Agrotechnology
ISSN: 2168-9881

Rivera et al., Agrotechnol 2015, 5:1
DOI: 10.4172/2168-9881.1000136

Case Report

Open Access

Effects of Polymicrobial Bioinoculant on Yield, Quality and *In Situ* Digestibility of Sorghum Sudangrass in South Mississippi

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Abstract

Sorghum-Sudangrass was used in randomized complete block to test the effects of a bioinculant (BI) and nitrogen (N) application on dry matter (DM) yield determination and forage quality parameters (CP, TDN, ADF, NDF Ca, and P) and 72 h *in situ* digestibility at two harvest periods (H1 and H2). No effects of BI or N (P>0.05) were noted in any of the quality, yield or digestibility variables. An effect of H was noted in all quality, DM yield and digestibility data (P<0.01). Samples obtained at H1 were higher in quality (P<0.01), yielded less DM (P<0.01) and had greater *in situ* digestibility (P<0.01) A harvest x N interaction (P<0.05) existed for DM yield and digestibility. Moreover, a harvest x BI interaction existed (P<0.05), for digestibility.

Keywords: Bioincoulant; Nitrogen fertilizer; Sorghum-Sudangrass; Digestibility; Forage quality

Introduction

With rising input costs, beef cattle producers are forced to make decisions based upon the return on investment. Chemical fertilizer application is a common used method by beef cattle producers in the southeastern United States to improve pasture yield and quality [1]. Application of 80 kg N/ha to smooth bromegrass pastures resulted in an increase of crude protein by two percentage points, and resulted in greater bodyweight gain per ha [2]. However, Mosier [3] noted that application of commercial fertilizer is often in excess of plant uptake, with the plant only fully utilizing 17-50%. With recent changes regarding commodity and fertilizer prices, the practice of fertilizer application falls under closer scrutiny. One potential method to help increase the utilization of N fertilizer may be the use of bioinocoulants. Bioincoulants are microbes that will work in conjunction with plants to increase nutrient uptake and in theory, increase yield or reduce fertilizer input. In a review, Kennedy [4] determined that certain bioincoulants could be used to crop production systems to reduce N use and enhance farm output. Hein [5] examined the use of a bioincoulant on production of rice in Vietnam, and determined that the addition of bioinoculant resulted in increases in yield of grain. In addition to the increased yield, Cong and Dung [6] demonstrated that the use of bioincoulants maintained yield with decreased fertilizer. Most of the data that exist, involve vegetables, and grains, however, it is unclear to what extent this product might enhance pasture productivity, since productivity (yield and quality) and affect animal performance [7,8]. Thus, our objective was to evaluate the effectiveness of this novel bioinoculant on DM yield, forage quality characteristics and digestibility of sorghum sudangrass.

Materials and Methods

Site establishment

The study was conducted on 6 acres at the White Sand Branch Unit, 6 km west of Poplarville, MS (30.80° N, 89.70° W, elevation 69 m). Soil is a Smithdale sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Hapludults). Winter (Lolium multiflorum) was physically grazed down by the cow-herd at White Sand Branch Experiment Station in early May 2012. On May 21, 2012, the soil was mechanically broken up with a disk and prepared for planting. The experimental design was a randomized complete block in a 3 × 2 factorial arrangement and replicated three times. Treatments were three levels of nitrogen fertilizer (0, 34 and 67 kg N/ha, using (34-0-0), 0N, 34N and 67N, respectively) with or without the inclusion of bioinoculant (Sumagrow, BI). Sorghum-Sudan hybrid (*Sorghum* X *drummondii;* SugarGrazer II variety) was planted using a modified strip-till system (Plant-O-Vator, Tarver Equip. Folsom, LA) at the rate of 34 kg/ha on May 23 and 24, 2012. Additionally, at the time of planting, fertilizer treatment (Ammonium Sulfate, 21-0-0-24S) was applied with the seed as well, via a fertilizer box attachment on the drill. Seed and fertilizer treatments were applied in rows, there were nine rows approximately 23-m wide, with a 9 m buffer between each row. In this treatment layout, a block consisted of three rows. With each row receiving a different rate of N application (0, 34 and 67 N/ha). Each row was approximately 109 m in length. Within each row, each individual treatment cell was approximately 18 m × 23 m. Within each row each treatment cell was randomly assigned to either receive BI or not receive BI (Control). Based upon back calculation of seed used, fertilizer used, and area covered it was determined that actual planting rate was about 36 kg/ha and actual N application was 0, 37, and 65 kg N/ha.

