Digestibility of stover of local corn varieties from the Poblano-Tlaxcalteca highlands

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Abstract

The quality of corn stover is an important aspect to improve its consumption in ruminants, and little is known about its variation in the diversity of varieties grown in the Mexican highlands. The objective was to know the quality of stover (leaf) in native populations of corn in two regions of the Poblano-Tlaxcalteca highlands. For each region, 144 cultivars were tested in two locations, of which 134 were native populations and six commercial cultivars recommended for temperate zones. In vitro digestibility, neutral detergent fiber, acid detergent fiber, earliness, and yield of forage and grain were quantified. The experimental design was a simple 12x12 lattice with two repetitions. In both regions, differences in digestibility (p < 0.01) were found between cultivars in an interval of 10 percentage units. The outstanding local varieties were those that presented the highest digestibility (62.8 and 57.7% averages per region), compared to the commercial cultivars (57.3 and 57.7%), a variable that was associated with lower concentrations of neutral detergentinsoluble fiber (68.8 and 75.7% vs 71.7 and 78.3%), mainly, Earliness, grain coloration, or grain yield were not associated with digestibility. In conclusion, there is diversity in the digestibility of the leaf in stover between cultivars; some local varieties had greater digestibility than the commercial hybrids tested. Differences in digestibility were related to differences in neutral detergent fiber concentration, where commercial varieties tended to be more fibrous. Some local varieties outstanding in digestibility had high production of grain and stover.

Keywords:

High Valleys, landrace corns, nutritional value.



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Introduction

In the areas of the Mexican highlands, corn stover is an important by-product in ruminant feeding because it provides economic stability to the production unit since it maintains the livestock subsystem during the period of forage scarcity, which covers at least half a year (Martínez-Loperena *et al.*, 2011; Mutsamba *et al.*, 2020). However, according to some studies, the nutritional value of corn stover is low (*in vitro* digestibility of 47.3-60.8%, digestibility of neutral detergent fiber of 67.9-89.1%, of acid detergent fiber of 36.6-56.5% and crude protein of 4.1-7.8%) (Russell, 1986; Undi *et al.*, 2001; Methu *et al.*, 2001; Xie *et al.*, 2009; Hansey and de Leon, 2011; Aya#an *et al.*, 2020).

Results from trials of corn varieties where genetic variability has been narrow show that there is variation in the nutritional quality of stover (Dhillon *et al.*, 1990; Lundvall *et al.*, 1994; Xie *et al.*, 2009; Lorenz *et al.*, 2010; Hansey and de Leon, 2011). In native corns, which are genetically diverse, information regarding the nutritional variability of stover is limited (Estrada-Flores *et al.*, 2006), so little has been researched on the nutritional characteristics of native corn populations of different colors (white, blue, and yellow) or different earliness (late, intermediate, and early).

In Mexico, practically in any of its regions, the genetic diversity of corn is extensive, therefore, it is feasible to find variations at the morphological, metabolic, and physiological levels reflected in the components of the nutritional value. Based on the above, it can be inferred that it is possible to identify varieties with better quality and quantity of stover and that, at the same time, combine acceptable grain production.

The present study assessed some characteristics of the nutritional value of native corn stover in the region of Libres-Serdán of the State of Puebla and the valley of the region of Huamantla, Tlaxcala, to provide decision elements in the selection of varieties that combine a greater production of grain and a better quality of stover.

Materials and methods

Description of the study area

Samples of native corn seeds were collected at an altitudinal range of 2 340 to 2 980 m in two regions comprising 19 municipalities in the state of Puebla and four in the state of Tlaxcala, Mexico. These two regions were named Libres-Mazapiltepec-Huamantla (L-M-H) and Serdán-Tlachichuca-Guadalupe Victoria (S-T-GV). The municipalities in L-M-H were Cuyoaco, Tepeyahualco, Ocotepec, Libres, Oriental, San José Chiapa, Rafael Lara Grajales, Nopalucan, Mazapiltepec, and Soltepec, in the state of Puebla; Altzayanca, Citlaltepec, Cuapiaxtla and Huamantla in the state of Tlaxcala. The S-T-GV region included the municipalities of Chalchicomula de Sesma, Aljojuca, Esperanza, La Fragua, San Juan Atenco, San Nicolás Buenos Aires, San Salvador El Seco, Tlachichuca and Guadalupe Victoria.

