

Comparison of nutrient composition, fermentation quality and *in vitro* ruminal fermentation parameters of silages from different maize genotypes

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ABSTRACT. The aim of this study was to compare the chemical composition, fermentation quality, and *in vitro* ruminal fermentation characteristics of several silages from different maize genotypes. For this purpose, maize plants of six genotypes (G1–G6) were grown in the same field, harvested at the same time, and then ensiled in laboratory silage bags. Significant differences ($P < 0.05$) were observed among silages in dry matter, neutral detergent fibre, non-fibre carbohydrates, pectin + sugars, crude protein, ash, and ether extract contents ($P < 0.05$). Pectin + sugar contents varied between 12.70 and 22.44% ($P < 0.05$). Lactic and acetic acid concentrations also differed among genotypes ($P < 0.05$). *In vitro* ruminal fermentation values, including total gas production after 24 h, metabolizable energy, organic matter digestibility, net energy for lactation and concentrations of acetic, propionic and butyric acids differed significantly between silages ($P < 0.05$). Crude protein content was positively correlated with haemicellulose ($P < 0.05$), while acid detergent fibre and cellulose contents were negatively correlated with pectin, sugars and lactic acid. Pectin and sugar content was positively correlated with gas production after 24 h and lactic acid levels. These findings indicate that differences in carbohydrate fractions between maize genotypes may influence silage quality and *in vitro* ruminal fermentation characteristics, and consequently affect energy values and digestive responses in ruminants.

Introduction

In dairy cattle, fibrous feed raw materials are indispensable as they promote rumen mat formation and optimal ruminal fermentation. In addition to maintaining ruminal pH through their buffering effect and stimulation of rumination, forages supply carbohydrates that support microbial protein synthesis and increase short-chain fatty acid (SCFA)

production and fibrolytic bacterial population (Hua et al., 2022). Maize silage, with its high starch content (20–35%), moderate level of crude protein (CP; 5–8%) and adequate neutral detergent fibre (aNDFom) and acid detergent fibre (ADFom) composition (40–50% and 20–25%, respectively), supports rumen mat formation owing to its pectin and sugar content and is efficiently fermented in the rumen (Kara, 2024). Its digestible carbohydrate

(starch + non-resistant starch), soluble dietary fibre (pectin) and sugar contents indicate higher digestibility compared with other conventional forages such as alfalfa, meadow hay, and wheat straw (NRC, 2001, NASEM, 2021; Kara, 2024). The amylose-to-amylopectin ratio in starch content influences its degradation (Gómez et al., 2016), while the high solubility of pectin (Chandel et al. 2022), which ferments rapidly, may alter the fermentation rates of different maize silage genotypes.

The ruminal degradability of cellulose and hemicellulose, which are the homo-polysaccharide and hetero-polysaccharide components of maize silage plant cell walls, ranges from approximately 20 to 90% (Hua et al., 2022; Kara, 2024). Differences in their content and the degree of covalent bonding between them in silages from different maize genotypes may account for variations in ruminal digestibility and energy values.

Previous studies have reported significant differences in the nutritional composition of maize silage produced from different genotypes, including CP, aNDFom, ADFom, starch, pectin, dry matter (DM), silage acids, e.g. lactic acid (LA), and ruminal digestibility (Loučka et al., 2018; Alvarado-Ramírez et al., 2023; Kitaw et al., 2024; Khan et al., 2024). Another study found that while *in vitro* metabolizable energy (ME) and short chain fatty acid (SCFA) levels of fresh whole maize crops did not vary among genotypes, fermented (silage) maize crops showed significant variation (Alvarado-Ramírez et al., 2024). Zicarelli et al. (2025) observed no differences in SCFA values after 72-h *in vitro* fermentation of five maize genotypes, but recorded differences in branched short-chain fatty acid (BSCFA) content. Genotypic variations are an important criterion for identifying maize varieties with better forage potential under specific regional conditions. The high energy accumulation associated with the grain-containing ear structure distinguishes maize silage from other forage crops. Moreover, high-quality silage can be produced without additives and with minimal nutrient loss during ensiling. Maize silage is preferred in dairy cattle rations due to its high DM yield per unit area, good palatability, easy incorporation into total mixed rations (TMR), suitability for mechanised harvesting, and its value as an important source of physically effective NDF (Bal et al., 2000; NASEM, 2021).

A wide variety of commercial maize genotypes are used for silage production, which remains the most common forage type on dairy farms worldwide. Based on this, it was hypothesised that maize silage used for silage differ in quality and digestibility, which affects ruminal fermentation. Therefore, this

study aimed to evaluate the fermentative profile and chemical composition of different maize genotypes and to determine their influence on gas production and ruminal fermentation, using an *in vitro* system.

Material and methods

Maize genotypes

Six commercial maize genotypes used for silage production were evaluated in this study. The hybrids were coded as genotype_1, genotype_2, genotype_3, genotype_4, genotype_5, and genotype_6 (G1, G2, G3, G4, G5 and G6, respectively).

