Effect of Spring Maize Genotypes on Fermentation and Nutritional Value of Whole Plant Maize Silage in Northern Pakistan

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Abstract: In the current study, we evaluated the growth, biomass yield, ensiling, and nutritional characteristics of spring maize genotypes grown under the climatic conditions of Northern Pakistan. Six promising spring maize genotypes were grown under uniform standard agronomic conditions in 72 plots (8 m × 10 m), blocked within three replicate fields. Maize crops were harvested at targeted dry matter (DM) content (33 g/100 g DM). Data were collected on plant phonological characteristics and biomass yield, and then the harvested crop of each plot was chopped, and subsamples were ensiled in three replicate 1.5 L laboratory silos (n = 12/genotype). After 90 days of ensiling, subsamples were analyzed for fermentation quality, nutrient composition, Cornell Net Carbohydrate and Protein System (CNCPS) carbohydrate subfractions, digestible nutrients, metabolizable energy (ME), and in vitro dry matter digestibility (DMD). Results revealed large differences (p < 0.001) among maize genotypes in the yields (tons/ha) of DM (13.0 to 17.9), crude protein (CP; 0.83 to 1.24), and starch (4.16 to 6.67). Except for total carbohydrates (CHO) and NH3-N, the contents of all measured chemical components varied (p < 0.001) among the spring maize genotypes. Similarly, all reported CNCPS subfractions varied (p < 0.01) among the genotypes, except for the non-digestible (CC) subfraction. Among the genotypes, there were large variations in the contents (g/100 g DM) of CP (6.60 to 8.05), crude protein (CP; 0.83 to 1.24), and starch (4.16 to 6.67). Except for total carbohydrates (CHO) and NH3-N, the contents of all measured chemical components varied (p < 0.001) among the spring maize genotypes. Similarly, all reported CNCPS subfractions varied (p < 0.01) among the genotypes, except for the non-digestible (CC) subfraction. Among the genotypes, QPM300 had the highest values and genotype Azam having the lowest values. It was concluded that QPM300 is the most suitable spring maize genotype for silage production in terms of yields and silage nutritional and fermentation quality under the environmental condition of Northern Pakistan.

Keywords: maize silage; genotype; fermentation; metabolizable energy; carbohydrate subfractions

1. Introduction

Globally, whole-crop maize silage is one of the common forage source for dairy animals due to its high yielding capacity under diverse environmental conditions, good ensiling...
quality, high starch content and metabolizable energy (ME), palatability, ease of incorporation in the total mix ration [1,2], and support for high dry matter (DM) intake and yields of milk and milk protein [3–5]. In Pakistan, the dairy sector has been rapidly changing from subsistence smallholders to commercial farming over the last decade. This has triggered an enormous increase in the production and feeding of maize silages. Khan et al. [6] conducted a multivariate analysis on all possible variables affecting maize silage quality and reported that most of the variation in maize silage quality is caused by the large differences in maturity at harvest and the genetic variation among the commercial maize genotypes. Other studies also suggest that the genotype and season of cultivation are the key factors that mainly influence the yield and chemical composition of maize silages [7,8].

Selection of suitable maize genotype for quality maize silage production is one of the most important factors [2], largely affecting the yield, starch:neutral detergent fiber (NDF) ratio, ruminal NDF degradability, site (rumen vs. small intestine) of starch digestion, and rate of starch fermentation in the rumen [2,9,10]. In the past, plant breeders put more emphasis on the high yield of maize grains, ignoring the nutritive value of the stover part [11]. For silage production, it is important to consider the degradability of the stover (NDF) fraction and evaluate the nutritional value of the whole crop for silage production. Whole-plant maize silage has higher nutritional value and supports higher intake and milk yield than other silages and is the most common feed for ruminants worldwide [2,5]. New maize genotypes have been developed for the production of silage with high grain-to-stover ratio [11–15] and NDF digestibility [2]. Although starch is completely digested in the whole tract of dairy cows, the site of digestion (rumen vs. small intestine) greatly varies due to maize genotypes that affect not only the rate and extent of starch fermentation in the rumen but also the total energy supply to the animal [2,14,15].

In Pakistan, maize is traditionally sown in autumn for grain production. However, for silage production, the crop is now grown in both the spring and autumn seasons. The growing conditions of spring maize are quite different from those of autumn maize, as the day length increases and there is also a very significant increase in temperature (up to 40 °C) with the progress in the growing season. To our knowledge, maize growth, changes in plant composition, and the subsequent changes in nutritional and fermentation quality and digestibility of the silages from different spring maize genotypes have not been evaluated under such conditions. Therefore, the current study was designed to evaluate promising (high-yielding) spring cultivars/hybrids for silage production in terms of (1) DM, starch, and crude protein (CP) yields; (2) silage fermentation quality; and (3) nutritive value, ME, and digestibility (in vitro) of the ensiled whole crop.

