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Nutritive Value and Silage Fermentation Characteristics of Forage Sorghum (*Sorghum bicolor* L.) Genotypes and Lablab (*Lablab purpureus* L.) Mixture

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Abstract

This study was conducted to assess the fermentation and nutritive value of sorghum silage mixed with lablab at different proportions. The treatments consisted of a combination of two sorghum genotypes *viz.* "Brown midrib" and "Brachytic dwarf" genotype of lablab and six population proportions viz. 150:0, 112.5:37.5, 75:75, 37.5:112.5, 0:150 and 150:150 × 10^3 plants·ha^{-1} sorghum genotypes and lablab, respectively totalling to 12 numbers. Sorghum genotypes and lablab were grown as monocrop and in intercropping systems in the same field. Forage sorghum was harvested at the late-dough stage and lablab at 20% bloom. They were cut and chopped together and ensiled. Lablab in the silage mixture was its actual contribution to the total forage mixture. For each mixture, a 1-L glass jar (mini-silo) was filled with 500 g of fresh material and replicated four times. Forage in mini silos was fermented for 60 days at room temperature (25˚C). Pre and post-silage dry samples were analysed for nutritive value and ensiled samples were analysed for fermentation characteristics. There was no significant difference in nutritive value between sorghum genotypes. The greatest impact of mixing lablab with sorghum genotypes was on crude protein (CP) and acid detergent fiber (ADF), but not on neutral detergent fiber (NDF). Across treatments, CP, ADF pH, lactic, and acetic acid concentrations increased as the proportion of LB was increased. The results indicated that lablab as an intercrop with sorghum for greater DM yield and forage and silage quality than respective monocrops.

Keywords

Brown Midrib, Brachytic Dwarf, Ensilage, Forage Quality, Intercropping, Lablab, Silage

1. Introduction

Ensiling is an ancient method used to preserve the nutritive value of forages by packing and storing forage in airtight conditions. Silage fermentation occurs naturally under anaerobic conditions. The combination of forage legumes with non-legumes under intercropping has shown benefits in terms of reduced N fertilizers, improved yield, and nutritive value in silage [1] [2]. Maize and sorghum are the predominant cereal forages for silage. The nutritional quality of sorghum has poor quality, particularly in protein concentration [2] [3] [4]. In forage-based production systems, no single species can provide high-quality forage as well as tonnage for the dairy industry across the world. Legumes are grown individually as green cover or forage, generally have lower yielding than cereals but supply herbage with higher N concentration and fiber digestibility [5]. Further, research efforts have been made to improve the adaptability of these forage legumes and identify optimal management practices with cereals [6]. In crop combinations, maize and sorghum with lablab/cowpea [7], and sorghum with several annual legumes [8] have shown benefits in improving dry matter yield and forage nutritive value. Begna *et al.* [9] reported that high forage yield with quality in terms of RFV and TDN) for animal nutrition can be achieved from the mixed cropping of canola and pea at 50:50 and 75:25 seeding ratios. Cultivated legumes are introduced into the cropping systems as a component crop with sorghum. Further, the CP concentration was increased by greater legume contribution in the mixture. When maize was ensiled with common bean pH, lactic acid, and total volatile fatty acids were greater in the mixtures than in monoculture maize [10]. It was shown that legumes alone cannot be ensiled due to their poor nutritive value and high pH and butyric acid potentially reduce their use. It was evident that mixing lablab with sorghum silage should increase CP concentration, but it would also increase other constituents, such as NDF, lactic acid, and total acids concentrations. Therefore, it is important to determine the proper combination of lablab and cereals that will result in an optimum mixture for nutritive value and fermentation. This research has been conducted on sorghum genotypes for silage along with lablab to assess the nutritive value and fermentation characteristics suitable for sustainable livestock production.