Herbage samples were collected from a commercial dairy farm in the Bünyan district of Kayseri province, Türkiye (38°56' N, 34°24' E). Maize was sown at a seeding rate of 2.5 kg per decare (1000m²) in June 2021 and harvested in September 2021, after 90 days of growth. Row spacing was 14 cm, and fertilisation included 40 kg/da of diammonium phosphate and urea. Irrigation was provided through a drip system 6 times at 1-week intervals. Representative samples were collected from three 1 m² areas. At harvest, all plants were at approximately the three-quarter milk line stage. The genotypes belonged to two FAO-700 maturity groups: G1, G3, and G6 were late-maturing, while G2, G4, and G5 were medium-late. Average plant heights for G1–G6 were 266, 258, 304, 299, 274, and 289 cm, respectively, and the heights of the first ear emergence were 115, 92, 121, 120, 127, and 105 cm, respectively.

Maize herbage samples were harvested 10 cm above ground level and chopped into approximately 2–3 cm pieces to produce laboratory-scale silages. Each polyethylene vacuum bag contained about 500 g of silage material and was sealed using a laboratory-type vacuum machine (Caso VC100, Caso Design, Sauerland, Germany). In total, 30 silages were prepared (6 genotypes × 5 replicates) and opened after 45 days of fermentation.

Determination of silage chemical composition

Maize silages were dried in a dryer (Binder, Germany) for 24 h at 60 °C and then 12 h at 105 °C to determine DM content. The dried samples were ground using a grinder mill (IKA Werke, Germany) to a maximum particle size of 1 mm. DM, ash, CP (nitrogen × 6.25), and diethyl ether extract (EE) contents were determined according to the AOAC International (1995) methods. aNDFom and ADFom were analyzed following van Soest et al. (1991), while non-fiber carbohydrates

(NFC) were calculated according to NRC (2001). All assays were carried out in triplicate. For each silage replicate, three chemical analyses were conducted, resulting in a total of 15 analyses per genotype (5 replicates \times 3 analyses).

Fermentative profile

The pH of opened silages was determined directly after opening. Wet maize silage samples (25 g) were homogenised for 15 s in a laboratory type blender (Waring, USA), then 100 ml of distilled water was added, and filtered mixture was measured using a digital pH meter (Mettler Toledo, S220 pH/ion meter, Ohio, USA).

For SCFA and BSCFA analysis, 1.5 ml of silage fluid was mixed with 0.3 ml of meta-phosphoric acid (25%, w/v) in a microcentrifuge tube and centrifuged at 15 000 rpm for 15 min. The supernatants were quantified using a gas chromatograph (GC, Thermo Trace 1300, Thermo Scientific, Waltham, Massachusetts, USA) equipped with FID detector. Separation was achieved on a polyethylene glycol column (60 m \times 0.25 mm \times 0.25 μ m, TG-WAXMS, Thermo Scientific). The operation procedure followed the protocol of Ersahince and Kara (2017).

In vitro ruminal fermentation test

The *in vitro* digestibility of maize silages was assessed using an *in vitro* gas production technique (Menke and Steingass, 1988). As the procedure utilised only fresh rumen content without live animal experimentation, the study was approved by the Local Ethics Committee for Animal Experiments of Erciyes University, Kayseri, Türkiye. Fresh rumen fluid was collected via the oesophageal tube from two Brown Swiss Simmental crossbreed cows to obtain a total of 1 l of inoculum. The cows were fed 9.8 kg/day DM consisting of 5 kg/day maize silage, 4.5 kg/day wet sugar beet pulp, 1.7 kg/day wheat straw, 1.5 kg/day lucerne hay, and a concentrated mix feed. The total mix feed contained 12.6% CP, 40.3% NDF in DM, and 2479 kcal/kg DM of ME (NRC, 2001). The buffer mixture used for *in vitro* fermentation included buffer solution, macro-mineral solution, resazurin solution, trace-mineral solution, and reducing solution. The oxidation-reduction potential and pH of the fermentation fluid were measured using a pH-Ion meter (Seven CompactTM pH/ Ion S220, Mettler-Toledo AG, Schwerzenbach, Switzerland) with InLab[®] Expert Pro-ISM sensor probes (Switzerland).

The samples were incubated in 100 ml anaerobic glass fermenters (Model Fortuna, Häberle

Labortechnik, Germany) containing 20 ml buffer mixture and 10 ml filtered rumen fluid, following the procedures by Menke et al. (1979). Each fermentation contained 200 \pm 10 mg of dried silage sample with 20 ml of buffer mixture and 10 ml of filtered rumen fluid. The fermenters were maintained at 39 \pm 0.5 °C and incubated for 24 h. Initial gas volumes were recorded at the start of incubation. For each silage replicate, three chemical analyses were conducted, resulting in a total of 15 analyses per genotype (5 replicates \times 3 analyses). Three blank fermenters containing only rumen fluid and buffer mixture were included to correct for background gas production.