In each region, the following was evaluated: 134 native populations, four commercial controls (32D06, Halcón, Z-60 and Sintética Serdán, for L-M-H and AS-722, Gavilán, Promesa and Sintético Serdán for S-T-GV) and six racial controls. Two experiments per region were established, which were located as follows.

For L-M-H, the localities were Buenavista de Guerrero, municipality of Cuyoaco, located at 19°38' 07" north latitude and 97° 30' 32" west longitude, with an altitude of 2 646 m with subhumid temperate climate with rains in summer, lower humidity (46.06%), of Phaeozem soils (INEGI, 2010), and Máximo Serdán, municipality of Lara Grajales, located at 9° 16' 36" north latitude and 97° 48' 19" west longitude, with an altitude of 2 402 m, with temperate climate with rains in summer, of greater humidity (100%), of Phaeozem soil (INEGI, 2010).

The accumulated rainfall in the experimental period was 572.2 mm. For S-T-GV, the localities were El Sabinal, municipality of Chalchicomula de Sesma, located at 18° 55' 19" north latitude and 97° 24' 07" west longitude, at an altitude of 2 540 masl, with semi-dry temperate climate and



Arenosol soil (INEGI, 2010), and Tlachichuca, municipality of Tlachichuca, located at 19° 06' 51" north latitude and 97° 25' 08" west longitude with an altitude of 2 600 m, of subhumid temperate climate with rains in summer, of medium humidity (43.13%) and Regosol soil (INEGI, 2010). The accumulated rainfall in the experimental period was 652.8 mm.

Experimental design and unit

The experimental design was a 12 x 12 lattice (Martínez, 1989) with two repetitions. Each variety was sown in two furrows 5 m long and 0.85 m wide. Three seeds were sown every 50 cm, thinning to two plants per bush to reach a population density of 40 000 plants ha⁻¹.

Crop management

In the L-M-H region, the sowing was carried out on April 14 in the locality of Buenavista de Guerrero and on April 21 in the locality of Máximo Serdán. In the S-T-GV region, the sowing was carried out on March 26 in the locality of El Sabinal and on April 3, 2020, in the locality of Tlachichuca. All four experiments depended exclusively on the rainy season. Details of crop work and other aspects of management are mentioned by Muñoz-Tlahuiz *et al.* (2013).

Variables evaluated

Three representative plants with full competition and ears were cut in the central parts of each experimental unit. The forage obtained was chopped with pruning shears, placed in paper bags, and dried in a forced air oven at 60 °C until reaching constant weight. The already dried forage was separated into leaf and stem, parts that were reduced to a smaller particle size in a blade mill. After the previous process, the material obtained was ground in a Foss[®] cyclonic mill with a 1 mm screen and stored in sealed Ziploc bags, stored in cardboard boxes also sealed.

Only the leaves were analyzed, to which the neutral detergent-insoluble fiber (NDF), acid detergent-insoluble fiber (ADF), and the *in vitro* enzymatic digestibility were determined, measurements that were carried out in the Laboratory of Physicochemical, Biochemical, and Biological Analysis of the College of Postgraduates, Puebla *Campus*. The determination of the NDF and the ADF were made in duplicate sequentially in an ANKOM 200/220 fiber analyzer, using the protocols of Ankom Technology[®] (Ankom Technology, 2006), excluding the step with alpha-amylase and that of acetone.

In vitro digestibility was done with a two-stage pepsin-cellulase technique (Jones and Hayward, 1975; Clarke *et al.*, 1982; Klein and Baker, 1993) with enzymes from the SIGMA-ALDRICH company. Pepsin (1:10000 porcine stomach mucosa) was dissolved in 0.125N hydrochloric acid at a ratio of 6.66 g liter. Cellulase Onozuka RS from *Trichoderma viride* (#5 000 units/g solid) was dissolved in acetate buffer (4.1 g of sodium acetate anhydrous and 2.9 ml of acetic acid per liter of distilled water) with cellulase:sample ratio of 1:100 (Clarke *et al.*, 1982).

