Effect of treatment with a mixture of bacteria and fibrolytic enzymes on the quality and safety of corn silage infested with different levels of rust

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ABSTRACT

This project aimed to determine if a dual-purpose bacterial inoculant could mitigate potential adverse effects of increasing levels of rust infestation on the quality, aerobic stability, and safety of corn silage. Corn plants with no rust infestation (NR), or medium (all leaves on the lower half of the plant affected, MR), or high (all leaves affected, HR) levels of southern rust infestation were harvested at random locations on a field, chopped, and ensiled without (control, CON) or with a dual-purpose inoculant applied at a rate that supplied 1×10^5 cfu/g of *Pediococcus pentosaceus* 12455 and 4×10^5 cfu/g of *Lactobacillus buchneri* 40788. Each treatment was prepared in quadruplicate in 20-L mini silos and ensiled for 97 d. As the level of rust infestation increased, the concentrations of dry matter (DM) and neutral detergent fiber increased, whereas DM digestibility decreased by up to 16%. Control HR silages also had lower 24-h neutral detergent fiber digestibility (NDFD; 36.2% of DM) than CON MR (39.8%) or NR silages (38.1%). Inoculation increased the NDFD of NR (43.4%) and MR silages (45.7%) but not HR silages (33.0%). Concentrations of lactate and volatile fatty acids decreased with increasing rust infestation in CON silages, but this trend was absent in inoculated silages. In HR silages, inoculation increased aerobic stability by 75% (77.3 vs. 44 h), and prevented production of aflatoxin (5.2 vs. 0 mg/kg). The concentration of aflatoxin in uninoculated HR silages exceeded action levels stipulated by the US Food and Drug Administration. In conclusion, increasing rust infestation was associated with reductions in the nutritive value and fermentation of corn silage. Inoculation reduced adverse effects of rust infestation on the fermentation, increased 24-h NDFD of NR and MR silages, and decreased aerobic spoilage and aflatoxin production in HR silages.

Key words: southern rust, silage inoculant, aflatoxin, corn silage

INTRODUCTION

Southern corn rust is an aggressive disease of corn caused by *Puccinia polysora* Underw. Pustules produced during the growth of the fungus cause severe damage to the leaf cuticle resulting in increased transpiration, premature leaf desiccation, and reduced photosynthesis and nutrient translocation (Lucas, 1998). The fungus can kill the hosts and cause yield losses of up to 45% as previously reported in cases of severe epiphytotic contamination in the United States (Rodriguez-Ardon et al., 1980) and other areas of the world (Rhind et al., 1952; Liu and Wang, 1999). The disease has several similar symptoms and effects to common rust, which is caused by *Puccinia sorghi*; however, cool wet conditions favor common rust, whereas hot humid conditions favor southern rust. Furthermore, southern rust has been reported to be the most destructive of the rusts that occur on corn worldwide (Raid et al., 1988) and it has caused yield loss as far north as Central Pennsylvania (Johnson et al., 1997). Despite the existence of resistant commercial corn hybrids, the unpredictable occurrence of Southern rust and its fast and aggressive development make it a real threat to dairy operations in the Southeast that feed corn silage.

Infestation of corn plants by *Puccinia polysora* can negatively affect the chemical composition of corn forage. Johnson et al. (1997) reported increases in concentrations of DM, NDF, and ADF and a decrease in DM digestibility of corn forage infested by southern rust. These effects partly reflect the fact that after infecting plants, *Puccinia* spp. rapidly convert photosynthates such as glucose and fructose into other fungus-specific products such as alcohols (Manners et al., 1982). Loss of such photosynthates, which are critical fermentation substrates, may inhibit or delay the acidification necessary for proper forage preservation during ensiling. Because many of the fermentation-inhibiting bacteria and spoilage-causing fungi thrive at high pH values (4.5 to 6; McDonald et al., 1991), delayed acidification due to rust infestation could potentially predispose silage

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to poor nutritional value, spoilage, and mycotoxin contamination (González Pereyra et al., 2008; Pedroso et al., 2010).

