_3–N as an end-product during the ensiling process (Saminathan et al., 2022). Increasing CP and NH_3–N in silage promotes the growth of ruminal microbiota when fed to animals (Sumadong et al., 2022).

Ensiling maize with urea increases NH_3 release, which in turn increases N concentrations in feeds rich in lignocellulosic matter and reduces cell wall fiber by reacting with lignocellulose. Moreover, urea serves as a delignifying agent through ammonification. This extensively disrupts fiber matrix and destroys the crystalline structures called microfibrils or lignocellulose biopolymers in maize silage during the ensiling process, thereby promoting microbial adhesion to the inner matrix of hemicellulose and cellulose (Zain et al., 2018).

The weak effects of urea treatment on ruminal total and individual VFA indicate improved synchronization between carbohydrate and N in the fermentation vessels resulting in speculating increased microbial protein synthesis. VFA concentration is balanced between feed nutrient degradation and majority uptake and incorporation into ruminal microbial protein synthesis (Herrera-Saldana et al., 1990).

Improving nutrient degradability coincides with decreasing fiber fraction contents and increased ruminal GP. Such effects may be associated with greater soluble sugar contents in treated maize cultivars, which is more accessible for rumen microbial degradation than in vitro fermentation of fiber. During ensiling, the treatment with urea treatment cleaves C–C bonds between lignin, cellulose, and other lignocellulosic compounds to convert them to simpler forms of carbohydrates or soluble sugar, resulting in increased digestibility of feed materials.

Decreasing the number of ruminal protozoa with some cultivars decreases predation of bacteria and facilitates N capture by bacteria (Jouany, 1996). Moreover, the lowered protozoal number may explain the lowered CH_4 production (Zhang et al., 2019). Urea treatment increased the concentrations of ruminal NH_3-N in almost all silages, which may be attributed to a great rate of urea breakdown in the incubation medium.

The inhibitory effects of urea treatment on CH_4 production might be associated with increased ammonium concentration in rumen fluids. Increasing ammonium concentration in the rumen could inhibit growth of methanogens, and thus reduce rumen methanogenesis (Zhang et al., 2018).

CONCLUSIONS

Maize cultivars showed a great effect on nutrient concentration, *in vitro* gas production, and nutritive value. Urea treatment of maize cultivars improved their chemical composition and *in vitro* fermentation, including improved DM and OM degradability and NH_3–N concentration and lowered CH_4 production. As observed for many parameters, the significant interactions between cultivars and urea treatment indicate that the effect of urea is cultivar dependent. The cultivars Pioneer 30N11, Pioneer 4444 and Pioneer 30K08 showed the greatest forage yield and nutritive value as a feed for large and small ruminants. Further research is needed to evaluate the effects of Pioneer 30N11, Pioneer 4444 and Pioneer 30K08 on milk production and beef production under farm conditions.

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