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Research Article

Silage Quality of Rations Based on *in situ Sorghum-Indigofera*

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Abstract

Background: Intercropping involves growing two or more crops on the same piece of land to produce rations for livestock, particularly ruminants. In this study, the silage quality of *in situ* rations produced from *Sorghum* intercropped with *Indigofera* was evaluated to determine which *Sorghum* variety produced the best silage. **Methodology:** The pH, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), ammonia-N (N-NH_3), Volatile Fatty Acids (VFA) and total bacteria in silage for use *in situ* rations were verified. Experiments were conducted using a Completely Randomized Design (CRD) with three replications of three factors: (1) *Sorghum* variety (Patir-37 and Citayam-33), (2) *Indigofera* composition (30, 40 and 50% *Indigofera*) and (3) Microbial inoculant (*Lactobacillus plantarum*, *Lactobacillus casei* and non-microbial inoculant). Data were analyzed using analysis of variance and HSD test. **Results:** For all rations tested, the pH and N-NH_3 values indicated good ensilage. Rations that included the *Sorghum* variety Citayam-33 had lower pH and N-NH_3 production relative to those with Patir-37. In whole crop silages, the inoculants did not significantly affect fermentation. Meanwhile, rations with higher amounts of *Indigofera* (up to 50%) had lower NDF and ADF. **Conclusion:** Together the results show that *in situ* rations made from intercropped *Sorghum* and *Indigofera* ensilage well and different compositions can be obtained directly from intercropped fields to produce rations that improve ruminant performance.

Key words: Composition, intercropping, *in situ* ration, silage, varieties

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inconsistent supplies of reliable, high-quality forage, especially during the dry season are a major constraint to improving ruminant performance. About 70% of forages used by farmers originate from local grasses that have low protein content (7-9%), high amounts of crude fiber and low digestibility. Rations that have crude protein contents below 6-7% depress microbial activity in the rumen due to a lack of N[1]. As such, farmers often add nutritional concentrates to ruminant rations to improve feed quality and nutrient intake. However, these concentrates are associated with regional increases in the price of raw feed materials and also have varying qualities. One alternative to these concentrates for complementing the low protein content of grass-based rations is legume forage that has high contents of crude protein, vitamins and specific minerals[2] such as P and Ca.

Sorghum (*Sorghum bicolor* L.) is a cereal plant of the Gramineae family that has great potential for supplementing fodder resources because of its wide adaptation, rapid growth and high green and dry fodder yields, as well as its high ratoon capacity. As such, *Sorghum* can be grown on poorer quality land to produce ruminant forage. Citayam and brown midrib (BMR) strains are genetically mutated *Sorghum* that have superior agronomic traits. One important *Sorghum* cultivar is the BMR mutant line like Patir-37, which has higher dry matter production and lower lignin content and is comparable to the BMR mutant[3] line Patir-3.1.

Legume forage has a high crude protein content (20-30%)[4] and is frequently used in ruminant feed. *Indigofera* legumes such as *Indigofera zollingeriana* have good growth with high production and nutritive value[5-7]. Moreover, incorporation of *Indigofera zollingeriana* increased the protein content, dry matter degradability and Volatile Fatty Acid (VFA) values of rations in an *in vitro* rumen model[8].

Legume and *Sorghum* crops are often grown separately and mixed at the time of feeding. Yet these crops can be grown simultaneously using intercropping, which is one of the most common cultivation practices used in sustainable agricultural systems and plays an important role in increasing land productivity and yield stability[9]. Furthermore, intercropping can conserve soil water by providing shade, buffering winds and increasing infiltration with mulch layers that improve soil structure. The enhanced productivity of multi-species agroecosystems through intercropping relative to that of monospecific agroecosystems (i.e., each species is grown alone) can be explained by complementarity and facilitation processes that result in improved resource use[9].