Pediococcus pentosaceus is a homolactic bacterium that often is used as a silage inoculant. Unlike *Lactobacillus plantarum*, *P. pentosaceus* can rapidly dominate the initial stages of the fermentation, when silage pH is between 6.5 and 5 and residual oxygen is still present (McDonald et al., 1991; Kung et al., 2003). *Lactobacillus buchneri* is a commonly used heterofermentative bacteria in silage inoculants because it converts lactic acid to acetic acid and thereby increases silage aerobic stability (Oude Elferink et al., 2001). To our knowledge, no study has evaluated if such inoculants can improve the quality and safety of disease-infested silage. Yet the safety of feeding silage to animals depends on the mold population, which can contaminate silage with harmful mycotoxins (Mallmann et al., 2009).

This study aimed to examine if bacterial inoculation could mitigate potential adverse effects of southern rust infestation on the fermentation, nutritive value, aerobic stability, and safety of corn silage. We hypothesized that rust infestation would decrease the quality of corn silage and inoculation would mitigate adverse effects of the disease on measures of silage quality and safety.

MATERIALS AND METHODS

Silage, Treatments, and Design

A corn hybrid (DeKalb 69-70; Monsanto Co., St. Louis, MO) was grown on a 65-ha field in August 2007 in Hague, FL as a summer silage crop. The corn was infested with southern rust at tasselling and treated with Abound fungicide (Syngenta Crop Protection LLC, Greensboro, NC) by aerial application using a crop duster airplane. Uneven coverage of the fungicide allowed rust to persist in some parts of the field. Consequently, in some areas, plants were completely disease free (no rust, **NR**) whereas in other areas, leaves in the lower half (medium rust, **MR**) or all leaves (high rust, **HR**; Figure 1) had the yellowish brown discoloration and dense occurrence of small orange pustules that typify the disease. Corn forage from representative parts of the field with each rust classification were harvested when the DM concentration of NR plants was 40% and chopped using a Jaguar 900 forage harvester (Claas of America Inc., Omaha, NE). The forages were placed in separate piles and either treated with 100 mL of deionized water (control, **CON**) or 100 mL of a solution of Buchneri 500 inoculant (**INOC**, LB500; Lallemand Animal Nutrition, Milwaukee, WI). As specified by the manufacturer, the inoculation rate delivered 1×10^5 cfu/g of *Pediococcus pentosaceus* 12455 and 4×10^5

cfu/g of *Lactobacillus buchneri* 40788 and it also contained enzymes resulting from *Trichoderma reesei* fermentation. Manufacturer-stipulated minimum activities were 400 mg of glucose/min per gram (β -glucanase), 1,140 mg of glucose/min per gram (xylanase), and 18 mg of glucose/min per gram (galactomannanase). Four replicates of each treatment were ensiled in 20-L plastic bucket silos at a density of 250 kg of DM/m³ for 97 d at ambient temperature (25°C) in an enclosed barn. The sequence of harvest and ensiling was as follows: NR, MR, and HR. Each of the 24 silos was sealed with airtight plastic lids secured with heavy-duty duct tape. Samples of freshly treated forage were stored at -20°C for chemical analyses. Weights of empty and full silos at ensiling were recorded for estimation of DM loss. At silo opening, the forage was transferred to a plastic bag for proper mixing, after which subsamples were collected for analysis of nutritive value, microbial counts, aerobic stability, and mycotoxins.

Laboratory Analysis

Corn forage and silage samples were dried at 60°C for 48 h in a forced-air oven. Dried samples were ground to pass the 1-mm screen of a Wiley mill (A. H. Thomas Co., Philadelphia, PA) and analyzed for DM (105°C for 16 h) and ash (512°C for 8 h). Concentrations of NDF and ADF were measured in an ANKOM 200 fiber analyzer (Ankom Technology Corp., Macedon, NY) using the method of Van Soest et al. (1991). Heat-stable α -amylase and sulfite were used in the NDF assay. Nitrogen was determined by rapid combustion using a macro elemental N analyzer (vario MAX CN, model ID 25.00–5003; Elementar Analysensysteme GmbH, Hanau, Germany) and CP was calculated by multiplying N concentration by a factor of 6.25. In vitro true DM digestibility (**IVTDMD**) was measured with the method of Van Soest et al. (1966) and NDF digestibility (**NDFD**) was estimated after analysis of dried samples and residues from the IVTDMD assay for NDF. Ammonia-N was measured by distillation (AOAC, 1985). Silage samples were also analyzed for aflatoxin, zearalenone, deoxynivalenol, and fumonisin by thin-layer chromatography (Scott et al., 1970) at Dairyland Laboratories Inc., Arcadia, WI).

