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Intrinsic and Extrinsic Factors Affecting Neutral Detergent Fiber (NDF) Digestibility of Vegetative Tissues in Corn for Silage

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Abstract: Dairy farming requires forages with high neutral detergent fiber (NDF) to maximize milk production, sustain cows' health, and ensure the economic and environmental sustainability of the dairy farm. The objectives of this study were to determine the effects of the brown midrib (BMR) genotype, agronomic environment, and maturity at harvest on the NDF digestibility (NDFD) and the composition of the cell wall of corn plant tissues. In this plot study, one conventional and one BMR corn hybrid were planted and subjected to an abundant (60,000 seeds/ha and 225 kg N/ha) and a limited (90,000 seeds/ha and 180 kg N/ha) environment. The ruminal NDFD was determined in vitro in leaf blades, leaf sheaths, and stem internodes. Cell walls from BMR corn had greater NDFD than cell walls from conventional corn on most tissues. Relative to the abundant environment, the limited environment had minimal effects on NDFD. As maturity advanced, NDFD decreased for various but not all tissues. In conclusion, under the conditions of this study, intrinsic characteristics of corn, such as genotype and maturity at harvest, had a greater effect on NDFD than environment or agronomic management.

Keywords: environment; cell wall; fiber; lignin; brown midrib; digestibility

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1. Introduction

Dairy farming requires forages with highly digestible cell walls (CW) or neutral detergent fiber (NDF) to maximize milk production, sustain cows' health, and ensure the economic and environmental sustainability of the dairy farm. Some pre- and post-harvest factors like hybrid selection [1–3], maturity at harvest [4], and cutting height [5] can affect the NDF digestibility (NDFD) of corn silage. Yet, limited information exists about the effects of other environmental and agronomical factors on NDFD [6–9], such as nitrogen (N) fertilization, seeding population, and their interactions with maturity.

Although it is sometimes (wrongly) referred to as a carbohydrate, lignin is a polyphenolic compound that limits the digestibility of other components of the CW like hemicellulose and cellulose [10–12]. Ruminant nutritionists have extensively studied the association between lignin concentration and the digestibility of the forage [6,10,13], and they associate decreases in the NDFD of the forage to increases in *lignification* of the CW. Although it seems self-explanatory, the term *lignification* has ambiguous connotations [9]. For example, increased lignification in forages may imply a greater proportion of more lignified tissues in the whole plant or, alternatively, a greater concentration of lignin within a plant tissue as a result of a greater rate of lignin synthesis and deposition (i.e., lignification rate). Distinguishing these two connotations is quite relevant when evaluating the effects of pre- and post-harvest factors on NDFD [9], as pre- and post-harvest factors might affect the structure of the plant, the chemical composition within plant tissues, or both [14].

In this study, we hypothesized that growing corn for silage in a challenging environment, defined as growing conditions with a large number of plants per unit of area and a less abundant N fertilization, decreases the NDFD of vegetative tissues of corn for silage

relative to growing corn in less challenging environments. We also hypothesized that such a decrease in NDFD is associated with a greater concentration of lignin in those tissues (i.e., lignification degree). Therefore, the objectives of this study were to determine the effects of the brown midrib (BMR) genotype, seeding population, N fertilization, and maturity at harvest on the NDFD and the CW composition of leaf blades, leaf sheaths, and stem internodes of corn for silage.

2. Materials and Methods

2.1. Experimental Design

A secondary study was performed from a previous experiment [2]. Briefly, one conventional variety (TMF17L86, Mycogen Seeds, Indianapolis, IN; hereafter **CON**) and one BMR variety (F2F817, Mycogen Seeds; hereafter **BMR**) of corn were planted in small plots (2.3 m wide and 25 m long) in two different fields of a 425-cow commercial dairy farm located in Crockett, VA (USA) during the spring of 2018.

For each corn variety and in each field, two plots (i.e., replicates) were planted according to two treatments that were classified as **ABUNDANT** and **LIMITED** environments. For the ABUNDANT treatment, corn was planted at a seeding population of 60,000 seed/ha and fertilized with 225 kg N/ha. For the LIMITED treatment, corn was planted at a seeding population of 90,000 seed/ha and fertilized with 175 kg N/ha. None of the plots' fields were subjected to supplemental irrigation.

