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Relationship Between Neutral Detergent Fiber and *In Vitro* Digestibility in Test Crosses of Maize Hybrids

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ABSTRACT

Objective: To relate the physio-technical parameters with the proportions of neutral detergent fiber (NDF) and *in vitro* dry matter disappearance (IVDMD) of test crosses of maize.

Design/methodology/approach: Crosses of early, intermediate, and late maize lines (total=75) from high valleys and subtropical/tropical testers were sowed in three regions of Central-North Mexico. Male and female flowering days (MFD, FFD); days to harvest (Dcor); plant and cob length (PL, CL); forage, corn stover (CS), and cobs humid base (HB) and dry matter (DM) yields; and NDF, acid detergent fiber (ADF), hemicellulose (Hem), crude protein (CP), and IVDMD were analyzed.

Results: High valleys lines had more MFD, FFD, Dcor, and PL, therefore better forage, MS, and cob yields. More MDF, FFD, and H were related to better HB and DM yields, and CP content, but also were related to more NDF, ADF, and Hem proportions, and therefore to a less IVDMD (r=0.47 to 0.98); however, crosslines with high cob yields also had high CS yields (r=0.57-0.68). Regression linear models showed that one unit of NDF might reduce 0.49 to 56% the IVDMD (R^2=0.59-0.78); additionally, NDF<68% was related to IVDMD>60% (R^2=0.63-0.78).

Limitations on study/implications: ADF correlated negatively with IVDMD in early lines; NDF composition should also be related to its degradability (NDFD).

Findings/conclusions: Maize-breeding might be directed to obtain hybrids with less NDF CS contents to use them in ruminant diets, maintaining the cob yields for human nutrition and resistance to plant lodging.

Keywords: Tested maize crosslines (*Zea mays* L.), linear modeling, neutral detergent fiber, dry matter digestibility, corn stover as ruminant feedstuff.

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INTRODUCTION

According to the FAO (2019), maize (*Zea mays* L.) cultivation is a global priority. Maize cultivation and consumption impact the social, environmental, and economic conditions in Mexico (Domínguez *et al.*, 2018; Ibarrola *et al.*, 2020). Consequently, along with the priority of funding social and environmental projects (IATP, 2022), public policies and actions are being implemented to ensure its production (Castro and Montpetit, 2017). Numerous previous studies have addressed objectives related to the effects of variations in agronomic management practices and the identification of maize hybrids to increase grain yield and composition (García-Lara *et al.*, 2020; Sánchez *et al.*, 2022). Additionally, alternatives have been sought to ensure the sustainability of maize cultivation, addressing not only the economic impact but also the environmental and social dimensions (Gayosso-Barragán *et al.*, 2021, 2023; FAO, 2024) in areas where producers are vulnerable due to reduced availability of productive land and water resources (Ibarrola *et al.*, 2020; Chávez-Aguilar *et al.*, 2023).

Genetic improvement can be directed towards increasing the content and quality of crude protein (CP), soluble and insoluble fibers, oils, sugars, and starch, as well as carotenoids and flavonoids in the grains (Yang and Zhai, 2010a, 2010b; Salinas-Moreno *et al.*, 2012, 2013). These outcomes contribute to enhancing the nutritional status of the majority of Mexicans (Vivek *et al.*, 2008). Other objectives of genetic improvement, such as increasing resistance to water stress, pests, or diseases, enhancing the earliness of varieties, and reducing lodging incidence, are crucial for arid or semi-arid regions, as they lead to better management of inputs and a greater margin between costs and profits (Medina-Cuéllar *et al.*, 2021). However, improving these characteristics can affect the composition of the stalks and leaves (corn stover (CS)) in terms of their cell wall proportion (primarily quantified by Neutral Detergent Fiber (NDF)) and the proportions of hemicellulose, cellulose, lignin, and CP, which will impact the ruminal digestibility of Dry Matter (DDM) and, therefore, the feasibility of using the forage (ears and CS) for ruminant feed (Tirado-Estrada *et al.*, 2021).

