Production of napiergrass as a forage and bioenergy feedstock with swine-lagoon effluent

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Abstract
Studies are lacking on the performance of napiergrass (Pennisetum purpureum Schum.) fertilized with swine (Sus scrofa domestica)-lagoon effluent. This study (2011–2013) determined biomass yield, nutrient removal, nutritive value, and ethanol yield in cultivar ‘Merkeron’ at a single, late-season harvest. Effluent irrigations provided 727 kg ha⁻¹ nitrogen (N) annually (3-yr average). Napiergrass removed 92% of N and 73% of phosphorus (P) applied in 2013, the peak year of production (58.9 Mg ha⁻¹). As compared to stems, leaves had greater (p < .01) crude protein (32 vs. 100 g kg⁻¹) and less acid detergent fiber (482 vs. 340 g kg⁻¹). Ethanol yield was approximately 36% lower in stems than leaves (98 vs. 153 g kg⁻¹), and xylose yield was 7% lower (170 vs 183 g kg⁻¹); however, stems account for a larger amount of lignocellulosic biomass for estimating bioethanol production than leaves. Ethanol yield potential was approximately 109 g kg⁻¹ grass biomass.

1 INTRODUCTION

Napiergrass (Pennisetum purpureum Schum.) is a dual-purpose, warm-season perennial forage and bioenergy crop candidate for the lower southeastern United States (Anderson et al., 2008; Chiluwal et al., 2019). Annual biomass yields over 25 Mg ha⁻¹ reported in Georgia (Dien et al., 2020; Knoll et al., 2013) indicate a high capacity for nutrient removal. This can benefit swine farms that apply lagoon effluent to summer forage grasses grown for hay (Adeli & Varco, 2001). Irrigation rates are determined by crop nutrient requirements coupled with soil nitrogen (N) and phosphorus (P) levels (Sistani et al., 2008). A revised nutrient management standard implemented in 2013 gives emphasis to soil P (McLaughlin et al., 2012). Because forage P concentration tends to fluctuate little relative to other nutrients, annual P removal is associated closely with biomass yield (Singh et al., 2015; Read et al., 2018).

Napiergrass has value as quality fodder for ruminant animals, particularly when harvested more than once per season (Woodard & Prine, 1991; Ishii et al., 2005; Chiluwal et al., 2019). Crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) are key components that determine forage nutritive value (i.e., nutrient supply and digestibility). Acid detergent fiber, or lignified cell wall concentration, potentially limits forage digestibility, and NDF, or total cell wall concentration, potentially limits voluntary intake by ruminants (Jung & Allen, 1995). In a comparison...
of digestibility in mature ‘Merkeron’ napiergrass and 12-wk-old ‘Tifton 85’ bermudagrass [Cynodon dactylon (L.) Pers.] grown in field nurseries and harvested in November, leaf ADF did not differ significantly, whereas napiergrass had greater stem ADF concentration (481 vs. 372 g kg⁻¹) (Anderson et al., 2008). In that study, values for leaf and stem NDF were lower in napiergrass.

Napiergrass generally meets the requirement of lignocellulose for biofuel production because of a low lignin content, particularly in leaves, and capacity for high biomass (Anderson, et al., 2008; Rengsirikul et al., 2011). We envision profitable production is supported through fertilization with swine effluent, as optimal growth requires relatively large inputs of major plant nutrients (Singh et al., 2015) and the per unit value of the raw lignocellulosic biomass is expected to be quite low. In studies on napiergrass conversion to ethanol using simultaneous saccharification and fermentation (SSF), Yasuda et al. (2013) reported ethanol production should be performed through the SSF process without the alkali pretreatment, which may simplify the conversion process. Ethanol yields of 268–300 L Mg⁻¹ biomass were reported for a single, late-season cutting of napiergrass forage in Georgia (Dien et al., 2020).

The objectives of this study were to determine napiergrass biomass yield, nutrient removal, and leaf and stem constituents that are indicative of forage nutritive value and ethanol yield from a single harvest of mature (full-season) plants in November after application of swine effluent. Results could provide a foundation for further study on harvest management for optimal production of high-quality forage and/or a feedstock for cellulosic bioethanol.