2.2. Sample Collection and Chemical Analyses

Stem internode, leaf sheaths, and leaf blade samples were collected from the 9th and 16th phytomers from each plot at two maturity stages (hereafter **LOWER** and **UPPER**, respectively). The latter included the tasseling (**VT**) and the 1/2 to 1/4 milk-line (**R5**) stages of maturity [15]. Tissue samples were immediately frozen on dry ice and transported to the laboratory. Depending on the tissue and the maturity at harvesting (e.g., internodes in the 16th phytomer at VT, when these internodes are elongating), the amount of sample collected was not sufficient to perform all analyses described below.

After drying (55 °C) for 48 h in a forced-air oven (Memmert UL 83; Wisconsin Oven Corporation; East Troy, WI, USA), the stem internodes, the leaf sheaths, and the leaf blades were ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). Ash-free neutral detergent fiber (aNDFom) concentration was determined using the Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY, USA) using sodium sulfite and α-amylase (Ankom Technology), as described previously [16]. After securing enough material to determine NDF concentration and in-vitro ruminal incubations (described below), tissue samples were reground to pass through a 0.5-mm screen of a cyclone mill (UDY Corporation, Inc., Fort Collins, CO, USA) for the extraction of CW from plant tissues, as described previously [9]. The concentrations of acetyl-bromide lignin **(ABL)**, arabinose **(ARA)**, xylose **(XYL)**, glucose **(GLU)**, and uronic acids **(UA)** in the CW were determined as described previously [9], and reported on a protein-free CW-basis.

2.3. In Vitro Disappearance

The 30-h ruminal in-vitro NDF digestibility (IVNDFD) was determined using the DaisyII rotating jar in-vitro incubator (Ankom Technology) following the procedures described by Ferreira and Mertens [17]. A composited inoculum was prepared with rumen fluid and rumen solids collected from 3 rumen cannulated lactating cows that were fed a diet containing (DM basis) 43% corn silage, 6% triticale silage, 4% alfalfa hay and 47% concentrate mix.

2.4. Statistical Analysis

The experiment was designed as a split-plot in a completely randomized design replicated in two fields. All variables were analyzed using the MIXED procedure of SAS (SAS version 9.4, SAS Institute Inc., Cary, NC, USA). The statistical model included the

random effect of field [1 degree of freedom (df)], the fixed effect of environment (1 df); the fixed effect of corn hybrid (1 df), the environment × hybrid interaction (fixed; 1 df), the field × environment × hybrid interaction (random; 3 df), the fixed effect of maturity (1 df), the environment × maturity interaction (fixed; 1 df), the hybrid × maturity interaction (fixed; 1 df), the environment × hybrid × maturity interaction (fixed; 3 df), and the random residual error (4 df). Due to the limited availability of sample, the effect of maturity and its interactions with environment and corn hybrid were not evaluated for some variables (e.g., IVNDFD).

3. Results

3.1. In-Vitro NDF Digestibility

Given that no interactions existed for IVNDFD among the main factors (Table 1), the description of results focuses only on the main effects. As we expected, BMR corn had a greater IVNDFD than CON corn in lower leaf blades (82.4 vs. 73.0%, $p = 0.04$), upper leaf blades (74.1 vs. 71.8%, $p < 0.01$), lower leaf sheaths (76.2 vs. 58.3%, $p = 0.02$), upper leaf sheaths (75.2 vs. 64.1%, $p < 0.01$), and lower stem internodes (56.6 vs. 36.5%, $p < 0.01$). For upper internodes, BMR corn tended to have greater IVNDFD than CON corn (65.2 vs. 50.3%, $p = 0.07$). Relative to the ABUNDANT environment, the LIMITED environment decreased IVNDFD in the upper leaf blades (78.5 vs. 67.5%, $p = 0.02$). The IVNDFD of lower leaf blades ($p = 0.72$), lower leaf sheaths ($p = 0.52$), upper leaf sheaths ($p = 0.15$), lower stem internodes ($p = 0.12$), and upper stem internodes ($p = 0.44$) did not differ between the two environments. Given the insufficient amount of tissue collected, we could not evaluate the effect of maturity on IVNDFD for lower blades, lower sheaths, and upper internodes. Relative to VT stage of maturity (Table 1), IVNDFD decreased substantially at R5 stage of maturity in the upper leaf blades (78.0 vs. 68.0%, $p < 0.01$), the upper leaf sheaths (76.6 vs. 62.6%, $p < 0.01$), and lower stem internodes (56.3 vs. 36.8%, $p < 0.01$).