Tropical forages are abundant during the wet season but usually are unavailable during the dry season. If forage is not harvested and consumed during the wet season, it will continue to grow and the nutritive value will decrease as the plants become more fibrous and lignified. Excess tropical forage available during the wet season can be conserved as silage for use during the dry period through ensiling[10]. The aim of this study was to determine the effect of ensiling on silage quality of rations produced from crops grown using intercropping of *Sorghum* and the legume *Indigofera*, as well as determine the composition of rations that optimizes nutritional value.

MATERIALS AND METHODS

Silage was produced from harvests of the *Sorghum* mutant lines Citayam-33 and BMR Patir-3.7 that were grown with *Indigofera* legumes in an intercropping system used at the research farm station of the Bogor Agricultural University Jonggol Animal Science Teaching and Research Unit of the Faculty of Animal Science. *Lactobacillus plantarum* and *Lactobacillus casei* were used as inocula (isolates from the Laboratory of PAU IPB).

Sorghum and *Indigofera* biomass were harvested simultaneously when 80% of the *Sorghum* was flowering (~90 days after planting). *Sorghum* plants were defoliated above the first node from the soil surface (~10 cm above ground). *Indigofera* plants were defoliated 100 cm above the soil surface. Whole *Sorghum* and *Indigofera* plant matter (stem, leaf and grain) was chopped into 2-3 cm lengths and then wilted for 12 h separately. Silage proportions were adjusted per 10 kg silage and active Lactic Acid Bacteria (LAB) were sprayed onto the silage at 1 mL kg^{-1} of forage or about 10^6 CFU g^{-1}. Polyethylene garbage bags (15 kg capacity) were used as silos and covered by 3 layers bags to prevent leaks. A vacuum pump was used to remove the air from the bags to produce an anaerobic atmosphere.

The mixtures were fermented for 30 days, after which the silage qualities were assessed. Immediately on opening the bags, the pH of 25 g of each silage mixture mixed with 100 mL distilled water was determined using a glass electrode coupled to a pH meter. Silage fiber components, including Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined using the methods described by van Soest. Ammonia-N (N-NH_3) concentrations were determined by micro-diffusion using the Conway method. The VFA was analyzed using gas chromatography and the total bacteria were counted using the Total Plate Count (TPC) method.

Experiments were conducted at the Field Laboratory, Faculty of Animal Science, Bogor Agricultural University [Jonggol Animal Science Teaching and Research Unit (UP3J)] between November, 2014 and April, 2015. The study used a completely randomized factorial design with three factors (2×3×3) and three replicates. The first factor was *Sorghum* variety [Patir-37 (S1) and Citayam-33 (S2)]. The second factor was *Indigofera* composition [30% *Indigofera* (I1), 40% *Indigofera* (I2) and 50% *Indigofera* (I3)]. The third factor was microbial inoculant [*L. plantarum* (B1), *L. casei* (B2) and non-bacterium (B3)]. The data were then statistically analyzed using analysis of variance (ANOVA) by means of MINITAB (Version 16). Honestly Significant Difference (HSD) was applied to determine the difference among treatments. Differences were considered significant at $p<0.05$.

RESULTS

pH and N-NH$_3$: The pH of the silage ranged between 3.80 and 4.53, whereas, the N-NH$_3$ values were between 0.958 and 1.557 mM (Table 1). These values indicate that the ensilage of the treatments was effective. There were significant differences ($p<0.01$) in pH and N-NH$_3$ among the treatments with different *Sorghum* varieties wherein silage composed of Citayam-33 had lower pH and N-NH$_3$ values than did Patir-37. There were no significant differences ($p>0.05$) in pH and N-NH$_3$ among compositions of *Indigofera*, microbial inoculant type and the interaction between these factors.

NDF and ADF: Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) are frequently used as standard forage testing techniques for fiber analysis. The average NDF of the silage ranged between 58.55 and 65.23% and the average ADF ranged between 43.72 and 50.04% (Table 2). There were significant differences ($p<0.01$) in the NDF and ADF contents among the silage with different *Indigofera* amounts such that silage with 30% *Indigofera* had higher NDF and ADF content than did silage with 40 and 50% *Indigofera*. Meanwhile, there were non-significant differences ($p>0.05$) in NDF and ADF among the different *Sorghum* varieties, bacterial inocula and among all interactions between these factors.

