J. Dairy Sci. 109:1386–1398
https://doi.org/10.3168/jds.2025-26982
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Assessing yield and nutritive value of corn varieties for silage production carrying the *brachytic2* mutation harvested at different stages of maturity

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ABSTRACT

The aim of this study was to evaluate the forage feeding value of short-stature corn (SSC) genotype characterized by a *brachytic2* (i.e., *br2*) mutation in comparison to tall-stature corn genotype (TSC) harvested at 5 different harvest moments (HM; i.e., 114, 119, 125, 135, and 149 d). In particular, 5 corn varieties selected for whole-plant silage were grown in an experimental field and harvested at 5 different HM. The field was divided into 15 experimental plots (30 m long × 4 rows × 0.75 m spacing between rows, with 1 row empty as a buffer zone); each variety was randomly seeded in 3 plots of experimental field. Notably, 2 corn varieties of TSC genotype (i.e., DKC7023 and DKC6731, respectively) were grown at a density rate of 9 plants/m^2, whereas 3 corn varieties of SSC genotype (i.e., DKC6648SC, DKC6646SC, and DKC7236SC, respectively) were grown at a density rate of 13 plants/m^2. The HM were based on the accumulated growing degree days values of 1,096, 1,172, 1,246, 1,398, and 1,596 (°C) using a 10°C base temperature. Data were analyzed as a split-plot in a randomized complete block design with 3 blocks (i.e., replicates) and 5 treatments (TRT). The whole-plot was TRT (i.e., 3 SSC and 2 TSC) and the subplot was maturity (HM; i.e., 114, 119, 125, 135, and 149 d after planting). An a priori contrast comparing SSC (3 varieties) and TSC (2 varieties) was run to evaluate the overall effect of genotype. Specific orthogonal contrasts were applied to evaluate linear and quadratic trends across HM. Interaction between HM and TRT showed significant effect on fresh yield and DM yield. Fresh yield decreased with the advance of maturity stage; however, DM yield increased among the HM. Fibrous fractions decreased with the advance of maturity. On average, SSC varieties had lower NDF, ADF, and ADL concentrations in comparison to TSC. Starch concentration increased with the advance of maturity stage, peaking at 149 d (i.e., 39.1% DM). Starch content was higher for SSC by 12.7% in comparison to TSC. The estimated energy content (i.e., kg milk/t DM) was different among genotypes, with SSC being characterized by higher value than TSC. A quadratic effect for in vitro NDF digestibility at 12 and 24 h after incubation was observed within the HM. In contrast, NDF digestibility linearly decreased after 30 h incubation with increasing HM. Varieties characterized by SSC genotype had higher NDF digestibility at 30, 48, and 120 h after incubation in comparison to TSC. The in situ starch disappearance (isSD) linearly decreased with increasing HM. The SSC had higher isSD compared with TSC. Concluding, cropping SSC may be a feasible strategy, in terms of agronomic response and nutritional composition, to maximize silage nutritional quality through greater starch concentration, lower fiber concentration, and improved starch and NDF digestibility, while maintaining similar DM yield with respect to TSC. Further studies are warranted to elucidate the agronomical advantages of these new corn hybrids.

Key words: corn varieties, *brachytic2* mutation, NDF digestibility, starch disappearance

INTRODUCTION

Whole-plant corn silage (**WPCS**) is the predominant forage used in diets for dairy cattle worldwide (Gallo et al., 2016; Ferraretto et al., 2018). The WPCS production has always been focused on planting hybrids with high grain yield characteristics or dual-purpose hybrids to allow farmers the possibility to harvest corn grain or WPCS based on feed stock availability (Ferraretto and Shaver, 2015). Traditionally, selection aimed to characterize

Received May 23, 2025.
Accepted October 26, 2025.
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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

traits, such as yield, pest and disease resistance, leafiness, standability, relative maturity, drought tolerance, stay-green ranking, and dry-down (Adesogan, 2006). The stay-green (**SG**) trait is an important factor that can influence the quality of whole-plant corn during the field operations. Notably, the SG trait refers to the ability of corn hybrid to delay the natural process of leaf senescence and maintain photosynthetic activity for a longer period, even as it matures (Saenz et al., 2025). For corn harvested as silage, SG is beneficial as it prolongs the period when the plant reaches the ideal moisture content for ensiling, improving the quality of the harvest (Arriola et al., 2012b). Generally, SG hybrids express their potential characteristics in the later maturity stage, such as black layer (Arriola et al., 2012a).

Over the years, specific corn hybrids for silage production have been selected to enhance the nutritional value of WPCS and match the requirements of high-producing dairy cows, considering factors related to digestibility and potential intake by animals. Altering the stalk or kernel characteristics or their ratio, or both, through hybrid selection can be considered as an opportunity to increase digestibility in WPCS (Ferraretto and Shaver, 2015). Notably, short-stature corn (**SSC**) genotype shows higher starch concentration compared with tall-stature corn (**TSC**) genotype, due to SSC genotype producing the same number of leaves and having similar grain yield, reducing the proportion of stalk in the silage mix (Kosola et al., 2023). Cell wall composition has an important role in forage digestibility that can affect stalk quality by affecting cell wall structure and strength. Maximizing digestible carbohydrate intake or enhancing NDF digestibility (**NDFD**), or both, from ensiled crops is crucial as the energy needed for maintenance and milk production exceeds the amount of energy that high-producing cows can consume, especially in early lactation (Refat and Yu, 2016). The NDFD is affected by plant maturity; in WPCS, the fibrous fraction is diluted with the increasing amount of starch with the advance of maturity stage (Coors et al., 1997), but lignin concentration remains unaffected from early dent to black layer stage (Owens, 2005).

Crop varieties affected by dwarfism or semidwarfism have different advantages, such as increased lodging resistance and higher harvest index (Hedden, 2003; Li et al., 2011). Many dwarf mutants have been classified by short internodes, which have been referred to as impaired internode elongation (Zhang et al., 2019). The stem/internode elongation is mainly regulated by different plant hormones, such as gibberellins, brassinosteroids, strigolactones, and auxins (Wang et al., 2018). Referring to corn, only the category of brachytic mutants expresses a short stature caused by a shortening of the internode length without a reduction in the number of internodes or the size of other organs (Pilu et al., 2007). Notably, corn hybrids carrying the *brachytic2* (*br2*) mutation are characterized by a reduced plant height through the shortening of internodes and more erect leaves and a greater standability, which allows them to prevent yield losses (Pilu et al., 2007; Barten et al., 2022). The *br2* mutation affects the lignin distribution within the stem of whole-plant corn, increasing NDFD and standability of SSC in comparison to TSC genotypes (Bourdoncle et al., 2023). Landoni et al. (2022) assessed that histochemical analysis did not reveal obvious differences in total amount of lignin between SSC and TSC. According to Bourdoncle et al. (2023), SSC tends to have less lignin concentration in the pith of the stalk compared with TSC, supposing this morphological difference can be beneficial for silage quality and digestibility.