Silage extract was prepared within 3 h of harvesting by mixing 25 g of corn silage with 225 mL of 0.1% peptone water in a stomacher for 3 min. The solution was filtered through 2 layers of cheesecloth and an aliquot was immediately used for fungal counts as described by Schmidt and Kung (2010). Yeasts and molds were enumerated after serial 10-fold dilutions by spread plating on malt extract agar (Oxoid CM59; Oxoid Inc., Basingstoke, UK). Silage pH was measured using a pH meter

Figure 1. Corn plants affected by different severities of southern rust. (A) No rust, (B) medium rust, and (C) high rust. Color version available in the online PDF.

(Corning model 12; Corning Scientific Instruments, Medfield, MA). An aliquot of 100 mL of silage extract was stored at -20°C , after its pH was reduced to 2 with 50% (wt/vol) sulfuric acid. Subsequently, such samples were used to quantify lactate and VFA with a high-performance liquid chromatograph (FL 7485; Hitachi Ltd., Tokyo, Japan) coupled to a UV detector (Spectroflow 757; ABI Analytical Kratos Division, Ramsey, NJ) set at 210 nm. The column was a Bio-Rad Aminex HPX-87H (Bio-Rad Laboratories Inc., Hercules, CA) with a 0.015 *M* sulfuric acid mobile phase and a flow rate of 0.7 mL/min at 45°C (Arriola et al., 2011).

Aerobic stability was measured by placing 2 kg of silage in an open-top polystyrene box. Temperature sensors (Hobo temperature data logger 64k; Onset Computer Corp., Cape Cod, MA) were placed in the geometric center of each silage sample and temperatures were recorded every 30 min for 14 d. Four additional sensors were placed in the room to record ambient temperature. Silages were covered with 2 layers of cheesecloth to avoid drying. Aerobic stability was denoted by the time that elapsed before the difference between silage and ambient temperature was above 2°C (Huisden et al., 2009). Samples of corn forage also were analyzed for DM, CP, NDF, ADF, IVTDMD, 24-h NDFD, aflatoxin, zearalenone, deoxynivalenol, and fumonisin as described above.

Statistical Analysis

This experiment was analyzed as a completely randomized design with a 3 (severity of rust: NR, MR, and

HR) $\times$ 2 (inoculation treatments: INOC vs. CON) factorial arrangement of treatments. The GLM procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC) and a model containing inoculant, rust, and interactions of these terms, was used to analyze the data. Mean separation was performed using the PDIFF procedure of SAS, which uses the Fisher F-protected least significant difference test. Polynomial contrasts were used to examine effects of rust, inoculant, and the interaction. Significance was declared at $P \leq 0.05$ and tendencies at $0.1 \geq P > 0.05$.

RESULTS AND DISCUSSION

The chemical composition of the freshly treated corn forage (Table 1) was similar to that of corn forage in other studies in Florida (Kim and Adesogan, 2006; Huisden et al., 2009) except that concentrations of NDF and ADF of MR and HR silages were greater. The chemical composition, IVTDMD, and NDFD of silages are shown in Table 2. For reasons that are unclear, the NDF and ADF concentration of the inoculated HR silages were greater than those of the control HR silages. Increasing rust infestation increased ($P < 0.001$, quadratic) the DM concentration of the silages and resulted in a 20-percentage-unit difference between NR and HR silages. Crude protein and ash concentrations decreased to a nadir in MR silage and then increased ($P < 0.001$, quadratic) with increasing rust infestation. The NDF and ADF concentrations increased linearly ($P < 0.01$), whereas IVTDMD decreased linearly ($P < 0.001$) as rust infestation increased. The decreases in

Table 1. Chemical composition (means $\pm$ SD) of untreated and inoculated corn forage with different levels of rust infestation (% of DM)¹