Improving the yield and DMD of CS as an alternative for animal feed could be a strategy that reduces the economic and environmental costs of animal production. Historically, genetic improvement programs and crop management have focused on maximizing grain production and quality while also improving CS composition (Peña *et al.*, 2006; Stendal *et al.*, 2006; Staton *et al.*, 2007; González-Manzano *et al.*, 2008). Specifically, studies have demonstrated how modifications in the structure and chemical composition of NDF can affect its degradability (Jung and Casler, 2006a, b) and, in turn, how increasing ruminal digestibility of NDF (DFDN) can promote reduced grain use in feed without compromising animal production potential (Oba and Allen, 1999). Increasing the DFDN helps improve animal productivity, reduce production costs, and lessen the environmental impact resulting from deforestation needed to maintain intensive grain production. Variations in the proportion and composition of NDF can be attributed to biological cycles, germplasm origin, and various environmental stresses (Tirado-Estrada *et al.*, 2021). Therefore, modeling can be useful in establishing the relationship between these factors, which affect the utilization of DM and, consequently, animal productive performance (Miranda-Romero *et al.*, 2020). In this study, linear models have been used to establish the relationship between physio-technical and performance variables, with the proportion of NDF and *in vitro* digestibility of DM (IVDMD) of early/intermediate/late maize crosses of subtropical origin and high valleys.

MATERIALS AND METHODS

Location

This study was conducted at the facilities of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) located in Pabellón de Arteaga, Aguascalientes, Mexico (102° 26' W, 22° 09' N, 1900 masl, average annual temperature (T) of 18 °C, 440 mm precipitation/year, xerosol and regosol soils); Torreón, Coahuila, Mexico (26° 44' N, 105° 10' W, 1510 masl, T of 14 °C, 501 mm precipitation/year, luvisol xerosol soils); and Las Delicias, Chihuahua, Mexico (28° 25' N, 105° 02' W, 1145 m asl, T of 18.3 °C, 334 mm precipitation/year, eutric regosols).

Genetic Material

Early, intermediate, and late S2 crosses with low endogamy, derived from simple crosses of testers provided by CIMMYT, INIFAP, and commercial sources, adapted to altitudes of 1600 to 2200 meters above sea level or to subtropical/tropical regions, were analyzed. The description and evaluation of the testers and test crosses were detailed by Peña *et al.* (2004).

Agronomic Management and Sampling

Total areas of 1 hectare (150 plots) were planted, blocked according to orientation and slope. Planting was conducted under irrigation conditions, with a density of 80,000 plants/ha. Fertilization was done using N-P-K doses of 200-90-00 kg/ha, applying half of the nitrogen and all of the phosphorus during the first weeding (between 25 to 30 days after planting) and the remaining nitrogen was applied between 25 and 30 days after the first weeding. Three supplementary irrigations were given to the group of early hybrids and four to the intermediate and late ones. Additionally, the corresponding agronomic management for maize cultivation was carried out in each region, including integrated pest, disease, and weed management. When the maize grains reached 58 to 60% dry matter (DM), a sample of 25 plants was randomly harvested from each experimental plot. The whole plants and ears were chopped and mixed, considering a particle size according to the method reported by Krause and Combs (2003).

Evaluated Variables

Data were collected on physio-technical variables: days to male flowering (DMF) and female flowering (DFF), days to harvest (Dcor), plant height (PH), and ear height (EH). During sampling, the fresh weights (FW) of the following were recorded: whole plants (Forage), stalks, leaves, and husks (corn stover (CS) and ears (cobs and grains)). Samples of 1 kg of whole plants, corn stover (CS), and ears were collected and placed in a forced-air oven at 60 °C until they reached a constant weight (DM) (González *et al.*, 2005). Subsequently, the dry samples of whole plants were ground using a Thomas-Willey mill (Laboratorios Mill) with a 1 mm screen. The proportions of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (Hem), and *in vitro* digestibility of dry matter (IVDMD) were determined. Based on the fresh and dry matter weights of corn stover (CS) and ears, the proportions of CS (%CS=kg of CS/kg of forage) and ears (%Ears=kg of ears/kg of forage) were calculated.