2. Materials and Methods

2.1. Experimental Design and Crop Management

The field study was conducted in the research fields of the University of Agriculture Peshawar (34°02’ North latitude, 71°48’ East longitude, and 347 m above the sea level), Pakistan. Peshawar has a semi-arid, subtropical climatic condition and is situated in the northwest part of the country. Six promising spring maize genotypes were selected for the study, namely P1543 and P1429 from Pioneer; DK 9108 from Monsanto; quality protein maize (QPM)200 and QPM300 from International Maize and Wheat Improvement Centre (CIMMYT); and Azam, a promising local genotype from Cereal Research Institute (Persabaq, KP, Pakistan). A total of 72 plots (8 m × 10 m) were blocked into three replicate fields, and within each field, each genotype was sown in 4 replicate plots according to the randomized complete block design. The seed of each genotype was sown in 12 replicate plots. Maize seed was sown manually by hand on 3 March 2016, in ridges, with a row-to-row spacing of 75 cm and plant-to-plant spacing of 20 cm at the seed rate of 66,000/ha plant population, for optimal production.
2.2. Land Preparation and Crop Management

The soil nutrient profile of the research field was tested on 16 February 2016 at the laboratory of soil science of the University of Agriculture Peshawar. Based on the soil nutrient profile, fertilization dose was computed. To provide favorable field conditions for a higher germination rate and seedlings, a seedbed was prepared using a rotavator after the field was plowed. Then, a tractor-drawn ridger was used to make ridges with a ridge-to-ridge distance of 75 cm. Before sowing, all the fields were fertilized with farmyard manures at a rate of 10 tons/ha on 28 February 2016 as per the soil nutrient profile and prescription of the agronomist. The plots were also fertilized with 30, 250, 90, and 90 kg/ha of N, P, and K, respectively, using diammonium phosphate, urea, and sulfate of potash, respectively. The K, P, and half of the urea fertilizers were applied at the time of sowing, and the remaining half was applied after the first irrigation. Maize is a drought-sensitive crop and requires frequent irrigation for successful vegetative and reproductive growth. Drought can affect many physiological processes, leading to reduced yields. Therefore, all the plots were first irrigated on 6 April 2016 and then irrigated after each growing week. Shoot flies and stem borers often attack spring maize plants after emergence. For their early control, Furadon powder (pesticide) was hand dropped on the shoot of maize plants at the rate of 20 kg/ha, 7 days after sowing, followed by irrigation. Weeds can reduce the yield of maize by up to 30%, as they compete with maize for nutrients in the soil. For complete weed control, Prime extra Gold 720SC herbicide was used at a rate of 1200 mL/ha after the first irrigation in a wet field. Besides herbicide application, manual weed removal was also carried out, when required. Data regarding temperature and rainfall during the research experiment are given in Figure 1.

![Figure 1](image_url)

Figure 1. Total monthly rainfall (mm) and minimum and maximum temperature (°C) of the experimental area from January to December 2016. Source: Department of Climate Change of Peshawar University head office Khyber Pakhtunkhwa 2010–2016.

2.3. Monitoring of Maize Growth, Maturity, and Sampling

Maize crop growth was monitored on a weekly basis by counting the number of fully grown leaves on a 1 m long strip of two randomly selected rows of each plot. The appearance of flowers (flowering) and silks (silking) was strictly monitored by counting the numbers of silking and total plants on a 1 m long strip of two randomly selected rows of each plot. The whole plot was considered flowered when 50% of the plants reached the flowering stage. The numbers of senescent leaves were counted every week after flowering for the determination of leaf senescence. Moreover, two weeks after the silking, the DM content of the whole crop was strictly monitored by oven drying randomly collected samples of each plot. Samples from each plot were harvested at a targeted whole-crop DM content of 33%. At harvest time, samples of a 1 m long strip of two consecutive rows were hand-harvested by cutting 5 cm above the land surface. For uniformity, the 1 m long
2.4. Laboratory-Scale Silage Preparation

After data collection and sampling, the remaining chopped whole-crop maize was used for silage production in laboratory silos (1.5 L capacity). The silos were labeled, and a small amount of the chopped maize of each replicate plot was gradually transferred to the corresponding silo and subsequently compacted with the help of a heavy steel pestle. Every effort was made to avoid oxygen being trapped in the bottle by proper pressing and complete filling of the bottles. After thorough compaction and filling of the lab silos, the lids were tightly closed and sealed with sealing tape. The silos were then shifted to a separate laboratory and stored for chemical analysis.

