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SHORT COMMUNICATION**Nutritive value of sweet pearl millet and sweet sorghum as influenced by N fertilization**

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Abbreviations: **ADF**, acid detergent fiber; **CHU**, corn heat units; **NDF**, neutral detergent fiber; **DM**, dry matter; **IVTD**, in vitro true digestibility of dry matter; **NDFd**, in vitro digestibility of NDF.

Abstract: Increasing N fertilization decreased the in vitro digestibility of dry matter (IVTD) and neutral detergent fiber (NDF), but increased N concentration of sweet sorghum [*Sorghum bicolor* (L.) Moench] and sweet pearl millet [*Pennisetum glaucum* (L.) R.BR.] in eastern Canada. Sweet sorghum had lower NDF, acid detergent fiber, and N concentrations, and greater IVTD than sweet pearl millet.

Key words: biomass, forage, nitrogen, nutritive value, digestibility

The yield potential of sweet pearl millet and sweet sorghum in eastern Canada is now well established (Bouchard et al. 2011; Leblanc et al. 2012; Thivierge et al. 2015a; dos Passos Bernardes 2015). The nutritive attributes of sweet pearl millet were shown to be affected by N fertilization in a study conducted in eastern Canada (Leblanc et al. 2012). However, the effect of N fertilization on the nutritive value of sweet sorghum and of the effect of organic N sources on the nutritive value of sweet sorghum and sweet pearl millet are not known. Thivierge et al. (2015a, b) recently reported the DM yield and N uptake response of sweet sorghum and sweet pearl millet to N fertilization but they did not provide any information on the nutritive value. The current study is a continuation of Thivierge et al. (2015a, b) with the objective of determining the response of several nutritive attributes of sweet pearl millet and sweet sorghum to (i) increasing mineral N fertilizer rates, (ii) mineral vs. organic N sources, and (iii) a single vs. a split application of mineral N fertilizer.

The study was conducted at two experimental sites: Sainte-Anne-de-Bellevue (Québec, Canada, 45°26'N, 73°56'W; 2901 to 3100 Corn Heat Units [CHU]; CRAAQ, 2012) in 2011 and 2012, and Saint-Augustin-de-Desmaures (Québec, Canada, 46°44'N, 71°31'W; 2501 to 2700 CHU; CRAAQ, 2012) in 2010 and 2011. Soil types were a St.

Bernard sandy loam at Sainte-Anne-de-Bellevue and a St. Antoine sandy loam at Saint-Augustin-de-Desmaures. Mean daily temperatures from seeding to harvest ranged from 18.6 to 21.9°C across the four site-years, while cumulated rainfall ranged 188 to 390 mm. A detailed description of the two experimental sites is presented in Thivierge et al. (2015a). A randomized complete block design with split-plot restriction with two species as main plots and eight N fertilization treatments as subplots was used with four replicates. A sweet pearl millet hybrid (CSSPM7; AERC Inc., Delhi, ON, Canada) and a sweet sorghum hybrid (CSSH45; AERC Inc., Delhi, ON, Canada) were compared. Mineral N fertilization treatments consisted of calcium ammonium nitrate (27-0-0) at rates of 0, 40, 80, 120 and 160 kg N ha⁻¹, with 40 kg N ha⁻¹ broadcast at seeding and the remaining N side-dressed at the four-leaf stage. These treatments are referred to hereafter as 0, 40, 80s, 120s, and 160s, where “s” stands for split. One additional mineral N fertilization treatment consisted of applying 80 kg N ha⁻¹ in a single application at seeding, a treatment referred to as 80. Two organic N sources, liquid swine manure (SM80) and liquid dairy manure (DM80), were broadcast at seeding at a target rate of 80 kg total N ha⁻¹. The actual amounts of applied N varied between 75 and 90 kg N ha⁻¹ (Thivierge et al. 2015a). All fertilizers (mineral and organic) applied at seeding were incorporated into the top 5 cm of soil with one pass of a harrow within two hours of application. For each of the two consecutive years at each site, a different plot area was used. Individual plots included seven 5-m rows at Sainte-Anne-de-Bellevue and nine 6-m rows at Saint-Augustin-de-Desmaures. Row spacing was 0.18 m with a seeding rate of 10 kg ha⁻¹ of viable seeds at a depth of 2.5 cm. Seeding and harvesting dates are presented in Thivierge et al. (2015a).

Both species were harvested when 3/4 of the inflorescence had emerged on at least 75% of the plants at Sainte-Anne-de-Bellevue and on at least 50% of the plants at Saint-Augustin-de-Desmaures. Five rows of 3.5 m in length in the center of each plot were hand harvested to a 5-cm stubble height. At Saint-Augustin-de-Desmaures in 2010, only two rows of 2 m in length were harvested in the center of each plot because of a lodging episode. The harvested biomass was weighed, chopped, and a subsample of 250 g was taken from each plot. All subsamples were dried at 55 °C until constant weight and ground using a Wiley mill (Standard model 3, Arthur H. Thomas Co., Philadelphia, PA) to pass through a 1-mm screen.