Statistical Analysis

The SAS statistical package (Statistical Analysis System, V. 9.4; 2013) was used to perform analysis of variance (ANOVA) employing general linear or mixed procedures (Proc GLM, Proc Mixed), considering random effects of lines within locations and fixed effects of biological cycle and germplasm origin, according to Model 1. Adjusted means (LSMeans), coefficients of determination, and variation (R^2 and CV) are reported.

$$Y_i = L(Loc)_{ij} + Cycle_k + Origin_l + (Cycle * Origin)_{kl} + \varepsilon_{ijkl} \qquad \text{Model (1)}$$

Where: Y_i=DFF, DMF, PH, EH; fresh and dry matter yields of forage, corn stover (CS), and ears; %DM; CP, IVDMD, NDF, ADF, Hem; $L(Loc)_{ij}$ =random effect of the *i*-th line within the *j*-th location; $Cicle_k$=fixed effect of the *k*-th biological cycle; $Origen_l$=fixed effect of the *l*-th germplasm origin; $(Cycle * Origin)_{kl}$ =interaction between factors; ε_{ijkl}=random error.

Correlation and Multiple Linear Regression Analysis

The simple relationships between the evaluated variables were analyzed by calculating Pearson correlations (Proc Corr). Simple linear regression models were obtained using the Stepwise (Forward) procedures (Proc Reg), including variables with $P<0.15$ and considering R^2 and Mallows' Cp as criteria for validity.

RESULTS

Table 1 shows the physiotechnical variables evaluated in the crop. The days to female flowering (DFF) and male flowering (DMF) were greater in late crosses ($P<0.0001$). However, more days were required for male flowering (DMF) and for harvesting (DCor) in lines from high valley testers for intermediate and late crosses ($P<0.01$). Plant height (PH)

Table 1. Physiotechnical variables evaluated in 75 maize hybrid test crosses.

Phenological cycle	Origin	FFD (d)	MFD (d)	DCor (d)	PH (cm)	EH (cm)
Early	High valleys	69.18	64.73	117.07	241.71	109.05
	Subtropical/Tropical	68.49	65.22	117.66	233.81	100.95
Intermediate	High valleys	77.15	73.43	124.94	246.88	117
	Subtropical/Tropical	76.60	71.72	123.38	245.7	112.95
Late	High valleys	80.41	76.68	129.76	260.2	114.71
	Subtropical/Tropical	79.94	75.11	128.68	248.23	111.56
R^2		0.88	0.92	0.95	0.72	0.35
CV (%)		3.53	3.15	2.44	6.49	13.56
Cycle		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Origin		0.51	0.11	0.36	0.16	0.03
C*O		0.97	<0.0001	0.01	0.03	0.53

DFF, days to female flowering; DMF, days to male flowering; DCor, days to harvesting; PH, plant height; EH, ear height; R^2, coefficient of determination; CV, coefficient of variation; C*O, cycle*origin interaction.

and ear height (EH) increased with the biological cycle and were generally higher in lines from high valleys (P<0.03).

In Table 2, the humid base and dry matter yields of forage, corn stover (CS), and ears (ear without husks) were better in lines from high valleys compared to those from subtropical/tropical origins (P<0.002). However, although early-cycle lines had lower yields in humid base and dry matter, intermediate-cycle lines had better forage, CS, and ear production than late-cycle lines (P<0.005). The proportions of ears relative to total forage ranged from 30.37% to 34.45% in humid base and between 47.46% and 52.82% in dry matter. However, the proportion in humid base was better in late hybrids, while in dry matter, it was better in early hybrids (P<0.02).