After 90 days of ensiling, the silos were opened, and samples were collected for fermentation quality evaluation, chemical analysis, and digestibility determination. For chemical analysis, the silage samples were oven dried at 60 °C in an air-dry oven. The dry samples were ground at a particle size of 1 mm and kept in airtight clean labeled bottles for chemical analysis. The contents of DM (method 930.15), ash (method 942.05), ether extract (EE, method 920.39), CP (method 984.13), and acid detergent fiber (ADF, method 973.18) were analyzed according to the standard procedures of AOAC [16]. The NDF content was determined according to the procedure of Van Soest et al. [17]. The neutral detergent-insoluble CP (NDICP) and acid detergent-insoluble CP (ADICP) contents were determined according to the method of Licitra et al. [18]. Non-protein nitrogen (NPN) content was analyzed by precipitating the true protein of feed samples with tungstic acid and calculated as the difference between total CP content and CP content of the residues after filtration [10]. Soluble CP (SCP) was analyzed by incubating samples with bicarbonate-phosphate buffer for 1 h at 39 °C and filtering the residues through Whatman #54 filter paper. The SCP content was calculated as the difference between the total CP content and the CP content of residues. The non-fiber carbohydrate (NFC) was calculated as 
\[
\text{NFC} = 100 - (\text{NDF} - \text{NDIP}) - \text{EE} - \text{CP} - \text{ash},
\]
and the total carbohydrate (CHO) content was calculated as 
\[
\text{CHO} = 100 - \text{EE} - \text{CP} - \text{ash}.
\]

2.5. Fermentation Quality

The fermentation quality of maize silages was evaluated in the PCSIR laboratory, Peshawar. Twenty-gram subsamples were suspended in 180 mL water, kept overnight at 4 °C, and then filtered through a double layer of nylon cloth. The filtrate was then used for the determination of pH and ammonia nitrogen (NH$_3$N) and organic acid contents. The previously established method was used for measuring the concentration of pH and NH$_3$N, lactic acid (LA), acetic acid (AA), and propionic acid (PA) in maize silages.

2.6. In Sacco Digestibility and Degradability

An in sacco digestibility study was conducted using fistulated animals. The standard protocol of in sacco degradability was used to determine the ruminal fermentation kinetics of starch, fiber, and dry matter of silages produced from the spring maize genotypes as described earlier [20], with slight modifications. Briefly, air-dried, ground (2 mm) 3 g samples of silage were placed in pre-weighted and numbered nylon bags. The nylon bags were then attached to the appropriate plastic tubes for incubation. The bags were then put in the rumen of a fistulated animal and knotted with canola through ropes. The bags were incubated in the rumen for 48, 24, 12, 8, 4, 2, and 0 h before being removed and washed in a bucket of cold water. The nylon bags were then removed from the plastic tubes and dried for 48 h at 60–65 °C. The nylon bags were weighed again after drying. The residues were analyzed for nutritional content.

For the determination of in vitro DM digestibility, the two-stage in vitro procedure was adopted according to the method of Tilley and Terry [21]. Fresh rumen fluid was collected from rumen-cannulated Sahiwal cows fed with a diet containing 30% con-
centrate mix, 25% berseem fodder, and 45% maize silage (DM basis). Buffer solution (3.72 g L\(^{-1}\) NaHPO\(_4\), 9.82 g L\(^{-1}\) NaHCO\(_3\), 0.47 g L\(^{-1}\) NaCl, 0.12 g L\(^{-1}\) MgSO\(_4\)-7H\(_2\)O, 0.57 g L\(^{-1}\) KCl, 1 mL L\(^{-1}\) CaCl\(_2\), and 0.15 g L\(^{-1}\) CH\(_4\)N\(_2\)O) and reducing solution were prepared in demineralized water under CO\(_2\) flux and stored at 39 °C. Rumen fluid was collected in the morning in hot (39 °C) thermostat bottles, filtered through double layers of muslin cloth, and mixed with buffer solution 1:2 v/v. In all steps in which rumen liquor was exposed to air, the fluid was flushed with CO\(_2\) to remove any trapped oxygen in the containers. The air-dried ground samples (1 ± 0.02 g) were transferred to in vitro tubes, and 30 mL of buffer–rumen fluid was added. All samples were incubated for 24 h in duplicates in two replicate runs in an automatic in vitro incubator. After 24 h, the tubes were centrifuged, and the residues were dried. The in vitro DM digestibility was calculated as the difference in mass over the incubation period.

2.7. The CNCPS Carbohydrate Subfractions, Digestible Nutrients, and Energy Values

The CNCPS carbohydrate subfraction composition of the silages was calculated according to Van Amburgh et al. [22]. Only CA4 (sugars), CB1 (starch), CB2 (soluble fiber), CB3 (available NDF), and CC (unavailable NDF) subfractions are reported in this study. The ruminal degradation rate (K\(_{d}\)) of CA4 is 0.40–0.60/h; CB1, 0.20–0.40/h; CB2, 0.20–0.40/h; and CB3, 0.01–0.18/h. The energy values of total digestible CP (tdCP), total digestible fatty acids (tdFA), total digestible NFC (tdNFC), total digestible NDF (tdNDF), total digestible nutrients (TDN), digestible energy (DE), and metabolizable energy (ME) for dairy cows were estimated from the chemical composition according to mathematical models of NRC [19].