Table 1. In-vitro NDF digestibility (IVNDFD) of corn tissues as affected by environment (ABUNDANT vs. LIMITED), corn genotype (CON vs. BMR), and maturity (VT vs. R5).

	ABUNDANT				LIMITED											
	CON		BMR		CON		BMR			*p* Value [2]						
Tissue [1]	VT	R5	VT	R5	VT	R5	VT	R5	SEM	E	H	EH	M	EM	HM	EHM
	IVNDFD, % NDF															
Lower blade	73.1	ND [3]	81.4	ND	72.9	ND	83.4	ND	3.55	0.76	0.04	0.72	NA [4]	NA	NA	NA
Upper blade	82.3	73.3	81.9	76.3	73.3	58.3	74.4	63.8	2.61	0.02	0.01	0.11	0.01	0.50	0.33	0.99
Lower sheath	58.1	ND	77.8	ND	58.2	ND	74.6	ND	3.51	0.52	0.02	0.50	NA	NA	NA	NA
Upper sheath	70.6	59.4	82.3	70.3	72.2	54.1	81.3	66.7	2.85	0.15	0.01	0.84	0.01	0.40	0.80	0.69
Lower internode	42.0	27.9	68.3	44.7	45.8	30.4	69.1	44.3	3.97	0.12	0.01	0.15	0.01	0.84	0.18	0.99
Upper internode	ND	54.1	ND	65.3	ND	46.5	ND	65.0	4.59	0.44	0.07	0.47	NA	NA	NA	NA

[1] Tissue are leaf blades, leaf sheaths, or stem internodes collected from the 9th (lower) or 16th (upper) phytomers of corn plants. [2] E: Environment (limited vs. abundant); G: genotype (conventional vs. brown midrib); EH: E × H interaction; M: maturity (VT vs. R5); EM: E × M interaction; HM: H × M interaction; EHM: E × H × M interaction. [3] Not determined. [4] Not applicable.

3.2. Acetyl Bromide Lignin

Given that no interactions existed among the main factors for ABL concentration in the CW of plant tissues (Table 2), the description of results focuses only on the main effects. Relative to CON corn, BMR corn had lower ABL concentrations in the CW of upper leaf blades (20.7 vs. 15.9%, $p < 0.01$) and tended to have lower ABL concentrations in the CW of upper stem internodes (18.3 vs. 14.7%, $p = 0.07$). Environment did not affect the concentration of ABL in the CW of any tissue ($p = 0.14$ or greater). The concentration of ABL in CW increased at the advanced stage of maturity in lower leaf blades (13.9 vs. 19.0%, $p = 0.04$) and upper stem internodes (10.7 vs. 22.3%, $p < 0.01$), and tended to increase at the advanced stage of maturity in upper leaf blades (16.7 vs. 19.9%, $p = 0.10$). Maturity did

not affect the concentration of ABL in CW of either lower and upper leaf sheaths or lower stem internodes.

Table 2. Concentration of acetyl-bromide lignin (ABL) in protein-free cell walls (PFCW) of corn tissues as affected by environment (ABUNDANT vs. LIMITED), corn genotype (CON vs. BMR), and maturity (VT vs. R5).