Bioincoulant was applied according to manufacturer's instructions. The BI used in the study consisted of 3% humic acid, 1% microbes, and 96% inert ingredients. The microbial make up was *Azorhizobium caulinodans* – 1 × 109 CFU/ml, *Bacillus subtilis* – 1 × 109 CFU/ml, *Pseudomonas fluorescens* – 1 × 109 CFU/ml, *Rhizobium meliloti* – 2 × 109 CFU/ml, *Rhizobium phaseoli* – 1 × 109 CFU/ml, and *Trichoderma virens* – 1 × 109 CFU/ml. The initial application of BI occurred immediately after planting May 24. Upon completion of application on Block 1, it was decided to postpone spaying until the next day due

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Received May 12, 2015; **Accepted** June 19, 2015; **Published** June 27, 2015

Citation: Rivera JD, Lemus RW, Gipson ML, Gipson RG (2015) Effects of Polymicrobial Bioinoculant on Yield, Quality and *In Situ* Digestibility of Sorghum Sudangrass in South Mississippi. Agrotechnol 5: 136. doi:10.4172/2168-9881.1000136

to increasing afternoon winds. On May 25, 2012, the remaining BI was applied (blocks 2 and 3). In all instances BI was applied via a sprayer boom attached to an all-terrain vehicle (ATV) at the rate of 9.4 L/ha

Plants were monitored throughout the month with height measurements taken every 2 wk. When it was determined that 75% of the plants or more were at least 66 cm tall, harvesting occurred (June 28, 2012). Four (9 m^2) random samples were taken from each treatment plot. Forage was clipped to approximately 7.5 to10-cm stubble height using a mechanical hedge trimmer. Total forage within the sample area was collected, weighed and dried at 50°C for 72 h. Total dry matter produced was extrapolated from the harvested samples and forage mass was determined. Following sampling, all forage was mechanically cut and removed from the test plot area. On July 2, 2012, following harvest and biomass removal, BI was applied to the treatment plots. Based upon typical management practices, a second application of N was going to be applied following harvest, however constant rain events following the harvest prohibited the application of N for fear of damaging the plots. On July 26, 2012, the second harvest of plots occurred. All procedures were similar to the first harvest.

All dried samples were ground through a 2-mm screen using a Wiley Mill. Ground samples were saved for subsequent NIR analysis for Crude Protein, Acid Detergent Fiber, Neutral Detergent Fiber,, TDN, Ca, and P using a FOSS 6500 (FOSS North America Inc., Eden Prairie, MN) and the hay equation of the NIRS Forage & Feed Testing Consortium (Madison, WI).

In Situ

The *in situ* procedure was approved by the Mississippi State University Institutional Animal Care and Use Committee. Approximately 1.5 g of ground dry sample from each test plot were weighed and sealed in individually pre-numbered and pre-weighed nylon digestion bags. Samples were done in triplicate and following sealing, bags were dried in a forced air oven for 48 h at 50°C, after which they were weighed. Samples were sorted by block and each block placed in a mesh laundry bag which was placed in the rumen of a cannulated heifer (3 heifers total). Heifers had access to warm season pastures (bahiagrass and bermuda grass). Following 72 h, samples were removed thoroughly hand washed until all residue was removed, samples were then dried for 48 h at 50°C after which they were weighed again. Dry matter disappearance was calculated as DM weigh prior to digestion-DM weight of residue corrected for the weight of the bag. Statistics data were analyzed using PROC MIXED in SAS, with block being a random effect. Harvest period, N fertilization level and BI application and their interactions were considered fixed effects. Additionally, based upon the stark contrast observed in weather patterns during each harvest period, individual rainfall, ambient temperature and soil temperature were analyzed within each harvest period using a linear model. Significance was declared at $P<0.05$.

