Inhibiting Dhurrin Biosynthesis Effects on Stocker Cattle Daily Gains

Gruss, S. M.*; Johnson, K. D.*; Lemenager, R. P. †; Tuinstra, M. R.*
*Department of Agronomy and †Department of Animal Sciences, Purdue University

Keywords: Dhurrin, sorghum, forage quality

Abstract. Climate change has made resilient crops more valuable in many agricultural production systems. Sorghum is an important resilient grain and forage crop due to its high drought tolerance and ability to thrive in low-N environments. All current commercial sorghum varieties produce the cyanogenic glucoside dhurrin. Cell maceration causes the conversion of dhurrin to hydrogen cyanide (HCN), which is toxic to animals. Toxicity symptoms range from labored breathing and convulsions to death within minutes. The dhurrin biosynthesis pathway was altered to inhibit dhurrin production by a mutation that inactivated CYP79A1, the first enzyme in the pathway. The dhurrin-free phenotype eliminates the risk of hydrogen cyanide (HCN) poisoning in animals; however, agronomic performance and livestock responses when the dhurrin-free is grazed has not been studied. This study focused on the impacts of the *cyp79a1* mutation on sorghum forage production and utilization. Over two months, weight gains were compared for stocker calves grazing either a dhurrin-free hybrid and a conventional hybrid (S&W SP4105). Presence or absence of HCN, nutritional quality, and yields were also determined before and throughout the grazing trial. The dhurrin-free hybrid and SP4105 had equivalent biomass yields (kg ha^{-1}) throughout the trial. The dhurrin-free hybrid's neutral detergent fiber and acid detergent fiber were 35 and 20 mg g^{-1} lower, respectively, when compared to SP4105 on the first sampling date (*P-value<0.1)*. Rumen degradable protein and total digestible nutrients were higher as well for the dhurrin-free, but the two hybrids were similar by the second sampling date for nutritional quality. Average daily gain was similar between the dhurrin-free hybrid and the conventional sorghum hybrid. Taken together, dhurrin-free sorghum has many beneficial aspects as a forage; no fear of HCN toxicity and competitive weight gains of stocker calves, with excellent biomass yields and forage quality.

Introduction

Sorghum bicolor ((L.) Moench) thrives in drought-prone and high temperature environments (Smith and Frederiksen 2000), making it a valuable grain and forage crop in a changing climate. Unfortunately, there is a concern about HCN toxicity. Dhurrin is a cyanogenic glucoside in sorghum and is the precursor to HCN release. A mutation in the *CYP79A1* enzyme inhibits dhurrin production, thereby creating dhurrin-free sorghum lines (Tuinstra et al. 2016). Lines carrying the *cyp79a1* mutation do not release HCN (Gruss et al. 2023) and have demonstrated favorable palatability when grazed by ewes (Gruss 2021).
This study compared stocker calves' average daily gains (kg day^{-1}) while grazing an experimental dhurrin-free sorghum x sudan hybrid compared to a conventional sorghum x sudan hybrid, SP4105 (S&W Seed Company). The hybrids were compared for HCN release, nutritional quality, and yield. We hypothesized that the dhurrin-free hybrid would perform as well as the commercial SP4105 hybrid in stocker calf weight gain, yield, and nutritional quality without the risk of HCN release.

Methods and Study Site

Experimental Layout

The performance trial was conducted at the Purdue University Scholer Beef Farm in Warren County, IN during the summer of 2021. The experimental design was a randomized complete block design with three replications. The treatments were the dhurrin-free sorghum x sudangrass hybrid *bmr6 cyp79a1* and the commercial sorghum x sudangrass hybrid SP4105 *bmr6* headless (photoperiod sensitive) (S&W Seed, Denver, Colorado). Planting occurred on June 17, 2021 at a rate of 664,800 seed per ha^{-1} for the dhurrin-free hybrid and 658,400 seeds ha^{-1} for the SP4105 hybrid. Application of 90 kg ha^{-1} N was applied on June 28, 2021.

Each plot (sorghum hybrid within a replication) was split into four equal-sized cells to allow a rotational grazing system. Four stocker calves (red Angus x Simmental) with an average weight of 282.4 kg were allocated to each plot (Fig. 1). Calf allocation was determined by weight, sex, age, and sire to minimize variation of the calves across plots. The same four stocker calves remained on the same plot for 62 days. All the calves were rotated to another cell within plot when there was minimal feedstock available for grazing. Initial stocking rate for each plot was 2.5 animal units for 0.77 ha.

