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Potentials to breed for improved fibre digestibility in temperate Czech maize (*Zea mays* L.) germplasm

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Abstract: Cell wall digestibility is an important quality trait of modern silage maize cultivars. The symbiotic relationship between microbes and ruminant livestock enables the efficient upcycling of otherwise for human consumption unsuitable rumen digestible fibre or cell wall components into highly nutritious milk and meat. Before entering the Czech National List of Plant Varieties, new silage maize germplasm is extensively tested for different cell wall digestibility parameters. Recently published, the undigestible neutral detergent fibre (uNDF) cell wall digestibility approach promises even greater practical relevance. The aim of our study was, therefore, to assess the potential of the uNDF method, compared with current standard procedures, using a vast set of official Czech plant variety trial evaluations and Czech silage analyses from the 2018 cropping season. The uNDF method yielded a twice as high phenotypic standard deviation, compared with the current standard approaches. This is good news for plant breeders, official variety testing organisations, and farm professionals alike, enabeling faster variety improvement and simpler variety selection. On the other hand, due to the low differentiation potential, we discourage the use of the absolute lignin content when selecting for digestible silage maize varieties. Since between the digestibility traits enzymatic soluble organic substance (ELOS) and cellulase digestibility (DCS), a Pearson correlation close to one was observed, the substitution of one of these analytics by the uNDF method, may render valuable additional information in a highly economical manner.

Keywords: cell wall digestibility; lignin; phenotypic variation; uNDF

Fibre, or in other words, cell wall digestibility is an important quality trait of feedstuffs rich in dietary fibre. Hereby, the cell wall proportion consists of the macromolecules; hemicellulose, cellulose, and lignin (Van Soest et al. 1991). Other than for monogastric species, the symbiotic relationship between the ruminants and microbes within the rumen allows for the efficient upcycling of the valuable fibre components of hemicellulose and cellulose, if their digestibility is adequate (Russell 2002). For instance, in maize, as one of the most important forage crops worldwide, both the absolute lignin content and the lignin structure have an influence on the cell wall digestibility (Barrière et al. 2003). Monogenic polymorphisms, like the brown midrib genotypes (e.g., bm1 and bm3) exist, showing reduced lignin contents and comparatively high cell wall digestibilities via their effect on the lignin biosynthesis pathway within the plant (Perry 1996). Despite their favourable cell wall digestibility, brown midrib hybrid varieties have not yet gained strong market relevance outside of the United States, due to their associated sub-optimal agronomic performances.

Nonetheless, since cell wall digestibility is a limiting factor for improving the feed value of maize, breeding companies and official state variety protection agencies extensively test new silage maize varieties for their cell wall digestibility parameters, as well as their overall agronomic performance (Ledencan et

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al. 2003; Li et al. 2019). For example, in the Czech Republic, currently, 391 different maize varieties are registered on the official Czech national list of maize varieties (National Plant Variety Office 2016). The assessed cell wall digestibility parameters include the *in vitro* digestibility of the non-soluble carbohydrates (IVDNSC or DINAG), the enzyme-soluble organic substance (ELOS), the cellulase digestibility (DCS) and the *in vitro* digestible organic matter (IVDOM). The mentioned *in vitro* cell wall and whole plant digestibility methods have gained broad application due to their reproducibility, and above all, since *in vivo* digestibility trials using animals (e.g., sheep or fistulated large ruminants) are considered time-consuming, laborious and expensive (Barrière et al. 2003). They also share a common ancestry, e.g., based on the two-stage pepsin and cellulase method, proposed by Tilley and Terry (1963) and Aufrère et al. (2007) to determine the ELOS and DCS, as well as modifications to assess the IVDOM, using rumen liquor (Moe & Carr 1984). Near-infrared reflectance spectroscopy (NIRS) calibrations are available, enabling the rapid assessment of these cell wall digestibility traits, whereas the DINAG constitutes a weighted criterion, shown by the calculation:

$$\text{DINAG} = \frac{100}{\text{NDF}} \times (\text{IVDOM} - (100 - \text{NDF}))$$

where the IVDOM and neutral detergent fibre (NDF) may be analysed via a NIRS calibration, to scale down the influence of the highly digestible non-fibre carbohydrates (Barrière et al. 2003).

