


## Forage production and rumen degradation kinetics of silage from brown midrib corn hybrids *versus* conventional corn hybrids



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### Abstract:

The purpose was to evaluate the production and content of dry matter (DM), bromatological composition, digestibility and degradation kinetics of DM and neutral detergent fiber (NDF), and the estimated milk production in the silage from brown midrib corn hybrids (BMHs) and conventional corn hybrids (CVHs). CVHs produced 21 % more DM than BMHs (27.4 *vs* 22.5 t ha<sup>-1</sup>, respectively), and there was an increase when harvesting at stage R5 (25.7 t ha<sup>-1</sup>) compared to stage R4 (24.3 t ha<sup>-1</sup>). The harvest stage did not affect the digestibility of DM or NDFD but the year and type of hybrid affected the fraction of indigestible NDF (iNDF, % DM), which was higher in 2020 (25.6 %) than in 2019 (13.8 %) and in CVHs (22.6 %) than in BMHs (16.8 %). Consequently, milk production per ton of DM (MIT) was higher in 2019 and in BMHs compared to 2020 and CVHs. In contrast, the year did not affect milk production per hectare (MIHA) as the higher DM production in 2020 compensated for the drop in MIT, but the lower DM production in BMHs reduced the value of MIHA compared to CVHs (29.1 *vs* 32.5 t ha<sup>-1</sup>, respectively). The MIHA estimate increased when harvesting at stage R5 (31.8 t ha<sup>-1</sup>) compared to R4 (29.7 t ha<sup>-1</sup>). Although the BMHs had

better digestibility and more milk production potential per ton of DM, the higher DM yield in CVHs compensated for the difference, and more milk was obtained per hectare.

**Keywords:** Digestibility, Milk, *Zea mays*.

Received: 01/04/2025

Accepted: 14/07/2025

## Introduction

In the main dairy basins of Mexico, corn silage accounts for between 30 and 45 % of the dry base of the dairy cow's diet<sup>(1,2)</sup>. This has put pressure on cropland to supply the demand for forage, which conflicts with water shortage<sup>(2)</sup>. Therefore, it is preponderant to increase the yield of forage corn with high grain content and adequate digestibility of neutral detergent fiber (NDFD)<sup>(3)</sup>. The use of outstanding hybrids and harvesting at an adequate stage of grain maturity can help obtain better quality silage<sup>(4)</sup>. Recent research has shown that harvesting between 33 and 38 % DM optimizes yield and nutritional quality without affecting NDFD<sup>(3,4)</sup>. On the other hand, feed intake is negatively associated with indigestible NDF (iNDF), which is determined at 240 h of *in vitro* or *in situ* incubation<sup>(5)</sup>. iNDF mainly represents lignified NDF that accumulates in stems and is influenced by an interaction between genotype and environment<sup>(6,7)</sup>. Forages with low iNDF improve feed intake, while increasing passage rate and reducing enteric methane production<sup>(8)</sup>. Thus, NDFD is related to rumen energy supply, and iNDF is associated with feed intake<sup>(9,10)</sup>.

Brown midrib hybrids (BMHs) with the *bm* mutation induce less lignin accumulation by reducing phenol synthesis in the cell wall<sup>(11)</sup>. Consequently, forage from BMHs has up to 8 percentage units more NDFD than that of conventional hybrids, but the latter have 10 to 15 % higher DM yield potential than the former<sup>(12)</sup>. In Mexico, it is unknown whether a genetic improvement program exists to obtain BMHs adapted to various agroclimatic conditions. In contrast, there is a growing interest in dairy farms to utilize BMHs to obtain silage with higher digestibility, even when domestic corn forage production is insufficient<sup>(13)</sup>. If it adds to the above a marked effect of agroclimatic variations on the iNDF and NDFD<sup>(4,14)</sup>, then there would be a greater deficit of forage with lower quality than expected. Therefore, the first hypothesis was to prove that, regardless of the harvest stage and the conditions of the evaluation year, the yield of conventional hybrids exceeds that of the BMHs, and even if the latter had better forage quality, the conventional hybrids would have more milk production per hectare, which would compensate for the difference between hybrids. The second hypothesis was to prove that the iNDF and the degradation rate of the NDF do not

decrease when harvesting at a later stage of plant or grain maturity, but the DM yield does increase, and consequently, so does milk production per hectare. Thus, the objective was to evaluate, over two consecutive years, the DM production and content of the forage, the bromatological profile, the digestibility and degradation kinetics of DM and NDF, as well as the milk production per ton of forage and hectare in BMHs and conventional hybrids harvested at two stages of grain maturity.

## Material and methods

### Study site

The research was conducted under irrigation conditions in the SS-2019 and SS-2020 cycles at the Pabellón Experimental Field, located at 1,907 m asl, 22.0936 ° N and 102.1733 ° W; semi-dry temperate climate with an average annual temperature of 17 °C and annual rainfall of 471 mm. The soil in which the experiment was established has a sandy loam texture, a pH of 7.9, an organic matter of 0.9 %, and an electrical conductivity of 1.2 dS/m.

### Experimental design and treatments

In each year (2019 and 2020), a randomized complete block design with four replications and arrangement in split plots was used, the large plot was the type of hybrid, BMH (*bm3*) or conventional (CVH), and the small plot was the state of maturity of the grain at harvest at 3/4 advance of the doughy phase and terminal milk line (R4 vs R5, respectively). The BMH group included the genotypes (AG-0924 and AG-0917; Anzu Seeds®, Waco, TX); in the CVH group, Hércules (Aspros®, Toluca, Mexico), Salamandra (Asgrow®, Guadalajara, Mexico), and P3026W (Pioneer®, Guadalajara, Mexico) were used. All the hybrids used were single-crossed, intermediate cycle ( $863 \pm 24$  GDD to male flowering); CVHs were white semident grained with plant heights between 300 and 330 cm, whereas BMHs were yellow dent grained with plant heights between 260 and 275 cm. The experimental plot ( $n=40$  per year) consisted of four rows, 0.75 m apart and 5.0 m long, with the useful plot being the two central rows.