Results and Discussion

Weather

Weather data are presented in Table 1. No differences ($P=0.87$) were noted for ambient temperature between H1 and H2, with ambient temperature being 32.5°C and 35.6°C for H1 and H2 respectively. However, rainfall differed ($P=0.003$) with H1 receiving 0.08 cm of rain compared to 0.91 cm of rain observed in H2. Moreover, rain in H2 occurred in 17 of the 29 d of the second period. There was a tendency ($P=0.06$) for increased soil temperature in H1 compared to H2; 31.8°C and 30.5°C, respectively, which was probably due to differences in soil moisture.

Forage dry matter yield

An effect of H (P<0.01) was noted for forage dry matter yield (Table 2). Greater yields were noted for the H2 compared to the H1. This is not surprising due to the lack of rainfall (Table 3) noted in June, followed by the increase in rainfall noted in July. Additionally, Ball [1] noted that greater yields in warm-season grasses will later in the growing season. No overall effect of N was observed (P=0.32); however, there was an N x H interaction (P=0.05), with greater response to N fertilizer observed during the first harvest. This difference was not surprising since N was applied at planting and rainfall constrains dater H1 impeded subsequent N application. Beyaert and Roy [9] sorghum-Sudangrass sudangrass responded favorably to N fertilization, with optimal use occurring at 100 kg/ha (over two applications). No effects of BI application ($P=0.46$), BI x N ($P=0.52$) or BI x H ($P=0.26$) interactions were noted on dry matter yield.

Forage quality

A significant difference (P<0.001) was noted for H on all quality parameters (CP, ADF, NDF, TDN, Ca and P), with greater quality associated with H1 compared to H2 (Table 4). The additional rain (Table 5) during the second period may have resulted in a more rapid growth period as demonstrated by the increased noted in DM yield. Greater DM yield has been inversely related to forage quality. Cusicanqui and Lauer [10] determined that increasing the forage DM yield resulted in a decrease in digestibility. Moreover, Hoveland [11] determined that IVDMD and CP decreased in alfalfa decreased thorough the growing season. No differences were noted with regards to N ($P>0.05$), which might be due to the lack of rainfall associated during H1, and the lack of N application in H2. Due to the weather

Item	Harvest 1[a]	Harvest 2[a]	SE[b]	P-value[c]
Ambient temperature (°C)	32.5	32.6	0.49	0.87
Soil temperature (°C)	31.5	30.5	0.41	0.06
Rainfall, cm	0.08	0.91	0.15	0.0003

[a]Harvest 1 growing period was May 25-June 28; Harvest 2 growing period was June 29-July 26.
[b]Pooled standard error of treatment least square means.
[c]Probability that least square means differ.

Table 1: Least square means for weather data during the two harvest periods.

Item	Harvest 1	Harvest 2	SE[a]	P-value[b]
Forage DM yield, kg/ha	2765.6	4680.4	171.1	0.0001
Acid Detergent Fiber, %	33.19	37.71	0.38	0.0001
Neutral Detergent Fiber, %	58.03	63	0.41	0.0001
Crude Protein, %	15.92	13.22	0.25	0.0001
TDN, %	63.04	59.52	0.29	0.0001
Ca, %	0.55	0.4	0.01	0.0001
P, %	0.27	0.25	0.002	0.0001
Mg, %	0.41	0.041	0.008	0.9
K, %	1.57	2.38	0.07	0.0001
In Situ Digestibility (72 h), %[c]	68.42	59.99	1	0.0001

[a]Pooled standard error of treatment least square means.
[b]Probability that least square means differ; NS=not statistically significant (P>0.10).
[c]*In situ* digestibility conducted over a 72 h period.