The objectives of this study aimed to (1) assess the nutritive value of 3 corn varieties of SSC carrying the *br2* mutation (i.e., DKC6648SC [**SSC1**], DKC6646SC [**SSC2**], and DKC7236SC [**SSC3**], respectively) in comparison to 2 corn varieties of TSC (i.e., DKC7023 [**TSC1**] and DKC6731 [**TSC2**], respectively) harvested at 5 different harvest moments (**HM**; i.e., being 114, 119, 125, 135, and 149 d from sowing); (2) evaluate the effect of the *br2* mutation on NDFD, starch digestibility, and lignification of SSC and TSC; and (3) estimate the energy content (kg milk/t DM) of corn varieties through a feed evaluation system.

MATERIALS AND METHODS

Five corn hybrids (Bayer AG, Leverkusen, Germany) selected for whole-plant silage were grown in an experimental field located in Cascina Alta-Cumignano sul Naviglio near Cremona, Italy (45°21′31.6”N 9°49′37.2”E). Notably, there were 2 varieties for TSC (i.e., TSC1 and TSC2, respectively) and 3 SSC varieties (i.e., SSC1, SSC2, and SSC3, respectively).

Field Trial Site and Harvest Management

Planting date was April 6, 2023. The experimental field was fertilized in preplanting period on April 2, 2023, and at mechanical hoeing on May 8, 2023, with 100 t/ha of swine slurry; no presowing manuring was applied. The experimental site was treated with pre- and postemergence herbicide to prevent weed development. The field was divided into 15 experimental plots (30 m long × 4 rows × 0.75 m spacing between rows, with 1 row empty as a buffer zone), and they were assigned to 1 of 3 blocks. In particular, blocks represented an area of experimental field that contained 5 flanked and consecutive experimental plots. Tested varieties (n = 5), nested within each plant genotype (i.e., tall and short), were seeded randomly in 1 of the 5 plots assigned to each block. Each

variety was seeded and replicated in 3 plots (i.e., statistical replicates) of experimental field to obtain statistical replicates. Tall-stature varieties were grown at a density rate of 9 plants/m^2 and the short-stature varieties at a density rate of 13 plants/m^2, according to manufacturer recommendations. The varieties were irrigated 3 times by flood on May 3, June 1, and July 10, 2023, respectively.

The HM were based on the accumulated growing degree days (**GDD**) values of 1,096, 1,172, 1,246, 1,398, and 1,596 (°C) using a 10°C base temperature, as adopted in corn maturity prediction systems (Roth and Yocum, 1997; Cox, 2006). The GDD were calculated using daily maximum and minimum air temperatures, with upper and lower thresholds of 30°C and 10°C, respectively. Temperature data were obtained from an automated meteorological station positioned near the experimental field. These HM were selected to cover the stages related to the kernel fill during the reproductive stages (Nleya et al., 2016). Whole-plant corn varieties were harvested progressively within the plots, chopping the 2 central rows. Notably, the fresh chopped whole-plant of each plot section was harvested and weighed to estimate biomass yield, following the HM schedule (i.e., 114, 119, 125, 135, and 149 d), with a progressive advancement of 6 m along the central rows (i.e., from section 1 to section 5). For each tested variety, samples of fresh chopped forage (i.e., 2 kg of fresh matter per sample) were collected in 3 plots and at 5 different HM. A total of 75 samples were collected during experiment (i.e., 5 varieties × 3 plots × 5 different HM). In particular, samples were harvested with a Kemper Champion C1200 (Maschinenfabrik, Stadtlohn, Germany), which was equipped with a kernel processor. The theoretical length of cut was 10 mm, and the roller gap was set at 2 mm to enhance kernel disruption.

Sample Preparation, Nutrient, and Yield Analysis

After harvesting, samples were dried in a forced-air oven set at 60°C for 48 h to determine DM concentration and ground to pass a 1-mm Wiley mill (Arthur H. Thomas, Philadelphia, PA) and to estimate DM yield. About 250 g of each dried and ground sample was sent to a commercial laboratory (Rock River Laboratory Inc.; Watertown, WI) for analysis of CP, soluble CP, acid detergent insoluble CP (**ADICP**), neutral detergent insoluble CP (**NDICP**), ADF, ADL, undigested NDF after 240 h of in vitro incubation (**uNDF240**), starch, water-soluble carbohydrates (**WSC**), ether extract (**EE**), and ash using near-infrared reflectance spectroscopy (**NIRS**) with a Foss 5000 (Foss North America Inc., Eden Prairie, MN). Calibration equations for NIRS analysis referring to CP, WSC, starch, ADF, ADL, soluble CP, ADICP, NDICP, EE, and ash were based on procedures described in AOAC International (2012) method 990.03, DuBois et al. (1956), AOAC International (2012) method 920.39, Hall et al. (2015), Möller et al. (2009), AOAC International (2012) method 942.05, and AOAC International (2012) method 920.29, respectively. Calibration equations for NIRS analysis of uNDF were based on in vitro incubation procedures reported in Goeser et al. (2009) for 240 h. Yield analysis data for the corn varieties were run through Milk2024 (Diepersloot et al., 2024) to estimate milk/ton (kg milk/t DM) and were converted to the metric system before statistical analysis. Notably, the milk/ton is estimated based on National Academies of Sciences, Engineering, and Medicine (NASEM, 2021) equations.

In Vitro NDF Digestibility Procedures

The in vitro NDFD (**ivNDFD**) was evaluated using rumen fluid inoculum collected and pooled from 2 ruminally cannulated multiparous lactating Holstein cows. Collection was performed following the method described by Goeser et al. (2009). The in vitro procedure was conducted under an approved protocol by the Animal Care and Use Committee of the College of Agriculture and Life Sciences, University of Wisconsin–Madison.

Further, ~0.5 g of dried, 1-mm ground whole-plant corn sample was weighed into tared, labeled filter bags with a mean pore size of 35 µm (F57, Ankom Technology, Macedon, NY), which then were heat-sealed. Samples were prepared in triplicate for the 0-h and each of the 5 digestion time points (12, 24, 30, 48, and 120 h). Blank filter bags were included for each time point in duplicate.