Zero point three grams of dry matter per sample was used, placing it in Ankom F57 bags in duplicate. In a Lumistell® Model ISO-45 orbital agitation incubator at 50 °C and 80 revolutions per minute, each digestion stage lasted 48 h. The dry matter yield of stover and grain and the days to 50% female flowering were estimated as complementary information.

The dry matter yield of stover was expressed per plant, in the same way, the grain yield, for which the yield per plot (on a dry basis) was divided by the number of plants in the plot, to do this, the grain and stover samples were dried in a Thermo Scientific[®] forced air oven at 80 °C until reaching constant weight and making the respective adjustments for moisture.

Statistical analysis

The analysis of variance was first performed individually by experiment, and subsequently, a combined analysis of variance between localities by region was performed using the GLM



procedure of the Statistical Analysis System (SAS) package version 9.4 (SAS, 2008). The linear model for the lattice was as follows:

$$Y_{ijkl} = \mu + \alpha_i + \gamma_j + \delta_{ij} + B(L)_{l(kj)} + \varepsilon_{ijkl}$$
, con i=1,2, ...144; j=1,2; k=1,2; l=1,2, ...12.

Where: Y _{ijkl} is the observation of the *i*-th variety in the *j*-th environment of the *k*-th repetition within the *I*-th block. μ is the overall mean. αi is the random effect of the *i*-th observation of the variety, γ_j is the random effect of the *j*-th environment. δ_{ij} is the random effect of the *i*-th variety in the *j*-th environment. $B(L)_{i(kj)}$ is the random effect of the *k*-th repetition of the *j*-th environment. ε_{ijkl} is the random effect of the *k*-th repetition of the *j*-th environment. ε_{ijkl} is the random error associated with the experimental unit Y _{ijkl}. The comparison between the means of the treatments was made using the test of the least significant difference ($\alpha = 5\%$).

Results

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The varieties were different regarding *in vitro* digestibility of the leaf (p< 0.01 and p< 0.0001 for L-M-H and S-T-GV, respectively). The mean value for the L-M-H region was 59.5%, and for S-T-GV, it was 54.9%, Tables 1 and 2. For this variable, the locality effect was detected only in the L-M-H region (p< 0.0001), where in Máximo Serdán, the varieties had an average digestibility of 55.9% compared to that of Buenavista, which reached 63.1% on average (Figure 1). In the localities of the S-T-GV region, the average was 55%.

Table 1. Local varieties corresponding to the top 10% in digestibility and commercial controls in the L-M-H region (average of two localities) with their values of fiber, dry matter of stover produced, dry grain,and days to 50% of female flowering.

Variety	Color	IVD (%)	NDF (%)	ADF (%)	DM (g plant ⁻¹)	DG (g plant ⁻¹)	DFF
41	White	64.2	66.9	35.7	145.3	61.3	129
78	White	64.2	66.3	35.5	114.3	68.8	126
42	Blue	63.6	65.5	34.5	95.4	41.7	122
16	White	63.5	72.3	37.4	118.4	53.6	127
76	White	63.2	66.3	36.3	109.3	58.9	126
133	White	63.1	69	36.9	87.5	62.1	126
83	Blue	62.8	69.5	36.6	94.9	57.1	123
12	Blue	62.7	70.5	38	79.3	40.6	121
70	Blue	62.7	68.7	36.9	112.8	45.5	122
97	White	62.5	68.1	34.6	105.9	49	128
142	White	62.1	71.6	39.8	44	22.3	105
126	White	62.1	68.1	35.5	114.9	62	125
19	Yellow	62	70.4	37.7	76.1	58.7	117
43	White	61.1	70.6	37.5	130.3	57.3	130
S. Serdán	White	61.1	69.9	36.3	96.2	63.6	127
Halcón	White	57.2	75.3	37.2	83.2	52.2	123
Z-60	White	56.9	70.1	37	78.8	44.8	125
32D06	White	54.2	71.8	38.4	71.9	56.6	121
LSD		4.35	4.19	3.7	31.82	19.65	5.46
Loc (Pr>F)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Var (Pr>F)		0.01	0.004	0.003	<0.0001	<0.0001	<0.0001
Loc*Var		0.13	0.1	0.001	0.13	0.69	0.24
(Pr>F)							
CV (%)		5.2	4.3	7.2	23.3	23.7	3.2
Mean		59.5	69.8	36.9	97.6	59.4	121



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Minimum	54.2	65.2	34.3	26.8	14.8	98
Maximum	64.2	75.3	43.1	155.5	81.3	132

IVD= *in vitro* digestibility; NDF= neutral detergent-insoluble fiber; ADF= acid detergent-insoluble fiber; DM= dry matter; DG= dry grain; DFF= days to 50% female flowering.