2 | MATERIALS AND METHODS

Experimental plots (each 4.88 by 6.08 m) were located in a swine-effluent spray field on a Brooksville silty clay loam (fine, smectitic, thermic Aquic Hapluderts) at a private farm near Crawford, MS (33°18′ N, 88°33′ W). The field received anaerobic lagoon effluent and produced summer hay from a mixed grass stand dominated by common bermudagrass and johnsongrass [Sorghum halepense (L.) Pers.]. Manure and urine were washed from pits beneath a slatted barn floor into open lagoons. Manure solids had been allowed to settle to the bottom and effluent was applied by a center-pivot irrigation system. All nutrients were provided in effluent irrigations (May–October), with amounts and timing governed by the farm manager, typically 0.3–0.6 cm ha⁻¹ per application, one to three times per week (Supplemental Table S1; Table 1). Prior to planting Merkeron napiergrass (Burton, 1989), vegetation was cleared from the experimental area using a lawn mower and glyphosate herbicide [N-(phosphonomethyl)glycine]. In April 2011, nine rooted cuttings were transplanted 1.22 m apart in rows 1.52 m apart in each of two experimental plots that consisted of three rows and were separated by a 2-m alley. Transplants were watered as needed until effluent irrigations commenced. Transplants grew vigorously and had approximately 28 and 40 tillers plant⁻¹ (n = 4) in mid-June and mid-July 2011, respectively.

In November of each year, four plants in each plot (n = 8) were cut by hand at 20-cm stubble height and fresh weights recorded. From each plant, individual stalks (n = 3, 2, and 4 in 2011, 2012, and 2013, respectively) were divided into leaves and stems (inclusive of the leaf sheath), weighed fresh,

| TABLE 1 | Biomass yield, leaf to stem ratio, tiller height, tiller number, and nutrient removal in biomass (mean ± standard deviation) for ‘Merkeron’ napiergrass plants harvested in late November and the rate of nutrients applied in swine-lagoon effluent in 2011, 2012, and 2013

| Parameter       | 2011          | 2012          | 2013          |
|-----------------|---------------|---------------|---------------|
| Dry biomass b, M g ha⁻¹ | 41.3 ± 7.8 | 57.2 ± 20.6 | 58.6 ± 19.9 |
| Leaf/stem biomass ratio | 0.36 ± 0.03 | 0.45 ± 0.11 | 0.18 ± 0.03 |
| Tiller height b, m | 2.45 ± 0.09 | 3.20 ± 0.09 | 2.94 ± 0.11 |
| Tillers, number plant⁻¹ | 67 ± 13 | 123 ± 44 | 120 ± 45 |
| N removal b, kg ha⁻¹ | 505 ± 126 | 1034 ± 551 | 815 ± 315 |
| P removal b, kg ha⁻¹ | 88 ± 19 | 191 ± 106 | 127 ± 48 |
| K removal b, kg ha⁻¹ | 1,111 ± 254 | 1,975 ± 993 | 1,644 ± 534 |
| N applied, kg ha⁻¹ | 750 | 548 | 883 |
| P applied, kg ha⁻¹ | 132 | 113 | 175 |
| K applied, kg ha⁻¹ | 877 | 699 | 1096 |

Note. Three manual gauges recorded effluent irrigations from May to October and these data, corrected for any attendant, localized rainfall (Supplemental Table S1), were used to derive N, P, and K application rates using regression coefficients for the relationship between total nutrient concentration in lagoon water and Julian day of the year (McLaughlin et al., 2012).

aDry biomass (kg plant⁻¹) was multiplied by 5,379.236 to convert to kg ha⁻¹. Values for individual plant biomass ranged from 5.74 to 10.40 kg in 2011, from 5.39 to 15.60 kg in 2012, and from 6.79 to 17.20 kg in 2013. Because the standard deviation for biomass yield was numerically greater in 2012 than 2011 and 2013 (34.9 vs. 7.8 and 19.9 Mg ha⁻¹, respectively), values for biomass yield and nutrient removal in 2012 are based on five “good” plants that had relatively low dispersion about the mean; otherwise, values are based on four plants per plot (n = 8).

bTiller height is based on 24, 16, and 32 observations in 2011, 2012, and 2013, respectively.