At ~0630 h on the day of incubation, 0.3125 g of primer was combined with 250 mL of buffer solution and 40 mL of reducing solution in each of five 1,000-mL side-arm Erlenmeyer flasks. As reported by Goeser et al. (2009), the primer consisted of 40% DM cellulose (Whatman no. 42 ashless filter paper, Whatman International Ltd., Maidstone, UK; ground to pass a 1-mm screen), 20% DM urea, 20% DM corn starch, and 20% DM cellobiose. The buffer and reducing solutions were prepared as described by Goering and Van Soest (1970). All 1,000-mL flasks were gassed with CO_2 for 15 min while rumen fluid inoculum was collected. At 0630 h, ~1 L of rumen fluid was collected from each of 2 ruminally cannulated lactating cows into prewarmed, glass-lined thermoses. The donor cows were fed a 60% forage (alfalfa and corn silage) and 40% concentrate diet ad libitum once daily at 0700 h. The rumen fluid inoculum was transported to the laboratory and, in a warm (39°C) room, strained through 4 layers of cheesecloth while being gassed continuously with CO_2. At 0645 h, 250 mL of strained, pooled rumen fluid was added to each of 1,000-mL flasks. All flasks were then sealed with rubber stoppers, set in an incubat-

ing (39°C) shaker, and allowed to reach 37.4 mm Hg of pressure, which correspond to 30 mL of gas production or 0.12 mL of gas production/mL of rumen fluid inoculum. The amount of gas production to reach 37.4 mm Hg was determined through manual calibration by forcing known amounts of gas into a sealed 1,000-mL side-arm flask and measuring corresponding pressures through an electronic pressure sensor. Once flasks averaged 37.4 mm Hg per flask, the contents of the 1,000-mL Erlenmeyer flasks were recombined under CO_2 and used to inoculate samples with 50 mL of inoculum solution per flask.

Each day at ~1500 h, all remaining corn bags were deflated using a plastic rod while purging the in vitro flask with CO_2. Samples were removed at 12, 24, 30, 48, and 120 h after inoculation. Fermentation was ended by rinsing the bags with ambient-temperature distilled water until the effluent was clear, as described by Eun et al. (2007). The NDF concentration (% DM) of samples and in vitro residues was determined by using a neutral detergent solution containing α-amylase and sodium sulfite, following the procedure reported by Goering and Van Soest (1970) adapted for an Ankom 200 Fiber Analyzer (Ankom Technology). The NDF percentages were determined using the following equation:

$$\text{NDF (\% of DM)} = [(\text{bag wt} + \text{residue}) - (\text{bag wt} \times \text{bag correction factor})]/[(\text{bag wt} + \text{sample}) - (\text{bag wt})] \times 100.$$

The bag correction factor is the weight of an empty, sealed bag divided by the weight of the same bag after undergoing the ruminal in vitro and NDF procedure. Neutral detergent fiber digestibility was determined using the following equation:

$$\text{NDFD (\% of NDF)} = 100 \times [(\text{NDF}_{0h} - \text{NDF}_{\text{residue}})/(\text{NDF0h})].$$

In Situ Starch Disappearance 7 h

Measurements of in situ starch disappearance (**isSD**) were carried out at the University of Wisconsin campus tiestall barn (Dairy Cattle Center, Madison, WI) under a protocol approved by the Animal Care and Use Committee of the College of Agricultural and Life Sciences. To evaluate isSD, the dried whole-plant corn samples were ground to pass through a 4-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Dacron polyester cloth bags (R1020, 10 × 20 cm and 50 ± 10 microporosity; Ankom Technology, Macedon, NY) containing 5.00 ± 0.25 g of DM of each variety were used. Two ruminally cannulated lactating cows were fed a diet containing 60% forage (alfalfa and corn silage) and 40% concentrate. The in situ bags were placed in a nylon laundry bag (30 × 40 cm) and then positioned in the ventral rumen for 7 h. Each laundry bag was attached to the inside of the rumen cannula with a 0.75-m-long nylon rope and contained a rubber weight to ensure they remained submerged in the ruminal contents. Bags were moistened in warm water before incubation. Blank bags were also incubated to allow correction for any infiltration of DM into sample bags. After removal, samples were soaked in ice water for 15 min to inhibit ruminal microbial activity and rinsed with room-temperature tap water to wash off any large particles adhered to the bags. The bags were then placed in clean laundry bags for further rinsing in a washing machine (Roper RTW4516F*, Whirlpool Corp., Benton Harbor, MI) set on the rinse and spin cycle with room-temperature water for 30 min. The bags were dried in a forced-air oven at 60°C for 48 h. Residues were ground through a 1-mm Udy mill screen (Udy Corp., Boulder, CO) for starch analysis. Duplicates within cows were composited into 1 sample before nutrient analysis. Samples were submitted to the same commercial laboratory and analyzed for starch as described previously. The mean starch disappearance value of the 2 bags on a treatment per incubation time was calculated for each cow.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The experiment was analyzed as a split-plot in a randomized complete block design with 3 blocks (i.e., replicates) and 5 varieties [i.e., treatment (**TRT**)]. The whole-plot was TRT (i.e., 3 SSC and 2 TSC) and the subplot was maturity (HM; i.e., 114, 119, 125, 135, and 149 d after planting). Plot served as the experimental unit (n = 15) and *HM* represented repeated observations within each plot. The statistical model was formulated as follows:

$$Y_{ijk} = \mu + Block_i + TRT_j + (Block \times TRT)_{ij} + HM_k + (TRT \times HM)_{jk} + \varepsilon_{ijk},$$

where Y_{ijk} is the observed response variable; μ is the overall mean; $Block_i$ is the fixed effect of block (i = 1–3); TRT_j is the fixed effect of treatment (j = 3 SSC and 2 TSC); $(Block \times TRT)_{ij}$ is the interaction between block and *TRT* (whole-plot error term); HM_k is the fixed effect of harvest moment (k = 114, 119, 125, 135, and 149 d after planting); $(TRT \times HM)_{jk}$ is the interaction between *TRT* and *HM*; and ε_{ijk} is the subplot error term. The LSM were estimated using the LSMEANS statement, and pairwise comparisons between TRT and HM were performed

using Tukey's adjustment test. A contrast comparing SSC (3 varieties) and TSC (2 varieties) was run to evaluate the overall effect of genotype. When HM resulted as significant, without the interaction effect between TRT and HM, specific orthogonal contrasts were applied to evaluate linear and quadratic trends across HM. When the interaction effect between TRT and HM was significant (i.e., $P < 0.05$) and biologically relevant, data were graphically reported to show effects of HM for each response. Being the HM levels not equally spaced, specific contrast coefficients were computed using the PROC IML (version 9.4; SAS Institute Inc., Cary, NC). Statistical significance was declared at $P < 0.05$ for both main effects and interaction.

RESULTS

Fresh Yield and DM Yield

Data for fresh and DM yields are reported in Table 1.

No effect of TRT on fresh ($P = 0.318$) and DM ($P = 0.848$) yields was found. Additionally, no differences within genotypes (i.e., SSC vs. TSC) on fresh ($P = 0.683$) and DM ($P = 0.906$) yields were observed. Interaction between HM and TRT showed significant effect on fresh yield ($P < 0.001$) and DM yield ($P = 0.002$; Figures 1A and 1B, respectively). Notably, the highest fresh yield (Figure 1A) was observed for SSC2 at 125 d (i.e., 103.2 t/ha); whereas at the same HM, the lowest value was reported for TSC2 (i.e., 78.7 t/ha). Regarding DM yield (Figure 1B), the highest value of DM yield was reported at 125 d for SSC2 (i.e., 34.3 t/ha); whereas at the same HM, the lowest value was observed for TSC2 (i.e., 27.2 t/ha).