Item	Control ²			Inoculant ³		
	NR	MR	HR	NR	MR	HR
DM	39.3 $\pm$ 1.2	40.7 $\pm$ 0.7	58.2 $\pm$ 1.2	39.0 $\pm$ 1.4	42.1 $\pm$ 0.3	58.6 $\pm$ 0.1
CP	7.40 $\pm$ 0.2	7.41 $\pm$ 0.4	8.07 $\pm$ 0.3	7.96 $\pm$ 0.1	6.82 $\pm$ 0.3	8.32 $\pm$ 0.2
NDF	44.7 $\pm$ 0.4	49.1 $\pm$ 0.3	55.1 $\pm$ 0.3	45.3 $\pm$ 0.4	51.1 $\pm$ 0.5	54.6 $\pm$ 0.3
ADF	25.5 $\pm$ 0.3	29.3 $\pm$ 0.3	32.9 $\pm$ 0.5	26.4 $\pm$ 0.1	28.4 $\pm$ 0.4	31.2 $\pm$ 0.1
Ash	4.43 $\pm$ 0.1	3.76 $\pm$ 0.1	4.79 $\pm$ 0.1	4.66 $\pm$ 0.2	3.86 $\pm$ 0.1	4.40 $\pm$ 0.1

¹NR = no-rust level; MR = medium rust level; HR = high rust level.²Control = no inoculant added.³Inoculant = 1×10^5 cfu of *Pedococcus pentosaceus* and 4×10^5 of *Lactobacillus buchneri*.

the nutritive value of corn silage with increasing rust infestation reflect the effects of the disease on corn forage (Johnson et al., 1997) and indicate that ensiling did not mitigate adverse effects of rust infestation on silage nutritive value. *Puccinia polysora* induces a premature desiccation of the plant (Rodriguez-Ardon et al., 1980), which could explain the adverse effects of increasing rust infestation on forage and silage DM concentration and nutritive value in this study. Johnson et al. (1997) reported that the DM, NDF, and ADF concentrations of 5 corn hybrids were increased by southern rust infestation, leading to decreased IVTDMD. Potter (1987) and Wilson et al. (1991) reported that rust infestation decreased the digestibility of 2 cultivars of ryegrass and pearl millet, respectively. In agreement, this study shows that rust infestation decreased the digestibility of corn forage and silage, largely by increasing the relative proportion of fiber in the silage. Others have reported that the lower digestibility reflects increased lignin deposition (Hammerschmidt, 1984), which is a defense mechanism against the invading fungus and the associated disease (Ride, 1978).

Johnson et al. (1997) reported that rust infestation had no effect on the CP concentration of corn plants.

Although a quadratic CP response to increasing rust infestation was detected in this study, the differences in CP concentration were very small. Based upon a literature review, Dimmock and Gooding (2002) stated that the effect of rust on wheat protein is variable even though rust may have a deleterious effect on N accumulation and partitioning within the plant.

Inoculation slightly reduced ($P < 0.001$) the DM concentration and increased ($P = 0.02$) the ADF concentration of silages, perhaps reflecting the use of water-soluble carbohydrates as substrates by the bacteria in the inoculant. Inoculation also increased the NDFD of silages of NR and MR silages (inoculant $\times$ rust interaction, $P = 0.0004$), probably reflecting the action of the fibrolytic enzymes in the inoculant as well as those that may have been released by *L. buchneri* (Kang et al., 2009). The absence of this effect in the HR silage is likely attributable to lack of sufficient moisture for enzyme action.

All silages had pH equal to or less than 4, indicating that the fermentations were satisfactory (Table 3). However, increasing rust infestation increased the pH values (quadratic, $P < 0.001$). The latter occurred largely because in CON forages, increasing rust infesta-

Table 2. Effect of rust infestation level and inoculant application on chemical composition of corn silage (% of DM or as stated)¹

Item	Control			Inoculant			SEM	<i>P</i> -value			Rust level (<i>P</i> -value)	
	NR	MR	HR	NR	MR	HR		Inoculant	Rust	Inoculant $\times$ rust	Linear	Quadratic
DM	38.0	40.6	58.3	37.2	39.6	56.5	0.29				<0.001	<0.001
CP	9.4	8.8	9.6	9.3	8.8	9.6	0.11	0.82	0.01	0.96	0.05	<0.001
NDF	44.1	47.7	48.5	43.4	48.8	51.3	1.50	0.38	0.002	0.51	<0.001	0.30
ADF	23.1	25.1	25.3	25.3	25.4	30.7	1.21	0.02	0.02	0.13	0.006	0.42
Ash	4.7	4.1	4.5	5.1	4.0	4.8	0.14	0.44	0.001	0.35	0.06	0.01
IVTDMD ²	66.9	63.2	60.1	68.9	64.2	57.5	1.41	0.93	<0.001	0.25	<0.001	0.80
NDFD ³	38.1	39.8	36.2	43.4	45.7	33.0	1.3	0.02	<0.001	0.004	0.001	0.001