Total bacteria and VFA: Total bacteria in the silage ranged between 8.27 and 8.72 $\log_{10}$ CFU mL^{-1} and the total VFA was between 54.99 and 72.03 mM (Table 3). There were non-significant differences ($p>0.05$) in VFA and total bacteria

Table 1: pH and N-NH$_3$ *in situ* ration silage

		Indigofera composition (%)			
Varieties	Inoculants	I1	I2	I3	Mean
pH					
Patir-37	LP	4.00±0.20	4.27±0.35	4.13±0.75	4.13±0.44
	LC	4.23±0.31	4.37±0.42	4.07±0.47	4.22±0.37
	NB	4.40±0.10	4.53±0.15	4.43±0.55	4.42±0.30
Mean		4.21±0.26	4.39±0.31	4.18±0.54	4.26±0.38^{A}
Citayam-33	LP	3.80±0.17	3.90±0.17	3.93±0.31	3.88±0.20
	LC	3.93±0.29	3.97±0.15	4.03±0.31	3.98±0.23
	NB	3.97±0.06	4.00±0.10	4.23±0.06	4.07±0.14
Mean		3.90±0.19	3.96±0.13	4.07±0.25	3.97±0.20^{B}
Mean inoculant	LP	3.90±0.20	4.08±0.32	4.03±0.52	4.01±0.36
	LC	4.08±0.31	4.17±0.36	4.05±0.36	4.10±0.33
	NB	4.18±0.25	4.27±0.31	4.28±0.35	4.24±0.29
Mean *Indigofera* composition		4.06±0.27	4.17±0.32	4.12±0.41	
N-NH$_3$ (mM)					
Patir-37	LP	1.137±0.413	1.529±0.589	1.674±0.754	1.447±0.574
	LC	1.199±0.374	1.550±0.744	1.302±0.591	1.350±0.534
	NB	1.385±0.189	1.591±0.621	1.653±0.719	1.543±0.499
Mean		1.240±0.315	1.557±0.568	1.543±0.626	1.447±0.522^{A}
Citayam-33	LP	0.868±0.215	1.033±0.189	1.095±0.258	0.999±0.218
	LC	1.033±0.095	1.033±0.199	1.219±0.719	1.095±0.387
	NB	0.971±0.156	0.951±0.218	1.095±0.449	1.006±0.270
Mean		0.958±0.158	1.006±0.180	1.137±0.447	1.033±0.292^{B}
Mean inoculant	LP	1.002±0.329	1.281±0.477	1.385±0.596	1.223±0.480
	LC	1.116±0.260	1.292±0.563	1.261±0.590	1.223±0.471
	NB	1.178±0.274	1.271±0.544	1.374±0.617	1.274±0.478
Mean *Indigofera* composition		1.099±0.282	1.281±0.497	1.340±0.567	

Means in the same column and species with different superscripts in uppercase highly differ significantly ($p<0.01$) and means in the same column and species with different superscripts in lowercase differ significantly ($p<0.05$), LP: *Lactobacillus plantarum*, LC: *Lactobacillus casei*, NB: Non-bacterium