Nutrient Composition and Milk2024 Calculation

Table 2 reports the effect of HM, TRT, and their interaction on nutrient composition and Milk2024 index calculation.

A quadratic effect ($P < 0.001$) on DM concentration was observed with the advance of maturity. An interaction between HM and TRT was detected ($P < 0.001$) for CP concentration (Figure 2). Notably, the highest CP value was observed at 125 d for SSC2 (i.e., 8.8% DM); whereas the lowest for TSC2 (i.e., 8.4% DM) at the same HM. A linear effect ($P < 0.001$) for soluble CP and NDICP was observed between the HM. A quadratic effect ($P < 0.001$) in NDF fraction was observed with the advance of maturity. Notably, the lowest NDF value was detected at 149 d (i.e., 38.7% DM). A linear effect ($P < 0.001$) on ADF and ADL among the different HM was detected. The lowest concentration for ADF was detected at 149 d (i.e., 21.0% DM); whereas the lowest ADL concentra-

Table 1. Effect of harvest moment (HM) and treatment (TRT; i.e., 3 SSC and 2 TSC varieties) on fresh yield, DM yield of whole-plant corn silage

Item	Unit of measurement	Harvest moment[1] (d)					Treatment[2]						*P*-value				Orthogonal contrast[3]	
		114	119	125	135	149	SSC1	SSC2	SSC3	TSC1	TSC2	SEM	HM	TRT	TRT × HM	SSC vs. TSC[4]	Linear	Quadratic
Fresh yield	t/ha	97.4^{c}	87.7^{b}	87.8^{b}	79.5^{a}	73.9^{a}	83.2	88.1	85.5	87.2	82.4	3.95	<0.001	0.318	<0.001	—	—	—
DM yield	t/ha	25.7^{a}	25.5^{a}	30.2^{b}	34.1^{c}	34.7^{c}	30.6	30.1	29.6	30.4	29.6	1.24	<0.001	0.848	0.002	—	—	—

$^{a-c}$Different superscripts within rows for harvest moment or treatment indicate that the specific means differed at $P < 0.05$. When the interaction was significant (i.e., $P < 0.05$) and biologically relevant, data were presented to show effects of harvest moment for each response.

[1]HM, harvest moment (i.e., 114, 119, 125, 135, and 149 d).

[2]SSC1, DKC6648SC corn variety of SSC; SSC2, DKC6646SC corn variety of SSC; SSC3, DKC7236SC corn variety of SSC; TSC1, DKC7023 corn variety of TSC; TSC2, DKC6731 corn variety of TSC.

[3]Orthogonal contrasts correspond to linear and quadratic effect of HM (i.e., 114, 119, 125, 135, and 149 d).

[4]SSC vs. TSC, genotypes were contrasted using Tukey's test. SSC, short-stature corn genotype; TSC, tall-stature corn genotype.

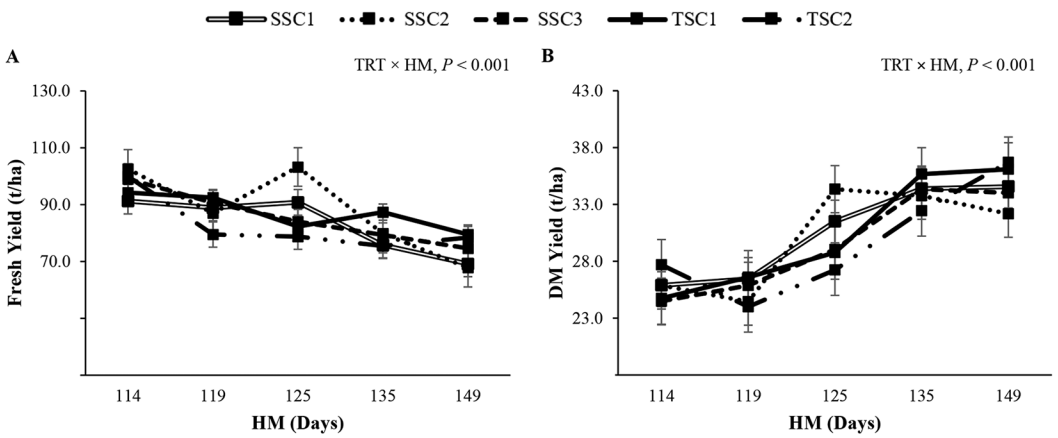


Figure 1. Effect of treatment (TRT; i.e., SSC1 [DKC6648SC corn variety of SSC]; SSC2 [DKC6646SC corn variety of SSC]; SSC3 [DKC7236SC corn variety of SSC]; TSC1 [DKC7023 corn variety of TSC]; TSC2 [DKC6731 corn variety of TSC]) and harvest moment (HM; i.e., 114, 119, 125, 135, 149 d) on fresh yield (A) and DM yield (B) of whole-plant corn silage. Error bars represent SEM.

tion was detected at 149 d (i.e., 3.5% DM). In contrast, a linear effect ($P < 0.001$) for starch was observed with the advancing stages of maturity. Starch increased among the stages of maturity, peaking at 149 d after planting (i.e., 39.1% DM). A linear effect ($P < 0.001$) on WSC content was observed; specifically, WSC decreased with the advance of maturity. A quadratic effect ($P < 0.001$) on EE was detected. A linear effect ($P < 0.001$) for ash was detected; indeed, ash decreased with the advance of HM. Predictions of Milk2024 (kg milk/t DM) had a linear effect ($P < 0.001$) as maturity stage progressed, reaching a peak at 149 d (i.e., 1,515.0 kg milk/t DM).

Concerning the effect of TRT, DM concentration differed among TRT ($P = 0.024$). Notably, SSC1 had higher DM concentration in comparison to SSC2, SSC3, and TSC1, whereas TSC2 had an intermediate value that did not differ from either group. Soluble CP differed among TRT ($P = 0.006$) and genotypes ($P < 0.001$). The NDF, ADF, and ADL concentrations were affected by TRT ($P < 0.001$) and genotype ($P < 0.001$). Overall, SSC varieties had lower concentrations of NDF, ADF, and ADL in comparison to TSC varieties. Differences in terms of starch concentration were reported for TRT ($P = 0.009$) and genotype ($P < 0.001$). On average, starch was higher by 12.7% for SSC varieties with respect to TSC varieties. The WSC concentration was affected by TRT ($P < 0.001$) and genotype ($P < 0.001$). In general, SSC varieties had lower WSC than TSC. Within TSC, WSC concentration was higher for TSC2 than TSC1 (i.e., 6.0% DM vs. 4.2% DM, respectively). The EE was affected by TRT ($P < 0.001$) and genotype ($P < 0.001$). Regarding predictions of Milk2024, differences for both TRT ($P < 0.001$) and genotype ($P < 0.001$) were observed. Overall, SSC varieties had higher energy content than TSC varieties by 5.4%.