Table 2: Effects of harvest date on forage DM yield and nutrient content of sorghum-sudangrass hybrid.

Treatment[a]						
	Item	N0	N34	N67	SSE[b]	PP-value[c]
Harvest 1						
	Forage DM yield, lb/ac	1895.0[x]	2886.80[y]	2626.0[y]	264.57	0.05
	Acid Detergent Fiber, %	32.74	33.44	33.4	0.5	NS
	Neutral Detergent Fiber, %	57.38	58.23	28.49	0.32	NS
	Crude Protein, %	16.17	15.9	15.7	0.43	NS
	TDN, %	63.4	62.85	62.88	0.39	NS
	Ca, %	0.56	0.55	0.55	0.02	NS
	P, %	0.27	0.27	0.27	0.003	NS
	Mg, %	0.42	0.4	0.41	0.01	NS
	K, %	1.61	1.57	1.51	0.1	NS
	In Situ digestibility (72 h), %[d]	67.50[x]	66.42[y]	71.34[x]	1.72	0.05
Harvest 2						
	Forage DM yield, lb/ac	4329.5	4041.1	4166.11	216.2	NS
	Acid Detergent Fiber, %	37.91	37.15	38.07	0.44	NS
	Neutral Detergent Fiber, %	62.2	62.46	63.34	0.53	NS
	Crude Protein, %	13.14	13.24	13.28	0.61	NS
	TDN, %	59.37	59.97	59.25	0.35	NS
	Ca, %	0.42	0.39	0.39	0.02	NS
	P, %	0.25	0.25	0.25	0.002	NS
	Mg, %	0.41	0.4	0.041	0.01	NS
	K, %	2.39	2.4	2.36	0.09	NS
	In Situ digestibility (72 h), %[d]	59.7	61.39	58.87	1.72	NS

[a]Treatments were : N0=0 kg N/ha (ammonium nitrate) applied at planting; N34=34 kg N/ha applied at planting; or N67=67 kg N/ha applied at planting, due to weather, no follow up N was applied following first harvest.
[b]Pooled standard error of treatment least square means.
[c]Probability that least square means differ; NS=not statistically significant (P>0.10).
[d]*In situ* digestibility conducted over a 72 h period.
[xy]Means without a common superscript differ (P<0.05).

Table 3: Effects of nitrogen application on forage DM yield and nutrient content of sorghum-sudangrass hybrid by harvest data.

TTreatment[a]					
	Item	BBI	Control	SE[b]	P P-value[c]
Harvest 1					
	Forage DM yield, lb/ac	2393.6	2544.9	216.02	NS
	Acid Detergent Fiber, %	33.01	33.37	0.45	NS
	Neutral Detergent Fiber, %	57.75	58.32	0.53	NS
	Crude Protein, %	16.22	15.63	0.34	NS
	TDN, %	63.18	62.91	0.36	NS
	Ca, %	0.56	0.55	0.01	NS
	P, %	0.27	0.27	0.001	NS
	Mg, %	0.41	0.41	0.01	NS
	K, %	1.57	1.56	0.1	NS
	In Situ Digestibility (72 h), %[d]	69.3	67.4	1.79	NS
Harvest 2					
	Forage DM yield, lb/ac	4091.09	4266.69	216.02	NS
	Acid Detergent Fiber, %	37.63	37.78	0.45	NS
	Neutral Detergent Fiber, %	62.71	63.29	0.53	NS
	Crude Protein, %	13.4	13.04	0.34	NS
	TDN, %	59.57	59.47	0.36	NS
	Ca, %	0.41	0.39	0.01	NS
	P, %	0.25	0.25	0.001	NS
	Mg, %	0.42	0.39	0.01	NS
	K, %	2.14	2.35	0.1	NS

[a]Treatments were: BI, bioinoculant applied at 11.2 L per ha following initial planting (1 d) and re-applied following first harvest (1 d); Control, no bioinoculant applied.
[b]Pooled standard error of treatment least square means.
[c]Probability that least square means differ; NS=not statistically significant (P>0.10).
[d]*In situ* digestibility conducted over a 72 h period.