Table 2: NDF and ADF *in situ* ration silage

		Indigofera composition (%)			
Varieties	Inoculant	I1	I2	I3	Mean
NDF					
Patir-37	LP	61.90±6.65	60.95±7.62	57.71±7.15	60.19±6.48
	LC	65.49±7.10	61.53±6.30	57.96±7.69	61.66±6.93
	NB	67.54±1.55	61.75±0.93	59.99±4.48	63.09±4.19
Mean		64.98±5.51	61.41±4.98	58.55±5.81	61.65±5.88
Citayam-33	LP	63.02±3.67	61.26±2.13	60.73±4.16	61.67±3.15
	LC	64.54±2.02	60.26±1.48	60.99±3.41	61.93±2.90
	NB	68.13±3.07	60.39±5.87	61.54±2.67	63.35±5.19
Mean		65.23±3.60	60.64±3.24	61.08±3.03	62.32±3.81
Mean inoculant	LP	62.46±4.84	61.10±5.01	59.22±5.49	60.93±5.00
	LC	65.02±4.70	60.90±4.15	59.48±5.58	61.80±5.11
	NB	67.84±2.56	61.07±3.83	60.76±3.41	63.22±4.58
Mean *Indigofera* composition		65.11±4.52^{A}	61.02±4.10^{B}	59.82±4.68^{B}	
ADF					
Patir-37	LP	48.07±0.81	47.03±2.37	42.89±2.73	46.00±3.01
	LC	48.19±3.21	47.10±1.47	43.07±1.84	46.12±3.07
	NB	50.78±4.00	47.40±1.87	45.21±4.09	47.80±3.87
Mean		49.01±2.92	47.18±1.69	43.72±2.86	46.64±3.32
Citayam-33	LP	47.30±2.22	45.96±2.48	47.20±0.83	46.82±1.83
	LC	49.68±2.93	46.57±4.56	47.62±2.24	47.96±3.24
	NB	53.16±1.84	47.91±6.36	47.73±4.21	49.60±4.75
Mean		50.04±3.28	46.81±4.19	47.52±2.43	48.12±3.54
Mean inoculant	LP	47.68±1.55	46.50±2.25	45.04±2.97	46.41±2.45
	LC	48.94±2.87	46.84±3.04	45.34±3.10	47.04±3.20
	NB	51.97±3.07	47.66±4.20	46.47±3.96	48.70±4.30
Mean *Indigofera* composition		49.53±3.06^{A}	47.00±3.11^{B}	45.62±3.23^{B}	

Means in the same column and species with different superscripts in uppercase highly differ significantly ($p<0.01$) and means in the same column and species with different superscripts in lowercase differ significantly ($p<0.05$), LP: *Lactobacillus plantarum*, LC: *Lactobacillus casei*, NB: Non-bacterium

Table 3: VFA and total bacteria of *in situ* ration silage

		Indigofera composition (%)			
Varieties	Inoculant	I1	I2	I3	Mean
VFA (mM)					
Patir-37	LP	68.91±15.81	68.73±5.95	58.42±5.95	65.36±10.36
	LC	58.42±11.90	61.86±10.31	61.86±10.31	60.71±9.57
	NB	61.72±10.53	65.15±15.94	61.72±10.53	62.86±11.04
Mean		63.02±12.13	65.25±10.38	60.67±8.12	62.98±10.12
Citayam-33	LP	58.42±5.95	58.42±5.95	54.99±5.95	57.28±5.43
	LC	58.42±5.95	68.73±15.75	54.99±5.95	60.71±10.87
	NB	58.28±12.03	63.58±7.87	72.03±18.11	64.63±13.03
Mean		58.38±7.34	63.58±10.31	60.67±13.13	60.87±10.34
Mean inoculant	LP	63.67±12.13	63.58±7.76	56.70±5.65	61.32±9.04
	LC	58.42±8.42	65.30±12.49	58.42±8.42	60.71±9.93
	NB	60.00±10.29	64.37±11.28	66.87±14.40	63.74±11.75
Mean *Indigofera* composition		60.70±10.02	64.41±10.07	60.67±10.59	
Total bacteria (log_{10} CFU mL^{-1})					
Patir-37	LP	8.45±0.41	8.43±0.43	8.54±0.17	8.47±0.31
	LC	8.38±0.41	8.61±0.27	8.52±0.16	8.51±0.28
	NB	7.97±0.31	8.58±0.29	8.59±0.27	8.38±0.40
Mean		8.27±0.40	8.54±0.30	8.55±0.18	8.45±0.33
Citayam-33	LP	8.66±0.21	8.61±0.31	8.78±0.26	8.68±0.24
	LC	8.53±0.28	8.63±0.31	8.72±0.22	8.63±0.25
	NB	8.58±0.19	8.58±0.34	8.67±0.27	8.61±0.24
Mean		8.59±0.21	8.61±0.28	8.72±0.22	8.64±0.24
Mean inoculant	LP	8.55±0.31	8.52±0.35	8.66±0.24	8.58±0.29
	LC	8.46±0.33	8.62±0.26	8.62±0.20	8.57±0.26
	NB	8.27±0.41	8.58±0.28	8.63±0.25	8.50±0.34
Mean *Indigofera* composition		8.43±0.35	8.58±0.28	8.64±0.22	