Despite the merits of the mentioned methods, the detailed assessment of the theoretical digestible fibre and as reciprocal view undigestible fibre (uNDF) promises a clearer perception of the nutritional fibre value for ruminant livestock (Raffrenato et al. 2018). The aim of our study was, therefore, to assess the potential of the uNDF method yielding additional information on fibre digestibility, contrasted by the currently used methods, using the complete set of official maize silage trials, conducted by the Czech National Plant Variety Office, as well as 112 maize silage samples from Czech dairy professionals, analysed following uNDF methodology, both from the cropping season 2018.

MATERIAL AND METHODS

Czech National Plant Variety Trials. From the 2018 silage maize variety trials, conducted by the Czech National Plant Variety Office, the detailed data of the complete set of 162 tested maize hybrids were available (Povolny et al. 2019). Measurements of the NIRS based quality traits of varieties included the dry matter (DM) content in %, the crude protein (CP) content in % DM, the crude starch (CS) content in % DM, the neutral detergent fibre (NDF) content in % DM and the acid detergent fibre (ADF) content in % DM. Associated NIRS fibre digestibility traits included the ELOS (% DM), IVDOM (% DM), DCS (% DM), and DINAG (%). Evaluated maturity groups of the 2018 silage maize variety trials spanned very-early (n = 50), early (n = 58), mid-early (n = 57) and mid-late (n = 13) maturity groups. Six hybrids were used as checks, two interconnecting the four maturity groups, respectively. Hybrid varieties, from the commercial breeding companies, Caussade (n = 12), Cezea (n = 5), Dow Agrosciences (n = 16), Euralis (n = 20), Hodowla Roslin (n = 4), KWS (n = 25), Limagrain (n = 13), Maisadour (n = 6), Monsanto (n = 19), Moreau Saatzucht (n = 3), Pioneer (n = 4), RAGT (n = 24), Saatbau Linz (n = 11), Saatzucht Gleisdorf (n = 5) and Syngenta (n = 9) were evaluated.

Maize silage analyses. From the cropping season 2018, a set of 112 maize silages from different Czech dairy farms (800 g per sample) were collected, vacuum sealed, and sent to the Sano-CVAS laboratory in Popovaca/Croatia for further analysis. The samples were air-dried at 60 °C for 24 h, ground, and subsequently analysed *in vitro*, following uNDF standard procedures via NIRS and proprietary Cumberland Valley Analytical Services (CVAS) European maize silage NIRS-calibration. The measured quality traits of the varieties included the DM content in %, the CP content in % DM, the CS content in % DM, the NDF content in % DM and the ADF content in % DM. Associated fibre digestibility traits included the neutral detergent fibre digestibility (NDFD30 in % NDF) after 30 h in rumen fluid and the undigestible neutral detergent fibre proportion (uNDF240 in % NDF) after 240 h in the rumen fluid. Statistical analyses were conducted using R (R Core Team 2018).

RESULTS AND DISCUSSION

State of the art. Within the 2018 Czech (CZ) maize silage variety evaluations, the reported methods to assess maize silage cell wall digestibility (ELOS, IVDOM, DCS, and DINAG) show intermediate to high and highly significant Pearson correlations (Figure 1). As expected, due to the mathematical property of

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the DINAG, via scaling of the total digestible matter for the non-fibre carbohydrates, Pearson correlation among the ELOS, IVDOM, and DCS resides on an upper-intermediate level. Visual inspection of the 112 CZ maize silage analysis results reveals a perceivable stratification of the indigestible fibre (uNDF240) content (Figure 2). In comparison to the uNDF240 (% DM), the visual differentiation of the lignin content among silages is low. As expected, the comparison of value determining components among CZ maize silage variety trials and CZ maize silage analyses yielded substantial similarities (Figure 3). Observed deviations may be attributed to the deviating harvest maturities within the 2018 harvest season. As has been shown in a broad phalanx of publications and feeding trials, the 30-h neutral detergent fibre digestibility (NDFD30 % NDF), as important fibre quality parameter, fulfils the highest accuracy standards (Higgs et al. 2015; Van Amburgh et al. 2015). As expected, following the reasoning of the uNDF methodology, NDFD30 (% NDF) and uNDF240 (% NDF) yield a high and highly significant Pearson correlation, also related to the absolute lignin content, but not quantitatively (Figure 4).