Nutrient Digestion

The effects of HM, TRT, and their interaction on NDFD and starch disappearance are reported in Table 3.

Considering the HM, a quadratic effect for NDFD after 12 h ($P = 0.004$) and 24 h ($P = 0.021$) of incubation was detected with the advance of maturity. The NDFD after 30 h decreased among the HM performing a linear effect ($P = 0.017$). The NDFD after 48 h was affected by HM ($P = 0.002$), showing the lowest value at 135 d after planting (i.e., 49.8% NDF). Interaction between TRT and HM was detected ($P = 0.027$) on NDFD after 120 h (Figure 3). Notably, the highest NDFD was detected at 135 d after planting for SSC1 (i.e., 66.3% NDF), whereas the lowest value was detected at the same HM for SSC2 (i.e., 57.6% NDF, respectively); additionally, the highest value was observed for SSC1 at 149 d after planting (i.e., 67.2% NDF), whereas at the same HM the lowest value was observed for TSC1 (i.e., 58.3% NDF). Starch disappearance decreased among the HM ($P < 0.001$) with a linear effect. Notably, the highest isSD was detected at 114 d (i.e., 73.2% starch), whereas the lowest at 149 d (i.e., 41.7% starch). The uNDF decreased ($P = 0.007$) with the advance of maturity, recording the lowest value at 149 d after planting (i.e., 9.3% DM).

An effect of TRT ($P = 0.038$) and genotype ($P = 0.003$) on NDFD after 30 h was observed. On average, the NDFD was higher for SSC varieties than TSC by 9.4%. A genotype effect ($P = 0.006$) for NDFD after 48 h was observed. Notably, the NDFD was higher for SSC genotype than TSC. Differences for the NDFD after 120 h were observed for TRT ($P < 0.001$) and genotype ($P < 0.001$). The NDFD was higher for SSC varieties than TSC by 8.8%. The isSD was affected by TRT ($P = 0.002$) and genotype ($P < 0.001$). The highest isSD value was

Table 2. Effect of harvest moment (HM) and treatment (TRT) on nutrient composition and Milk2024 calculation of whole-plant corn silage

		Harvest moment[1] (d)					Treatment[2]						*P*-value				Orthogonal contrast[3]	
Item[4]	Unit of measurement	114	119	125	135	149	SSC1	SSC2	SSC3	TSC1	TSC2	SEM	HM	TRT	TRT × HM	SSC vs. TSC[5]	Linear	Quadratic
DM	%	26.5^{a}	29.1^{b}	34.5^{c}	43.0^{d}	47.1^{e}	37.7^{b}	35.3^{a}	35.4^{a}	35.2^{a}	36.5^{ab}	0.84	<0.001	0.024	0.084	0.543	<0.001	<0.001
CP	% DM	9.2^{c}	8.9^{b}	8.7^{b}	8.3^{a}	8.4^{a}	8.9	8.8	8.5	8.7	8.6	0.14	<0.001	0.078	<0.001	—	—	—
Soluble CP	% CP	43.1^{c}	36.7^{ab}	35.9^{ab}	39.8^{bc}	35.4^{a}	35.5^{a}	36.4^{a}	36.5^{a}	40.4^{ab}	42.1^{b}	2.38	<0.001	0.006	0.920	<0.001	<0.001	0.166
ADICP	% DM	0.54	0.51	0.52	0.51	0.57	0.55	0.52	0.53	0.54	0.51	0.040	0.567	0.369	0.191	—	—	—
NDICP	% DM	1.47^{bc}	1.49^{c}	1.53^{c}	1.25^{a}	1.31^{ab}	1.39	1.36	1.33	1.48	1.48	0.105	0.019	0.686	0.674	—	<0.001	0.641
NDF	% DM	46.6^{c}	44.1^{c}	41.5^{b}	40.0^{ab}	38.7^{a}	40.5^{a}	40.8^{a}	40.7^{a}	44.3^{b}	44.6^{b}	1.47	<0.001	<0.001	0.661	<0.001	<0.001	<0.001
ADF	% DM	23.8^{b}	22.9^{ab}	23.1^{b}	23.2^{b}	21.0^{a}	21.7^{a}	21.7^{a}	21.8^{a}	24.6^{b}	24.2^{b}	1.14	0.003	<0.001	0.874	<0.001	<0.001	0.197
ADL	% DM	4.1^{b}	3.9^{b}	3.8^{ab}	3.9^{b}	3.5^{a}	3.6^{a}	3.5^{a}	3.6^{a}	4.3^{b}	4.2^{b}	0.18	<0.001	<0.001	0.852	<0.001	<0.001	0.393
Starch	% DM	29.6^{a}	33.2^{b}	33.7^{b}	34.3^{b}	39.1^{c}	35.6^{b}	35.6^{b}	35.4^{b}	31.8^{b}	31.4^{a}	1.81	<0.001	0.009	0.887	<0.001	<0.001	0.952
WSC	% DM	5.4^{c}	3.5^{ab}	3.7^{ab}	4.2^{b}	3.1^{a}	3.2^{a}	3.1^{a}	3.5^{ab}	4.2^{b}	6.0^{c}	0.53	<0.001	<0.001	0.364	<0.001	<0.001	0.112
EE	% DM	2.4	2.5	2.7	2.8	2.7	2.7^{bc}	2.8^{c}	2.8^{c}	2.5^{a}	2.5^{ab}	0.09	<0.001	<0.001	0.805	<0.001	<0.001	<0.001
Ash	% DM	4.2^{b}	4.3^{b}	4.3^{b}	4.0^{a}	3.8^{a}	4.0	4.2	4.0	4.3	4.0	0.13	<0.001	0.179	0.129	—	<0.001	0.105
Milk2024	kg milk/t DM	1,450.0^{a}	1,466.0^{a}	1,481.0^{ab}	1,471.0^{ab}	1,515.0^{b}	1,503.0^{b}	1,509.0^{b}	1,516.0^{b}	1,419.0^{a}	1,436.0^{a}	25.65	0.003	<0.001	0.806	<0.001	<0.001	0.647

$^{a–d}$Different superscripts within rows for harvest moment or treatment indicate that the specific means differed at a $P < 0.05$. When the interaction was significant (i.e., $P < 0.05$) and biologically relevant, data were presented to show effects of harvest moment for each response.

[1]HM, harvest moment (i.e., 114, 119, 125, 135, 149 d).

[2]SSC1, DKC6648SC corn variety of SSC; SSC2, DKC6646SC corn variety of SSC; SSC3, DKC7236SC corn variety of SSC; TSC1, DKC7023 corn variety of TSC; TSC2, DKC6731 corn variety of TSC.