	ABUNDANT				LIMITED											
	CON		BMR		CON		BMR			*p* Value [2]						
Tissue [1]	VT	R5	VT	R5	VT	R5	VT	R5	SEM	E	H	EH	M	EM	HM	EHM
	ABL, % PFCW															
Lower blade	13.8	21.0	13.2	18.1	14.4	18.5	14.1	18.5	2.03	0.81	0.23	0.31	0.04	0.61	0.79	0.71
Upper blade	16.9	24.0	13.9	16.9	20.1	21.9	15.7	16.9	1.62	0.22	0.01	0.76	0.10	0.32	0.49	0.59
Lower sheath	15.5	18.4	15.1	18.7	17.9	15.5	15.2	15.3	1.51	0.32	0.41	0.44	0.44	0.14	0.54	0.72
Upper sheath	15.9	30.7	11.7	15.4	16.7	17.1	13.9	19.7	5.45	0.68	0.25	0.25	0.22	0.51	0.75	0.39
Lower internode	22.8	25.3	20.1	24.0	24.2	28.3	17.8	20.0	3.41	0.84	0.14	0.33	0.28	0.99	0.96	0.76
Upper internode	14.5	24.8	11.4	20.5	9.5	24.4	7.2	19.6	1.69	0.14	0.07	0.95	0.01	0.15	0.44	0.76

[1] Tissues are leaf blades, leaf sheaths, or stem internodes collected from the 9th (lower) or 16th (upper) phytomers of corn plants. [2] E: Environment (limited vs. abundant); H: hybrid (conventional vs. brown midrib); EH: E × H interaction; M: maturity (VT vs. R5); EM: E × M interaction; HM: H × M interaction; EHM: E × H × M interaction.

3.3. Arabinose, Xylose, Glucose, and Uronic Acid

Environment did not affect the concentration of ARA in the CW of any of the tissues analyzed (Table 3). Relative to CON corn, BMR corn had greater concentrations of ARA in lower (2.0 vs. 2.6%, $p < 0.01$) and upper (4.3 vs. 4.9%, $p < 0.01$) stem internodes. An interaction tended to exist between environment and hybrid for the concentration of ARA in CW ($p < 0.07$). The reduced concentration of ARA in CW of the CON corn at the ABUNDANT environment explains the interaction. The concentration of ARA did not differ between CON and BMR varieties in leaf tissues ($p > 0.25$). As maturity advanced, the concentration of ARA decreased in lower leaf blades (5.5 vs. 3.8%, $p < 0.02$), lower leaf sheaths (5.0 vs. 3.8%, $p < 0.03$), and upper stem internodes (6.6 vs. 2.7%, $p < 0.01$), and tended to decrease in upper leaf blades ($p < 0.08$).

Cell walls from the upper sheaths of plants grown in the ABUNDANT environment had a slightly lower concentration of XYL than those grown in the LIMITED environment (29.1 vs. 30.6%, $p < 0.05$; Table 3). Relative to the CON hybrid, the BMR hybrid had a greater concentration of XYL in CW from the upper sheaths (28.5 vs. 30.5%, $p < 0.01$) and the lower stem internodes (24.7 vs. 27.7%, $p < 0.01$). As maturity advanced, the concentration of XYL decreased in CW of lower leaf sheaths (25.2 vs. 22.5%, $p < 0.05$) and tended to increase in CW of upper stem internodes (26.1 vs. 30.6%, $p < 0.06$).

An interaction between environment and hybrid existed (Table 3) for the concentration of GLU in CW of the upper leaf blades ($p < 0.01$), in the lower stem internodes ($p < 0.08$), and in the upper stem internodes ($p < 0.01$). For upper leaf blades, the BMR hybrid in the LIMITED environment and the CON hybrid in the ABUNDANT environment had the greatest concentrations of GLU (42.0 and 39.6%, respectively), whereas the BMR hybrid in the ABUNDANT environment and the CON hybrid in the LIMITED environment had the lowest concentrations of GLU (36.0 and 34.7%, respectively). For lower stem internodes, the BMR hybrid in the LIMITED environment had a lower concentration of GLU relative to other treatments (41.5 vs. 49%). For the upper stem internodes, the BMR hybrid in the ABUNDANT environment had the greatest concentration of GLU (45.8%), the CON hybrid in the ABUNDANT environment and the BMR in the LIMITED environment had an intermediate concentration of GLU (39.9%), and the BMR hybrid in the LIMITED environment had the lowest concentration of GLU (36.6%). As maturity advanced, the concentration of GLU decreased only in upper leaf sheaths (48.0 vs. 39.1%).