2.8. Statistical Analysis

The effects of spring maize genotypes on the yields of DM, CP, and starch; contents of chemical components; silage fermentation quality parameters; in vitro DMD; CNCPS carbohydrate subfractions; digestible nutrients; and energy values were determined by PROC MIXED procedure of Statistical Analysis System (SAS Inst., Inc., Cary, NC, USA). The following model was used:

\[
Y_{ijk} = \mu + G_{ei} + \epsilon_{ijk}
\]

where \(\mu\) is the fixed effect of the population mean for the variable; \(G_{ei}\) is the fixed effect of maize genotype, and \(\epsilon_{ij}\) is the random error associated with the observation \(ij\). Replication was considered as a random effect. Post hoc analyses were carried out using the Tukey–Kramer test to compute pairwise differences in the means. Means with different letters were obtained with “pdmix 800SAS macro”.

3. Results

3.1. Growth and Phonological Characteristics

Data on growth and phonological characteristics of spring maize genotypes are summarized in Table 1. There was a large variation \((p < 0.001)\) in days to flowering (DTF) among the genotypes, varying from 57 DTF (Azam) to 62 DTF (P1543). Similar variability was also observed for days to silking (DAS). There was a large genetic variation \((p < 0.001)\) in plant height (m), ranging from 1.83 m (Azam) to 2.45 m (DK9108). The leaf senescence per plant (LS/P) differed \((p < 0.05)\) among the maize genotypes. The maximum rate of LS/P was recorded for Azam (4.50), and the minimum rate of LS/P was recorded for QPM300 (3.30) at harvest maturity. The number of cobs per plant varied \((p < 0.001)\) among the maize genotypes, ranging from 1.58 (QPM300) to 1.07 (P1429).

3.2. Biomass and Nutrient Yield

The yields (tons/ha) of DM, CP, NDF, and starch had marked variation \((p < 0.001)\) among the spring maize genotypes (Table 2). Among the genotypes, QPM300 had the highest \((p < 0.05)\) yields (tons/ha) of DM (17.9), CP (1.24), starch (6.67), and NDF (7.42), while Azam had the lowest \((p < 0.05)\) yields (tons/ha) of DM (13.0), CP (0.83), starch (4.16), and NDF (6.12).
Table 1. Growth and phological characteristics of spring maize genotypes evaluated for silage production.

| Genotypes | Growth Characteristics | Phonological Parameters |
|-----------|------------------------|------------------------|
|           | DTF (days) | DTS (days) | Height (m) | LS/P | C/P |
| P1429     | 59 b       | 64 b       | 2.14 d     | 3.90 bc | 1.07 d |
| Azam      | 57 d       | 61 d       | 1.83 e     | 4.50 a  | 1.14 cd |
| DK9108    | 59 c       | 62 c       | 2.45 a     | 3.80 c  | 1.30 bc |
| QPM200    | 61 b       | 64 ab      | 2.22 c     | 3.80 c  | 1.33 b  |
| QPM300    | 60 ab      | 64 ab      | 2.37 b     | 3.30 d  | 1.58 a  |
| P1543     | 62 a       | 65 a       | 2.15 d     | 4.10 b  | 1.25 c  |
| SEM       | 0.40       | 0.3        | 0.11       | 0.18    | 0.06    |
| SEM       | 0.40       | 0.3        | 0.11       | 0.18    | 0.06    |

Means with different superscripts (abcde) within a column differ at p < 0.05. DTF, days to flowering; DTS, days to silking; LS/P, leaf senescence/plant; C/P, cobs per plant.

Table 2. Dry matter and nutrients yield of spring maize genotypes.

| Genotypes | DM (g/kg) | Yield (tons/ha) |
|-----------|-----------|----------------|
|           | DM CP Starch NDF |
| P1429     | 326       | 14.9 b 1.02 c 5.25 c 6.60 d |
| Azam      | 339       | 13.0 c 0.83 d 4.16 c 6.12 e |
| DK9108    | 329       | 16.0 b 1.06 c 5.76 b 7.29 b |
| QPM200    | 323       | 16.3 ab 1.20 a 5.41 b 7.04 c |
| QPM300    | 320       | 17.9 a 1.24 a 6.67 a 7.42 a |
| P1543     | 335       | 15.9 b 1.06 c 6.67 a 7.31 b |
| SEM       | 0.31      | 0.34     0.03 | 0.11    | 0.37    |

Means with different superscripts (abcde) within a column differ at p < 0.05. SEM, standard error of mean; NS, non-significant; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber.

3.3. Chemical Composition

Data on the proximate chemical composition, CP fractions, and in vitro DMD of the silages produced from the spring maize genotypes are summarized in Table 3. Except for total carbohydrates (CHO), the contents of all measured chemical components varied (p < 0.001) among the spring maize genotypes. Among the genotypes, QPM300 had highest (p < 0.05) contents (g/100 g DM) of CP (8.05), starch (37.3), NFC (43.8), and in vitro DMD (67.9) and the lowest (p < 0.05) contents of NDF (41.5 g/100 g DM) and SCP (41.1 g/100 g CP). The local cultivar Azam had the lowest (p < 0.05) contents (g/100 g DM) of CP (6.60), starch (32.0), NFC (40.3), and in vitro DMD (61.5) and the highest (p < 0.05) contents of NDF (47.1 g/100 g DM) and SCP.