Total N concentration was determined by dry combustion (LECO CNS-1000, Leco Corp., St. Joseph, MI). The acid detergent fiber (ADF) concentration was determined according to AOAC (1990), while the neutral detergent fiber (NDF) concentration was determined following Mertens (2002) with the addition of sodium sulfite and heat-stable α -amylase. These fiber extractions were done using the Ankom filter bag technique (Ankom Technology Corp., Fairport, NY, USA). The in vitro digestibility of NDF (NDFd) was measured using the method of Goering and Van Soest (1970) based on a 48-h incubation with buffered rumen fluid followed by a NDF determination of the postdigestion residues. The rumen fluid incubations of the selected samples were performed in two consecutive batches within 2 wk using Ankom F57 filter bags, in an Ankom DaisyII incubator, with the filter bag method recommended by Ankom Technology (Fairport, NY, USA). The in vitro true digestibility of the DM (IVTD) (g kg^{-1} DM) and NDFd (g kg^{-1} NDF) were calculated as in dos Passos Bernardes et al. (2016).

Data were assessed by analyses of variance (ANOVA) using the GENSTAT statistical software (VSN International 2011). Sites and years were considered random effects in order to establish the average response over a large section of agricultural areas in Québec. Single-degree of freedom contrasts were used to test the response of all variates to N rates and N sources. Differences were considered significant when $P \leq 0.05$. A principal component analysis (PCA) was used to assess the relationships among variates (DM yield and nutritive attributes) and how variations in these variates were related to the N treatments and species. The PCA was performed on the least squares means of the treatments using the correlation matrix method to give equal weight to all variates using the GENSTAT statistical software (VSN International 2011). The contribution of each variate to a principal component axis can be seen from its loadings.

Increasing mineral N fertilization decreased NDFd and IVTD of both species but it increased N concentration (Table 1). Dry matter yield also increased with N fertilization up to a maximum with rates between 107 and 121 kg N ha⁻¹ (Thivierge et al. 2015a). The decrease in IVTD with increasing mineral N fertilization was greater in sweet pearl millet than in sweet sorghum (significant Species $\times$ N interaction; Table 1). Increasing mineral N fertilization affected the NDF concentration differently for the two species (significant Species $\times$ N interaction; Table 1); it decreased the NDF concentration of sweet sorghum but had little effect on the NDF concentration of sweet pearl millet. Increasing mineral N fertilization did not significantly affect the ADF concentration. In a previous study on sweet pearl millet conducted at the same sites, increasing N rates also resulted in a decrease in NDFd, an increase in N concentration, and a small decrease in NDF concentration along with an increase in DM yield (Leblanc et al. 2012). Our results

confirm those previously reported for sweet pearl millet and extend this effect of N fertilization to the nutritive attributes of sweet sorghum.

The concentrations of ADF and NDF, and IVTD were similar with mineral N and organic N sources (80 vs. SM80 and DM80; Table 1) and between organic N sources (DM80 vs. SM80; Table 1). The N concentration, however, was greater and the NDFd was less with mineral N than with organic N sources (80 vs. SM80 and DM80; Table 1). The DM yield was also greater with mineral N than with organic N sources (Thivierge et al. 2015a). Splitting the mineral N fertilization between seeding and the four-leaf stage did not affect most nutritive attributes (80 vs. 80s; Table 1). To our knowledge, the effect of organic N sources on the nutritive value of those two species has never been reported.

The first component of the PCA was defined mostly by forage DM yield and N concentration on the positive side, and by IVTD and NDFd on the negative side (Fig. 1). Attributes within the same group on each side were positively correlated, while attributes in opposing groups were negatively correlated. This first component of the PCA mostly defined differences among N treatments. High rates of mineral N fertilization ($\geq 80 \text{ kg N ha}^{-1}$) were opposed to low rates (0 and 40 kg N ha^{-1}), which were more closely related to organic N sources. Similarities between low mineral N rates and organic N sources are in agreement with Thivierge et al. (2015b) who used ^{15}N -tracing to demonstrate that crop uptake of manure-derived N was low (10 to 19 kg ha^{-1} with DM80 and 19 to 26 kg ha^{-1} with SM80) but comparable to crop uptake of fertilizer-derived N (23 kg ha^{-1}) applied at 40 kg ha^{-1} . This low recovery of manure N was attributed to the low concentration of available N in manure, and to possible N immobilization or losses of manure available N through ammonia volatilization (Thivierge et al. 2015b). This first component of the PCA

confirms that the response of IVTD and NDFd to N fertilization is related to a large extent to the effect of N fertilization on DM yield. The decrease in IVTD and NDFd with increasing N availability, either through increasing mineral N rates or by using mineral N rather than organic sources at same rate of total N, can be attributed to their negative relationship with DM accumulation, which is well documented for perennial forage species (Bélangier et al. 2001). Our results suggest that this negative relationship with DM accumulation also exists in sweet sorghum and sweet pearl millet.