Table 4: Effects of Sumagrow on forage DM yield, nutrient content, and *in situ* digestibility of sorghum-sudan hybrid by harvest data.

Treatment[a]				
Item	Sumagrow	No Sumagrow	SE[b]	PP-value[c]
Forage DM yield, lb acre	3242.4	3405.8	890.7	NS
Acid Detergent Fiber, %	35.33	35.57	2.27	NS
Neutral Detergent Fiber, %	60.22	60.8	2.5	NS
Crude Protein, %	14.81	14.33	1.36	NS
TDN, %	61.38	61.19	1.78	NS
Ca, %	0.46	0.48	0.07	NS
P, %	0.26	0.26	0.01	NS
Mg, %	0.4	0.41	0.01	NS
K, %	1.98	1.96	0.41	NS
In Situ Digestibility (72h)[d]	64.97	64.43	1.05	NS

[a]Treatments were: BI, bioinoculant applied at 11.2 L per ha following initial planting (1 d) and re-applied following first harvest (1 d); Control, no bioinoculant applied.
[b]Pooled standard error of treatment least square means.
[c]Probability that least square means differ; NS=not statistically significant (P>0.10)
[d]*In situ* digestibility conducted over a 72 h period.

Table 5: Effects of Sumagrow on forage DM yield, nutrient content, and *in situ* digestibility of sorghumsudangrass hybrid, overall data.

pattern noted in the present study, N may not have been effectively utilized thereby negating any effect. Jaleel [12] determined that yields and quality characteristics are reduced in drought stressed plants. No effects of BI (P>0.10) were noted on any quality parameters. Similarly no interactions (HxN, HxBI, BIxH, BIxN, or BIxHxN) were noted in the present study (P>0.05)

In situ digestibility

In situs were done in triplicate and all had coefficient of variation (CV, %) less than 4%. There was an effect of H (P<0.001) on *in situ* digestibility, with greater digestibility noted in H1. Similar to other forage quality measurements, we hypothesize that the increase in digestibility is probably due to the potential differences in maturity at the two harvests. However, a tendency for interaction was noted for harvest x nitrogen application (P=0.10). In the first harvest, plants fertilized with N67 had greater digestibility than the other two fertilizer treatments (P<0.05). As expected, no differences were noted during the second harvest. Additionally, an H x BI interaction tendency (P=0.09) was also detected; while there were no differences noted in the first harvest, in the second harvest, a tendency (P=0.07) in decreasing *in situ* digestibility was noted with application of BI. It is unclear in what manner the BI might have decreased digestibility; however, there may be no biological explanation for this.

Conclusions

The application of BI to sorghum-sudangrass did not affect DM yield, forage quality parameters, nor *in situ* digestibility. Nitrogen increased DM yield and improved *in situ* digestibility for the first 28 d; however, due to weather issues, it was not feasible to reapply N to plots following the first harvest. Therefore, it was difficult to measure any effects during the second growth phase. Moreover, no interactions were noted regarding BI and N application. Time of harvest was significant and did interact with some other variables. The environmental conditions between the two growth periods were so diverse that this difference was not surprising. Further studies are needed to determine treatment effects of BI on forage biomass and quality.

Acknowledgement

This project was funded in part by a grant from Bio Soil Enhancers, Hattiesburg, MS and -the Mississippi Agricultural and Forestry Experiment Station.

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