Table 3. Chemical composition (g/kg DM) and in vitro dry matter digestibility (DMD) of maize after ensiling for 90 days.

| Genotypes | g/100 g Dry Matter CP CHO Starch ADF NFC NDF SCP NDICP ADICP | g/100 g CP | IVDM (g/100 g) |
|-----------|------------------|-------------------|-------------------|
| P1429     | 6.30 d 84.2 35.3 b 25.7 a 42.0 b 44.3 bc 44.1 a 13.2 abc 47.0 c 65.7 b |
| Azam      | 6.04 e 84.7 32.0 d 27.2 a 40.3 c 47.1 a 42.1 bc 11.5 c 5.70 b 61.5 c |
| DK9108    | 6.60 c 84.9 36.0 a 26.6 a 41.9 bc 45.6 ab 43.8 a 13.3 a 5.65 b 65.8 b |
| QPM200    | 7.51 b 83.8 33.6 c 26.1 a 42.6 ab 43.2 c 43.6 a 12.9 abc 6.00 b 65.8 b |
| QPM300    | 8.05 a 84.0 37.3 a 23.6 b 43.8 a 41.5 d 41.1 c 12.3 bc 7.01 a 67.9 a |
| P1543     | 6.60 c 85.3 34.9 bc 25.6 a 42.3 ab 44.9 bc 43.0 ab 20.1 c 5.42 b 66.7 ab |
| SEM       | 0.11 0.33 0.36 0.40 0.42 0.37 0.67 0.44 0.22 0.47 |

Means with different superscripts (abcde) within a column differ at p < 0.05. NFC, non-fibrous carbohydrates; CHO, total carbohydrates, SCP, soluble CP; NDICP, neutral detergent-insoluble crude protein; ADICP, acid detergent-insoluble crude protein, DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADIC, acid detergent fiber; SEM, standard error of mean; NS, non-significant; ***, p < 0.001.
3.4. Rumen Degradation Characteristics of Maize Silages of Different Maize Genotypes

The ruminal starch degradation kinetics and effective rumen degradability of maize silages of six promising maize genotypes for maize silage are presented in Table 4. Our findings showed large differences in soluble (W; \( p < 0.0 \)), potentially rumen-degradable (D; \( p < 0.001 \)), and rumen undegradable (U; \( p < 0.05 \)) degradation characteristics; rate of degradation (\( K_d; p < 0.001 \)); effectively rumen-degradable starch (EDstarch; \( p < 0.001 \)); and undegradable starch (UDstarch; \( p < 0.001 \)). In QPM300, the values of W, \( K_d \), and EDstarch were higher (\( p < 0.05 \)) but the values of D, U, and UDstarch were lower (\( p < 0.05 \)) as compared to other maize genotype silages.

### Table 4. Effect of spring maize genotypes on ruminal starch degradation kinetics and effective rumen degradability of maize silages.

| Genotypes | Rumen Degradation Characteristics | EDstarch | UDstarch |
|-----------|----------------------------------|----------|----------|
|           | W & D & U & \( K_d \)            |          |          |
| P1429     | 33.6 \( ^{cd} \) & 53.0 \( ^{ab} \) & 5.71 \( ^a \) & 19.4 \( ^a \) & 88.0 \( ^b \) & 12.0 \( ^b \) |
| Azam      | 37.9 \( ^c \) & 50.0 \( ^b \) & 2.55 \( ^c \) & 09.7 \( ^c \) & 85.7 \( ^b \) & 14.6 \( ^{ab} \) |
| DK9108    | 40.4 \( ^b \) & 37.8 \( ^c \) & 2.80 \( ^c \) & 14.5 \( ^b \) & 94.2 \( ^a \) & 05.81 \( ^d \) |
| QPM200    | 31.0 \( ^d \) & 55.4 \( ^a \) & 5.85 \( ^a \) & 19.1 \( ^a \) & 83.8 \( ^c \) & 16.2 \( ^{a} \) |
| QPM300    | 46.2 \( ^a \) & 37.6 \( ^c \) & 4.66 \( ^b \) & 22.1 \( ^a \) & 95.8 \( ^a \) & 09.22 \( ^{c} \) |
| P1543     | 41.6 \( ^b \) & 21.5 \( ^{d} \) & 1.28 \( ^d \) & 11.2 \( ^c \) & 94.4 \( ^a \) & 02.60 \( ^{e} \) |
| SEM       | 1.12 & 0.56 & 0.89 & 0.57 & 2.22 & 1.60 |
| \( p \) value | ** *** * *** *** *** |

Means with different superscripts (abcde) within the columns differ at \( p < 0.05; *, p < 0.05; **, p < 0.01; *** , p < 0.001; \) SEM, standard error of the mean. W, washable fraction; D, potentially rumen-degradable fraction; U, undegradable fraction; \( K_d \), rate of degradation; EDstarch, effectively rumen-degradable starch; UDstarch, undegraded starch.