Core Ideas

- Napiergrass is a highly productive forage and bioenergy crop with swine-effluent fertilization.
- With full-season growth, plants utilized 80% of N and 70% of P applied in lagoon effluent.
- With full-season growth, forage nutritive value is greater in leaves than stems.
- Stems have low ethanol yield but account for more cellulosic biomass than leaves.
dried to completion at 60 °C, and weighed again to determine dry matter (DM) content and leaf-to-stem ratio. Leaf and stem samples were ground to pass a 1-mm screen in a Wiley mill (Thomas Scientific). Total N was determined by dry combustion (Elementar Americas, Inc.) according to Bremner (1996). Total P and potassium (K) were determined using an inductively coupled argon plasma optical emission spectrometer (Thermo Jarrell Ash) according to Read et al. (2018). Nutrient removal (kg ha⁻¹) was calculated as the product of plant DM and weighted-average nutrient concentration in leaves and stems. Additionally, ash, total N, ADF, NDF, xylose, and potential ethanol concentrations were estimated using near-infrared reflectance spectroscopy (NIRS) with ISIS-can software (FOSS NIRSSystems, Laurel, MD) according to Westerhaus et al. (2004). The NIRS equation is based on napiergrass samples analyzed for ADF and NDF by Ankom filter bag method (Ankom Technology Corp.) and xylose and ethanol by SSF techniques using Saccharomyces cerevisiae D5A and subsequent quantification of released xylose and ethanol fermentation using high-performance liquid chromatography (Vogel et al., 2010; Anderson et al., 2019). A. cerevisiae strain unable to ferment xylose was used so that released xylose and cellulose conversion to ethanol could be measured separately. The Global H test ranged from 2.4 to 10.7, with 10 of 36 samples (28%) fitting the equation (H < 3.0) (Murray & Cowe, 2004).

Means and standard deviations were computed by year for agronomic production parameters. A paired t test was used to detect difference between leaf and stem tissues for forage nutritive value and bioenergy parameters using PROC GLIMMIX procedures in SAS (SAS Institute, 2013). Tissue type was considered a fixed effect; plant, year, and their interaction were considered random effects. The tissue × year interaction was an important source of variability but did not affect any variable (p > .36).

3 | RESULTS AND DISCUSSION

Biomass production increased 38% between 2011 and 2012 in association with increases in tillers per plant (84%) and plant height (31%) (Table 1). Full-season biomass yield for Merkeron napiergrass of 52.4 Mg ha⁻¹ (3-yr average) exceeds the mean production level of 26.8 Mg ha⁻¹ observed in northern Florida (Woodard & Prine, 1991) and supports its use in northeast Mississippi as a lignocellulosic biomass crop.

Estimates for N, P, and K removal in 2011 were 505, 88, 1,111 kg ha⁻¹ and increased considerably in 2012 to 1,034, 191, and 1975 kg ha⁻¹, respectively, despite reduced N–P–K fertilization (Table 1). The apparent high K requirement for growth would be supported from regular swine-effluent applications. In a study from Georgia, Merkeron napiergrass was provided N–P–K rates of 100, 40, and 90 kg ha⁻¹ yr⁻¹, respectively, and removed approximately 225, 26, and 535 kg ha⁻¹ in biomass in the peak year of production (30.3 Mg ha⁻¹) (Knoll et al., 2013). In 2011 and 2013, napiergrass removed approximately 80, 70, and 138% of the N, P, and K applied in effluent, whereas recovery of N and P in 2012 was 189, 169, and 282%, respectively. Because land application rates are dependent on plant nutrient utilization, the increased recovery of N and P in 2012 means reduced potential for surface and groundwater impairment. In 2012, the carry-over (residual) N and P from the previous year accounts for a maximum of 23% to that removed in biomass.

Leaves harvested from mature plants in November are a forage product that would meet nutritional standards as animal fodder, but corresponding stems had lower forage nutritive value (p < .01), as reflected by lower concentrations of CP and greater ADF and NDF (Table 2). Averaged across years, stems had approximately 142 g kg⁻¹ more ADF than leaves and approximately 61 g kg⁻¹ more NDF. Lower amounts of ADF in leaves than stems suggest greater potential digestibility of leafy (less mature) forage (Woodard & Prine, 1991; Isha et al., 2005). For example, Chiluwal et al. (2019) reported greater CP and less ADF and NDF in 42-d-old napiergrass regrowth forage after an October cutting than a single cutting in November. A proxy for leafiness is leaf/stem biomass ratio, which was rather low in the mature plants (Table 1). Data on seasonal harvests of a group of tillers indicated that the reduced leaf/stem ratio between October and November was chiefly a function of increased stem biomass rather than leaf loss through senescence, particularly in 2013. Averaged across 2012 and 2013 (n = 8), plants had a leaf/stem biomass ratio of 0.85 ± 0.19 in mid-June, 0.41 ± 0.04 in mid-August, and 0.37 ± 0.05 in late-October (data not presented). A forage CP level of 105 g kg⁻¹ is considered moderate in meeting the CP requirements for different classes of beef cattle (NRC, 1996). In the present study, leaf CP in 2011, 2012, and 2013 was approximately 86, 97, and 117 g kg⁻¹, respectively, whereas stem CP averaged 32 g kg⁻¹ across years (Table 2). Across 30 napiergrass genotypes harvested in autumn, leaf and stem CP averaged 59 and 18 g kg⁻¹, respectively; Merkeron had the greatest leaf CP (82 g kg⁻¹) and ranked 10th among genotypes for stem CP (20 g kg⁻¹) (Anderson et al., 2019).