¹Control = no inoculant added; inoculant = 1×10^5 cfu of *Pedococcus pentosaceus* and 4×10^5 of *Lactobacillus buchneri*; NR = no-rust level; MR = medium rust level; HR = high rust level.²IVTDMD = 48-h in vitro true DM digestibility (%).³NDFD = NDF digestibility obtained after 48-h incubation in ruminal fluid (%).

tion progressively decreased concentrations of lactate, acetate, propionate, and total VFA, whereas these trends were mostly reversed by INOC (rust $\times$ inoculant interaction, $P < 0.01$). These results demonstrate that rust infestation worsens the fermentation of corn silage but INOC can mitigate such effects. It is also noteworthy that INOC was most effective at improving the fermentation of HR silages.

In CON silages, ammonia-N concentrations decreased to a nadir in MR silages as rust infestation increased but they increased progressively in INOC silages (rust $\times$ inoculant interaction, $P < 0.001$). Butyrate was only detected in HR silages and CON HR silages had less ($P = 0.023$) butyrate than INOC HR silages. These results suggest that secondary clostridial fermentation occurred in HR silages (McDonald et al., 1991), but it was inhibited at least partly by INOC.

Yeasts were not detected in the silages and INOC did not affect DM losses. Mold counts decreased (quadratic, $P < 0.05$) as the level of rust infestation increased likely because of the concomitant increases in DM concentration reduced the water activity of the silages. Aerobic stability increased (quadratic, $P < 0.01$) as the level of rust infestation increased, reflecting the corresponding decrease in mold counts. Inoculation had no effect ($P = 0.13$) on mean mold counts, although counts were 3.5 times greater in CON HR than in INOC HR silages (3.40 vs. 0.95 cfu/g). The latter explains the 75% improvement in the aerobic stability of CON compared with INOC HR silages (44 vs. 77 h). The increase in aerobic stability with increasing rust infestation was more pronounced in INOC than CON silages (rust $\times$ inoculant interaction, $P = 0.01$), further justifying inoculation of the silages.

Aflatoxin was only detected in the CON HR silage (rust $\times$ inoculant interaction, $P = 0.10$; Table 4) and the level (5.2 mg/kg) was above the action level of 20 μ g/kg stipulated by the US Food and Drug Administration for dietary ingredients for dairy cattle. Consequently, the CON HR silage would be unsafe to feed. High levels of aflatoxin in corn silage are notable but not unique. González Pereyra et al. (2008) reported an aflatoxin concentration of 156 μ g/kg in corn silage, yet silage is expected to have low concentrations of aflatoxin because the toxin is primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which have low tolerance for the anaerobic conditions and low pH in silage (Holmquist et al., 1983). However, the latter factors do not prevent the survival of these organisms in silage. Garon et al. (2006) successfully isolated *A. parasiticus* from corn silage samples with a high aflatoxin concentration (34 μ g/kg).

That no toxin was found in the inoculated HR silage suggests that inoculation prevented accumulation

Table 3. Effect of rust infestation level and inoculant application on fermentative parameters, microbial counts, aerobic stability, and mycotoxins in corn silage (% of DM or as stated)¹