Table 3 shows that there were differences between lines of different biological cycles in terms of dry matter (DM), crude protein (CP), and *in vitro* dry matter digestibility (IVDMD) proportions, as well as in the composition of neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (Hem). Although intermediate-cycle lines had higher CP contents, early-cycle lines exhibited better IVDMD and lower proportions of NDF, ADF, and Hem (P<0.002). IVDMD was higher in lines from subtropical/tropical testers compared to lines from high valleys testers (P<0.001).

Lines from high-valley testers had higher FFD, MFD, DCor, and plant height (PH) than those from subtropical/tropical origins. Additionally, they showed better yields of forage, CS and Ear in both humid base (HB) and dry matter (DM). Late varieties had lower yields compared to intermediate crosses. Early crosses and those from subtropical/tropical testers had lower contents of NDF, ADF, and Hem and better IVDMD. Intermediate lines had higher CP content compared to the other crosses. Longer biological cycles may favor better

Table 2. Humid base (HB) and dry matter (DM) yields in 75 maize hybrid test crosses.

Phenological cycle	Origin	Wet yield (kg WB/ha)						DM Yield (kg DM/ha)			
		Forage	Stover	Cob	%S	%Cob	Forage	Stover	Cob	%S	%Cob
Early	High valleys	69824	48320	21504	69.63	30.37	25952	13344	12576	51.67	48.33
	Subtropical/ Tropical	62464	41152	21312	65.58	34.42	24192	11424	12736	47.18	52.82
Intermediate	High valleys	79648	53984	25632	67.79	32.21	30912	16096	14816	51.83	48.17
	Subtropical/ Tropical	68512	46688	21760	68.2	31.80	26208	13728	12448	52.52	47.48
Late	High valleys	72800	48000	24736	65.63	34.37	29472	15392	14048	52.54	47.46
	Subtropical/ Tropical	64736	43840	20896	67.08	32.92	25664	13440	12256	52.39	47.61
R^2		0.71	0.64	0.71	0.33	0.33	0.48	0.38	0.55	0.42	0.42
CV (%)		15.72	18.66	16.33	6.3	6.3	15.18	20.86	17	12.46	12.46
Cycle		<0.0001	<0.0001	<0.0001	0.02	0.02	<0.0001	<0.0001	0.005	<0.0001	<0.0001
Origin		0.002	0.005	0.006	0.48	0.48	0.001	0.005	0.02	0.46	0.46
C*O		0.32	0.33	<0.0001	<0.0001	<0.0001	0.008	0.79	<0.0001	0.001	0.001

CS, corn stover; Forage, whole plants; CS, corn stover; Cob, cobs without husks; R^2, coefficient of determination; CV, coefficient of variation; C*O, cycle*origin interaction.

Table 3. Dry matter (DM) proportion, *in vitro* digestibility, and composition of test crosses of early/intermediate/late lines from high valley or subtropical/tropical testers.

Phenological cycle	Origin	DM (g/100 of WB yield)			Composición (g/100 MS)				
		Forage	Stover	Cob	CP	IVDMD	NDF	Hem	ADF
Early	High valleys	37.62	28.04	60.39	8.00	71.83	56.85	27.35	29.51
	Subtropical/Tropical	39.70	28.61	60.34	8.37	74.04	55.68	27.69	27.98
Intermediate	High valleys	39.99	31.15	59.09	8.57	69.19	60.78	29.51	31.27
	Subtropical/Tropical	39.36	30.49	58.15	8.51	72.74	59.47	30.75	28.73
Late	High valleys	43.33	34.91	58.82	8.06	66.83	60.16	28.56	31.6
	Subtropical/Tropical	41.73	33.05	59.46	8.37	70.24	61.3	30.91	30.39
R^2		0.60	0.58	0.63	0.89	0.43	0.31	0.33	0.28
CV (%)		12.63	19.27	7.4	8.29	4.04	7.91	9.82	11.28
Cycle		<0.0001	<0.0001	0.002	0.006	<0.0001	<0.0001	<0.0001	<0.0001
Origin		0.99	0.73	0.82	0.45	0.001	0.66	0.20	0.09
C*O		0.02	0.31	0.4	0.09	0.23	0.22	0.11	0.42