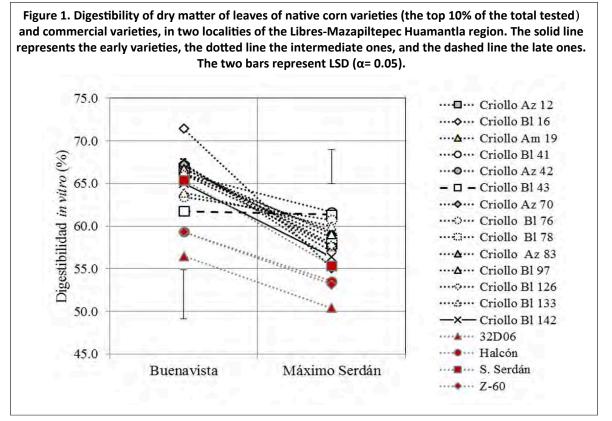
Table 2. Local varieties corresponding to the top 10% in digestibility and commercial controls in the S-T-GV region (average of two localities) with their values of fiber, dry matter of stover produced, dry grain,and days to 50% of female flowering.

Variety	Color	IVD (%)	NDF (%)	ADF (%)	DM (g plant ⁻¹)	DG (g plant ⁻¹)	DFF
36	Yellow	60.4	76.5	40.5	91.1	59.8	126
77	White	58.6	75.2	39.2	99.6	57.6	136
20	White	58.4	75.9	38.2	77.7	60.7	128
81	Blue	57.9	74	40	100.1	58.9	128
9	Blue	57.7	75.4	38.7	82.4	66.4	126
34	White	57.6	76.2	37.3	81.9	71	127
15	White	57.6	75	38.7	104.8	67.8	130
39	White	57.4	76.2	37.8	111.6	66.7	134
92	White	57.4	76.7	40.3	95.4	73.7	124
5	White	57.3	75.2	38.7	117.9	65.5	138
123	White	57.3	75.4	37.4	86.6	77.9	125
67	White	57.1	77.6	41.2	126.1	77.8	124
26	White	57.1	74.9	38.6	120.1	71.5	135
68	White	57	75.9	39.1	99.7	66.3	131
Promesa	White	55.8	77.5	42	97.2	67.6	128
Gavilán	White	55	79	41.8	86.1	57.9	125
AS722	White	51.6	79	41	60	64.9	116
S. Serdán	White	50.8	77.8	39.6	62.7	45.1	129
LSD		3.54	4.01	3.75	31.41	25.89	7.3
Loc (Pr>F)		0.539	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Var (Pr>F)		<0.0001	0.3619	0.5138	<0.0001	0.0017	<0.0001
Loc*Var		0.85	0.74	0.79	0.84	0.29	0.81
(Pr>F)							
CV (%)		4.6	3.7	6.7	24	30.3	4.4
Mean		54.9	77.2	40.5	90.5	61.4	128.2
Minimum		50.8	73.7	37.3	32.5	36.6	100
Maximum		60.4	81.2	44.6	127.7	87	144
IVD= <i>in vitro</i> digestibility; NDF= neutral detergent-insoluble fiber; ADF= acid detergent-insoluble fiber; DM= dry matter; DG= dry grain; DFF= days to 50% female flowering.							



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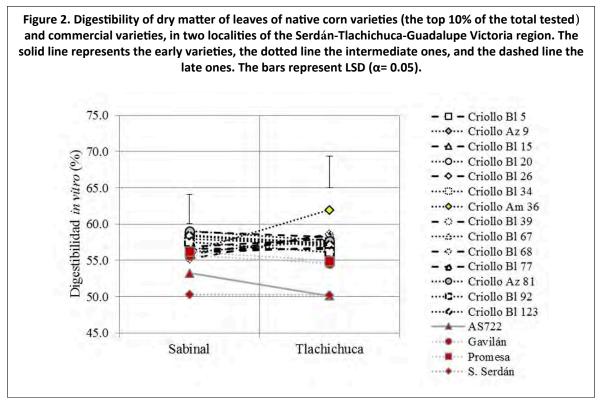


In both regions, the commercial controls had, in general, lower leaf digestibility than the native varieties (Figures 1 and 2; Tables 1 and 2). Blue and yellow varieties that were equally digestible as the white ones were found, although there was a predominance of the latter. Likewise, regarding the time of development of the plants measured through the days to 50% of female flowering, representation was found in the upper group of digestibility for early, intermediate, and late varieties.