Sowing was carried out in dry soil on 05/19/2019 and 05/15/2020, using an experimental planter (Almaco®, Nevada, IO). After planting, an irrigation tape line was placed in each furrow, and irrigation was provided until the moisture reached the seed. At 20 d post-emergence, a uniform population of 92,400 plants  $\text{ha}^{-1}$  was left, equivalent to 7 plants per linear meter. Total fertilization was 303 N-86 P-00 K + 16 Mg, applying 30 % of the N, all P, and all Mg at sowing and the rest of the N in four weekly applications through irrigation from d 35 post-sowing. Irrigation was applied twice a week to meet or exceed 100 % of the

potential evaporation ( $EV_0$ ) requirement recorded at a National Water Commission station located 170 m from the work site.

### Harvesting and sample processing

The plots were harvested when the grain reached the R4 (grain 3/4 doughy phase) or R5 (doughy grain with terminal milk line) stage. The days to harvest in 2019 were 128/133 and 134/140, whereas in 2020, they were 123/131 and 133/142 d in the BMHs and CVHs in the R4 and R5 phases, respectively. In each useful plot, all the plants were cut to a height of ~20 cm above ground level; the total fresh weight was then recorded, and a random sample of five complete plants was taken for grinding in a mill (DPM-500, Nogueira, Brazil). The chopped fresh forage was manually homogenized, and two sub-samples were taken, one of ~200 g to dry in a forced-air oven at 55 °C to constant weight to determine DM and calculate the forage yield (on a dry basis) at harvest; and another 300 g sample to make microsilos in 20 × 40 cm embossed bags, and the air was extracted with a vacuum machine (Nesco VS-02, Two Rivers, WI). The microsilos were left to ferment for 90 days in a cool, dark place.

### Laboratory analyses

After the fermentation period, the microsilos were opened, and each one was emptied onto a flat surface, where the content was homogenized and quartered to reduce to a 150 g sample, which was dried at 55 °C to determine DM. The dried sample was then ground to pass through a 1 mm sieve (Arthur P. Thomas, Philadelphia, PA), and then the bromatological analyses were performed following the standardized AOAC<sup>(15)</sup> and *in situ* digestibility methodologies. NDF, ADF, and lignin contents were sequentially determined in F-57 bags on the A-200 fiber analyzer (Ankom Tech, Macedonia, NY). The NDF was determined using  $\alpha$ -amylase and sodium sulfite, then ADF was determined in a 1N solution of H<sub>2</sub>SO<sub>4</sub> and CTAB, and finally, the lignin content was analyzed by immersing the bags for 3 h in a 72 % H<sub>2</sub>SO<sub>4</sub> solution. The total N content was determined using the semi-automated Kjeldahl procedure, digesting 1.0 g of sample in H<sub>2</sub>SO<sub>4</sub> at 400 °C for 1 h and then distilling the reaction (Tecator-2508 and Kjeltex-8100; Foss, Hilerrød, Denmark) to be titrated with 0.5N HCl; the crude protein (CP) content was calculated as % N × 6.25. The starch content was analyzed with the enzymatic-colorimetric release procedure<sup>(16)</sup>, where 1.0 g of sample was incubated for 60 min at 100 °C in 30 mL of 100 mM acetate buffer solution + 100  $\mu$ L of  $\alpha$ -amylase; then the reaction was incubated for 2 h in 3 mL of GOPOD solution for 50 min (Megazyme Ltd., Wicklow, Ireland), and the absorbance of the reaction was read at 505 nm in a visible-light spectrophotometer (Genesis 10S, Thermo Sci., Madison WI). The ash content was determined in 2.0 g of sample incinerated in a crucible at 550 °C in a muffle. Finally, the ethereal extract was assumed to be 2.8 % for all samples due to the minimal variation that exists in corn silage<sup>(9)</sup>. The non-fibrous carbohydrate (NFC) content was obtained by

difference [NFC= 100 – (% NDF + % CP + % ash + % ethereal extract)] as calculated in the NASEM model<sup>(9)</sup>.

### Rumen degradation kinetics

For each year of evaluation (n= 2), a composite sample was obtained from each hybrid and harvest stage from the four replications (n= 40). From each sample, approximately 4.5 g was introduced into Dacron R1020 bags (Ankom Tech., Macedonia, NY) and incubated in duplicate at 12, 24, 36, 48, and 240 h in the ventral sac of two rumen fistulated cows. All bags were simultaneously removed and manually rinsed until clear water was obtained; the bags at 0 h to measure fraction *a* (*fa*) were immersed for 10 min in a bucket containing previously heated tap water at 37 °C and then rinsed. All bags, including those of 0 h, were dried in a forced-air oven at 55 °C until they reached a constant weight. DM digestibility (DMD) was calculated as the difference between the final and initial weights; the NDF digestibility (NDFD) was determined by analyzing the bag residue using the procedure described above for NDF. The degradation rate (*kd*) of DM and NDF was calculated using linear regression, based on the natural logarithm of the ratio between DMD or NDFD at 12, 24, 36, or 48 h and the digestible fraction at 240 h<sup>(17,18)</sup>. The slope was then calculated using the four logarithms calculated to obtain the *kd* with a linear regression in Excel (Microsoft®, Redmond, WA). The effective rumen degradation (ERD) of DM and NDF was calculated using the following model<sup>(19)</sup>:

$$\text{ERD} = 100 \times \{ fa + fb \times [ kd / (kd + kp) ] \}$$

Where *fa* is the percentage of DM or NDF disappeared at 0 h; *fb* is the potentially digestible percentage of DM or NDF calculated as 100 – % *fa* – % *fc*, where *fc* is the percentage of DM or NDF not digested at 240 h; *kd* is the calculated degradation rate of DM or NDF; and *kp* is the passage rate of DM estimated in the NASEM<sup>(9)</sup> model using the bromatological profile analyzed, it was assumed that the *kp* of NDF was 37 % lower than that of MS<sup>(5)</sup>.