*HB, humid base; DM, dry matter; total, whole plant; CS, corn stover; Cob, cobs without husks; CP, crude protein; IVDMD, *in vitro* dry matter digestibility; NDF, neutral detergent fiber; Hem, hemicellulose; ADF, acid detergent fiber; R^2, coefficient of determination; CV, coefficient of variation; C*O, cycle*origin interaction.

humid base (HB) and dry matter (DM) yields and higher crude protein (CP) content but negatively impact in vitro dry matter digestibility (IVDMD) by increasing the amount of neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (Hem) (r=0.47 to 0.98; P<0.0001). However, varieties that produce a higher amount of grain could also be useful in terms of corn stover (CS) yield (r=0.57 to 0.68; P<0.0001).

NDF contributes negatively by 63 to 78% to IVDMD in early crosses, from high valley testers, and from subtropical/tropical origins (R^2=0.63 to 0.78; P<0.0001) (Table 4). In general, to achieve an IVDMD of >60 g/100 DM, the NDF should be less than 68%. In early and late crosses, and those from testers adapted to high valleys or from subtropical/tropical origins, the proportion of NDF is negatively related by 59 to 78% to IVDMD. According to these models, an increase of one unit of NDF would result in a reduction of 0.49 to 0.56% in IVDMD (R^2=0.59-0.78; P<0.0001). However, in some lines, such as intermediate ones, a unit increase in ADF can reduce IVDMD by up to 0.66% (R^2=0.70; P<0.0001). Other variables such as MFD, DCor, or the proportions of CS or cob may be related to IVDMD; these contribute less to the R^2 of the models (4 to 7%).

DISCUSSION

In this study, we found that later maturing hybrids might increase the production of DM from forage, stover, and ears. However, they can also reduce in vitro digestibility of DM (IVDMD) by favoring the increase of neutral detergent fiber (NDF) and acid detergent fiber (ADF); this is possible because as the maize maturity period extends, the proportion and composition of lignin and the types of bonds linking it with some hemicellulose structures change, making NDF a less digestible compound (Jung and Casler, 2006a, b). Nevertheless, the intermediate hybrids evaluated not only showed

Table 4. Multiple Linear Regression Models for Neutral Detergent Fiber (NDF) and *In Vitro* Dry Matter Digestibility (IVDMD).

$Y_i = NDF$	$Y_i = \beta_0 + X_i\beta_i + \varepsilon_{ij}$	R^2
Early	$Y_i = 136.10 - 1.13\ IVDMD +$ ij	0.63
Intermediate	$Y_i = 26.34 + 1.20\ ADF + \varepsilon_{ij}$	0.71
Late	$Y_i = 30.22 + 1.03\ ADF + \varepsilon_{ij}$	0.60
High valleys	$Y_i = 156.85 - 1.40\ IVDMD + \varepsilon_{ij}$	0.78
Subtropical/Tropical	$Y_i = 167.64 - 1.54\ IVDMD + \varepsilon_{ij}$	0.70
	$Y_i = 110.27 - 1.05\ IVDMD + 0.75\ Hem + \varepsilon_{ij}$	0.88
$Y_i = IVDMD$		R^2
Early	$Y_i = 102.58\text{-}0.55\ NDF + \varepsilon_{ij}$	0.63
	$Y_i = 112.8\text{-}0.48\ NDF + -0.21\ MFD + \varepsilon_{ij}$	0.69
Intermediate	$Y_i = 88.96 - 0.66\ ADF + \varepsilon_{ij}$	0.70
	$Y_i = 93.52 - 0.46\ ADF - 0.17\ NDF + \varepsilon_{ij}$	0.73
	$Y_i = 117.11 - 0.40\ ADF - 0.24\ NDF - 0.15\ HT + \varepsilon_{ij}$	0.77
Late	$Y_i = 98.30 - 0.49\ NDF + \varepsilon_{ij}$	0.59
	$Y_i = 102.24 - 0.49\ NDF - 0.2\ Stover\ WB + \varepsilon_{ij}$	0.65
High valleys	$Y_i = 103.19 - 0.56\ DNF + \varepsilon_{ij}$	0.78
	$Y_i = 105.43 - 0.50\ NDF - 0.63\ Cob\ DM + \varepsilon_{ij}$	0.84
Subtropical/tropical	$Y_i = 97.12 - 0.45\ NDF + \varepsilon_{ij}$	0.70
	$Y_i = 97.45 - 0.31\ NDF - 0.31\ ADF + \varepsilon_{ij}$	0.75
	$Y_i = 108.68 - 0.23\ NDF - 0.36\ ADF - 0.11\ HT + \varepsilon_{ij}$	0.79