2. Methodology

2.1. Experimental Site and Crop Management

Field and laboratory experiments were conducted at New Mexico State University's Agricultural Science Center at Clovis (34˚N, 103˚12'W, and 1348 m elevation), with annual precipitation of 445 mm and Olton clay loam soil. Forage sorghum brown midrib cv. Dairy Master BMR (BMR-LB) and brachytic dwarf sorghum cv. 26,837 (BDS-LB) types were intercropped with lablab cv. Rio Verde. Each sorghum variety was mixed with the legume using six population density ratios. Treatment combinations were BMR-LB seeding rates at 150 - 0, 112.5 - 37.5, 75 - 75, 37.5 - 112.5, 0 - 150 and 150 - 150 ($\times 10^3$ plants·ha^{-1}) similarly for

BDS-LB combinations. Crops were fertilized according to soil test recommendations. The total planting density of sorghum and lablab was at 150,000 plants·ha^{-1} except in one treatment having equal density of 150,000 plants·ha^{-1} (**Table 1**). Forage sorghum was harvested at late-dough maturity stage of the grain development and lablab at 20% bloom stage. Field trial was irrigated to prevent water stress during the growing season. There was no serious problem with pests and disease incidence during the season. Both sorghum and lablab were combined and harvested at ground level from random locations within each field approximately 114 and 110 days after sowing, respectively in 2010 and 2011. Ensiling process for all the crops and crop mixtures were ensiled at the same time. Lablab was wilted for 18 h before chopping. All the crops were chopped simultaneously to a theoretical particle size of 15 mm with a two-row pull-type forage harvester (John Deere, Moline, IL). After chopping, approximately 20 kg of fresh material of sorghum and LB together were collected in separate plastic buckets and taken to the laboratory for ensilage.

2.2. Ensilage of Forage Mixture

Individual 500 g fresh mixtures were made for each treatment and placed in a 0.5-L glass jar with four jars per treatment. Quantity of lablab mixed in each treatment was depends on its contribution to forage mixture details of each treatment (**Table 1**). Mini-silos were fermented for 56 days at room temperature (25˚C). Before ensiling, two 250 g subsamples for each treatment were placed in a paper bag and oven dried at 60˚C for 48 h for dry matter determination. Subsamples were powdered and analysed for pre-ensiling chemical composition. At opening, each mini-silo was dumped into an ethanol-disinfected plastic container and mixed uniformly. A 250 g subsample was placed in a plastic vacuum pouch of 20 × 30 cm (Doug Care equipment, Springville, CA), immediately vacuum sealed using a fast vacuum machine (Doug Care equipment, Springville, CA) and frozen to −18˚C for later analysis for fibre and fermentation characteristics. The remaining 250 g sample was frozen as a backup sample.

2.3. Forage Quality Analysis

The biomass samples from the final harvest were used to analyze forage quality served as pre silage sample. Crude protein, NDF, ADF, IVTD, and NDFD were determined using a near-infrared spectroscopy system with equation calibrations used for sorghum types. The pH, acetic, butyric, propionic, and iso-butyric acids, lactic acid were determined using standard procedure [10]. All these analyses were conducted at Dairy One Laboratory (Ithaca, NY).

3. Statistical Analysis

The data were analysed by using SAS PROC MIXED software programme version 9.4 (SAS Institute, 2020). For significant mixtures and mixture x, crop effects were used to assess the order of the response trend as well as the nature of the interaction with the crop. Significance was defined at $P < 0.05$.

Table 1. Quantity of dry matter contribution (%) to total mixture and mixed with sorghum during ensilage.