Variety improvement potential. Due to the substantial similarities of value determining components in the CZ variety trials and CZ maize silage data sets, the direct comparison of associated cell wall digestibility traits can be conducted as a straight forward procedure (Figure 3). Of interest, the comparison of the measured digestibility parameters yielded the highest phenotypic standard deviation (σ_p) for the uNDF240 (% NDF) digestibility trait, about twice as large as the phenotypic standard de-

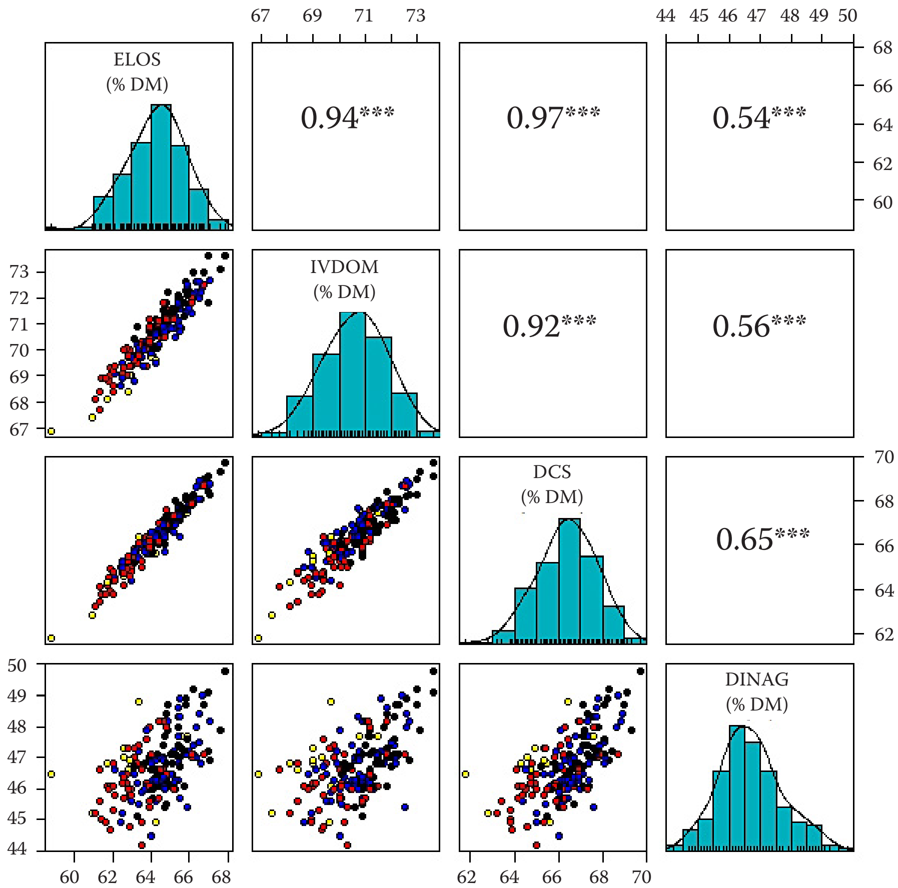

Figure 1. Cropping season 2018 comparison of measured dry matter (DM) digestibility traits within the CZ maize variety trials, including the enzyme-soluble organic substance (ELOS in % DM), the *in vitro* digestible organic matter (IVDOM in % DM), the cellulase digestibility (DCS in % DM) and the digestibility of the non-soluble carbohydrates (DINAG in %); the upper triangular matrix shows the Pearson correlation (***, $P < 0.001$) between the traits, the diagonals show the traits frequency distribution, respectively; the lower diagonal matrix shows the colour-coded harvest maturity groups: very early = black; early = blue; mid-early = red; mid-late = yellow

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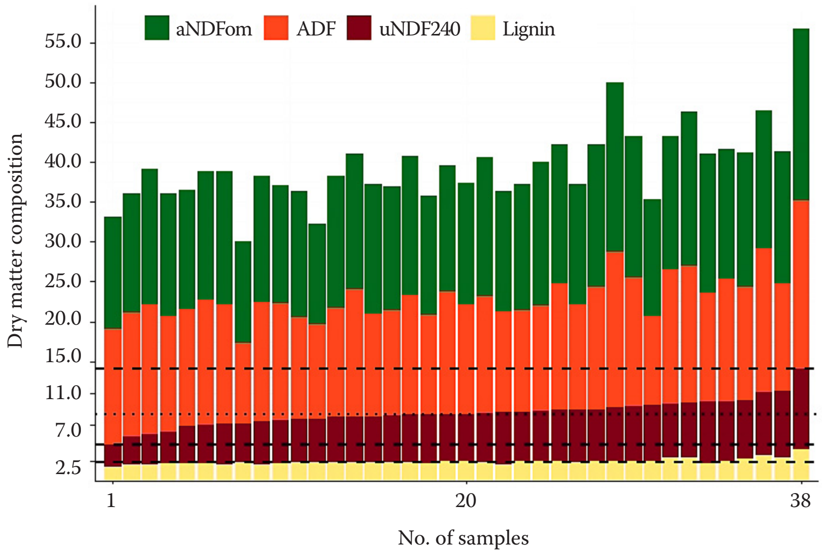