Table 3. Concentration of arabinose (ARA), xylose (XYL), glucose (GLU), and uronic acids (UA) in protein-free cell walls (PFCW) of corn tissues as affected by environment (ABUNDANT vs. LIMITED), corn genotype (CON vs. BMR), and maturity (VT vs. R5).

	ABUNDANT				LIMITED											
	CON		BMR		CON		BMR			*p* Value [2]						
Tissue [1]	VT	R5	VT	R5	VT	R5	VT	R5	SEM	E	H	EH	M	EM	HM	EHM
	ARA, % PFCW															
Lower blade	5.7	4.1	5.9	3.8	5.2	4.0	5.2	3.3	0.55	0.40	0.46	0.88	0.02	0.63	0.40	0.98
Upper blade	5.1	4.3	5.2	4.8	4.4	4.2	4.7	4.6	0.25	0.18	0.25	0.92	0.08	0.21	0.37	0.74
Lower sheath	5.1	3.5	5.1	4.7	4.7	3.5	5.1	3.3	0.49	0.27	0.37	0.47	0.03	0.49	0.66	0.23
Upper sheath	5.1	5.9	5.1	5.6	5.1	5.9	5.3	5.1	0.40	0.91	0.34	0.73	0.25	0.65	0.41	0.65
Lower internode	1.9	1.6	2.7	2.7	2.4	2.0	2.5	2.4	0.16	0.18	0.01	0.07	0.46	0.46	0.20	0.56
Upper internode	5.7	2.6	6.5	2.9	6.3	2.7	7.7	2.6	0.63	0.12	0.05	0.73	0.01	0.33	0.33	0.61
	XYL, % PFCW															
Lower blade	24.7	22.5	30.0	21.9	23.9	24.0	28.3	19.4	2.10	0.57	0.48	0.43	0.04	0.83	0.08	0.64
Upper blade	36.2	30.8	31.5	40.0	30.9	31.0	35.1	33.2	2.80	0.77	0.54	0.34	0.20	0.72	0.13	0.04
Lower sheath	24.5	20.9	25.1	24.6	23.8	22.2	27.4	22.1	1.29	0.93	0.11	0.82	0.05	0.52	0.90	0.15
Upper sheath	28.7	27.3	30.0	30.4	29.5	27.9	36.1	29.4	1.07	0.05	0.01	0.15	0.08	0.13	0.45	0.15
Lower internode	24.2	24.4	25.2	32.4	24.3	25.9	25.0	28.2	1.42	0.50	0.05	0.20	0.05	0.60	0.12	0.28
Upper internode	28.4	28.5	25.6	33.1	27.0	31.4	23.2	29.5	2.16	0.46	0.52	0.25	0.06	0.68	0.26	0.48
	GLU, % PFCW															
Lower blade	41.4	41.5	39.6	35.2	40.5	50.3	38.5	39.3	5.81	0.56	0.30	0.80	0.71	0.41	0.45	0.80
Upper blade	38.4	40.7	35.0	36.9	34.0	35.3	44.8	39.1	3.58	0.02	0.01	0.01	0.99	0.55	0.60	0.64
Lower sheath	52.4	48.2	44.9	47.1	45.5	50.5	51.8	50.1	3.33	0.58	0.77	0.18	0.90	0.97	0.58	0.22
Upper sheath	47.6	38.4	44.1	40.8	47.6	38.4	52.5	38.9	3.26	0.34	0.99	0.83	0.03	0.49	0.85	0.20
Lower internode	47.3	47.4	51.3	47.6	51.1	49.2	39.9	43.0	2.27	0.28	0.20	0.08	0.63	0.34	0.81	0.12
Upper internode	40.9	38.4	44.7	46.9	38.5	41.9	38.8	34.3	1.53	0.02	0.22	0.01	0.81	0.88	0.55	0.07
	UA, % PFCW															
Lower blade	4.5	3.9	6.3	3.5	5.3	3.8	4.0	3.6	0.88	0.62	0.98	0.37	0.09	0.52	0.67	0.25
Upper blade	4.5	3.3	4.1	3.0	3.6	2.9	3.2	3.0	0.31	0.07	0.26	0.70	0.06	0.29	0.57	0.82
Lower sheath	4.2	4.3	3.8	4.3	3.8	4.5	3.7	4.3	0.48	0.83	0.68	0.98	0.23	0.60	0.86	0.75
Upper sheath	3.4	4.0	3.7	4.3	3.8	5.3	3.8	3.9	0.53	0.27	0.56	0.19	0.19	0.85	0.48	0.48
Lower internode	3.6	2.7	3.7	3.2	3.9	2.7	3.5	3.0	1.19	0.86	0.41	0.28	0.01	0.73	0.09	0.73
Upper internode	5.7	3.3	7.0	3.3	6.6	3.0	7.8	3.2	0.55	0.16	0.04	0.85	0.01	0.27	0.20	0.84