### Estimated milk production

For each observation (n= 80), the bromatological profile was captured in the Milk-2024 model, using the NDFD at 48 h and the iNDF at 240 h calculated as the percentage of *fc* multiplied by the percentage of NDF in the total DM. The above was used to obtain the kilograms of milk per ton of dry forage (MIT), which was multiplied by the DM yield (t ha<sup>-1</sup>) to estimate the milk production per hectare (MIHA).

## Statistical analysis

All data were analyzed in the R program (R-Studio Inc., Boston, MA) for an analysis of variance using the *agricolae* package with the following statistical model:

$$Y = \mu + A_i + R_j + T_k + \beta_{ijk} + E_l + (E \times T)_{kl} + (A \times E \times T)_{ikl} + E_{ijkl}$$

Where:

$Y$  is the response variable,

$\mu$  is the overall mean,

$A$  is the fixed effect of the  $i$ -th year of evaluation ( $i = 1$  to  $2$ ),

$R$  is the random effect of the  $j$ -th replication ( $j = 1$  to  $4$ ),

$T$  is the fixed effect of the  $k$ -th type of hybrid ( $k = 1$  to  $2$ ),

$\beta$  is the experimental error associated with the large plot,

$E$  is the fixed effect of the  $l$ -th harvest stage ( $l = 1$  to  $2$ ),  $E \times T$  is the interaction between harvest stage and hybrid type,

$(A \times T \times E)$  is the interaction between year, hybrid type, and harvest stage,

$E_{ijkl}$  is the residual error.

Data on degradation kinetics, DMD, and NDFD at 12, 24, 36, and 48 h were analyzed using the same model, but with the random effect of replication removed and replaced by the random effect of the cow ( $n=2$ ). The values reported in the tables and figures are least squares means obtained with the EMMEANS package. Statistical significance was declared at  $P \leq 0.05$ ; if considered significant, the separation of means was done with Tukey's test with  $\alpha = 0.05$  using the TukeyHSD command.

## Results and discussion

### Agrometeorological conditions

Table 1 presents the temperatures, precipitation, and growing degree-days (GDD) recorded in 2019 and 2020, along with the historical average for a 30-yr period (1989 – 2018). Compared to the historical average, the temperatures of 2019 were more similar than those recorded in 2020, which was considered cooler, especially in the second quarter. It is worth mentioning that 2019 and 2020 are recorded as neutral in the El Niño-Southern Oscillation (ENSO) Status<sup>(20)</sup>; therefore, they are considered normal years without extreme drought or humidity events. The rainfall recorded in the SS cycle was 288 mm in 2019 and 315 mm in 2020, whereas the historical average rainfall is 395 mm for the same period, which represents a deviation of -27 and -20 %, respectively. The distribution of rainfall differed between years; in 2019, the highest rainfall was recorded in August and September, which coincided with

grain filling (R1 – R4). In contrast, in 2020, the highest rainfall occurred in July and August, which coincided with flowering (VT) and the beginning of grain filling (R0 – R1). In general, it is inferred that the evaluation years differed in the thermal regimes, which affected the accumulation of GDD to some degree. Although there was only an 8 % difference in precipitation between the two years, the distribution of rainfall contrasted in the cycles evaluated.

**Table 1:** Temperatures, precipitation, and growing degree-days recorded in the Pabellón Experimental Field in 2019 and 2020 and the 30-yr historical average in the same locality

Year / month	Maximum T. (° C)	Minimum T. (° C)	Average T. (° C)	Precipitation (mm)	GDD
2019					
May	31	11	21	0	11.1
June	32	14	24	7	13.1
July	29	14	22	31	11.6
August	29	15	22	85	11.9
September	28	13	21	152	10.8
October	27	12	19	13	9.5
2020					
May	30	10	20	0	10.5
June	31	15	22	4	12.6
July	29	15	22	155	11.7
August	27	14	21	125	10.5
September	26	12	19	31	9.3
October	25	9	17	0	7.6
Historical <sup>1</sup>					
May	31	13	22	11	11
June	30	15	22	75	12
July	28	14	21	105	11
August	28	14	21	89	11
September	27	13	20	81	10
October	27	11	18	34	9