Figure 2. Value determining components of CZ maize silages, harvested in the year 2018, including neutral detergent fibre (aNDFom, % DM), amylase treated and adjusted for ash content, acid detergent fibre (ADF, % DM), undigestible neutral detergent fibre (uNDF240, % DM) after 240 h in the *in vitro* rumen fluid essay and lignin (% DM); to improve the perception, a subset (n = 38), every third sample out of the complete set of 112 maize silage analysis results is shown; from the bottom to the top, the dashed lines represent the arithmetic mean of lignin (% DM), the minimum, the arithmetic mean, and the maximum of the uNDF240 (% DM)

viation of the ELOS (% DM). Absolute lignin content (% DM) yielded the lowest σ_p (Table 1). Since silage maize germplasm/genetics was identified as one

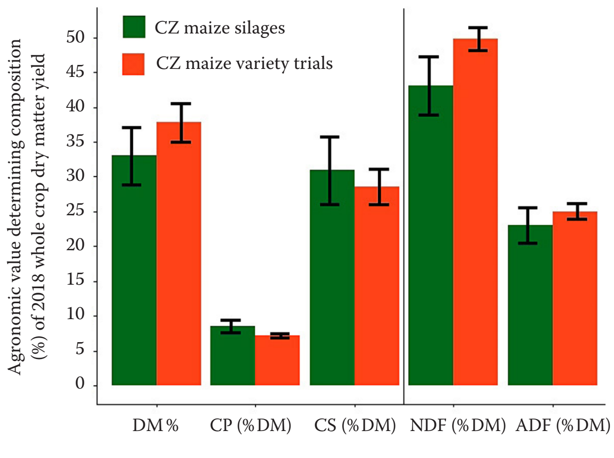

Figure 3. Consistency comparison of the value determining agronomic components of 178 whole plant near-infrared reflectance spectroscopy (NIRS) analysis results (CZ Variety trials) and 112 CZ NIRS silage analyses, both from the 2018 cropping season; compared traits include dry matter content (DM %), crude protein (CP %), starch (CS %), neutral detergent fibre (NDF %DM) and acid detergent fibre (ADF %DM); whiskers show the trait-specific standard deviation

key source of variation in NDF digestibility, with typically high heritabilities (Barrière et al. 2003), selection for improved cultivars might, therefore, be highly promising, and conducted applying classic and modern breeding methodologies (Meuwissen et al. 2001; Geiger 2009). One main driver of variety improvement efforts is the observable and measurable variability of the breeding germplasm. In this respect, the expected progress or response to selection (R) can be calculated as follows:

$$R = i \times h^2 \times \sigma_p \quad (1)$$

where:
i – the selection intensity,
h^2 – the heritability,
σ_p – the phenotypic standard deviation of the trait (Falconer & Mackay 1996).

Following this fundamental knowledge, using the uNDF methodology, the uNDF240 (% NDF) assessment may double the progress of selection for cell wall digestibility in silage maize under *ceteris paribus* conditions. This is good news for plant breeders and farm professionals alike. Ruminant livestock farmers may especially profit from the uNDF240 (% NDF) assessment, since the comparatively broader value range simplifies proper variety