Treatment		Lablab contribution to total mixture %		Quantity of lablab with sorghum during ensilage	
		2010	2011	2010	2011
Population density ('000 ha^{-1})					
Brown mid rib sorghum	Lablab				
150	0	0.0	0.0	0	0
112.5	37.5	17.4	10.2	87	51
75	75	29.8	14.3	149	71.5
37.5	112.5	39.8	21.9	199	109.6
0	150	100	100.0	500	500
150	150	26.6	16.8	133	84.1
Brachytic dwarf sorghum	Lablab				0
150	0	0.0	0.0	0	0
112.5	37.5	14.2	5.6	71	28
75	75	14.0	14.7	70	73.4
37.5	112.5	24.8	20.9	124	104.6
0	150	100	100.0	500	500
150	150	19.5	8.9	97.5	44.5

4. Results and Discussion

4.1. Nutritive Value

The Dry matter concentration at the time of ensiling was below 300 g·kg^{-1} for sorghum and lablab. The sorghum was harvested at the maturity stage recommended for ensiling and lablab was wilted 18 h before ensilage. Dry matter concentration was in a lower range between 270 and 277 g·kg^{-1}. Even at these high moisture conditions, both sorghum genotype silage mixtures fermented well. Adding lablab to sorghum affected the nutritive value of silage mixture (Table 2). Lablab was intercropped with sorghum to increase the crude protein content of the silage. Addition of crude protein rich lablab was increased in the mixture. The effect of lablab on crude protein was similar in both the sorghum genotypes. Earlier studies also reported that maize and legume mixture increased crude protein concentration from 12.9% to 29.0%. The NDF content of the silage was varied from 29.8% to 40.2%. The presence of lablab in the ensiled mass increased ADF (377 - 383 g·kg^{-1} DM) and but decreased NDF (553 - 529 g·kg^{-1} DM) levels (Table 2). The digestibility of NDF was also decreased by increased concentration of lablab in association with sorghum (643 - 565 g·kg^{-1} DM). Similar results were also with total digestible nitrogen (Table 2). The calcium concentration was increased by 3.1% with increased concentration of lablab in the mixture. It was consistent with the sorghum genotypes. The relative feed quality of inter-

cropping forage was not significantly influenced by lablab inclusion. Htet, *et al.* [11] reported that the maize intercropped silages increased pH, and CP contents ($P < 0.05$), whereas decreased NDF, ADF, and ash contents. No difference ($P > 0.05$) was found in K contents of nutrient composition of silage among the four treatments. The Ca contents in the intercrop silages were higher than the SM silage.

Table 2. Pre-silage forage content of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), NDF digestibility (NDFD), total digestible nutrients (TDN), calcium and relative feed quality (RFQ) of legumes with sorghum genotypes at different seeding ratios.

Treatment		#CP	ADF	NDF	NDFD	TDN	Ca	RFQ
		g·kg^{-1} DM						
Sorghum types (S)								
Brown Mid-Rib (BMR)		96	397	551	613	571	6.6	112
Brachytic Dwarf (BDS)		104	368	485	638	603	6.5	130
LSD (0.05)		NS	28	49	NS	31	NS	18
Sorghum population '000 plants·ha^{-1}) (P)								
Sorghum	***Lablab***							
150	0	61.3	377.1	552.6	653.8	595.4	2.5	111.3
112.5	37.5	78.9	372.9	528.8	642.5	599.6	4.0	117.0
75	75	86.8	383.4	533.0	636.3	588.3	4.5	116.6
37.5	112.5	103.3	398.3	527.4	625.0	571.3	5.6	115.6
0	150	179.1	382.1	437.5	565.0	577.9	18.0	148.5
150	150	89.1	383.1	528.9	630.0	588.8	4.6	119.0
LSD (0.05)		9.7	15.7	21.2	16.7	17.7	0.7	8.2
S × P interactions								
BMR	**Lablab**							
150	0	54.5	396.0	605.5	642.5	574.9	2.3	100.3
112.5	37.5	73.3	384.0	564.8	635.0	587.3	4.1	109.0
75	75	89.5	393.5	555.3	622.5	576.8	5.1	111.5
37.5	112.5	102.8	404.5	545.5	617.5	564.0	6.1	112.8
0	150	169.3	410.0	477.3	545.0	548.0	17.0	127.8
150	150	84.8	395.8	559.3	612.5	574.3	4.7	112.8
BDS	**Lablab**							
150	0	68.0	358.3	499.8	665.0	616.8	2.7	122.5
112.5	37.5	84.5	361.8	492.8	650.0	612.0	3.9	125.0
75	75	84.0	373.3	510.8	650.0	599.8	3.8	121.8
37.5	112.5	103.8	392.0	509.3	632.5	578.5	5.1	118.5
0	150	189.0	354.3	397.8	585.0	607.8	19.0	169.3
150	150	93.5	370.5	498.5	647.5	603.3	4.6	125.3
LSD (0.05)		NS	NS	26.7	NS	NS	0.9	10.4