ADF, acid detergent fiber; Dcor, days to harvest; DM ear, ear yield on a humid base; DM, dry matter; CS, corn stover; BH, humid base; R^2, coefficient of determination.

better proportions of crude protein (CP) but, in some cases, also better yields of forage, stover, and ears, and even better *in vitro* digestibility of DM (IVDMD) than the late varieties. This demonstrates that it is possible to direct maize genetic selection towards obtaining earlier hybrids with similar yields of ears and stover (Medina-Cuéllar *et al.*, 2021) without reducing their IVDMD.

According to Van Soest *et al.* (1991), the determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) allows for the differentiation of some basic cell structures, such as the total content of cell walls (NDF) and the combined content of cellulose, lignin, tannins, and silicates (ADF). In line with the findings of the present study, the selection of hybrids with better nutritional characteristics for the formulation of ruminant diets has been directed towards hybrids with lower contents of NDF (Stendal *et al.*, 2006; Staton *et al.*, 2007).

In previous studies, we found that, in addition to the proportion of NDF, it is important to differentiate the contents of cellulose, hemicellulose, and lignin, which can vary depending on the germplasm origin. Some of the hybrids generated in the present study, originating from highland testers, may contain more lignin than hybrids from subtropical or tropical testers, affecting the *in vitro* digestibility of DM (DIVDM) even though they had lower

NDF contents (Tirado-González *et al.*, 2016). Several studies conducted by Oba and Allen (1999, 2000a, b, c, 2003) demonstrated that, in addition to the NDF content, its individual digestibility should also be considered. They found that NDF digestibility (NDFD) is directly related to the potential for milk production (corrected to 4% fat). At the ruminal level, small variations in the composition of NDF can modify ruminal fermentation and the potential for fiber utilization (Soufizadeh *et al.*, 2018; Miranda-Romero *et al.*, 2020). This means that, in addition to composition, variations in the quantity and types of bonds between hemicellulose and lignin modify the ruminal potential for obtaining energy from cellulose and hemicellulose (Tirado-González *et al.*, 2018, 2021; Carrillo-Díaz *et al.*, 2022). These aspects have been considered relevant for increasing the proportion of fiber in ruminant diets (Soufizadeh *et al.*, 2018; Tirado-Estrada *et al.*, 2021). Furthermore, seeking the inclusion of higher proportions of CS without reducing the productive potential of ruminants is a strategy that can promote the sustainability of meat and milk production, considering the reduction of economic and environmental costs (Adesogan *et al.*, 2019; Tirado-Estrada *et al.*, 2021; FAO, 2024).

CONCLUSIONS

The simple regression models reported in this study show that for hybrids from testers of highland or subtropical/tropical germplasm origins, an increase of one unit of NDF can reduce IVDDM by between 0.49% and 0.56%. To ensure an IVDDM greater than 60%, NDF must be less than 68%. Although the results of these models should be validated in the future, considering their R^2 values, we can assume that at least in Mexican lines from testers of highland or subtropical/tropical origins, reducing NDF may be a viable strategy to improve the IVDDM of CS. The results found in this study may be helpful in identifying maize hybrids whose CS can be efficiently used in ruminant feeding.

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