[3]Orthogonal contrasts correspond to linear and quadratic effect of HM (i.e., 114, 119, 125, 135, 149 d).

[4]ADICP, acid detergent insoluble CP; NDICP, neutral detergent insoluble CP; SSC, short-stature corn genotype; TRT, treatment (i.e., 3 SSC and 2 TSC varieties); TSC, tall-stature corn genotype; WSC, water-soluble carbohydrates.

[5]SSC vs. TSC, genotypes (i.e., short-stature and tall-stature) were contrasted using Tukey's test.

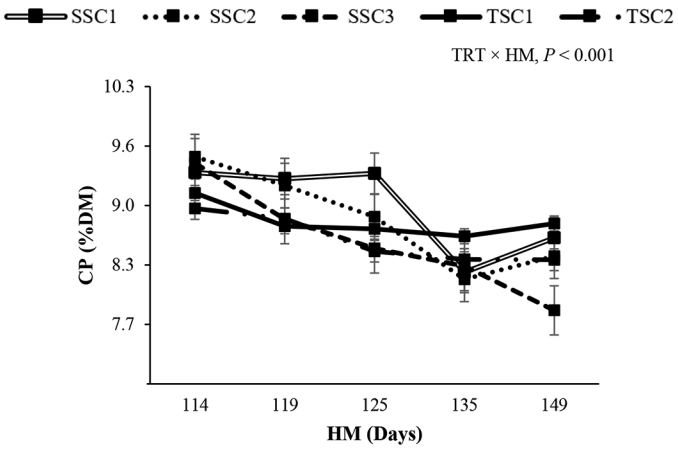


Figure 2. Effect of treatment (TRT; i.e., SSC1 [DKC6648SC corn variety of SSC]; SSC2 [DKC6646SC corn variety of SSC]; SSC3 [DKC7236SC corn variety of SSC]; TSC1 [DKC7023 corn variety of TSC]; TSC2 [DKC6731 corn variety of TSC]) and harvest moment (HM; i.e., 114, 119, 125, 135, 149 d) on CP of whole-plant corn silage. Error bars represent SEM.

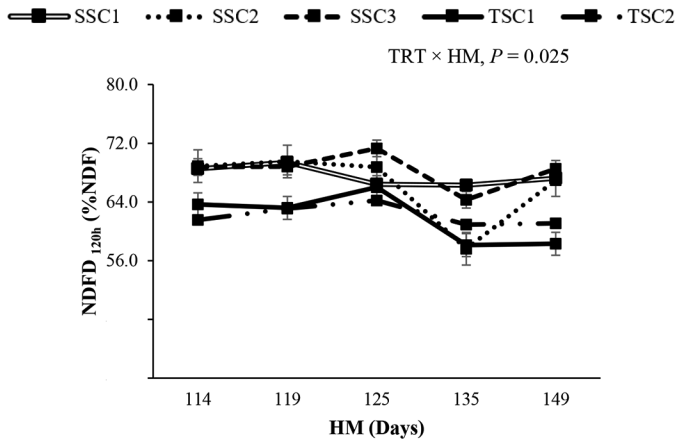


Figure 3. Effect of treatment (TRT; i.e., SSC1 [DKC6648SC corn variety of SSC]; SSC2 [DKC6646SC corn variety of SSC]; SSC3 [DKC7236SC corn variety of SSC]; TSC1 [DKC7023 corn variety of TSC]; TSC2 [DKC6731 corn variety of TSC]) and harvest moment (HM; i.e., 114, 119, 125, 135, 149 d) on $NDFD_{120h}$ of whole-plant corn silage. Error bars represent SEM.

recorded for SSC2 (i.e., 66.4% starch), whereas the lowest for TSC2 (i.e., 54.4% starch). The uNDF after 240 h of in vitro incubation was affected by TRT ($P < 0.001$) and genotype ($P < 0.001$). Overall, the uNDF value was lower by 34.4% for SSC varieties in comparison to TSC.

DISCUSSION

Fresh Yield and DM Yield

Maturity at harvest influences several crop factors, such as yield, nutrient composition, digestibility, and fermentation of the crop (Ferraretto et al., 2018). Fresh yield decreased among the HM for both genotypes, whereas DM yield increased as maturity progressed. This trial aimed to compare varieties characterized by SSC genotype to varieties of TSC genotype planted at different density rates (i.e., 13 plant/m^2 for SSC vs. 9 plant/m^2 for TSC) to maximize yield in high plant production system and compensate for the biomass deficit (i.e., shorter height) produced by the genotype SSC. Notably, whereas higher densities can increase total biomass, they can also reduce individual plant growth (Paponov et al., 2005). Corn varieties characterized by SSC genotype can be planted at higher densities, potentially increasing DM yield, optimizing the space and resource use—including light—due to a more favorable leaf architecture and a higher leaf area index (Barten et al., 2022; Kosola et al., 2023). However, no differences for fresh and DM yields were observed for the tested varieties in this study. Similar results were reported in trials in which corn varieties from different genotypes were compared. For instance, Peña et al. (2023) evaluated the effects of corn-planting population, comparing different corn varieties characterized by BMR and conventional genotype. Even if authors reported similar DM yield for both genotypes planted at different rates, corn varieties characterized by different genotypes can be planted at higher density without penalizing the yields. It should be highlighted that in the current study the effects of plant density and genotype were not analyzed separately. This made it impossible to distinguish between these 2 factors; thus, this aspect represents a future area of investigation.

Nutrient Composition and Milk2024 Calculation

During the maturation of the corn plant, sugars in kernels are converted to starch, increasing the DM concentration of kernels and whole-plant (Allen et al., 2003). In this trial, a linear increase in DM concentration was observed among the HM. Moreover, harvesting whole-plant corn hybrids for silage at a DM concentration ranging from 32% to 36% was recommended to optimize nutritive value and nutrient digestibility of the ensiled crop and dairy cow performance (Ferraretto et al., 2018). Within the growing conditions of this study, our results suggest that whole-plant corn for silage should be harvested at least 125 d from planting.