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This variable was negatively correlated (p= 0.007 and 0.003) with NDF at -0.61 and -0.66 for the L-M-H and S-T-GV regions; respectively, while with ADF there was no significance in the correlation. Digestibility was positively correlated (p= 0.05 and 0.02) with dry matter production per plant at 0.45 and 0.43 for the L-M-H and S-T-GV regions, respectively.

Differences between varieties in NDF concentration were only detected in the L-M-H region (p< 0.004). In both regions, there was a marked effect of locality (p< 0.0001). In Máximo Serdán, the general average was 73.2%, higher than that recorded in Buenavista, which reached 66.4%. In the S-T-GV region, the differences between localities were smaller (76.2% in El Sabinal and 78.2% in Tlachichuca) (Figures 3 and 4).





Figure 3. Contents of neutral detergent-insoluble fiber of the dry matter of leaves of native corn varieti es (the top 10% of the total tested) and commercial varieties, in two localities of the Libres-Mazapiltepec-Huamantla region. The solid line represents the early varieties, the dotted line the intermediate ones, and the dashed line the late ones. The bars represent LSD (α = 0.05).

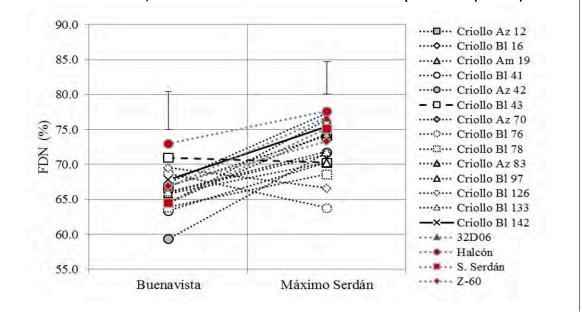
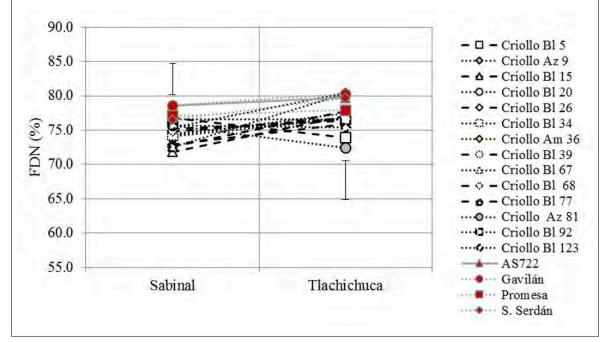


Figure 4. Contents of neutral detergent-insoluble fiber of the dry matter of leaves of native corn varieti es (the top 10% of the total tested) and commercial varieties, in two localities of the Serdán-Tlachichuca-Guadalupe Victoria region. The solid line represents the early varieties, the dotted line the intermediate ones, and the dashed line the late ones. The bars represent LSD (α= 0.05).



The commercial controls were in the group of varieties with the highest values, confirming their greater fibrosity compared to the local varieties, mainly in the L-M-H region. In the L-M-H region, the NDF was negatively correlated (p= 0.05) with the amount of dry matter produced per plant (-0.46), while in the S-T-GV region, a strong negative trend (p= 0.06) was detected at -0.44.



Regarding the ADF concentration (Figures 5 and 6), the varieties were different (p> 0.002), but only in the L-M-H region, where the overall average value was 36.9%, 33% for Buenavista and 40.9% for Máximo Serdán. The same did not happen in the localities of the S-T-GV region, in which an average value of 40.5% was reached. This characteristic was influenced by locality (p< 0.0001) in both regions.