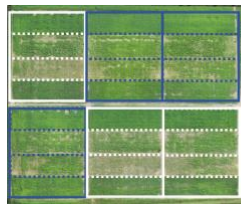

Figure 1: The layout of the trial. Areas with a white border represent the dhurrin-free hybrid treatments and areas with a blue border represent SP4105 treatments. Solid lines represent the whole plots, while the dotted lines are the cells within the plots.

HCN Release, Biomass, and Nutritional Quality Collection

Before initiating the trial, sorghum plants within each plot were sampled for HCN release using a Feigl Anger (FA) Assay (Feigl and Anger 1966). Leaf blade tissue samples were approximately 2.5 cm long and were harvested from the youngest emerged leaf of six plants within a plot. The FA assay was conducted as described by Gruss et al. (2022).

Biomass data were collected on the two hybrids before rotating calves to a new cell. Biomass collection consisted of four subsamples 1 meter in length and 10 cm above the soil surface from an interior row. Samples were placed in brown paper bags, dried at 60 °C for one week, and then weighed to determine dry weight.

Nutritional quality was analyzed from biomass samples from two dates. The two collection dates were before flowering of the dhurrin-free hybrid to reduce variation in maturity between the two hybrids. On the first sampling date, both hybrids were in the vegetative growth stage. On the second date, both hybrids were in the vegetative growth stage, but the dhurrin-free hybrid was entering the boot stage (R0). Dried subsamples from the biomass collection were ground with an Udy Cyclone mill to 1 mm. The four subsamples from each plot were combined to have a single plot sample at each sampling time. The samples were submitted to Cumberland Valley Analytic Services (Waynesboro, PA) for wet chemistry analysis. Forage analyses included crude protein (CP), soluble protein (SP), ruminant digestible protein (RDP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN).

Daily Average Gains

Stocker calves were individually weighed the day the experiment began and on days 34 and 62. The first and last calf weight measurements were the average of two consecutive days. Full weights were taken. Average daily gain (ADG) was determined from day 0 to 62.

Results and Discussion

HCN Release

HCN release was tested across the plots prior to the entry of calves on the study. No HCN release was detected in the dhurrin-free hybrid, while the SP4105 had multiple plants releasing HCN in each of the three plots (fig. 2).

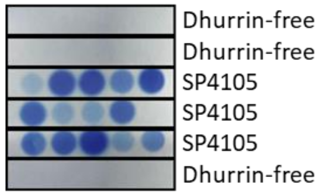

Figure 2: Blue coloring indicates HCN release, while cells that remain white indicate there was no HCN release.

Biomass Comparison

Biomass data collected throughout the trial was similar between the two hybrids (*P-value* > 0.1). The two hybrids are genetically different, but this demonstrates the sorghum x sudangrass hybrid containing the *cyp79a1* mutation can produce as much biomass as an available commercial hybrid (Fig. 2).

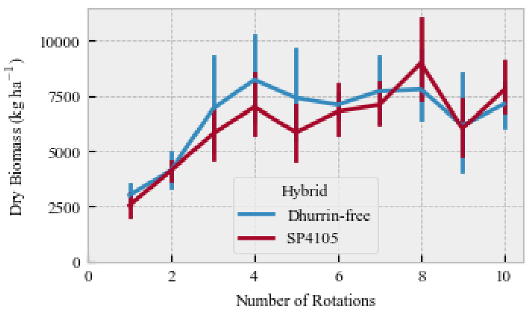

Figure 3: Biomass (kg ha^{-1}) comparison with a 95 percent confidence interval between the hybrids for each cattle rotation in the trial (p-value < 0.1).

Nutritional Quality

Nutritional quality can be used to predict potential livestock productivity (Coleman and Moore 2003). These hybrids had different growth habits, photoperiod insensitive and photoperiod sensitive. Nutritional quality is primarily affected by the growth stage, with a decline in quality as the plant matures. Quality was examined at two time points before flowering to compare the samples at similar growth stages. At the first sampling date, both hybrids were in vegetative growth with strictly leaf material. The second sampling date occurred when the dhurrin-free hybrid entered the boot stage, and the SP4105 remained in vegetative growth with no flag leaf present. When examining the quality, there is some variation among the hybrids. On the first sample date (July 26, 2021), the dhurrin-free hybrid had higher TDN and RDP with lower NDF and ADF ((*P-value* < 0.1; Table 1). By the second sampling date, most of the variation in quality had diminished, except NDF remained 37 mg g^{-1} lower in the dhurrin-free hybrid as compared to SP4105 (Table 1). Over our two sampling dates the dhurrin-free hybrid had slightly better nutritional quality.