ME, organic matter digestion (OMD), and net energy lactation (NEL) values of silages were calculated using the equations from Menke and Steingass (1988).

In vitro ruminal fermentation parameters

The total gas volume produced after 24 h of *in vitro* incubation was recorded, and 10 ml of digestion fluid were frozen at -20 °C until analysis. Samples were centrifuged at 15 000 rpm for 15 min in a micro-centrifuge (Gyrozen 1524, Gyrozen Co. Ltd., Daejeon, Korea). Supernatant was mixed with 25% (w/v) metaphosphoric acid (1.25:0.25 ml). SCFA and BSCFA were analysed using the same GC-FID methods and instrumentation previously described for the silage samples.

The ammonia-nitrogen concentration in the *in vitro* fermentation fluid was determined using a commercial assay kit (Megazyme, K-AMIAR 02/20, Wicklow, Ireland). Briefly, 0.10 ml of fermentation fluid was mixed with 2.0 ml of distilled water, 0.3 ml of buffer containing 2-oxoglutarate and sodium azide (0.02% w/v), and 0.2 ml of NADPH. Absorbance was read at 340 nm, after which glutamate dehydrogenase suspension was added, and absorbance was measured again at 340 nm. Ammonia-nitrogen content (mg/l) was calculated according to the kit instructions (Megazyme, K-AMIAR 02/20, Wicklow, Ireland).

Statistical analysis

Statistically significant differences between maize genotypes for silage quality and *in vitro* digestion variables were evaluated using one-way analysis of variance. The Duncan multiple comparison test was applied to separate means at a significance level of $P < 0.05$. Multivariate analyses were performed using General Linear Model procedures assuming homogeneous variances.

Pearson's correlation coefficients were calculated to assess the relationships among variables examined. All statistical procedures were executed using SPSS 17.0 software (IBM Corp., Armonk, NY, USA). Differences between medium-late and late varieties were evaluated using an independent samples t-test. Mean differences (Late – Medium-late) and their 95% confidence intervals were calculated and visualized using a forest plot.

Results

Chemical composition and fermentative profile of silages

The different maize genotypes grown for silage in the same season and field, and harvested at the same phenological stage, differed in DM, aNDFom, NFC, pectin + sugars, CP, ash, and EE contents. The highest DM value was 30.84% in G2 (medium-late variety), and the lowest was 23.85% in G5 (medium-late variety, $P < 0.001$). The lowest aNDFom content was 40.70% in G2, while the highest values were 49.67, 49.11, and 48.95% in G6 (late variety), G5, and G4 (medium-late variety), respectively (Table 1) ($P < 0.05$).

The ash contents of the silages ranged from 5.96 to 7.87% and the EE content from 0.79 to 1.54%. The lowest ash and EE contents were found in G1 (late variety) ($P < 0.001$). Starch and lignin contents did not show statistically significant differences between maize silages from different genotypes (Table 1).

No differences were recorded in silage pH. However, differences were found between the genotypes regarding LA and AA levels. The highest silage LA content was 7.72% in G2 (medium-late variety) and the lowest was 2.74% in G3 (late variety) ($P < 0.05$). The AA concentrations in G1, G2, and G6 (late varieties) were lower than in G5 ($P < 0.05$). PA, BA, VA, IBA, and IVA concentrations were similar in all silages ($P > 0.05$) (Table 2).

Gas production and ruminal fermentation parameters

G2 had the highest *in vitro* total gas production, ME, OMD, and NEL values, which were comparable to those of G1 and G6. However, *in vitro* total gas production in G2 was higher than in G3, G4, and G5 ($P < 0.05$). The ammonia-N concentration in the *in vitro* fermentation fluid of the six different maize silages did not differ significantly ($P > 0.05$) (Table 3).