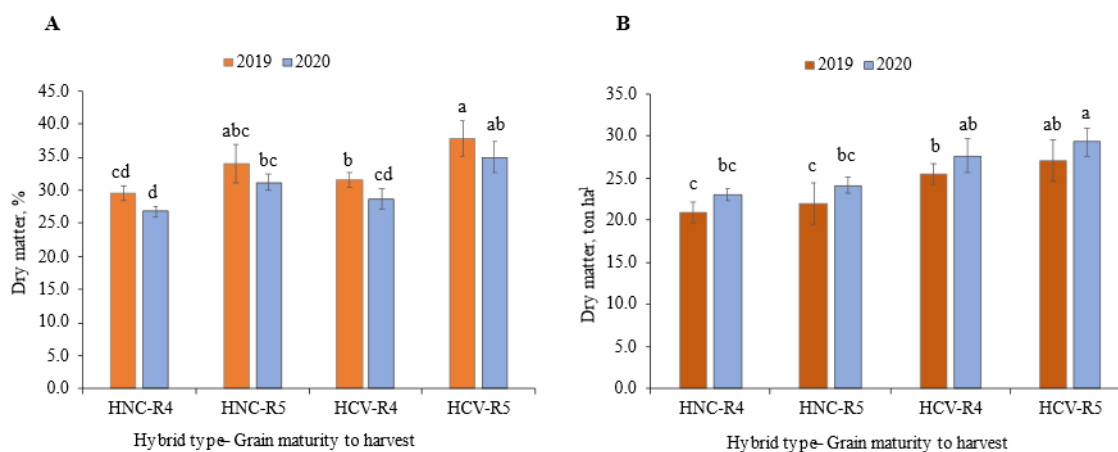
Historical average (1989 – 2018) obtained courtesy of Dr. Arturo Corrales Suastegui, INIFAP-CEPAB (<https://clima.inifap.gob.mx/lnmysr/>).

T= temperature; GDD= growing degree-days calculated as the difference between the Average T. and the base temperature of corn (i.e., 10 °C).

## Dry matter content and forage production

DM content was affected by the interaction of year  $\times$  hybrid type  $\times$  harvest stage ( $P < 0.01$ ) that is shown in Figure 1A. In 2019, BMHs had lower DM content than CVHs harvested in the R4 phase, whereas in R5, both types of hybrids had a similar DM percentage. The opposite was true in 2020, where DM content was similar between BMHs and CVHs harvested in both R4 and R5. DM content at harvest was higher in 2019 than in 2020 ( $33.3 \pm 3.4$  vs  $30.4 \pm 3.5$  %, respectively;  $P < 0.01$ ); this may be associated with the fact that 2019 was a slightly warmer year than 2020 (Table 1), and that could have accelerated the loss of moisture from the plant or promoted a greater accumulation of DM by having more GDDs<sup>(4,21)</sup>. CVHs had a higher percentage of DM at harvest than BMHs ( $33.3 \pm 1.6$  vs  $30.4 \pm 2.4$  %, respectively;  $P < 0.01$ ). Similar results, showing lower DM content in BMHs compared to CVHs, have been reported in other studies<sup>(12,22)</sup>; in contrast, other reports have documented similar DM content or even a higher percentage of DM when harvesting at the same stage of grain maturity<sup>(23,24)</sup>. As expected, the dry matter content was higher when harvesting in the R5 phase than in R4 ( $34.5 \pm 2.7$  vs  $29.2 \pm 2.0$  %, respectively;  $P < 0.01$ ), which is associated with the increase in grain percentage<sup>(4)</sup>.

**Figure 1:** A) Dry matter content and B) Dry matter production of brown midrib corn hybrids (HNC=BMH) and conventional corn hybrids (HCV=CVH) harvested at two stages of grain maturity



<sup>abcd</sup> Bars with different letters differ ( $P \leq 0.05$ ) and in each type of bar, BMH ( $n = 8$ ) and CVH ( $n = 12$ ).

As shown in Figure 1B, forage production was affected by the interaction of year  $\times$  type of hybrid  $\times$  harvest stage ( $P < 0.01$ ); in both years of evaluation, DM yield was similar among BMHs harvested in both R4 and R5; in contrast, in CVHs, forage production did differ between the evaluation year and the harvest phase. DM yield was  $2.2 \text{ t ha}^{-1}$  lower in 2019 compared to 2020 ( $23.9 \pm 2.9$  vs  $26.1 \pm 2.9 \text{ t ha}^{-1}$ , respectively;  $P = 0.02$ ), which is attributed

to an interaction among genotype, management, and environment<sup>(14)</sup>. In the present study, management is ruled out since it was practically identical in 2019 and 2020, and the genotypes were also the same in both cycles. It is possible that the more temperate environment in 2020 was more favorable for the vegetative development of corn, as it decreases its yield when grown in warmer environments<sup>(14)</sup>. CVHs produced 4.8 t ha<sup>-1</sup> more DM than BMHs (27.4 ± 1.6 vs. 22.6 ± 1.4 t ha<sup>-1</sup>, respectively;  $P < 0.01$ ), which is consistent with different documented works<sup>(17)</sup>. The mutation of the *bm* gene that confers the brown midrib trait generates plants of shorter height than their conventional isogenic counterparts<sup>(11)</sup>, which would explain the lower yield. On the other hand, DM production tended to be higher when harvesting in the R5 grain phase than in R4, with a difference of 1.4 t ha<sup>-1</sup> ( $P = 0.10$ ). This is due to grain accumulation, although the corn plant has its maximum grain and starch deposit between R2 and R4<sup>(4)</sup>.