[1] Tissues are leaf blades, leaf sheaths, or stem internodes collected from the 9th (lower) or 16th (upper) phytomers of corn plants. [2] E: Environment (limited vs. abundant); G: genotype (conventional vs. brown midrib); EH: E × H interaction; M: maturity (VT vs. R5); EM: E × M interaction; HM: H × M interaction; EHM: E × H × M interaction.

Relative to the CON hybrid, the BMR hybrid had a greater concentration of UA in CW of upper stem internodes (4.7 vs. 5.3, $p < 0.04$). Furthermore, as maturity advanced, the concentration of UA decreased in lower (3.7 vs. 2.9%, $p < 0.01$) and upper (6.8 vs. 3.2%, $p < 0.01$) stem internodes.

4. Discussion

Neutral detergent fiber concentration in a forage and forage NDFD are the two most important determinants of forage quality [18]. In the context of climate change, understanding the effects of environment on NDFD of forages is critical to maximize forage production and nutrient utilization by livestock. Both climate and agricultural practices determine the growing environment of a crop and, hence, their yield and composition. However, our understanding of the effects of climate [8,9] and agricultural practices on NDFD is limited.

In the current study, we determined the effects of growing corn in a limiting environment with high corn planting population (90,000 seeds/ha) and a lower N fertilization (175 kg N/ha) relative to an abundant environment with low corn planting population (60,000 seeds/ha) and a greater N fertilization (225 kg N/ha) on NDFD and lignin concentration in corn vegetative tissues. In general terms, NDFD decreased when corn was grown in a limited environment, although we only observed this in the upper and not the lower tissues within the plant. Differently, Ferreira and Teets reported similar IVNDFD for whole-plant corn planted at 55,000 to 100,000 seeds/ha [19], and Diepersloot et al. [20] reported similar IVNDFD for whole-plant corn planted at 63,000 and 73,000 seeds/ha. The lack of differences in the latter two studies is likely related to the type of sample analyzed (i.e., individual tissues vs. whole plant). In this experiment, we also contrasted conventional against BMR genotypes and early against late harvests as common factors affecting NDFD and lignin concentration. As expected, and in agreement with previous

studies [1,3,4,21], we observed greater NDFD coefficients in most tissues obtained from the BMR and the early-harvested corn. All these data highlight that pre-harvest factors like planting population and N fertilization may affect NDFD at the plant tissue level, although other pre-harvest factors like genotype or maturity at harvest have greater impacts on NDFD, especially at the whole-plant level.