In general, lower concentration of fibres in the DM of legumes in relation to grasses. However, Dawo *et al.* [1] found that a greater increase in crude protein would be expected when the legume was at 50% of the normal plant density. Sorghum species variation and maturity at harvest could have caused lower crude protein values in this study. Contreas-Govea *et al.* [10] reported that cell wall structural carbohydrates were not affected during fermentation and lactic acid bacteria consumed the pool of water-soluble carbohydrates. Dry matter concentration of the pre-ensiling mixtures was below 300 $g \cdot kg^{-1}$ for sorghum and lablab mixtures.

The pH across the mixtures was ranged from 3.79 to 4.58, which were below those reported by Jones *et al.* [12]. While lactic acid concentration increased as the quantum of lablab increased in the mixture indicating acidic conditions were conducive to cell wall hydrolysis, which decreased NDF and to some degree ADF. Apparently, ADF fraction was also susceptible to hydrolysis, but at a lower degree than NDF fraction. The ADF of ensiled mixtures with greater proportion of sorghum in 150:0, 112.5:37.5 planting density was lower (**Table 3**). The ADF concentration increased as the quantum of lablab increased in the mixture. In contrast, NDFD was reduced as the concentration of lablab increased (0:150) in the mixture while it was in sorghum alone (**Table 1**). These results in agreement with those reported by Contreras-Govea *et al.* [10] in which NDFD declined by adding beans to maize. In addition, our IVTDMD values were similar in magnitude to those reported by Contreras-Govea *et al.* [13]. Hence, positive impact of adding lablab in mixture with sorghum could be greater in low-quality sorghum than in high-quality sorghum cultivars. A summative approach that considers the true digestibility of CP, NDF, non-fiber carbohydrates and fatty acids was used to determine TDN concentration. Across treatment TDN (630 to 755 $g \cdot kg^{-1}$) was greater when lablab increased from 0 to 75 but then declined when lablab increased from 75 to 150 seeding ratios (**Table 2**). The IVTDMD ranged from 747 to 840 $g \cdot kg^{-1}$ across forage mixtures. These results may be attribute high crude protein concentration in legumes, which is conducive to greater proteolysis than sorghum types. In addition, legumes have greater buffering capacity than grasses.

4.2. Fermentation Profile

The most predominant acids in the silage mixtures were lactic and acetic acids and whereas propionic, butyric and iso-butyric acids were very low. Lactic acid was higher in sorghum mixtures then increased linearly with increased lablab proportion. The concentration of lactic acid was highest in sorghum genotype "Brown mid rib" and it increased as the lablab proportion increased from 0 to 1,12,500 $plants \cdot ha^{-1}$ (56 to 83 $g \cdot kg^{-1}$). Similar trends were also observed for acetic acid and total acids. However, the lactic acid: acetic acid ratio showed a reverse trend and it decreased as acetic acid increased. Legumes have larger organic acid concentrations than grasses; therefore, in general legume silages have higher pH

Table 3. Fermentation products in terms of crude protein (CP), lactic acid (LA), acetic acid (AA), total acids (TA), *in-vitro* dry matter digestibility (IVTD) of sorghum mixed with different proportion of lablab bean mixtures planned at different population levels (Mean of two years).