Table 1. Chemical compositions of silages from different maize genotypes

	Precocity (FAO-700)	DM	aNDFom	NFC	CHO	ADFom	starch	HC	CEL	pectin+sugars	CP	ADL	ash	EE
G1	Late variety	29.88 ^{ab}	47.59 ^{ab}	38.11 ^{ab}	85.70	25.16 ^{ab}	20.67	22.42 ^a	20.08 ^{ab}	17.43 ^{ab}	7.55 ^a	5.08	5.96 ^d	0.79 ^d
G2	Medium-late variety	30.84 ^a	40.70 ^b	44.09 ^a	84.79	21.75 ^b	21.64	18.94 ^{ab}	15.16 ^b	22.44 ^a	7.07 ^a	6.60	7.25 ^b	0.89 ^d
G3	Late variety	26.90 ^{bc}	48.19 ^{ab}	38.45 ^{ab}	86.64	31.28 ^a	22.22	16.91 ^b	24.03 ^a	16.23 ^{ab}	4.90 ^b	7.25	6.91 ^c	1.54 ^{ab}
G4	Medium-late variety	27.02 ^{bc}	48.95 ^a	34.37 ^b	83.32	26.17 ^{ab}	22.30	22.77 ^a	20.88 ^{ab}	14.73 ^{ab}	7.83 ^a	5.29	7.87 ^a	0.98 ^d
G5	Medium-late variety	23.85 ^c	49.11 ^a	35.91 ^{ab}	85.02	30.58 ^a	20.63	18.53 ^{ab}	23.35 ^a	15.27 ^{ab}	6.41 ^{ab}	7.24	7.34 ^b	1.23 ^{bc}
G6	Late variety	29.78 ^{ab}	49.67 ^a	34.31 ^b	83.98	27.42 ^{ab}	22.61	22.25 ^a	20.96 ^{ab}	12.70 ^b	7.07 ^a	6.46	7.30 ^b	1.66 ^a
SD		3.22	3.64	4.11	2.43	1.62	1.50	3.67	2.10	3.98	1.19	2.59	0.60	0.35
SEM		0.44	0.86	0.97	0.45	0.29	0.35	0.87	0.38	0.93	0.28	0.61	0.14	0.08
P-values		<0.001	0.021	0.025	0.145	0.022	0.479	0.035	0.033	0.026	0.008	0.895	<0.001	<0.001

DM – dry matter as % in wet basis, aNDFom – neutral detergent fibre without ash and analysed with α -amylase as % in DM, NFC – non-fibre carbohydrate as % in DM, CHO (carbohydrates) – aNDFom + NFC, as % in DM, ADFom – acid detergent fibre without ash as % in DM, HC – haemicellulose (NDF – ADF) as % in DM, CEL – cellulose (ADF – ADL) as % in DM, CP – crude protein as % in DM, ADL – acid detergent lignin as % in DM, EE – diethyl ether extract as % in DM, pectin + sugars – pectin + monosaccharides + disaccharides + sugar alcohols (polyols) as % in DM; G1, G2, G3, G4 G5 and G6 - Genotype 1, 2, 3, 4, 5 and 6, respectively; SD – standard deviation, SEM – standard error of means. ^{ab} – means within a column with different superscripts are significantly different

The highest NFC content was 44.09% in G2, and the lowest values were 34.31% in G6 and 34.37% in G4 ($P < 0.05$). The pectin+sugar contents varied between 12.70 and 22.44% among silages, with the highest value determined in G2 ($P < 0.001$). The CP content of the silages ranged from 4.90 to 7.83%, with G3 (late variety) showing the lowest value ($P < 0.01$) (Table 1).

The highest AA molarity in the *in vitro* fermentation fluid was determined in G2 (47.37 mmol/l). The AA molarity of G2 in the *in vitro* fermentation fluid was similar to that of G4, but statistically higher than those of other silages ($P < 0.05$) (Table 4).

The molarities of PA and BA in the *in vitro* fermentation fluid of G2 were highest in G2 among

Table 2. Fermentation acids in silages from different maize genotypes

	pH	LA	AA	PA	BA	VA	IBA	IVA
		DM, %						
G1	3.40	5.20 ^{ab}	0.50 ^b	0.13	0.16	0.01	0.01	0.02
G2	3.49	7.72 ^a	0.51 ^b	0.14	0.16	0.01	0.01	0.02
G3	3.38	2.74 ^b	0.58 ^{ab}	0.15	0.17	0.01	0.01	0.02
G4	3.38	3.89 ^b	0.62 ^{ab}	0.15	0.19	0.02	0.01	0.02
G5	3.49	3.93 ^b	0.79 ^a	0.20	0.21	0.02	0.01	0.02
G6	3.57	2.86 ^b	0.50 ^b	0.14	0.15	0.01	0.01	0.02
SD	0.190	2.26	0.132	0.033	0.034	0.003	0.001	0.003
SEM	0.03	0.53	0.031	0.008	0.008	0.001	0.000	0.001
<i>P</i> -values	0.105	0.030	0.011	0.062	0.295	0.147	0.091	0.254

LA – lactic acid, AA – acetic acid, PA – propionic acid, BA – butyric acid, DM – dry matter, VA – valeric acid, IBA – *iso*-butyric acid, IVA – *iso*-valeric acid; G1, G2, G3, G4 G5, and G6 – genotype 1, 2, 3, 4, 5 and 6, respectively; SD – standard deviation, SEM – standard error of means; ^{ab} – means within a column with different superscripts are significantly different

Table 3. *In vitro* gas production, estimated ruminal degradation parameters and ammonia-nitrogen concentration of silages from different maize genotypes