Item	Control				Inoculant				P-value			Rust level (P-value)		
									SEM	Inoculant	Rust	Inoculant $\times$ rust		
	NR	MR	HR		NR	MR	HR					Linear	Quadratic	
pH	3.65	3.71	3.97		3.68	3.69	4.00		0.02			<0.001	<0.001	
NH ₃ -N	0.29	0.19	0.25		0.17	0.27	0.42		0.03			0.002	0.034	
NH ₃ (% total N)	3.05	2.18	2.55		1.87	2.98	4.24		0.28			0.004	0.18	
Lactate	4.99	4.02	2.28		2.27	3.17	3.50		0.21			0.004	0.077	
Total VFA	6.37	4.59	4.02		4.04	5.31	6.31		0.36			0.92	0.45	
Acetate	1.53	1.35	1.38		1.27	1.89	2.67		0.20			0.007	0.62	
Propionate	1.16	0.90	0.58		0.94	0.91	0.74		0.05			<0.001	0.25	
Butyrate	0.00	0.00	0.51		0.00	0.00	0.71		0.03			<0.001	<0.001	
DM loss (%)	1.13	1.26	0.93		1.07	0.97	0.78		0.42			0.33	0.54	
Molds (log cfu/g)	5.24	4.96	3.40		4.93	5.20	0.95		0.64			<0.001	0.018	
Aerobic stability (h)	26.0	27.5	44.0		27.5	23.8	77.3		3.77			<0.001	0.005	

¹Control = no inoculant added; inoculant = 1×10^5 cfu of *Pediococcus pentosaceus* and 4×10^5 of *Lactobacillus buchneri*; NR = no-rust level; MR = medium rust level; HR = high rust level.

Table 4. Effect of rust infestation level and inoculant application on mycotoxin concentration of corn silage (mg/kg of DM)¹

Item	Control			Inoculant			SEM	P-value		
	NR	MR	HR	NR	MR	HR		Inoculant	Rust	Inoculant × rust
Aflatoxin	0.00	0.0	5.20	0.00	0.0	0.00	1.50			
Zearalenone	0.64	0.0	0.0	0.471	0.0	0.0	0.080	0.303	<0.001	0.303
Deoxynivalenol	0.00	0.0	0.00	0.00	0.0	0.00	0.00	NA ²	NA	NA
Fumonisin	0.00	0.0	0.00	0.00	0.0	0.00	0.00	NA	NA	NA

¹Control = no inoculant added; inoculant = 1×10^5 cfu of *Pediococcus pentosaceus* and 4×10^5 of *Lactobacillus buchneri*; NR = no-rust level; MR = medium rust level; HR = high rust level.

²NA = not applicable.

of the toxin in silage. Future research should validate this finding and determine effects of rust infestation on populations of *A. parasiticus* and *A. flavus* on corn forages before and after ensiling.

No deoxynivalenol or fumonisin was detected in the silages. However, unlike rust-infested silages, CON and INOC NR silages had high concentrations of zearalenone (0.65 vs. 0.47 mg/kg, respectively). The Food and Drug Administration (Silver Spring, MD) does not have action advisory levels for zearalenone; however, the concentrations of zearalenone in this study were below the guidance level (2,000 µg/kg) stipulated by the European Commission legislation (European Commission, 2006). The presence of zearalenone in the CON NR silage indicates that summer or second planting corn silage in the Southeast could be infested with this mycotoxin, possibly because of the hot, humid conditions.

No data was found in the literature on how inoculation affects the quality of disease-infested silage. However, effects of bacterial inoculation on the quality of ensiled healthy corn plants are well documented (Pedroso et al., 2010; Schmidt and Kung 2010; Arriola et al., 2011). Huisden et al. (2009) reported that the aerobic stability of corn silage was increased by the same inoculant used in this study (LB500; 143 vs. 389 h). Schmidt and Kung (2010) demonstrated that *L. buchneri* alone or in combination with *P. pentosaceus* increased the concentration of acetate, which decreased the population of spoilage fungi and increased aerobic stability of corn silage. In this study, mean concentrations of antifungal acids (acetate and butyrate) were increased by inoculation and, hence, mean aerobic stability was increased. The inoculated HR silage had the greatest acetate concentration, the lowest mold counts, and the best aerobic stability. These results reflect the ability of *L. buchneri* in the inoculant to synthesize the antifungal organic acid, acetate, by using lactate as a substrate (Oude Elferink et al., 2001).

CONCLUSIONS

Increasing levels of rust infestation were associated with reduced nutritive value and poorer fermentation of corn silage. Silages with the highest level of rust infestation had an aflatoxin concentration that exceeded the Food and Drug Administration action level for livestock feeds, which implies that they were unsafe to feed. Inoculation with the dual-purpose inoculant decreased most negative effects of rust infestation on the nutritive value and fermentation of the silage and prevented accumulation of aflatoxin in the highly infested silage.

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