Figure 5. Contents of acid detergent-insoluble fiber of the dry matter of leaves of native corn varieties (the top 10% of the total tested) and commercial varieties, in two localities of the Libres-Mazapiltepec-Huamantla region. The solid line represents the early varieties, the dotted line the intermediate ones, and the dashed line the late ones. The bars represent LSD (α = 0.05).

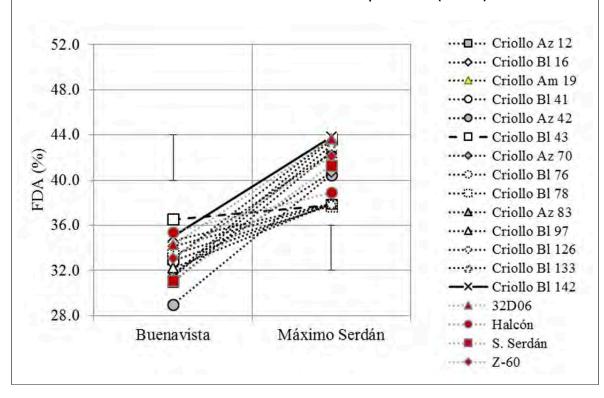
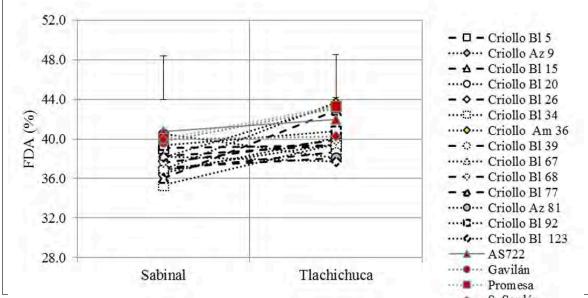




Figure 6. Contents of acid detergent-insoluble fiber of the dry matter of leaves of native corn varieties (the top 10% of the total tested) and commercial varieties, in two localities of the Serdán-Tlachichuca-Guadalupe Victoria region. The solid line represents the early varieties, the dotted line the intermediate ones, and the dashed line the late ones. The bars represent LSD (α= 0.05).



Tables 1 and 2 show the averages of 10% of the local varieties outstanding in leaf digestibility, their fibers, yields of dry matter and dry grain, and days to reach 50% of female flowering. In the L-M-H region, the female flowering interval for these cultivars was 25 days, there was a dominance of intermediate cycle cultivars, and only one was early and one late. In this case, the commercial controls presented, on average, the same cycle as the outstanding local cultivars.

In dry matter production per plant, the range was 101 g, with a dominance of local varieties to have a higher amount of DM, while commercial controls tended to have a low average (82.5 g plant⁻¹). Regarding the dry grain yield per plant, the interval was 46.5 g, with similarities in the averages between the local varieties and the commercial controls. In this region, the local varieties that showed the ability to produce stover and grain, as well as better digestibility, were varieties 41, 78, and 126.

In the S-T-GV region, the female flowering interval for local cultivars was 22 days, there was a dominance of intermediate cycle cultivars, and there were six late and one early (a commercial control). In dry matter production per plant, the interval was 66 g, with a dominance of local varieties to have more DM compared to commercial controls (97.6 *vs* 76.5 g plant⁻¹).

Regarding the dry grain yield per plant, the interval was 32.8 g, where the highest averages were for the local varieties compared to the commercial controls. In this region, the local varieties that showed the ability to produce stover and grain with greater digestibility were varieties 5, 15, 26, 39, 67, and 77. Dry matter production was positively correlated with dry grain production and days to female flowering in both regions; for the L-M-H region, there was a correlation of 0.58 (p= 0.01) and 0.8 (p< 0.0001), while in the S-T-GV region, the correlation was 0.47 (p= 0.04) and 0.58 (p= 0.01), respectively.

Discussion

Corn improvement in experimental fields has generally been conducted exclusively for grain production or silage production (Bertoia *et al.*, 2002; Xie *et al.*, 2009; Santiago *et al.*, 2018) and practically nothing for the generation of dual-purpose varieties in which the production of better quality stover is included. Currently, countries such as the United States of America seek to improve dual-purpose corn for cellulosic ethanol production, trying to find plants that have



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a high concentration of glucose in the cell wall, which can be released, and that have a low concentration of lignin (Hansey *et al.*, 2010; Lewis *et al.*, 2010).