3.5. Fermentation Characteristics

Silage fermentation parameters of the six promising maize genotypes are presented in Table 5. Our results demonstrated that the QPM300 contained a higher (\( p < 0.05 \)) concentration of lactic acid than other maize genotypes. Silage produced from the Azam maize genotype exhibited higher (\( p < 0.05 \)) acetic acid content and pH and no significant effect on the concentration of total NH\(_3\)-N. Additionally, after 45 days of fermentation, pH values ranged between 3.60 and 4.20, while no butyric acid was detected.

### Table 5. Fermentation parameters of different maize genotypes for maize silage production.

| Genotypes | pH | Lactic Acid\% | Acetic Acid\% | Propionic Acid | NH\(_3\)-N (g/100 g N) |
|-----------|----|---------------|---------------|----------------|-------------------|
| P1429     | 4.00 \( ^{ab} \) | 4.72 \( ^{b} \) | 2.55 \( ^{c} \) | 0.90 \( ^{c} \) | 9.20 |
| Azam      | 4.20 \( ^{a} \) | 3.95 \( ^{d} \) | 3.03 \( ^{a} \) | 1.18 \( ^{a} \) | 10.1 |
| DK9108    | 3.90 \( ^{b} \) | 4.71 \( ^{b} \) | 2.85 \( ^{b} \) | 1.02 \( ^{b} \) | 9.60 |
| QPM200    | 3.90 \( ^{b} \) | 4.17 \( ^{c} \) | 2.70 \( ^{bc} \) | 0.98 \( ^{bc} \) | 9.40 |
| QPM300    | 3.60 \( ^{c} \) | 5.19 \( ^{a} \) | 2.24 \( ^{d} \) | 0.80 \( ^{d} \) | 9.10 |
| P1543     | 3.60 \( ^{c} \) | 4.95 \( ^{ab} \) | 2.69 \( ^{bc} \) | 0.90 \( ^{c} \) | 9.60 |
| SEM       | 0.31 | 0.40 | 0.11 | 0.10 | 0.43 |
| \( p \) value | ** ** * * NS |

Means with different superscripts (abcd) within a column differ at \( p < 0.05; * p < 0.05; **, p < 0.01; *** , p < 0.001; \) SEM, standard error of mean, NS, non-significant; * \( p < 0.05; ** , p < 0.001; \) NH\(_3\)-N: ammonia nitrogen.

Data on the CNCPS carbohydrate subfraction composition of the silages prepared from spring maize genotypes are summarized in Table 6. Except for the non-digestible (CC) subfraction, all other reported CNCPS subfractions varied (\( p < 0.001 \)) among the maize silage genotypes. The QPM300 had the highest (\( p < 0.05 \)) values of CB1 and CA4 subfractions. In contrast, Azam had the highest (\( p < 0.05 \)) contents of CB2 and CB3 subfractions.
Table 6. The effect of spring genotypes on Cornel Net Carbohydrate and Protein System (CNCPS) carbohydrate subfractions of maize silages.

| Genotypes | Carbohydrate Subfraction |
|-----------|--------------------------|
|           | CA4 | CB1 | CB2 | CB3 | CC |
| P1429     | 5.32 | 35.3 | 10.77 | 40.8 | 3.66 |
| Azam      | 5.77 | 32.0 | 11.35 | 43.3 | 3.80 |
| DK9108    | 5.16 | 36.0 | 9.84  | 42.0 | 3.64 |
| QPM200    | 5.40 | 33.6 | 11.48 | 39.6 | 3.59 |
| QPM300    | 6.79 | 37.3 | 8.80  | 38.0 | 3.50 |
| P1543     | 6.11 | 34.9 | 11.26 | 39.6 | 3.46 |
| SEM       | 0.098 | 0.36 | 0.12  | 0.37 | 0.09 |

Significance ** *** ** *** NS

Means with different superscripts (abcd) within a column differ at \( p < 0.05 \). CA4, sugar (Kd 0.40–0.60/h); CB1, starch (Kd 0.20–0.40/h); CB2, soluble fiber (Kd 0.20–0.40/h); CB3, digestible fiber (Kd = 0.01–0.18/h); CC, indigestible fiber; SEM, standard error of mean; NS, non-significant; **, \( p < 0.01 \); ***, \( p < 0.001 \). 

3.6. Total Digestible Nutrients and Estimated Energy Values

Except for tdFA, all measured digestible nutrients and energy values varied (\( p < 0.05 \)) among the maize genotypes (Table 7). Moreover, the QPM300 had the highest values (\( p < 0.05 \)) of tdNFC, tdCP, TDN, DE, and ME. In contrast, Azam had the highest (\( p < 0.05 \)) value of tdNDF.