### Bromatological composition

No interaction was detected for any of the parameters evaluated (Table 2). NDF and NFC contents, which together accounted for approximately 84 % of DM, were influenced by hybrid type (both  $P < 0.05$ ), but not by year or harvest phase. BMHs had 2.9 fewer units of NDF and 2.6 units more of NFC than CVHs, resulting in an inversely proportional relationship. This could be associated with a lower plant height in the BMHs than in the CVHs (274 vs 314 cm, respectively; *data not shown*), which concentrates a greater proportion of the ear of corn in the DM and dilutes the NDF. The lignin content was affected by the type of hybrid ( $P < 0.01$ ), but not by the year of evaluation or harvest phase. In the BMHs, the lignin content was 0.9 units lower than in the CVHs (2.4 vs 3.3 %, respectively). This difference is consistent with what has been reported in other studies comparing *bm3* BMHs and conventional hybrids<sup>(10,25)</sup>. On the other hand, starch content was not affected by the type of hybrid, but tended to be slightly higher when harvesting in the R5 phase than in R4 ( $P = 0.06$ ). In contrast, the year of study affected ( $P < 0.01$ ) the starch content, which was lower in 2019 (26.4 ± 1.9 %) than in 2020 (30.8 ± 1.5 %). This result is possibly due to the fact that 2020 was a more temperate year, which may have promoted better grain filling, which increases starch accumulation<sup>(26)</sup>. Finally, the concentration of NEL was 0.13 units higher in 2019 than in 2020, and there was an identical difference in favor of BMHs compared to CVHs (1.53 vs 1.40 Mcal kg<sup>-1</sup>, respectively; both  $P < 0.01$ ). This result is explained by consistently higher NDFD and lower iNDF in 2019 compared to 2020, as explained in detail below.

**Table 2:** Bromatological composition of corn silage from brown midrib hybrids (BMHs) and conventional hybrids (CVHs) harvested at two stages of grain maturity

Variable	Composition, % DM							NeL Mcal kg <sup>-1</sup>
	CP	NDF	ADF	ADL	ASH	NFC	STA	
BMH (n = 32)								
R4-2019	7.9 <sup>a</sup>	48.0	24.2	2.1	4.5	36.7	25.2 <sup>b</sup>	1.61
R4-2020	9.2 <sup>b</sup>	49.2	23.9	2.3	4.5	34.2	31.6 <sup>a</sup>	1.47
R5-2019	7.3 <sup>a</sup>	46.3	22.5	2.5	4.8	38.7	29.0 <sup>b</sup>	1.59
R5-2020	8.6 <sup>b</sup>	47.5	23.1	2.6	4.7	36.3	30.4 <sup>a</sup>	1.46
CVH (n = 48)								
R4-2019	7.3 <sup>a</sup>	51.6	21.6	3.2	4.3	33.9	24.3 <sup>b</sup>	1.44
R4-2020	8.6 <sup>b</sup>	51.7	20.9	3.4	4.6	32.2	30.7 <sup>a</sup>	1.30
R5-2019	7.9 <sup>a</sup>	49.4	22.5	3.1	4.8	35.0	27.2 <sup>b</sup>	1.49
R5-2020	8.2 <sup>b</sup>	49.7	22.1	3.3	4.8	34.4	30.6 <sup>a</sup>	1.35
SEM <sup>1</sup>	0.18	1.59	0.91	0.22	0.40	1.63	1.79	0.08
Year	< 0.01	0.43	0.88	0.43	0.76	0.51	< 0.01	< 0.01
TH	0.11	0.04	0.29	< 0.01	0.58	0.05	0.45	< 0.01
Har	0.09	0.65	0.79	0.80	0.88	0.08	0.06	0.96
TH × Har	0.57	0.57	0.08	0.28	0.91	0.52	0.80	0.27

SEM= standard error of the mean; TH= type of hybrid (BMH vs. CVH); Har= harvest phase (R4 vs. R5); TH × Har= interaction between type of hybrid and harvest phase.

CP= crude protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin; ASH= ashes; NFC= non-fibrous carbohydrates; STA= starch; NEL= net energy for lactation calculated at three times the maintenance with the determined bromatological profile.

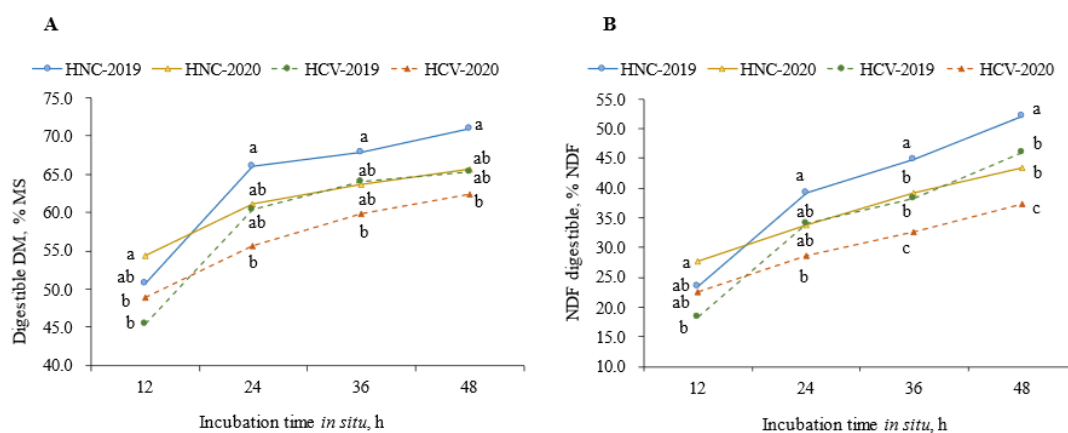
<sup>a,b</sup> Means with different letters differ ( $P \leq 0.05$ ).