The two species significantly differed in all nutritive attributes, except for NDFd (Table 1). Sweet sorghum had lower ADF, NDF, and N concentrations than sweet pearl millet and a greater IVTD. Our results confirm those from a previous study conducted at the same sites in which sweet sorghum had lower ADF and NDF concentrations, and DM yield, but greater IVTD than sweet pearl millet (dos Passos Bernardes et al. 2015, 2016). The second component of the PCA mostly defined differences among species (Fig. 1). This second component was defined mostly by forage ADF and NDF concentrations, and NDFd on the positive side, and by IVTD, N concentration, and DM yield on the negative side. Interspecific differences in NDF concentration and IVTD varied with N treatments (significant Species $\times$ N interaction; Table 1) and were greater at high than at low N rates. This interspecific difference can also be seen in the PCA analysis where N treatments with high mineral N rates for sweet pearl millet and sweet sorghum were further apart than those with low mineral N rates or with organic N sources.

Our results clearly indicate that increasing N fertilization decreases the digestibility of sweet pearl millet and sweet sorghum but increases their N concentration. This negative effect of N fertilization on biomass digestibility can be attributed to its negative

relationship with DM yield, which was increased by high N rates. Both DM yield and nutritive attributes should therefore be considered when determining the economically optimum N rates for those two species. Our results also confirm that sweet sorghum has a greater nutritive value than sweet pearl millet.

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Table 1. Concentrations of acid detergent fibre (ADF), neutral detergent fibre (NDF) and N, and in vitro digestibility of the NDF (NDFd) and in vitro true digestibility of the dry matter (IVTD) along with the probability values for the effects of species and N treatments, and their interaction. Data are average values across two sites and two years.

N treatments ^a	ADF		NDF		NDFd		IVTD		N ^b	
	(g kg ⁻¹ DM)		(g kg ⁻¹ DM)		(g kg ⁻¹ NDF)		(g kg ⁻¹ DM)		(g kg ⁻¹ DM)	
	Millet	Sorghum	Millet	Sorghum	Millet	Sorghum	Millet	Sorghum	Millet	Sorghum
0	385	363	627	577	657	645	786	795	7.9	6.9
40	386	352	627	556	645	622	777	791	8.3	7.8
80s	399	345	633	542	614	594	756	780	10.7	9.8
120s	402	341	633	543	598	607	744	787	13.3	12.6
160s	395	337	625	538	600	599	749	784	15.9	13.4
80	392	341	624	549	617	623	761	793	10.0	10.2
SM80	385	354	633	561	649	623	778	789	8.8	8.3
DM80	384	358	622	570	639	646	776	799	7.8	7.5
Mean	391	349	628	554	627	620	766	790	10.3	9.6
<i>P values</i>										
Species	<0.001		<0.001		0.38		0.002		0.002	
N treatments	0.72		0.024		<0.001		<0.001		<0.001	
N linear ^c	0.24		0.001		<0.001		<0.001		<0.001	
N quadratic ^c	0.74		0.49		0.002		0.033		0.041	
80 vs. SM80 and DM80	0.39		0.061		0.002		0.064		<0.001	
DM80 vs. SM80	0.78		0.88		0.35		0.43		0.050	
80 vs 80s	0.27		0.86		0.025		0.062		0.77	
Species × N	<0.001		0.002		0.043		0.004		0.26	

^a0, 40, 80s, 120s, and 160s: mineral N fertilizer was split with 40 kg N ha⁻¹ at seeding and the balance side-dressed at the four-leaf stage; SM80: liquid swine manure at 80 kg total N ha⁻¹ applied at seeding; DM80: liquid dairy manure at 80 kg total N ha⁻¹ applied at seeding; 80: mineral N fertilizer (80 kg N ha⁻¹) applied at seeding.

^bSome data of N concentration were presented in Thivierge et al. (2016b) but not for each species.

^cLinear and quadratic components for the mineral N fertilizer (0, 40, 80s, 120s, and 160s).

Fig.1. Diagram of the first two principal components of a principal component analysis to illustrate the relationship among forage attributes (ADF, acid detergent fiber concentration; DM yield; NDF, neutral detergent fiber concentration; IVTD, in vitro true digestibility of dry matter; N concentration; NDFd, in vitro digestibility of NDF) averaged across two sites and two years for two species (sweet pearl millet and sweet sorghum) and eight N treatments [0, 40, 80s, 120s, and 160s: mineral N fertilizer split with 40 kg N ha⁻¹ at seeding and the balance side-dressed at the four-leaf stage; SM80: liquid swine manure at 80 kg total N ha⁻¹ applied at seeding; DM80: liquid dairy manure at 80 kg total N ha⁻¹ applied at seeding; 80: mineral N fertilizer (80 kg N ha⁻¹) applied at seeding]. The proportion of total variation explained by each component is indicated with the title axes.