The CP concentration decreased with the advance of maturity, with high concentration at 114 d and a low content at 149 d. Indeed, crops harvested too early present low concentrations of DM, high CP and fiber content, and low levels of starch (Ferreira and Brown, 2016). Referring to the tested varieties, values for CP ranged among the data present in interested literature (Gallo et al., 2016; Tharangani et al., 2021). The TRT and HM

Table 3. Effect of harvest moment (HM) and treatment (TRT) on nutrient digestion of whole-plant corn silage

Item[4]	Unit of measurement	Harvest moment[1] (d)					Treatment[2]						*P*-value				Orthogonal contrast[3]		
		114	119	125	135	149	SSC1	SSC2	SSC3	TSC1	TSC2	SEM	HM	TRT	TRT × HM	SSC vs. TSC[5]	Linear	Quadratic
$NDFD_{12h}$	% NDF	19.4^{b}	19.0^{ab}	15.1^{a}	15.0^{a}	16.7^{ab}	17.1	16.5	19.0	16.3	16.3	2.25	0.005	0.364	0.867	—	0.027	0.004
$NDFD_{24h}$	% NDF	30.8^{b}	29.1^{ab}	25.9^{a}	26.4^{a}	26.4^{a}	28.1	28.7	29.5	25.0	27.2	2.47	0.007	0.205	0.236	—	0.005	0.021
$NDFD_{30h}$	% NDF	39.7^{ab}	41.8^{b}	38.9^{ab}	38.7^{ab}	37.7^{a}	41.0^{b}	40.2^{b}	41.0^{b}	37.4^{a}	37.1^{a}	2.08	0.043	0.038	0.603	0.003	0.017	0.976
$NDFD_{48h}$	% NDF	52.0^{ab}	54.5^{b}	55.8^{b}	49.8^{a}	52.9^{ab}	55.4	54.4	54.6	50.2	50.5	2.49	0.002	0.082	0.149	0.006	0.269	0.979
$NDFD_{120h}$	% NDF	66.3^{bc}	66.8^{bc}	67.3^{c}	61.4^{a}	64.4^{b}	67.6^{b}	66.3^{b}	68.3^{b}	61.9^{a}	62.2^{a}	1.50	<0.001	<0.001	0.027	—	—	—
isSD	% starch	73.6^{d}	68.0^{c}	60.9^{b}	59.5^{b}	41.7^{a}	60.2^{abc}	66.4^{c}	64.0^{bc}	58.6^{ab}	54.4^{a}	2.76	<0.001	0.002	0.565	<0.001	<0.001	0.186
$uNDF_{240h}$	% DM	10.3^{ab}	9.9^{ab}	9.6^{a}	10.7^{b}	9.3^{a}	8.9^{a}	8.8^{a}	9.1^{a}	11.4^{b}	11.0^{b}	0.58	0.007	<0.001	0.882	<0.001	0.257	0.213

$^{a-d}$Different superscripts within rows for harvest moment or treatment indicate that the specific means differed at a $P < 0.05$. When the interaction was significant (i.e., $P < 0.05$) and biologically relevant, data were presented to show effects of harvest moment for each response.

[1]HM, harvest moment (i.e., 114, 119, 125, 135, 149 d).

[2]SSC1, DKC6648SC corn variety of SSC; SSC2, DKC6646SC corn variety of SSC; SSC3, DKC7236SC corn variety of SSC; TSC1, DKC7023 corn variety of TSC; TSC2, DKC6731 corn variety of TSC.

[3]Orthogonal contrasts correspond to linear and quadratic effect of HM (i.e., 114, 119, 125, 135, 149 d).

[4]isSD, in situ starch disappearance 7 h; $NDFD_{12,24,30,48,120h}$ (NDF digestibility after 12, 24, 30, 48, and 120 h of incubation); SSC, short-stature corn genotype; TRT, treatment (i.e., 3 SSC and 2 TSC varieties); TSC, tall-stature corn genotype; $uNDF240h$, indicates the part of the fiber that has not been digested after 240 h.

[5]SSC vs. TSC, genotypes (i.e., short-stature and tall-stature) were contrasted using Tukey's test.

affected soluble CP concentration. The SSC varieties had lower soluble CP than TSC. This variation may be explained by considering some different mutations in endosperm due to different zein levels that may affect protein-starch complexes of WPCS (Varela et al., 2023). The highest soluble CP value was found at 114 d. Rondahl et al. (2011) similarly observed a decrease in soluble CP with advancing maturity.

The WPCS quality depends on stover portion, as 40% to 50% of the whole-plant consists of stover, which is high in NDF, ADF, and lignin (Hunt et al., 1992). The fibrous fractions decreased with the advance of maturity. Xu et al. (1995) documented the change in yield, plant physical and chemical composition, and whole-plant and stover digestibility for WPCS hybrids harvested at 3 stages of maturity. The authors concluded that as maturity advanced from milk to black layer, the NDF, ADF, and lignin decreased. Considering the TRT effect, in this study, SSC exhibited lower NDF and ADF concentrations than TSC hybrids. Similar results have been observed by Bourdoncle et al. (2023), in which SSC had a lower NDF and ADF content than TSC and BMR hybrids. The lignin concentrations were lower in SSC varieties compared with TSC. Lignin has always been considered a limiting factor in ruminant nutrition due to its relationship with cellulose and hemicellulose, which creates a physical barrier, obstructing the degradation process of the rumen microorganisms (Hatfield, 1993). In the current study, the low lignin concentration in SSC varieties may find an explanation in the suppression of the internode elongation, which is operated by the *br2* mutation. Notably, the *br2* has the ability to encode a P-glycoprotein1 protein, regulating the auxin flux from node to internode and avoiding the auxin accumulation in the intercalary meristem region to suppress the elongation of internodes (Zhang et al., 2019). Bourdoncle et al. (2023) observed that the lignin composition in corn varieties carrying the *br2* mutation was 4.3% points lower than TSC corn.

Starch concentration increased with the advance of maturity due to the conversion of sugars in kernels. As maturity progresses, the kernel proportion increases, and sugar in the amyloplast cells of kernels is converted to starch during ear development until physiological maturity, at which time the kernel reaches its maximum in terms of DM weight (Gusmão et al., 2021). Plant density can influence nutrient composition of WPCS, such as starch concentration (Allen et al., 2003). Starch concentration was higher in SSC than TSC. The change in starch composition is related to the selection of hybrids with starch with a higher amylopectin content than amylose or hybrids with a more floury endosperm (Giuberti et al., 2014). To the best of our knowledge, only Bourdoncle et al. (2023) evaluated the nutrient composition of these SSC, which present higher starch concentration

in comparison to BMR and TSC. The concentration of WSC declined with the advance of maturity, due to their conversion to starch within the kernel, followed by an increase in DM of the crop (Allen et al., 2003). Considering WSC composition between the tested genotypes, varieties of TSC genotypes had a higher WSC concentration in comparison to varieties of SSC; in contrast, this difference can explain the lower starch concentration in the TSC with respect to SSC.

However, data for EE presented differences between HM and TRT effects, but differences were small and not considered biologically meaningful. Ash slightly decreased among the HM. Nevertheless, ash content differed between TRT; these differences were also small and of minor biological relevance, even if ash content ranged among the data reported by Hoffman (2005).

Energy content of corn varieties (kg milk/t DM) was predicted using the Milk2024. Notably, SSC reported a higher energy content (kg milk/t DM) in comparison to TSC. Bourdoncle et al. (2023) reported no difference in terms of energy content between SSC and BMR.