### Digestibility of DM and NDF

DMD and NDFD were sensitive ( $P < 0.05$ ) only to the type of hybrid and year at each incubation time (Figures 2A and 2B). DMD was consistently higher in BMHs than in CVHs for 12, 24, 36, and 48 h incubation (52.6 vs 47.1 %, 63.7 vs. 58 %, 65.8 vs 62.0 %, and 68.3 vs 63.9 % of DM, respectively; all  $P < 0.05$ ). NDFD was higher in BMHs than in CVHs at 12, 24, 36, and 48 h of incubation (25.7 vs. 20.4 %, 36.6 vs 31.3 %, 42.0 vs. 35.4 %, and 47.8 vs 41.7 % of NDF, respectively; all  $P < 0.05$ ). It is known that the higher NDFD in brown midrib hybrids is due to the lower lignification of NDF conferred by the *bm* gene<sup>(11)</sup>. In the present study, BMHs had 4.9 % lignified NDF compared to 6.4 % of CVHs (calculated using data from Table 2). Except at 12 h, both DMD and NDFD were higher in 2019 than in 2020. Discrepancies in both digestibilities at 12 h may be due to the lag phase of NDFD, which refers to the time it takes before NDF degradation begins<sup>(19,27)</sup>. This derives from an interaction between the morphophysiology of the plant and the structural biochemistry of the fibers<sup>(7,27)</sup>. Thus, it is possible that, compared to the 2019 harvest, the 2020 silages had a

shorter lag time, which favored the higher NDFD at 12 h of incubation. On the other hand, it has been documented that warmer conditions during the vegetative development of the plant lead to greater NDFD, which is due to the shortening of internodes and an increase in stem thickness<sup>(14)</sup>. As 2019 was slightly warmer than 2020, that could explain the higher NDFD at 24, 36, and 48 h of incubation. In summary, the differences observed in DMD are mainly due to NDFD, where both followed a similar increase at the four incubation times.

**Figure 2:** A) Dry matter (DM) digestibility and B) Neutral detergent fiber (NDF) digestibility of silage from two types of corn hybrids (TH), brown midrib (BMH) and conventional (CVH), harvested at two stages of grain maturity



<sup>abcd</sup> Points with different letters differ in each incubation time ( $P \leq 0.05$ ) and in each type of line, BMH ( $n = 4$ ) and CVH ( $n = 6$ ).

## Rumen degradation kinetics

No interactions were detected for any of the parameters determined. As shown in Table 3, the fraction *a* (*fa*) of DM was 3.0 units higher in 2019 than in 2020 (9.2 vs 6.2 %, respectively;  $P < 0.01$ ). The *fa* of DM is associated with the content of non-protein nitrogen (NPN), simple sugars, and water-soluble phenolic compounds<sup>(9)</sup>. It is very likely that the sugar content in 2019 was higher than in 2020, as the NFC content was similar between years, but the difference in starch percentage suggests that the remainder was sugars. The *fb* of DM was 8.3 units higher in 2019 than in 2020 (69.4 vs 61.1 %, respectively;  $P < 0.01$ ), and differences were also detected between BMHs and CVHs, where the former had 3.1 units more *fb* than CVHs (66.8 vs 63.7 %, respectively;  $P = 0.02$ ). The fraction *b* (*fb*) is the proportion of DM (or NDF) potentially digestible in the rumen and is a function of retention time and degradation rate<sup>(19,27)</sup>. The CP (NPN and true protein) and NFC fractions have high degradation rates ( $kd > 8 \% h^{-1}$ ), so they are retained in the rumen for less than 24 h<sup>(9)</sup>. Therefore, after 12 h, the *fb* of DM largely depends on the size of the *fb* of NDF, which can be verified by observing both parameters in Table 3. The fraction *c* (*fc*) of DM was 11.2 units

lower in 2019 than in 2020 and 3.8 units lower in BMHs than in CVHs (32.7 vs 21.5 % and 29.0 vs 25.2 %, respectively; both  $P < 0.01$ ). The *fc* of DM refers to the indigestible part after 48 h and is mainly linked to the *fc* of NDF, which represents indigestible fiber. Considering the *fa*, *fb*, and *fc*, the effective rumen degradation (ERD) of DM contrasted between BMHs and CVHs, with a difference of 5.9 percentage units (43.0 vs 37.1 %, respectively). The harvest stage affected the ERD of DM, being 2.9 units higher in R5 (41.5 %) than in R4 (38.6 %). However, the *kd* of DM was not altered and averaged  $4.7 \pm 0.5 \text{ h}^{-1}$ . In general, although the year of evaluation had an impact on fractions *a*, *b*, and *c*, ultimately, the ERD was strongly influenced by the type of hybrid and, to a lesser degree, by the harvest stage.

**Table 3:** Rumen degradation kinetic parameters of corn silage from brown midrib hybrids (BMHs) and conventional hybrids (CVH) harvested at two stages of grain maturity