	Gas24h	ME	OMD	NEL	Ammonia-N
G1	71.58 ^{ab}	11.98 ^{ab}	78.89 ^{ab}	7.91 ^{ab}	72.14
G2	74.60 ^a	12.39 ^a	81.56 ^a	8.25 ^a	70.75
G3	58.34 ^c	10.16 ^c	67.01 ^c	6.38 ^c	74.41
G4	61.83 ^{bc}	10.65 ^{bc}	70.25 ^{bc}	6.79 ^{bc}	60.93
G5	59.83 ^{bc}	10.38 ^{bc}	68.40 ^{bc}	6.55 ^{bc}	72.38
G6	64.85 ^{abc}	11.06 ^{abc}	72.90 ^{abc}	7.13 ^{abc}	69.88
SD	8.12	1.11	7.24	0.94	7.79
SEM	1.91	0.26	1.71	0.22	1.84
<i>P</i> -values	0.042	0.041	0.041	0.041	0.380

DM – dry matter, Gas24h – total gas production as ml/0.2 g DM silage maize for 24 h *in vitro* ruminal incubation, ME – metabolic energy calculated from Gas24h, as MJ/kg DM, OMD – organic matter digestion calculated from Gas24h, % in DM, NEL – net energy lactation for dairy cattle calculated from Gas24h, as MJ/kg DM; Ammonia-N – ammonia nitrogen of the *in vitro* ruminal fermentation fluid; G1, G2, G3, G4 G5, and G6 – genotype 1, 2, 3, 4, 5 and 6, respectively; SD – standard deviation, SEM – standard error of means; ^{ab} – means within a column with different superscripts are significantly different

Table 4. *In vitro* ruminal fermentation parameters of silages from different maize genotypes, mmol/l

	AA	PA	BA	VA	IVA	IBA	TSCFA	BSCFA	SCFA
G1	35.57 ^b	7.90 ^{ab}	7.43 ^{ab}	0.60	0.78	0.37 ^{ab}	52.65 ^{ab}	1.15 ^{ab}	51.51 ^{ab}
G2	47.37 ^a	9.75 ^a	7.73 ^a	0.67	0.87	0.44 ^a	66.83 ^a	1.31 ^a	65.52 ^a
G3	34.57 ^b	7.40 ^{ab}	6.20 ^b	0.52	0.68	0.34 ^b	49.71 ^b	1.02 ^b	48.70 ^b
G4	37.22 ^{ab}	7.39 ^{ab}	6.96 ^{ab}	0.55	0.73	0.36 ^{ab}	53.22 ^{ab}	1.10 ^{ab}	52.12 ^{ab}
G5	34.25 ^b	7.33 ^{ab}	6.72 ^{ab}	0.48	0.71	0.34 ^b	49.85 ^b	1.06 ^b	48.79 ^b
G6	32.29 ^b	7.15 ^b	6.14 ^b	0.53	0.74	0.35 ^b	47.22 ^b	1.09 ^b	46.13 ^b
SD	6.03	1.20	0.44	0.08	0.09	0.05	8.15	0.14	8.04
SEM	1.42	0.28	0.12	0.02	0.02	0.01	1.92	0.03	1.89
<i>P</i> - values	0.006	0.039	0.008	0.067	0.141	0.055	0.015	0.106	0.015

AA – acetic acid, PA – propionic acid, BA – butyric acid, VA – valeric acid, IVA – *iso*-valeric acid, IBA – *iso*-butyric acid, TSCFA – total short chain fatty acids (TSCFA = BSCFA + SCFA), BSCFA – branched short chain fatty acids (BSCFA, mmol/l = IBA + IVA), SCFA – straight short chain fatty acids (SCFA, mmol/l = AA + PA + BA + VA). G1, G2, G3, G4 G5, and G6 – genotype 1, 2, 3, 4, 5 and 6, respectively; SD – standard deviation, SEM – standard error of means; ^{ab} – means within a row column with different superscripts are significantly different

the maize genotypes grown for silage. The PA molarity differed between G2 and G6 ($P < 0.01$), while no statistical differences were observed for other silages ($P > 0.05$). Iso-acid molarities in the *in vitro* ruminal fermentation fluid of maize silages varied among genotypes, withdiffered, and the IBA and BSCFA molarities

of ruminal fermentation fluid in G2 were higher than those of G3, G5, and G6 ($P < 0.05$) (Table 4).