Table 7. Total digestible nutrients and estimated energy values (Mcal/kg).

|          | tdNDF | tdNFC | tdCP | tdFA | TDN | DE | ME  |
|----------|-------|-------|------|------|-----|----|-----|
| P1429    | 24.4  | 41.2  | 6.59 | 2.22 | 70.2| 3.03| 2.41|
| Azam     | 25.8  | 39.5  | 6.43 | 2.08 | 68.1| 2.80| 2.28|
| DK9108   | 25.0  | 40.1  | 6.65 | 2.22 | 69.8| 3.02| 2.41|
| QPM200   | 22.9  | 41.7  | 7.50 | 1.98 | 69.7| 3.02| 2.40|
| QPM300   | 21.8  | 42.9  | 8.04 | 2.16 | 70.6| 3.07| 2.44|
| P1543    | 24.0  | 41.5  | 6.89 | 2.12 | 70.3| 3.04| 2.42|
| SEM      | 0.32  | 0.40  | 0.11 | 0.10 | 0.24| 0.01| 0.99|

Significance *** *** ** NS **

Means with different superscripts (abcd) within a column differ at \( p < 0.05 \); tdNDF, total digestible NDF; tdNFC, total digestible non-fiber carbohydrates; tdCP, total digestible CP; tdFA, total digestible fat; TDN, total digestible nutrients; DE, digestible energy; ME, metabolizable energy; SEM, standard error of mean; NS, non-significant; *, \( p < 0.05 \); **, \( p < 0.01 \); ***, \( p < 0.001 \). 

4. Discussion

In several tropical countries such as Pakistan, silage production from spring maize has increased over the last few years. The growing conditions of spring maize are quite different from those of autumn maize, as the day length and temperature increase (up to 40 °C) with the progress of the growing season. Moreover, the ensiling condition is also quite different, characterized by high ambient temperature and humidity. All these factors contribute to the variation in the nutritional and fermentation quality of maize silages [2,23,24]. This study presents the first-time dataset on the biomass, starch and CP yields, silage fermentation characteristics, nutrient composition, carbohydrate subfractions, TDN, DMD (in vitro), and ME content of promising spring maize genotypes under uniform experimental conditions. This dataset, produced from a well-replicated experimental design using standard protocols and advanced analytical techniques, can be used by dairy farmers for the selection of maize genotypes for high biomass and good-quality silage production. Moreover, the QPM300 maize silage genotype has higher yields of DM, CP, and starch; lactic acid content; nutritive value; DMD (in vitro); TDN and estimated energy values (Mcal/kg); and rumen degradation kinetics and lower values of pH, ADF, NDF, CB2 and CB3 CNCPS subfractions, acetic acid, and NH3-N than all other maize silage genotypes.
The variation in growth and phonological characteristics of maize plants affects biomass yield, fermentation quality, and nutritional value of silages. In the present study, the DTS varied from 61 to 65 among the spring maize genotypes, which is in line with the ranges (57 to 62 DTS) reported in [25] and [26] for 20 commercial spring maize genotypes. Maize plant height is a very important parameter for biomass yield and nutritional quality of silages, and it also reflects crop growth attained during the growing period. The range of plant height (1.83 to 2.45 m) in our study was consistent with the results (1.97 to 2.27 m) of [27]. The number of leaves and the number of cobs per plant in a maize crop affect the yield, nutritional value, and silage fermentation quality. The ranges of the number of cobs (1.07 to 1.58) and the number of leaves (13 to 17) in our study are consistent with literature values [25,28]. The variation observed in growth and phonological characteristics among the maize genotypes could be related to differences in genetic makeup such as days to germination, nitrogen uptake capacity, adaptation to a certain soil and sowing temperature, and maturity period of these genotypes. Early-maturing genotypes produce a minimum number of leaves and reach a lower height than those having late maturity stages like QPM300 in the current study.

The selection of suitable maize genotypes for silage production is key for obtaining high biomass yield, fermentation quality, fiber digestibility, and nutritional value [2,23,29]. In the current study, a large variation (13.0 to 17.9 tons/ha) in DM yield was recorded among the spring maize genotypes. A similar variation (11.0 to 16.7 tons/ha) was reported by Guyader et al. [30] for eight maize genotypes. This genotypic variation in DM yield was mainly due to variations in plant height, number of leaves, cobs/grains per plant, and maturity pattern of the maize plant. The range in CP yield (0.83 to 1.24 tons/ha) was consistent with values reported earlier for spring maize genotypes [30–32]. In this study, the stay-green and leafy maize genotype QPM300 had a high CP yield as compared to early-maturing genotypes such as Azam. The high CP yield of QPM300 may be related to the greater contribution of CP from the leaf fraction and a higher CP content of the grains [13]. The variation in starch yield (4.16 to 6.67 tons/ha) among the six spring genotypes was consistent with literature values [32,33]. Cone et al. [14] reported that the yield of starch was highest in late-maturing genotypes due to their high kernel fraction and maximum time for starch deposition.