	Dry matter (DM)					Neutral detergent fiber (NDF)				
	<i>fa</i>	<i>fb</i>	<i>fc</i>	EDR % DM	<i>kd</i> % h <sup>-1</sup>	<i>fa</i>	<i>fb</i>	<i>fc</i>	EDR % NDF	<i>kd</i> % h <sup>-1</sup>
BMH (n= 16)										
R4-2019	10.4 <sup>a</sup>	70.8 <sup>a</sup>	18.8 <sup>b</sup>	42.8 <sup>B</sup>	4.0	9.8	68.4 <sup>a</sup>	21.8 <sup>b</sup>	44.2 <sup>a</sup>	3.0 <sup>b</sup>
R4-2020	7.4 <sup>b</sup>	62.6 <sup>b</sup>	30.0 <sup>a</sup>	40.1 <sup>B</sup>	5.1	10.3	44.6 <sup>b</sup>	45.1 <sup>a</sup>	34.6 <sup>b</sup>	3.5 <sup>a</sup>
R5-2019	8.7 <sup>a</sup>	71.1 <sup>a</sup>	20.3 <sup>b</sup>	46.7 <sup>A</sup>	5.4	8.8	66.0 <sup>a</sup>	25.2 <sup>b</sup>	41.9 <sup>a</sup>	3.0 <sup>b</sup>
R5-2020	5.7 <sup>b</sup>	62.8 <sup>b</sup>	31.5 <sup>a</sup>	42.4 <sup>A</sup>	6.6	9.3	42.2 <sup>b</sup>	48.5 <sup>a</sup>	32.2 <sup>b</sup>	3.5 <sup>a</sup>
CVH (n= 24)										
R4-2019	9.1 <sup>a</sup>	64.5 <sup>a</sup>	26.4 <sup>b</sup>	37.1 <sup>B</sup>	3.6	6.5	60.5 <sup>a</sup>	33.0 <sup>b</sup>	36.3 <sup>a</sup>	2.9 <sup>b</sup>
R4-2020	6.1 <sup>b</sup>	56.3 <sup>b</sup>	37.6 <sup>a</sup>	34.5 <sup>B</sup>	4.8	7.3	36.4 <sup>b</sup>	56.3 <sup>a</sup>	26.8 <sup>b</sup>	3.4 <sup>a</sup>
R5-2019	8.7 <sup>a</sup>	71.1 <sup>a</sup>	20.3 <sup>b</sup>	39.5 <sup>A</sup>	3.6	7.4	59.8 <sup>a</sup>	32.8 <sup>b</sup>	36.8 <sup>a</sup>	2.9 <sup>b</sup>
R5-2020	5.7 <sup>b</sup>	62.8 <sup>b</sup>	31.5 <sup>a</sup>	37.4 <sup>A</sup>	4.8	5.8	38.0 <sup>b</sup>	56.2 <sup>a</sup>	26.1 <sup>b</sup>	3.4 <sup>a</sup>
SEM	1.65	1.88	1.22	1.37	0.7 5	1.81	1.96	2.15	2.58	0.2 5
Year	< 0.01	< 0.01	< 0.01	0.08	0.0 6	0.81	< 0.01	< 0.01	< 0.01	0.0 3
TH	0.45	0.02	< 0.01	< 0.01	0.0 8	< 0.01	< 0.01	< 0.01	< 0.01	0.6 7
Har	0.30	0.50	0.60	0.04	0.4 2	0.65	0.48	0.55	0.55	0.9 3
TH × Har	0.97	0.88	0.52	0.80	0.3 4	0.94	0.29	0.40	0.82	0.9 6

*fa*= fraction A disappeared at 0 h of incubation; *fb*= digestible fraction obtained by difference [100 - (*fa* + *fc*)]; *fc* = fraction not digested at 120 h of *in situ* incubation; *kd*= degradation rate; EDR= effective digestibility in rumen.

SEM= standard error of the mean; TH= type of hybrid (BMH vs CVH), Cos= harvest stage (R4 vs R5).

<sup>a,b</sup> Means with different lowercase letters differ statistically in year of evaluation.

<sup>A,B</sup> Means with different capital letters differ statistically in harvest phase.

As shown in Table 3, no interactions were detected, nor was any effect of harvest stage on any of the degradation kinetic parameters of NDF. The *fa* only differed between hybrid types and was 2.8 units higher in BMHs than in CVHs (9.5 vs 6.7 %, respectively;  $P < 0.01$ ). Usually, the *fa* of NDF is associated with non-degradable soluble phenolic compounds related to the synthesis of lignin and other water-soluble fiber fractions<sup>(28)</sup>. In the *fb*, differences were detected between the year of evaluation and the type of hybrid, and it was 1.6 times higher in 2019 than in 2020, and there was a difference of 6.6 percentage units between BMHs and CVHs (63.7 vs 40.3 % and 55.3 vs 48.7 %, respectively; both  $P < 0.01$ ). This was strongly influenced by the differences observed in NDFD, where the climatic conditions of 2019 may have promoted a more digestible NDF than in 2020, whereas the lower lignified NDF of BMHs explains the difference from CVHs. Consequently, the *fc* also differed between years of evaluation and type of hybrid (both  $P < 0.01$ ), being 1.8 times higher in 2020 than in 2019 and 9.4 percentage units higher in CVHs than in BMHs (51.5 vs 28.2 % and 44.6 vs 35.2 % NDF, respectively). Consequently, the ERD of NDF was 9.9 percentage units higher in 2019 than in 2020, and there was a difference of 6.7 units more in BMHs than in CVHs (39.8 vs 29.9 % and 38.2 vs 31.5 %, respectively; both  $P < 0.01$ ). The *kd* of NDF only differed between years of evaluation ( $P = 0.03$ ), and was 3.5 % h<sup>-1</sup> in 2020 and 2.9 % h<sup>-1</sup> in 2019; these values are within the expected range for corn silage<sup>(9)</sup>.