LP: *Lactobacillus plantarum*, LC: *Lactobacillus casei*, NB: Non-bacterium

content among the treatments with different *Indigoflera* compositions, microbial inoculants and the interaction between these factors. In whole crop silages, the finding that inoculants did not significantly affect fermentation could be due to the higher numbers of epiphytic LAB used and the good ensiling characteristics of the crop materials.

DISCUSSION

All silages examined in this study had pH<4, which is required for stability during fermentation. In well-preserved silages general practice specifies that pH values should be <4.5. Ensiling is a preservation method for most crops that is based on natural lactic acid fermentation under anaerobic conditions, whereby Lactic Acid Bacteria (LAB) convert Water Soluble Carbohydrates (WSC) into organic acids, mainly lactic acid. The lactic acid concentration of inoculated silages typically ranges between 83 and 85.9% of total silage acid[11]. Successful silage production depends on anaerobic storage of material that contains adequate levels of WSC that can be fermented by LAB into lactic acid, which preserves the materials due to a rapid reduction in pH. Thus to improve LAB growth, adequate amounts of Water Soluble Carbohydrate (WSC) should be available as a fermentation substrate for LAB or aerobic bacteria during ensiling[12]. The pH value of silage produced from *Sorghum* Citayam-33 intercropped with *Indigofera* was lower than that for Patir-37, likely because of higher amounts of WSC in the former treatment. Meanwhile, our finding that the inoculant type did not influence the silage pH was consistent with earlier studies using maize silage[13].

The N-NH_3 content of the inoculated silage was between 0.29 and 0.43 mM[11]. The N-NH_3 content in silage reflects the degree of protein degradation and is an indicator of the total amount of N degraded during ensiling. As such, the N-NH_3 content of the silage can be used to determine its quality. In well-preserved silages, the N-NH_3 content is typically below 100 g kg^{-1} total N. The best silage has NH_3 <50 g N kg^{-1} total N whereas, good silage has NH_3 contents between 50-100 g N kg^{-1} total N. High concentrations of ammonia arise from excessive protein breakdown in the silo that is caused by a steady decrease in pH or excessive Clostridia or enterobacteria growth. In general, silage with higher water contents has higher ammonia concentrations. The ammonia concentration of silage is also an indicator of silage crop damage, because ammonia can increase silage pH. The higher pH value we saw for Patir-37 *Sorghum* treatments was likely due to its higher N-NH_3 content. During ensiling, plant proteases degrade proteins to peptides and free amino acids, which are in turn degraded to ammonia and non-protein nitrogenous fractions largely by Clostridia proteases. The N-NH_3 is an indicator of the proportion of total N that has been completely degraded during ensiling and can be used to assess secondary fermentation. The lower content of N-NH_3 of silage produced from Citayam-33 intercropped with *Indigofera* was likely due to its low pH value that would inhibit the growth of proteolytic Clostridia and reduce the amount of protein degradation.

The NDF approximates the total cell wall constituents including hemicellulose, whereas, ADF primarily represents cellulose and lignin. The NDF and ADF represent the fibrous portions of plant material and influence digestibility and energy availability from forage. As such, NDF and ADF can be used to predict intake potential and calculate digestibility, respectively. As the fiber content increases, the forage quality declines. The dynamics of ADF content are consistent with that of the NDF content during generative development of plants. As plants mature, the crude protein decreases concurrent with increases in starch and NDF[14]. Here the NDF values ranged from 54.30-61.28% and ADF ranged from 31.72-38.40%[15]. The NDF and ADF values were highest for the treatment with 30% *Indigofera*, which had a higher structural carbohydrate concentration relative to treatments with 40 and 50% *Indigofera*. Mature plants are typically higher in fiber and have lower Non-Structural Carbohydrate (NSC) content relative to immature plants. As such, environmental conditions that restrict growth (NSC utilization) to a greater extent than photosynthesis (NSC synthesis) would increase the amount of NSC in plant herbage. Starch, a storage carbohydrate, is present in low amounts in young vegetative tissues (tiller to flowering stages) and then increases during maturation[14], the fiber fraction of plants that excludes hemicellulose from the total fiber can be expressed as Acid Detergent Fiber (ADF). Relative Feed Value (RFV) increases as the fiber (NDF or ADF) values decline. The NSC content and type depends on the plant species, plant part and development stage, as well as environmental conditions such as root and shoot temperature during growth. Other factors that affect NSC are light intensity and duration, plant nutrient availability and water status[14].