Table 1: Nutritional quality of the dhurrin-free and SP4105 sorghum x sudangrass hybrids at two sampling dates testing for differences in crude protein (CP), soluble protein (SP), ruminal digestible protein (RDP), acid detergent fiber (SDF, neutral detergent fiber (NDF), and total digestible nutrients (TDN) (mg g^{-1}, *P-value*<0.1)

	Hybrids	**CP**	**SP**	**RDP**	**ADF**	**NDF**	**TDN**
		mg g^{-1}	**mg g^{-1}**	**mg g^{-1}**	**mg g^{-1}**	**mg g^{-1}**	**mg g^{-1}**
7/26/2021	**Dhurrin-free**	116	33	75 a	357 a	601 a	617 a
	SP4105	94	20	57 b	376 b	636 b	595 b
8/9/2021	**Dhurrin-free**	110	33	71	376	614 a	597

SP4105	106	31	69	367	651 b	604

Average Daily Gain

Average daily gain of the stocker calves was similar between the two hybrids throughout the trial (*P-value* < 0.1; Table 2). Calf weights from 0-34 days and 34-62 days were similar between the two hybrids with only a 2 kg difference in average total gain between the hybrid treatments. These weight gains were expected after examining the similarity in nutritional quality between the hybrids. Any nutritional quality advantage the dhurrin-free hybrid had was expected to be diminished as the dhurrin-free hybrid matured into reproductive growth. The dhurrin-free hybrid began to flower approximately 34 days into the experiment.

Table 2: Cattle weights (kg) for each weigh day and the overall average daily gain (kg day^{-1}) for the dhurrin-free and SP4105 sorghum x sudangrass hybrids.

Hybrids	**Average Calf Weight (kg)**			**Average Daily Gains (kg day^{-1})**
	Day 0	Day 34	Day 62	
S&W SP4105	284	309	343	0.94
Dhurrin-free	280	306	337	0.92

Conclusions

This study demonstrates the value of dhurrin-free sorghum as a forage crop. Inhibiting dhurrin production results in no HCN release. Dhurrin-free sorghum provided stocker cattle weight gains equivalent to a commercially available hybrid. Along with consistent average daily gain, the dhurrin-free hybrid also sustained similar biomass production and had similar or better nutritional quality. Overall, the dhurrin-free hybrid is a significant development in sorghum forage production by eliminating the fear of HCN release with no decrease in quality or stocker-calf performance.

References

Blomstedt, Cecilia K., Roslyn M. Gleadow, Natalie O'Donnell, Peter Naur, Kenneth Jensen, Tomas Laursen, Carl Erik Olsen, Peter Stuart, John D. Hamill, Birger Lindberg Møller, and Alan D. Neale. 2012. "A Combined Biochemical Screen and TILLING Approach Identifies Mutations in Sorghum Bicolor L. Moench Resulting in Acyanogenic Forage Production: Acyanogenic Forage Sorghum Plants." *Plant Biotechnology Journal* 10(1):54–66. doi: 10.1111/j.1467-7652.2011.00646.x.

Coleman, Sam W., and John E. Moore. 2003. "Feed Quality and Animal Performance." *Field Crops Research* 84. doi: doi:10.1016/S0378-4290(03)00138-2.

Feigl, F., and V. Anger. 1966. "Replacement of Benzidine by Copper Ethylacetoacetate and Tetra Base as Spot-Test Reagent for Hydrogen Cyanide and Cyanogen." *The Analyst* 91(1081):282. doi: 10.1039/an9669100282.

Gruss, Shelby M. 2021. "THE EFFECTS OF INHIBITING DHURRIN BIOSYNTHESIS IN SORGHUM." thesis, Purdue University Graduate School.

Gruss, Shelby M., Keith D. Johnson, Manoj Ghaste, Joshua R. Widhalm, Sandy K. Johnson, Johnathon D. Holman, Augustine Obour, Robert M. Aiken, and Mitchell R. Tuinstra. 2023. "Dhurrin Stability and Hydrogen Cyanide Release in Dried Sorghum Samples." *Field Crops Research* 291:108764. doi: 10.1016/j.fcr.2022.108764.

Smith, C., and Richard Frederiksen. 2000. *Sorghum: Origin, History, Technology, and Production*. 1st ed. Wiley.

Sohail, Muhammad N., Cecilia K. Blomstedt, and Roslyn M. Gleadow. 2020. "Allocation of Resources to Cyanogenic Glucosides Does Not Incur a Growth Sacrifice in Sorghum Bicolor (L.) Moench." *Plants* 9(12):1791. doi: 10.3390/plants9121791.

Tuinstra, Mitchell R., Kartikeya Krothapalli, Brian Dilkes, and Elizabeth Buescher. 2016. "Genetic Mutations That Disrupt Dhurrin Production in Sorghum."