Some tissues from the BMR genotype had lower concentrations of ABL than tissues of the conventional genotype (e.g., upper blades and upper internodes; Table 2). Additionally, a few tissues from corn harvested early had lower concentrations of ABL than tissues from the corn harvested late (e.g., lower blade and upper internode; Table 2). However, we expected to find differences in many more tissues throughout the corn plant than we did. Despite the different ABL concentrations between genotypes and maturities, we did not find differences in the concentration of ABL between the different environments. Similarly, Ferreira et al. [9] reported slightly different ABL concentrations (14.1 vs. 16.1% ABL) in leaf blades of corn from different irrigation regimes, although ABL concentration did not differ in stem internodes. Overall, tissue type (i.e., stem internode vs. leaf blades or leaf sheaths) had a much greater impact on ABL concentration than all pre-harvest factors, and this agrees with previous observations [9].

In this study, we measured and reported lignin as acetyl-bromide lignin (ABL) and not as acid detergent lignin (ADL), and this is worth highlighting. Acid detergent lignin is the most common empirical procedure to determine lignin concentration in animal feeds [6,22]. Even though both ABL and ADL are empirical procedures [22], ABL measures phenolic compounds of the cell wall more comprehensively than ADL [23–25]. This means that phenolic compounds crosslinking the arabinoxylans to other phenolic compounds of the CW that are not captured by the ADL procedure (namely ferulic and p-coumaric acids) may be included in the ABL procedure. As the synthesis of precursors to synthesize phenolic compounds is diminished in varieties containing the BMR mutation [26], we considered it critical to include ferulic and p-coumaric acids in the ABL component of the feed to better reflect the differences in CW composition of different genotypes.

In general terms, lignin is a major determinant of IVNDFD [6] for at least two reasons. First, lignin is a very stable component that is minimally digested by rumen microbes. Second, lignin has hydrophobic properties, therefore limiting the access of fibrolytic enzymes that degrade other components of the CW like cellulose and hemicellulose. Despite this, observations from this clearly suggest that IVNDFD is beyond lignin concentration. For example, upper leaf blades from the CON corn grown in an abundant environment and harvested at a late stage of maturity had a 73.3% IVNDFD while having 24.0% ABL, whereas upper leaf blades from the BMR corn grown in a limited environment and harvested at a late stage of maturity had a 74.4% IVNDFD while having 15.7% ABL (Tables 1 and 2). These data reflect a quite similar IVNDFD with quite variable ABL concentrations. Alternatively, upper internodes from conventional corn grown in the abundant environment and harvested at the late stage of maturity had a 54.1% IVNDFD while having 24.8% ABL, whereas lower internodes from conventional corn grown in the abundant environment and harvested at the late stage of maturity had a 27.9% IVNDFD while having 25.3% ABL (Tables 1 and 2). These data reflect a quite different IVNDFD with similar ABL concentrations. Figure 1a shows that a linear relationship exists between IVNDFD and ABL concentration ($r = -0.64$; $p < 0.01$) when analyzing the whole plant. However, when analyzing samples by tissue type, maturity stage, and position within the plant, we observed weaker or non-existing correlations (Figure 1b). More specifically, no correlation existed between IVNDFD and ABL for leaf blades harvested at VT from the 9th phytomer ($p = 0.79$), leaf blades harvested at VT from 16th phytomer ($p = 0.32$), leaf sheaths harvested at VT from the 9th phytomer ($p = 0.52$), leaf sheaths harvested at R5 from the 16th phytomer ($p = 0.61$), stem internodes harvested at VT from the 9th phytomer ($p = 0.11$), stem internodes harvested at R5 from the 9th phytomer ($p = 0.24$), and stem internodes harvested at R5 from the 16th phytomer ($p = 0.17$). Alternatively, moderate correlations existed between IVNDFD and ABL within leaf blades harvested at VT from the 9th phytomer ($r = 0.78$;

$p < 0.03$) and within leaf sheaths harvested at VT from the 16th phytomer (r = 0.67; $p < 0.07$). All these observations indicate that factors beyond ABL might affect IVNDFD.