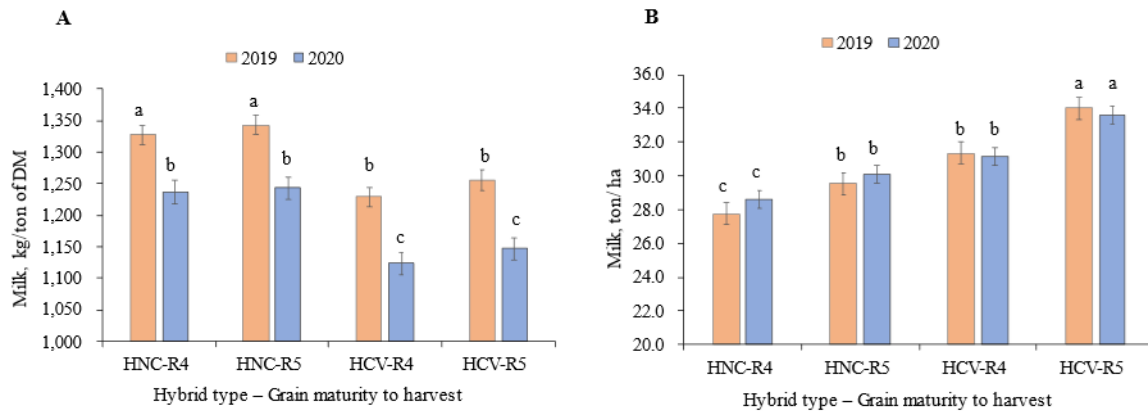
The *fc* of NDF is also known as the iNDF, expressed as a percentage of DM, and can be estimated by multiplying the *fc* by the percentage of NDF. The importance of iNDF is that it is negatively associated with feed intake in dairy cows, and therefore, with milk production<sup>(29)</sup>. In the present research, the values of *fc* or iNDF are higher than those reported in other studies<sup>(5,14,30)</sup>. In contrast, a study conducted in the region reported iNDF values of 23 % DM in corn forage from four conventional hybrids<sup>(4)</sup>. This is similar to the iNDF estimated in 2020 ( $25.6 \pm 3.5$  %) or CVHs ( $22.6 \pm 3.4$  %), but differs from the iNDF in 2019 ( $13.8 \pm 3.3$  %) or in BMHs ( $16.8 \pm 3.1$  %). Broadly speaking, the degradation kinetics of NDF were primarily influenced by the size of the *fc*, which may have been altered by meteorological differences between the two years of evaluation and the type of hybrid, but not by the interaction of these factors.

## Milk production

The interaction between year and type of hybrid affected the estimation of kilograms of milk per ton of dry forage (MIT;  $P < 0.01$ ). As shown in Figure 3A, the CVH-2019 had a similar MIT value as the BMH-2020 ( $1,241 \pm 10.9$  kg t<sup>-1</sup> DM), whereas the opposite occurred in the BMH-2019, which outperformed their counterparts in MIT, and the CVH-2020, which had the lowest value (1,335 vs 1,135 kg t<sup>-1</sup> DM, respectively). The harvest stage did not affect the value of MIT, but it was higher in 2019 (1,289 kg t<sup>-1</sup> DM) than in 2020 (1,188 kg). Likewise, the MIT value was higher in BMHs (1,288 kg t<sup>-1</sup> DM) than in CVHs (1,189 kg). The lower

NDF content, the higher NDFD, and the lower *fc* in BMHs compared to CVHs explain the higher value of MIT. The reduction in the value of MIT in BMH-2020 and its similarity to CVH-2019 can be associated with the increase in the *fc* of NDF in the former. This implies that, despite a higher starch content in 2020, the *fc* (or iNDF) has a marked negative impact that is not compensated by higher grain content.

**Figure 3:** A) Prediction of milk in kilograms per ton of dry forage and B) Prediction of milk in tons per hectare in brown midrib corn hybrids (BMH) and conventional corn hybrids (CVH) harvested at two stages of grain maturity



<sup>abcd</sup> Bars with different letters differ ( $P \leq 0.05$ ), and in each type of bar, BMH ( $n = 8$ ) and CVH ( $n = 12$ ).

In milk production per hectare (MIHA;  $t\ ha^{-1}$ ), an interaction between the type of hybrid and stage of harvest was detected ( $P < 0.01$ ). As shown in Figure 3B, in both years of study, CVH-R5 outperformed all others in MIHA ( $33.8 \pm 0.5\ t\ ha^{-1}$ ), whereas BMH-R4 had a lower MIHA value ( $28.2 \pm 0.6\ t$ ). For their part, BMH-R5 and CVH-R4 had a similar MIHA value ( $30.5 \pm 0.8\ t$ ) and differed from their counterparts. The year of study did not affect the estimated value of MIHA, but the higher potential for DM production ( $t\ ha^{-1}$ ) in CVHs resulted in them having a higher value of MIHA than BMHs ( $32.5 \pm 1.4$  vs  $29.1 \pm 1.0\ t\ ha^{-1}$ , respectively;  $P < 0.01$ ). On the other hand, harvesting at grain maturity in the R5 phase increased the MIHA estimate by  $2.1\ t\ ha^{-1}$  compared to harvesting in the R4 phase ( $31.8$  vs  $29.7\ t$ , respectively;  $P = 0.01$ ). Although the harvest stage had little effect on the bromatological composition and did not affect forage digestibility, the increase of  $1.4\ t\ ha^{-1}$  in DM production explains the difference in the estimated value of MIHA.

### Conclusions and implications

In the present study, regardless of the year and stage of harvest, forage yield was higher in conventional hybrids than in brown midrib hybrids; for their part, the year and the type of hybrid changed the nutritional composition and digestibility of the forage, where the brown

midrib hybrids were consistently superior to the conventional ones. This resulted in the former having higher milk production per ton of DM, but the higher forage yield in conventional hybrids counterbalanced that effect, and they had higher estimated milk production per hectare. The iNDF values obtained suggest that agronomic strategies should be considered to reduce this parameter, so future research may focus on improving forage digestibility in this regard.

### Acknowledgements

The authors are grateful to the National Institute of Forestry, Agricultural, and Livestock Research for the funding provided to conduct the study, as well as the team of field workers who helped care for the experiments. Likewise, thanks are extended to the National School of Sustainable Dairying S.C. de R.L. [ENLS (for its acronym in Spanish), Zapotlanejo, Jalisco] for allowing the use of the fistulated cows and for caring for them during the digestibility studies. Engineer Cassandra de la Cruz Espino is also thanked for her care and dedication in the laboratory analyses.

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