The highest TSCFA and SCFA levels in the *in vitro* ruminal fermentation fluid of the examined corn silage species were detected in G2. The molarities of TSCFA and SCFA in G2 ruminal

Table 5. Pearson's correlation coefficients between silage chemical composition and *in vitro* digestion variables examined of silages from different maize genotypes

	EE	CP	aNDFom	ADFom	ADL	CEL	HC	NFC	Starch	Pectin+sugars	Gas24h	LA
Ammonia-N	0.178	-0.503*	-0.255	0.125	0.380	-0.082	-0.429*	0.405	0.079	0.060	0.058	0.191
EE		-0.471*	0.401	0.564*	0.229	0.404	-0.275	-0.348	0.355	-0.540*	-0.360	-0.604**
CP			0.031	-0.358	-0.073	-0.293	0.481*	-0.271	-0.053	-0.020	0.466	0.159
aNDFom				0.639**	-0.062	0.624*	0.272	-0.956**	0.234	-0.891**	-0.621**	-0.527*
ADFom					0.130	0.858*	-0.566	-0.538	0.093	-0.617**	-0.357	-0.508*
ADL						-0.397	-0.229	0.049	-0.058	-0.035	0.026	0.100
CEL							-0.406	-0.523	0.116	-0.553*	-0.344	-0.521*
HC								-0.351	0.135	-0.183	-0.219	0.070
NFC									-0.263	0.884**	0.507*	0.501*
Starch										-0.502*	-0.275	-0.128
Pectin+sugars											0.537*	0.561*
Gas24h												0.438*

DM – dry matter, EE – diethyl ether extract as % in DM, CP – crude protein as % in DM, aNDFom – neutral detergent fibre without ash and analysed with α -amylase as % in DM, ADFom – acid detergent fibre without ash as % in DM, ADL – acid detergent lignin as % in DM, CEL – cellulose, HC – haemicellulose as % in DM, NFC – non-fibrous carbohydrate as % in DM, Gas24h – total gas production as ml/0.2 g DM silage maize for 24 h *in vitro* ruminal incubation, LA – lactic acid; * – correlation is significant at the 0.05 level, ** – correlation is significant at the 0.01 level

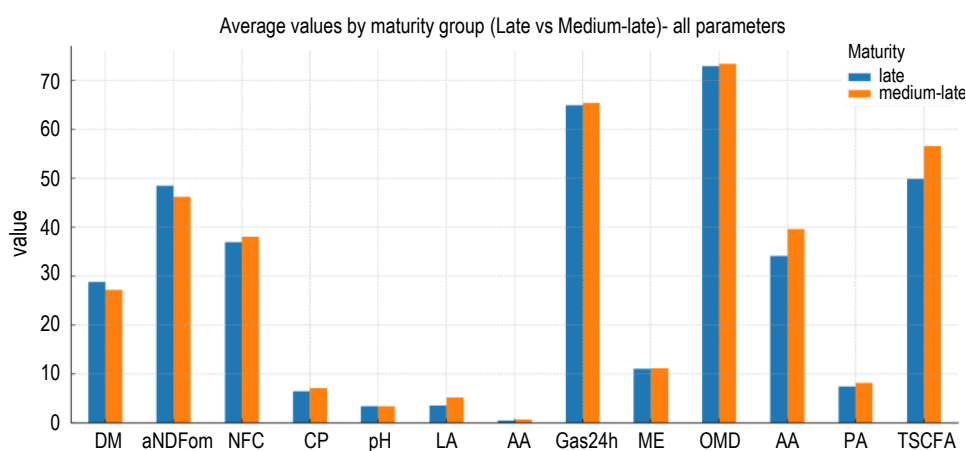
fermentation fluid were higher than those of G3, G5, and G6 ($P < 0.05$) (Table 4).

The ammonia-N concentration of the *in vitro* rumen fluid was negatively correlated with the aNDFom ($P < 0.05$) and HC ($P < 0.05$). The EE content of silages was negatively correlated with CP ($P < 0.05$), pectin + sugars ($P < 0.05$), and the LA ($P < 0.05$). The silage CP content was positively correlated with HC content ($P < 0.05$). Silage aNDFom content was positively correlated with ADFom and CEL, but negatively correlated with NFC, pectin + sugars, Gas24 and LA ($P < 0.05$). Silage ADFom and cellulose contents were negatively correlated with pectin + sugars and silage LA ($P < 0.05$). Silage NFC content was positively correlated with pectin + sugars, Gas24h

and LA ($P < 0.05$). Silage pectin + sugars content was positively correlated with Gas24 and LA. Gas24 value of silages ($P < 0.05$) was positively correlated with silage LA content (Table 5).

Maturity class of maize silage genotypes

The comparison of corn silage from medium-late and late varieties showed limited differences in chemical composition and fermentation characteristics. Only ash content was significantly higher in medium-late varieties $P = 0.005$. No significant differences were observed for ammonia, CP fibre fractions (aNDFom, ADF, ADL, cellulose, haemicellulose) or energy-related parameters (ME, OMD, and NEL) ($P > 0.05$) (Figure 1).