Nutrient Digestion

Differences in ruminal ivNDFD at 12, 24, 30, 48, and 120 h were detected for the HM effect. The NDFD is affected by the stage of maturity because the tissues of the crop have different digestibility rates in the early and advanced phases (Adesogan et al., 2019). The NDFD of whole-plant corn declines with the advance of maturity, which is related to greater stover lignification among the maturation of the whole-plant corn (Buxton and O'Kiely, 2003). In the current study, lower NDFD values were reported at 135 and 149 d after planting. Therefore, delaying maturity at harvest may affect NDFD in whole-plant corn harvested for silage production (Allen et al., 2003). Regarding the TRT effect, differences in NDFD at 30, 48, and 120 h were observed. Bourdoncle et al. (2023) observed the high NDFD in SSC is related to a higher leaves/stalk ratio and a different deposition of lignin. Particularly, the authors observed that SSC had a higher ivNDFD at 30 h when compared with TSC. The different distribution of lignin in the tissues of whole-plant would favor NDFD (Méchin et al., 2005). In particular, the higher NDFD of SSC may find an explanation in the different repartition of lignin. Particularly in SSC varieties, lignin deposition is more pronounced at a lower rate during the senescence, and it is distributed in the cortical part of the stem rather than in the pith, allowing a better microbial digestibility by increasing the accessible degradable area that can be attacked by rumen microbes (Jung et al., 2000; Méchin et al., 2005; Bourdoncle et al., 2023). Additionally, this condition may contribute to having a longer harvest window for SSC varieties in comparison to TSC varieties (Barten et al., 2022). Catellani et al. (2026) evaluated the NDFD after 48 h of rumen incubation testing a corn variety corn hybrid carrying the *br2* mutation in comparison to a TSC. The authors observed an 8% increase in NDFD 48 h for the SSC in comparison to TSC. The TRT affected the uNDF at 240 h. Considering the genotype effect, SSC had lower values than TSC for uNDF at 240 h. The low uNDF concentration can be explained by considering the lower lignin levels in smart hybrids that might explain the greater NDFD (Oba and Allen, 1999). Low uNDF contents were reported by Ferraretto et al. (2015) in BMR corn hybrids used to formulate dairy cow diets in comparison to floury-leafy corn hybrids. Referring to the data reported by our study, the low uNDF concentration of SSC was measured even when this parameter was reported on NDF basis in comparison to TSC (i.e., 23.7% vs. 26.8% NDF, respectively).

The isSD declined with the advance of maturity. Late stages of maturity in WPCS impair starch digestibility in lactating dairy cows (Ferraretto and Shaver, 2012), which is explained by the increase in kernel vitreousness (Correa et al., 2002) and the vitreous-to-floury endosperm ratio, which might be related to increased zein proteins cross-linked to starch granules (Giuberti et al., 2014). Jensen et al. (2005) evaluated the effects of maturity stage of WPCS harvested with 3 different levels of DM on digestion of starch and NDF. The authors stated that increased maturity at harvest corresponded to a high starch content, but starch digestibility decreased with maturity. Similar results have been reported by Saylor et al. (2021), stating a greater isSD for the early maturity stage (1/4 milk line) in comparison to the other harvest maturities (1/2 and 3/4 milk line, respectively). The isSD was also affected by TRT. Particularly, SSC had greater starch degradability. Variations in isSD between corn varieties for snaplage production with different proportions of vitreous and floury endosperm were reported by Gusmão et al. (2021). Particularly, the authors observed that varieties with low vitreous endosperm had greater starch degradability. To our knowledge, there are no studies evaluating starch disappearance in corn varieties carrying *br2* mutation; therefore, more studies are needed to clarify this scenario.

In summary, forage feeding value for dairy cattle of 3 SSC varieties was evaluated in comparison to 2 TSC varieties. With the planting densities used in this study, no differences between genotypes were detected for the yield parameters. The SSC had higher energy content (kg milk/t DM) and starch concentrations and lower NDF compared with TSC. The ivNDFD at 30, 48, and 120 h was higher in SSC than TSC. The SSC had a greater

isSD than TSC, which may be related to a better conversion within the kernel fractions that makes starch of SSC more digested than TSC genotypes.

CONCLUSIONS

Considering the aforementioned results, cropping SSC may be considered as a feasible strategy, in terms of agronomic response and nutritional composition, to enhance silage nutritional quality and properly cover the high energy requirements of high-producing lactating dairy cows. However, it is important to assert that these outcomes may vary in different climate conditions and with agronomic practices. For instance, despite SSC and TSC varieties being planted at different density rates (i.e., 13 plants/m^2 vs. 9 plants/m^2, respectively), this management aspect deserves future investigations to elucidate how high-density rates can influence WPCS quality. Therefore, future research is needed to elucidate the performance, both in qualitative or quantitative terms, of the tested corn varieties at different plant densities or in distinct areas, highlighting the agronomical advantages/disadvantages and their effects on lactation performance.

NOTES

This work was supported by Bayer Crop Science S.r.l. (Bayer AG, Leverkusen, Germany). Part of the study was conducted in the framework of projects supported by "Fondazione Romeo and Enrica Invernizzi" (Milano, Italy) as part of "Safety of Silage" and NODES ("Nord Ovest Digitale e Sostenibile"), which has received funding from the MUR – M4C2 1.5 of PNRR with grant agreement n. ECS00000036. Appreciation is extended to graduate and visiting students of Ferraretto Ruminant Nutrition Laboratory for their valuable help during the analysis and John Goeser and all the staff of the Rock River Laboratory (Watertown, WI). The in vitro procedure was conducted under an approved protocol by the Animal Care and Use Committee of the College of Agriculture and Life Sciences, University of Wisconsin–Madison. Measurements of in situ starch disappearance were carried out at the University of Wisconsin campus tiestall barn (Dairy Cattle Center, Madison, WI) under a protocol approved by the Animal Care and Use Committee of the College of Agricultural and Life Sciences. Authors Jean-Luc Pellet, Emanuele Badalotti, and Michela Battisti are employees at Bayer Crop Science, and they played no role in collection, analysis, and interpretation of data. The other authors have not stated any conflicts of interest.

Nonstandard abbreviations used: ADICP = acid detergent insoluble CP; EE = ether extract; GDD = growing degree days; HM = harvest moments; isSD = in situ starch disappearance; ivNDFD = in vitro NDF digestibility; NDFD = NDF digestibility; NDICP = neutral detergent insoluble CP; NIRS = near-infrared reflectance spectroscopy; SG = stay-green; SSC = short-stature corn; SSC1 = DKC6648SC; SSC2 = DKC6646SC; SSC3 = DKC7236SC; TRT = treatment; TSC = tall-stature corn; TSC1 = DKC7023; TSC2 = DKC6731; uNDF240 = undigested NDF after 240 h of in vitro incubation; WPCS = whole-plant corn silage; WSC = water-soluble carbohydrates.

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