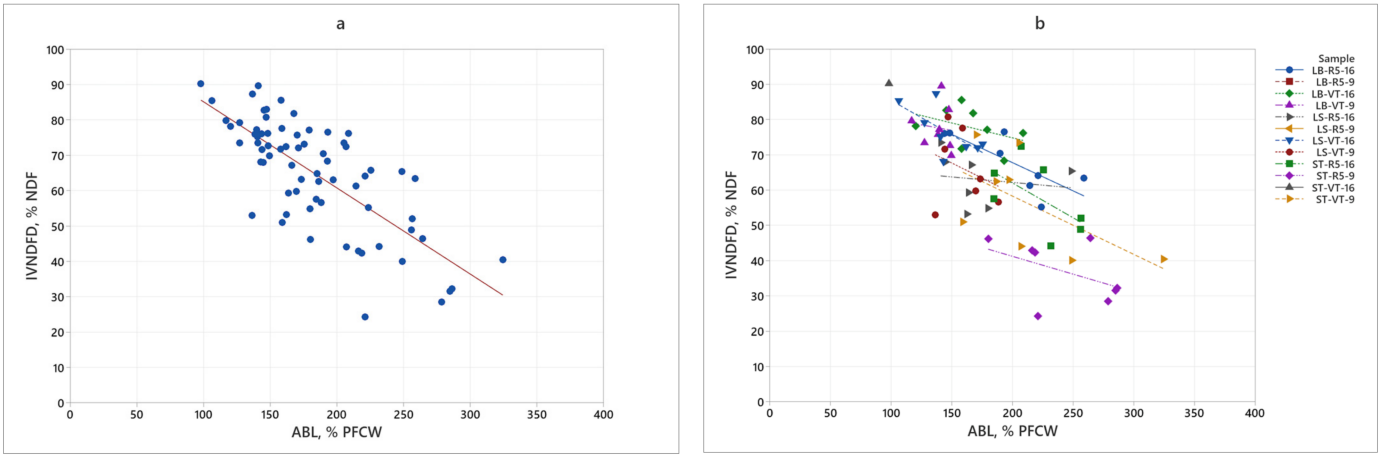

Figure 1. Relationship between in-vitro neutral detergent fiber digestibility (IVNDFD, % NDF) and acetyl-bromide lignin (ABL) concentration in the protein-free cell wall (PFCW) of corn plant tissues. Stem internodes (ST), leaf sheaths (LS), and leaf blades (LB) were collected from the 9th (9) and 16th (16) phytomers of corn plants at tasseling (VT) and $\frac{1}{4}$ to $\frac{1}{2}$ milkline (R5) stage of maturity. (**a**) describes the relationship between IVNDFD and ABL considering all samples, and (**b**) describes the relationship between IVNDFD and ABL within each tissue and maturity stage.

Differences in IVNDFD among different tissues (i.e., internodes vs. leaf sheaths vs. leaf blades) and between tissues of different strata within the plant (i.e., lower vs. upper tissues) may be related to other components of the CW. Screening the different tissues, we observed that the stem internodes had substantially lower concentrations of ARA than the leaf sheaths and blades (Table 3). Arabinose is a component of the arabinoxylans that may crosslink with lignin. In this crosslinking, ARA forms ester bonds with ferulic and p-coumaric acids. Plausibly, a greater linkage between ARA and ferulic acid or p-coumaric acid diminishes the concentration of ARA in the CW, especially in lower stem internodes. Under this rationale, we observed that the prediction of IVNDFD was improved after subjecting the data to multiple linear regression analysis, as described in Equation (1):

$$\text{IVNDFD}_{(\%\ \text{NDF})} = 61.01 + 0.52 \times \text{ARA}_{(\%\ \text{PFCW})} - 0.11 \times \text{ABL}_{(\%\ \text{PFCW})};\ r^2 = 0.57 \quad (1)$$

5. Conclusions

We concluded that environmental stress could affect IVNDFD in corn for silage, although such an effect is tissue-dependent and less impactful than anticipated. Specifically, a limited environment with a high number of plants and lower N fertilization decreased the IVNDFD of upper leaf blades only. This observation might be relevant to understand the effects of other stressing factors, such as drought or heat stress, on the digestion of fiber. Still, pre-harvest factors like corn genotype, maturity at harvest, and plant structure (i.e., proportion of tissues) have a much greater and consistent impact on NDFD than environment.

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