**Figure 1.** Comparison of medium and medium-late maize genotypes based on silage characteristics

DM – dry matter ($P = 0.430$; SD = 3.51), aNDFom – acid detergent fibre ($P = 0.478$; SD = 3.43); NFC – non-fibrous carbohydrate ($P = 0.039$; SD = 3.48); CP – crude protein ($P = 0.034$; SD = 1.19); pH – pH value ($P = 0.874$; SD = 0.19); LA – lactic acid ($P = 0.014$; SD = 2.26); AA – acetic acid of silage ($P = 0.540$; SD = 0.13); Gas24h – total gas production for 24 h ($P = 0.040$; SD = 8.12); ME – metabolizable energy ($P = 0.031$; SD = 1.11); OMD – organic matter digestibility ($P = 0.031$; SD = 7.24); AA – acetic acid of fermentation fluid ($P = 0.057$; SD = 6.03); PA – propionic acid of fermentation fluid ($P = 0.058$; SD = 1.20); TSCFA – total short chain fatty acids ($P = 0.017$; SD = 8.15)

Discussion

Chemical composition and fermentative profile of silage

In the present study, although all silage genotypes were harvested in the same phenological stage, it was observed that silage DM values vary between approximately 24 and 31%. Genotypes with higher DM also showed higher *in vitro* digestibility, NFC and pectin + sugar contents. Consistent with these results, previous studies reported that DM values increased from 20.1 to 37.5% for soft dough, early dent, $\frac{1}{2}$ milkline or $\frac{3}{4}$ milkline phenological stages of different silage types, and differed between genotypes for $\frac{3}{4}$ milkline phenological stage (Silva et al., 2020). It is well documented that as DM in maize silage increases from 25 to 40%, starch digestibility decreases from approximately 80 to below 50% (Michalet Doreau and Philippeau, 1999). In this experiment, starch content remained stable across the 24–31% DM range for the same phenological stage, but *in vitro* ruminal digestion parameters differed between individual genotypes. Degener and Kappas (2015) observed that although rising temperatures were generally beneficial for late varieties, prolonged exposure above the optimal 25–30°C range during the growing season reduced photosynthesis rates, and the negative impact of summer temperatures on yield was expected to increase over time.

The differences in the CP content of maize silages observed in this study are consistent with previous reports showing variation between genotypes (Jiang et al., 2022; Loučka et al., 2018). This study identified genotypes with both low (4.9%), and high CP (7.83%) values, which was consistent with the range reported in the existing literature. Supporting the current results, a study conducted in Türkiye reported that CP content in silages from 15 different mid-late maize genotypes (FAO 650-700) varied between 6.29 and 11.25% (Taş and Uçak, 2020). In contrast, Kitaw et al. (2024) reported lower CP levels for different maize silages than those observed here. These discrepancies in CP content between studies can be generally attributed to differences in the leaf, stem and ear ratios in individual genotypes (Loučka et al., 2018; Jiang et al., 2022; Khan et al., 2024).

Haemicellulose, cellulose and pectin are components of the microfibril structures in the primary and secondary cell walls of plants (Kara, 2024). Pectin, located in the intercellular junction and the

outer layer of the primary cell wall, is a soluble carbohydrate that, together with sugars, contributes to carbon dioxide production and helps establish optimal fermentation acidity in maize silage. In the present study, pectin + sugar contents varied between 13% and 22% among maize genotypes. A previous study reported pectin and sugar contents in maize silage in an 8–12.5% range (Kara et al., 2022). It is important to note that the term ‘sugar contents’ in this context includes mono-saccharides, di-saccharides and sugar alcohols. Cone et al. (2008) reported slight variation in sugar proportions of maize silages in the range of 3.4–4.7%, depending on the harvest time. Genotype G2, with high pectin and sugar contents, also showed elevated *in vitro* ruminal fermentation, total gas production, ME, OMD, and NEL values. Pectin is an NFC component, and NFC content in this study ranged from 34 to 44% among genotypes, which was consistent with previous reports (Jiang et al., 2022; Khan et al., 2024). NFC positively influences both ensiling fermentation process and subsequent *in vitro* ruminal fermentation (Ersahince and Kara, 2017; Kara et al., 2022). The present correlation analysis demonstrated that the *in vitro* total gas production of the different maize silages increased in relation to their pectin content. This positive relationship also affected silage lactic acid content, thereby directly influencing its acidity. Consequently, pectin and sugars may additionally increase silage acidity and its oxidative stability.

Fermentation of silages is necessary to achieve optimal acidity, which requires the presence of fermentable carbohydrates for lactic acid bacteria. In the study, the total carbohydrate content of maize silages ranged from 83 to 87%, with starch, hemicellulose, pectin and sugars, present at sufficient levels to support proper fermentation. Starch content did not differ significantly among the genotypes, ranging from approximately 21 to 23%. This finding contrasts with previous studies which reported significant variations in starch content between silage prepared from different maize genotypes (Loučka et al., 2018; Jiang et al., 2022; Khan et al., 2024). Silva et al. (2020) observed that starch content of silages increased from 5.5 to 25.8% as two maize genotypes progressed through soft dough, early dent, $\frac{1}{2}$ milkline, and $\frac{3}{4}$ milkline stages. In the current study, the starch content of individual silages at the $\frac{3}{4}$ milkline stage was comparable to the results of Silva et al. (2020).