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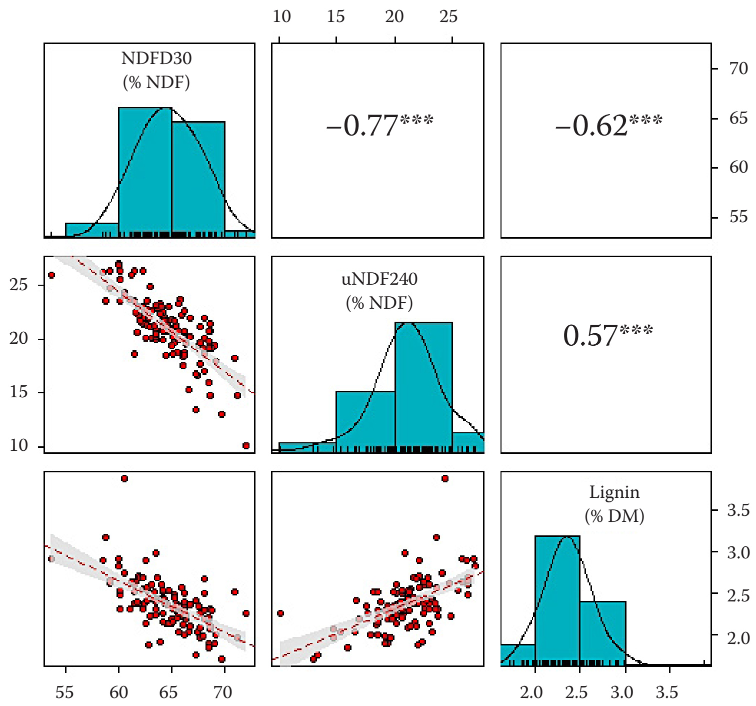

Figure 4. Significant (***, $P < 0.001$) Pearson correlations among the digestible neutral detergent fibre proportion (NDFD30 in % NDF after 30 h in rumen fluid), the undigestible neutral detergent fibre proportion (uNDF240 in % DM, after 240 h in rumen fluid) and the lignin content (% DM) using 112 CZ maize silages from the 2018 cropping season

The red dotted line shows the linear regression, and the grey area covers the corresponding confidence interval

choice (Table 1, Figure 2). This property, therefore, suggests the uNDF methodology to satisfy the key prerequisite of a broad value range, to be an appropriate method for comparing the digestibility of forages, and providing meaningful information across a wide range of animals and crops (Allen et al. 2003). Additionally, due to its focus towards the NDF fraction, the uNDF methodology offers the chance to screen for yet unknown favourable alleles within locally adapted landraces using modern pre-breeding schemes (Mudry & Kraic 2007; Geiger 2009). Modern maize germplasm, fortified with such favourable alleles, might, next to the agronomic potential, also have a substantial impact within the global warming context. Focusing just on emissions, grass, pasture, and silage, maize fed dairy cows contribute a vital part of the total greenhouse gases emitted by the agricultural sector (FAO 2010). Since the Food and Agriculture Organization of the United Nations also stated fodder digestibility to be an influential critical parameter in improving the economic and ecological sustainability of

Table 1. Cropping season 2018 comparison of measured maize whole crop dry matter (DM) digestibility trait phenotypes within the CZ variety trials, including the enzyme-soluble organic substance (ELOS in % DM), the *in vitro* digestible organic matter (IVDOM in % DM), the cellulase digestibility (DCS in % DM), the digestibility of the non-soluble carbohydrates (DINAG in %), and the CZ maize silage traits, including lignin (% DM) and the undegradable neutral detergent fibre proportion after 240 h (uNDF240 in % NDF)

Trait	ELOS	IVDOM	DCS	DINAG	Lignin	uNDF240 (% NDF)
			(% DM)			
No. of observations	178	178	178	178	112	112
Minimum	58.8	66.9	61.8	44.2	1.7	10.1
Mean	64.3	70.6	66.4	46.7	2.4	21.0
Median	64.5	70.7	66.4	46.6	2.4	21.2
Maximum	67.9	73.6	69.7	49.8	3.9	27.0
Range	9.1	6.7	7.9	5.6	2.2	16.9
Phenotypic SD (σ_p)	1.5	1.2	1.4	1.0	0.3	3.0

SD – standard deviation

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dairy farming, focusing on higher digestible silage maize cultivars may supply substantial leverage potential and reductions in dairy sector greenhouse gas emissions. In the light of the proposed global increase in consumption of dairy products, future maize silage cultivars, improved for their cell wall digestibility, may contribute substantially towards more sustainable farming practices (Schönleben et al. 2020). The presented results, applying the uNDF methodology, are, therefore, highly encouraging and good news for the environment, plant breeders, end-customers, and ruminant livestock farmers, in the combined quest towards modern maize silage cultivar development and sustainable farming. On the other hand, due to the comparatively lowest σ_p and differentiation potential, we discourage the use of the absolute lignin content (% DM) when selecting for digestible silage maize varieties (Table 1, Figure 2). Furthermore, since the Pearson correlation between the ELOS (% DM) and DCS (% DM) approaches one (Figure 1), the substitution of one of these analytics through the uNDF240 (% NDF) may render valuable additional information for plant breeders, farmers/end-customers and official variety testing agencies alike, in a highly economical manner.

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