The main source of energy for ruminants is VFA originating from microbial fermentation of carbohydrates in the rumen. The VFA are absorbed from the rumen wall into the circulation. Lower VFA values indicate a lower rate of carbohydrate degradation by microbes. In this study, the total VFA produced was still below normal levels (70-150 mM)[4]. The treatments did not have different effects on VFA production due to the overhaul of silage dry matter. During both fermentation and respiration, organic matter is hydrolyzed

into CO_2, H_2O and energy. The EFSA[16] reported that additives containing *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Lactobacillus casei* did not improve silage production. Moreover, the total VFA for legume silage mixed with Citayam and BMR 36 *Sorghum* was 52.5 and 59.28 mM, respectively[17].

Thomas *et al.*[15] reported that total LAB counts for silage grass-legume mixtures ranged from 2.84×10^9 CFU mL^{-1} with legume to 1.62×10^9 CFU mL^{-1} without legume, the addition of LAB to 40% legumes resulted in lower bacterial counts (2.53×10^7 to 3.9×10^7 CFU mL^{-1}). Legumes with 70% LAB ranged from 2.84×10^8 to 5.98×10^8 CFU mL^{-1} and the total bacteria was between[15] 5.76 and 8.76 log CFU g^{-1}. Higher numbers of LAB can arise from high levels of WSC in the silage. The higher bacterial population could also be due in part to an increase in the numbers of amylolytic bacteria when more fermentable substrates are available. Bacterial proliferation rates increase with increasing supplies of carbohydrates that are more readily fermentable[18]. The count of epiphytic LAB, yeast and enterobacteria was approximately 10^8 CFU g^{-1} in the crop material. Therefore, bacteria in the inoculants would not dominate the ensiling process or affect fermentation before pH declined to values needed for silage stability. Biochemical differences in plant tissue composition could affect the composition of microbial communities in silage[19]. Furthermore, the pH from lactic acid production can inhibit the growth of other microbes such as yeast, bacilli, enterobacteria and Clostridia and eventually even LAB themselves[20].

CONCLUSION

Citayam-33 *Sorghum* had lower pH and N-NH_3 production than did Patir-37 *Sorghum*. The silage pH ranged between 3.80 and 4.53 and the N-NH_3 content was between 0.958 and 1.557 mM, indicating that the treatments ensilaged very well. In whole crop silages, inoculants did not significantly affect fermentation because of the higher numbers of epiphytic LAB and the good ensiling characteristics of this crop. Incorporation of up to 50% *Indigofera* resulted in lower NDF and ADF. The results also revealed that a combination of *Sorghum* varieties with different *Indigofera* compositions and inoculant bacteria in an intercropping system did not affect VFA or total bacteria levels.

SIGNIFICANCE STATEMENT

A comprehensive study of silage for use in *in situ* rations made from intercropped *Sorghum* and *Indigofera* will be beneficial for improving ruminant performance. Ensilage of the different treatments was good and could produce a healthy rumen environment with a pH that optimizes microbial activity. Improving microbial activity in the rumen will enhance feed intake, digestibility and feed metabolism. Together these factors can contribute to better production performance and efficiency as well as animal health. Commercial *in situ* rations produced from intercropped *Sorghum* and *Indigofera* could also increase ruminant industry profits and support sustainability of ruminant production.

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