Cellulose is a polymer composed of several thousand D-glucose units linked by β -(1→4)-glycosidic bonds (Niwiska, 2012). Haemicelluloses are complex

heteropolymers that vary considerably in their composition and degree of branching and can be classified as xylans, xyloglucans, mannans and mixed-linked β -glucans (Kara, 2024). Haemicellulose contains both readily fermentable and more resistant components, particularly xylan groups, which can associate with cellulose microfibrils during plant development and limit its microbial degradation. In maize harvested for silage at the end of the phenological period, haemicellulose content was approximately 17–23%, but it did not adversely affect ruminal fermentation *in vitro*. Changes in aNDFom and ADFom levels among different genotypes were consistent with the results of Loučka et al. (2018).

Lignin, a complex phenolic polymer, is indigestible by rumen microorganisms, but its presence decreases the digestibility of structural carbohydrates. The primary mechanism for this reduction is the formation of strong covalent bonds between lignin and cell wall polysaccharides, which creates a physical barrier that hinders access for microbial hydrolases (Liu et al., 2018; Kara, 2024). In the current experiment, lignin content did not differ between the silages and remained low, reflecting the limited lignification of the plants at the harvest stage.

The pH value, a key indicator of silage acidity, was found to range from 3.38 and 3.57 between silages, while a previous study reported values of 3.60–4.20 for six different maize silages (Jiang et al., 2022; Khan et al., 2024). LA, produced through sugar fermentation by homofermentative lactic acid bacteria, is the most abundant organic acid formed during optimal fermentation (Broberg et al., 2007). Differences in LA content may result from variations in genotype, chemical composition, climate, soil structure and season. Here, LA content, essential for ideal silage fermentation, ranged from 2.86 to 7.72% of DM. Loučka et al. (2018) reported LA levels of 7.1 and 7.2% of DM in two different maize silages. On the other hand, Jiang et al. (2022) and Khan et al. (2024) also observed variation in LA content of silages produced from different maize genotypes. The silage with the highest LA content in the present study had the lowest acetic acid content, which was in line with previous findings (Jiang et al., 2022). AA and PA are present in silage and contribute to mould inhibition and other silage-specific properties, but their excessive levels are undesirable. In the presented study, AA content was below 1% of DM, with slight variation between the silages.

Gas production and ruminal fermentation parameters

The *in vitro* gas production technique provides insight into the ruminal degradation rates of carbohydrates in feedstuffs. In this study, gas production, an indicator of *in vitro* digestibility, was highest in G1 and G2, likely reflecting their elevated NFC and pectin + sugar contents. Digestible and readily fermentable carbohydrates increase ruminal gas production more significantly and consequently elevate the calculated digestion parameters, namely ME, NEL, and OMD. This interpretation is supported by the positive correlation determined between the silages' LA content, their pectin and sugars contents, and *in vitro* ruminal gas production. Genotype G2, a medium-late variety, contained high NFC and low NDFom values, resulting in increased gas production and *in vitro* ruminal fermentation. These findings suggest that higher levels of soluble carbohydrates accelerated silage degradation during *in vitro* ruminal fermentation and increased gas production. Although cumulative gas production over 24 h was not measured, and thus gas kinetics could not be determined, the observed differences in total gas production were attributed to variations in soluble carbohydrate contents. It cannot be conclusively stated that late and medium-late varieties differ consistently in terms of *in vitro* gas production and digestibility. Nevertheless, the results demonstrate that superior genotypes exist within both maturity periods.

Carbohydrates fermented during *in vitro* ruminal fermentation are broken down into monomers and subsequently catabolised into SCFA and BSCFA (Kara et al., 2024). Thus, a positive relationship is expected between the TSCFA concentration in the *in vitro* ruminal fermentation fluid and total gas production. In this study, G2 had the highest concentrations of TSCFA, SCFA, BSCFA, AA, PA, and BA, as well as the highest *in vitro* gas production and digestion values, which was an expected result. Differences in TSCFA concentrations between genotypes was similar with the results reported for maize silages of different genotypes in a previous study (Silva et al., 2020).

Maturity class of maize silages

The results indicate that corn silage maturity had a limited on most nutritional and fermentation parameters, as no significant differences in mineral accumulation during plant development. Overall both maturity groups showed comparable feeding value, suggesting flexibility in harvest timing without major changes in silage quality.

Conclusions

Considerable differences were observed in the structural (cellulose, haemicellulose), digestible (starch), and fermentable-absorbable (pectin and sugars) carbohydrate fractions of the six silages produced from individual maize genotypes, which corresponded to variations in silage acidity and *in vitro* ruminal fermentation characteristics. The differing carbohydrate profiles and protein contents of maize silage genotypes are factors that may subsequently impact gut function and overall energy availability. At the same harvest time, medium-late and late genotypes showed comparable forage quality with only minor differences in specific compositional traits.

Conflict of interest

The Authors declare that there is no conflict of interest.

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