In Mexico, corn is a source of vast diversity and a generator of rural well-being (Polanco and Flores, 2008), which is linked to livestock production and human consumption. As this is a fundamental part of ruminant feeding, it is highly likely that producers will consider how accepted the stover is by the animal in the selection of varieties. The results found in the leaves of the local varieties regarding average digestibility ranged in the interval of ten percentage units (54.2 to 64.2% in the L-M-H region and from 50.8 to 60.4% in S-T-GV) and differed from the commercial varieties.

Therefore, it can be considered that the differences found in digestibility and concentration of fibers between local varieties with respect to commercial varieties are due to what the producer has selected cycle after cycle in their varieties, maintaining a softer stover. Russell (1986) and Methu *et al.* (2001) report an *in vitro* leaf digestibility range for a hybrid grown over several years of 55.4 to 62.1% and 52.2 to 58.4%, respectively, which indicates that the digestibility of a variety can varies from year to year according to the climatic conditions that occur.

Lundvall *et al.* (1994) reported an *in vitro* leaf digestibility interval of 51.7 to 60.2% in 45 corn lines in which three lines with the characteristic of brown veins were included, which synthesize less lignin than normal genotypes, making the estimate at a stage very close to physiological maturity. Lower values of *in vitro* leaf digestibility of grain corn of four hybrids were reported by Gutierrez-Ornelas and Klopfenstein (1991) in a range of 33.5 to 51.1%. With the results of the present study, it is confirmed that there is a considerable variation in digestibility between landrace varieties, and it is detected that there is a variation by locality.

These digestibility values are in the range initially reported by other authors. Buxton (1996) mentions that, between sites and between years, variations in digestibility are common since some environmental factors, such as temperature, can vary, and that can affect fiber concentrations, as can be detected mainly in the L-M-H region. Small increases in *in vitro* digestibility have been found to be reflected in increases greater than its magnitude in daily weight gains in the animal (Casler and Vogel, 1999; Krämer-Schmid *et al.*, 2016), hence the importance of selecting more digestible materials, not letting them lose and improving them.

In NDF for leaf, Russell (1986) reported a range of 56.6 to 61.2%; Lundvall *et al.* (1994) placed the range between 51.7-60.2%. Higher values were reported by Hansey *et al.* (2010), with an average of 71.6% for 23 hybrids, and Methu *et al.* (2001) from 80.9 to 82.1% also for one hybrid. In 24 landrace varieties of corn from the Toluca Valley, Mexico, Estrada-Flores *et al.* (2006) reported an average of 71.3% for the NDF of the leaf.

The results found for NDF in the present study range from 65.2 to 75.3% in the L-M-H region and from 73.7 to 81.1% in the S-T-GV region, agreeing with the results by Estrada-Flores *et al.* (2006). According to the correlation found between NDF concentration and *in vitro* digestibility, cultivars must have differences in cell wall composition, mainly in hemicellulose. In ADF for leaf, the average value reported by Hansey *et al.* (2010) was 34.6%, those by Methu *et al.* (2001) were in the range of 49.8 to 52%, similarly, Gutierrez-Ornelas and Klopfenstein (1991) reported an interval of 43 to 55.1%, these last two studies with values above those found in the L-M-H and S-T-GV regions (34.3 to 43.1% and 37.3 to 44.6%, respectively).

Estrada-Flores *et al.* (2006) report an average of 42.4% for ADF of the leaf of landrace varieties, agreeing with the values found in the present research. Probably, these results contrast with those of other authors because of environmental differences and because they have worked with hybrid corns that have been selected for grain production with archetypes designed to withstand high densities (Duvick, 2005; Perez *et al.*, 2019). The characteristic of erect leaves in hybrids is related to higher concentrations of fiber, as it implies an increase in the leaf veins with changes in the pattern of veins and sclerenchyma, as reported by Ford *et al.* (2018).



There is diversity in local populations regarding digestibility and fiber concentrations in the leaves, differing from commercial controls, which were generally more fibrous and with lower digestibility. Among this variation in dry matter quality, it was found that some local varieties have a production of stover and grain that exceeds commercial controls under rainfed conditions. It is, therefore, feasible to start from these materials to begin selection combining the production of grain, stover, and digestibility to derive dual-purpose materials.

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