Increased N fertilization in 2013 was associated with increased leaf ash and CP and decreased leaf ADF and NDF (Tables 1 and 2). This supports studies on warm-season grasses of increased forage nutritive value with N fertilization (Monson & Burton, 1982; Johnson et al., 2001; Read et al., 2018). As compared to 2011 and 2012, leaves in 2013 had approximately 22 g kg⁻¹ less ADF and 28 g kg⁻¹ less NDF. In contrast, stem ADF and NDF had much smaller interannual difference, probably because napiergrass stems are primarily lignocellulose with virtually no nonstructural carbohydrate (Duke, 1983).
TABLE 2  Ash, crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), xylose, and potential ethanol concentrations in leaf and stem (inclusive of leaf sheath) tissues collected from a group of tillers cut from 'Merkeron' napiergrass plants (n = 6) in late November

| Parameter and tissue | 2011     | 2012     | 2013     | Mean  |
|----------------------|----------|----------|----------|-------|
| Ash                  | 97 ± 5   | 114 ± 10 | 116 ± 5  | 109   |
| CP                   | 86 ± 5   | 97 ± 8   | 117 ± 9  | 100   |
| ADF                  | 113 ± 4  | 136 ± 44 | 92 ± 8   | 114   |
| NDF                  | 351 ± 12 | 355 ± 18 | 315 ± 13 | 340   |
| Ethanol              | 193 ± 2  | 183 ± 7  | 173 ± 4  | 183   |
| t value              | -5.48**  | -1.04 ns | 4.94**   | -0.16 ns |
| t value              | 22.93**  | 12.77**  | 21.42**  | 7.32** |
| t value              | -12.22** | -15.09** | -16.08** | -3.76** |
| t value              | -8.08**  | -15.09** | -10.49** | -4.62** |
| t value              | 6.61**   | 3.31**   | 3.64**   | 1.67 ns |
| t value              | 155 ± 2  | 154 ± 4  | 151 ± 2  | 153   |
| t value              | 103 ± 6  | 98 ± 20  | 94 ± 11  | 98    |
| t value              | 16.58**  | 6.15**   | 12.06**  | 3.84** |

Note. Values are based on a near-infrared reflectance spectroscopy (NIRS) equation developed from napiergrass leaf and stem samples. The standard error of cross validation (g kg⁻¹ dry matter) was 5.679 for ash (n = 59), 0.520 for N (n = 64), 8.759 for ADF (n = 60), 8.656 for NDF (n = 59), 8.677 for xylose (n = 172), and 10.939 for ethanol (n = 167).

Paired t test significant at the 5% probability level.
**Paired t test significant at the 1% probability level.
*ns, not significant (ns).

Each year, xylose and ethanol yields were greater (p < .05) in leaves than stems (Table 2). Averaged across years, leaves yielded 8% more xylose and 56% more ethanol per gram of DM than for stems. Similarly, Anderson et al. (2019) reported 5% greater xylose and 59% greater ethanol yields for leaves than stems from 30 napiergrass genotypes. Xylan is a major plant structural polymer, second only to cellulose in natural abundance; xylan is typically 20–30% of the total cell wall in grasses (Hatfield et al., 2017; Kongkeitkajorn et al., 2020). While in this study cellulose conversion to ethanol and xylose saccharification were measured separately, there are presently engineered yeast strains suitable for fermenting both sugars into ethanol (Wang et al., 2019). A study on different chemical pretreatments and fermentation processes concluded ethanol amounts for 90- to 150-d-old napiergrass forage were in the range of 171–187 g kg⁻¹ DM (Kongkeitkajorn et al., 2020). In the present study that sampled tillers in mature plants after full-season growth, potential ethanol had a range of 151–155 g kg⁻¹ in leaves and 94–103 g kg⁻¹ in stems, depending on year (Table 2). Assuming leaves and stems sampled from individual tillers represent production-scale harvest of napiergrass, ethanol amounts were in the range of 102–116 g kg⁻¹ total DM, which corresponds to potential ethanol yields of 130–147 L Mg⁻¹ biomass.

In conclusion, napiergrass is a highly productive crop with swine-effluent fertilization. With variable rates and timings of effluent, the plants utilized more N and P than applied in 2012 and approximately 80% of N and 70% of P applied in 2011 and 2013. A single harvest in November indicated greater forage nutritive value and ethanol yield in leaves than stems; however, stems account for a larger amount of lignocellulosic biomass for bioethanol production.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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