In the current study, the contents of CP ranged from 6.3 to 7.5 g/100 g DM in silage produced from spring maize genotypes. In agreement with our findings, earlier studies have reported CP values of 6.4 to 8.0 g/100 g DM among different maize genotypes [30,34,35]. Not only the CP content but also the CP chemical profile, SCP, NDICP, and ADICP varied in silages made from the spring maize genotypes, which is supported by earlier observations [12,36]. Starch is traditionally the most important nutrient in maize silage, and a large variation in starch contents (32.2 to 37.3 g/100 g DM) among the genotypes could have a large influence on the nutritional value of maize silage [2,36]. The genetic variation in starch content could be related to variations in the rate of kernel filling, number of cobs, and mass of grains, as well as to the stover fraction and composition of the whole-crop plant [30,31,34]. The NDF content varied from 41.5 to 47.1 g/100 g DM among spring maize genotypes. The differences in NDF contents may be due to variations in leaf:stem ratio and grain-to-stover ratio. A high grain:stover ratio can indirectly decrease the fiber content of maize silages. For high production, dairy animals need to be fed with forages that are highly digestible and support high intake [5,37,38]. The DM digestibility value of the maize silage made from spring maize genotypes ranged from 61.5 to 67.9%, which is consistent with the literature values [30,34,39]. Due to the high digestibility and energy value and smaller particle size, maize silage supports high DM intake and production in dairy cows [2].

A lower pH value is usually an indicator of increased lactic acid concentration, thereby implying better fermentation of silages during the ensiling period; our results fall into the 3.60 to 4.20 range [40]. The lactic acid in silage results from the fermentation of sugar by homofermentative lactic bacteria [41] and is the most abundant organic acid produced by ideal fermentation; our results were within the recommended range (4–6%) [42], similar to those obtained by Nennich et al. [43], suggesting the maize silages were well fermented.
in this experiment. Additionally, the NH3-N indicates the degradation of CP and is a good indicator of quality fermentation during the ensiling process. In the current study, the contents of NH3-N were less than 10 g/100g of the total N among the spring maize genotypes. Earlier studies reported that the NH3-N content should not exceed more than 10 g/100g of total N for quality silages [2,5]. Butyric acid was not detected in this experiment, and it has been argued that butyric acid concentration is low and biologically negligible in whole-plant corn silage [12]. Millner et al. [28] reported that low compaction and delayed sealing adversely impact silage quality.

The CNCPS system is an animal nutrition model that allows the formulation of feed in such a way that closely matches dairy cattle requirements [22]. Apart from the non-digestible (CC) subfraction, all other CNCPS carbohydrate subfractions varied among the maize genotypes. Similarly, the TDN ranged from 68.1 to 70.6% and the ME ranged from 2.28 to 2.44 Mcal/kg among the maize genotypes. The CNCPS subfractions and TDN and ME values were calculated from the chemical profile data [19,36], and thus the variations observed in the CNCPS subfractions and TDN and ME values were strongly explained by the differences in chemical composition among the maize genotypes. Next to variation in chemical composition, the CNCPS and in situ data also highlight genetic variation in rumen-degradable dry matter, CP, starch, and NDF [2,44]. These results indicate that genetic variations should be considered for optimizing the nutritional value of maize silages produced from spring crops.

5. Conclusions

Suitable maize genotype selection for silage production under a particular growing condition not only influences the biomass and nutrient yields, but also affects the digestibility, silage fermentation quality, and nutritional value. Large differences in DM yield; contents of CP, starch, and non-fiber carbohydrates; in vitro DMD; total digestible nutrients; and metabolizable energy were observed within the six maize genotypes. It was concluded that QPM300 is the most suitable spring maize genotype for silage production in terms of yields, digestibility, and silage nutritional and fermentation quality under the environmental conditions of Northern Pakistan.

Author Contributions: Conceptualization, M.J., Y.M. and N.K.; writing—original draft preparation, M.Z.K., Y.M., M.J., N.A.K. and N.K.; editing and technical review, Y.M., M.J., N.K., N.A.K., R.U.K., A.A., M.K. and M.Z.K.; visualization, M.Z.K. and N.K.; supervision, N.A.K. and N.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the International Livestock Research Institute under The Agriculture Innovation Programme and Pakistan Research Council project grant (AID-BFS-G-11-00002). Moreover, the study was also financially supported by Effects of different types of corn by-product bio-fermented feed on finishing pigs; Heilongjiang Agriculture Economics Vocational College; College Project, No. NJKY202102; and Effect of corn secondary product-type fermented feed on growing and finishing pigs No. JSFZAFSY2021121599.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors pay thanks to Wouter Hendriks and John Cone of Wageningen University, The Netherlands, for assisting in the designing of the work and reading of the manuscript. The laboratory staff of the Animal Nutrition Group, University of Agriculture Peshawar, is gratefully acknowledged for technical assistance. Financial support was provided by the Higher Education Commission of Pakistan under the Indigenous PhD fellowship and Pakistan Agriculture Research Council (PARC) through Agriculture Linkage Program. Finally, the authors also acknowledged the financial support provided by Effects of different types of corn by-product bio-fermented feed on finishing pigs; Heilongjiang Agriculture Economics Vocational College and Effect of corn secondary product-type fermented feed on growing and finishing pigs.

Conflicts of Interest: The authors declare no conflict of interest.
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