Treatment		DM	CP	LA	AA	L/A ratio	TA	IVTD	pH
				g·kg DM					
Sorghum types (S)									
Brown midrib (BMR)		229	98.3	68.8	25.9	2.8	95.2	808.3	4.0
Brachytic dwarf (BDS)		271	110.5	54.1	23.4	2.8	77.8	772.1	4.2
LSD @ 5%		8.1	7.8	13.5	1.6	NS	15.4	18.3	NS
Population density ('000 plants·ha^{-1}) (P)									
Sorghum	***Lablab***								
150	0	282	135.3	58.6	35.0	2.3	93.9	793.8	4.4
112.5	37.5	255	83.3	53.1	17.6	3.3	71.3	808.8	3.9
75	75	263	93.1	59.0	20.3	2.9	79.5	782.5	3.9
37.5	112.5	246	94.4	67.7	21.6	3.1	89.4	798.8	3.9
0	150	189	92.5	63.5	21.9	2.9	85.8	786.3	3.9
150	150	263	127.5	66.8	31.5	2.4	99.1	771.3	4.4
LSD (0.05)**		7.4	6.1	3.1	2.1	0.4	3.5	21.1	0.05
Population density ('000 plants·ha^{-1})									
BMR	**Lablab**								
150	0	257	79.8	59.6	16.6	3.6	76.3	840.0	3.80
112.5	37.5	234	84.5	59.9	22.4	2.7	83.1	837.5	3.80
75	75	241	94.5	67.6	22.5	3.0	90.3	795.0	3.90
37.5	112.5	228	87.5	76.1	24.1	3.2	100.2	822.5	3.83
0	150	189	162.5	85.9	46.1	1.9	133.4	752.5	4.63
150	150	225	80.8	63.6	23.5	2.7	87.5	802.5	3.83
BDS	**Lablab**								
150	0	306	82.0	46.4	12.8	3.9	59.4	780.0	4.00
112.5	37.5	277	91.8	50.4	18.1	2.8	68.7	770.0	3.95
75	75	286	101.3	59.2	19.2	3.1	78.7	775.0	4.05
37.5	112.5	265	104.3	63.4	20.4	3.1	84.0	770.0	4.03
0	150	188	190.8	57.6	53.4	1.1	111.4	747.5	4.95
150	150	302	93.3	47.6	16.9	2.8	64.7	790.0	4.08
LSD@ 5%		11.5	9.3	5.2	2.9	0.7	5.5	32.0	0.08

because of the higher buffering capacity caused by the organic acids. Earlier studies on fermentation analysis of legume silages like kura clover, lucerne, faba bean and soybean, indicated that these crops having higher values of lactic acid and acetic acid concentration [13] [14]. This study showed that the concentration of lactic acid, acetic acid and total acid increased with the increase in the proportion of lablab bean in the mixture (Table 4). Earlier studies by Contreas-Govea *et al.* [13] on fermentation analysis of legume silages also showed higher values of lactic acid and acetic acid concentration due to high buffering capacity. Adding

legumes to grasses was found to extend the fermentation time resulting in increased accumulation of fermentation end products. The results of the current study were agreed with previous studies [1] [13]. Lactic acid is a stronger acid as compared to other acids (acetic, propionic, butyric) and thus contributes most to the drop in silage pH. It increased linearly in both Brown mid rib-lablab (3.8 to 4.63) and Brachytic dwarf sorghum-lablab (4.0 to 4.95) mixtures as lablab increased from 0 to 150 seeding ratios (Table 4).

Table 4. Post silage content of crude protein (CP), acid detergent fibre (ADF), neutral detergent fiber (NDF), NDF digestibility (NDFD), total digestible nutrients (TDN), relative feed quality (RFQ) of sorghum and lablab seeded at different population ratios.

Treatment		CP	ADF	NDF	TDN	IVTD	NDFD	RFQ
		g·kg^{-1} DM						
Sorghum cultivars (S)								
Brown midrib (BMR)		113	340	508	670	812	630	149.2
Brachytic dwarf (BDS)		117	346	500	630	786	570	138.8
LSD (P = 0.05)		**NS**	**NS**	**NS**	**15**	**18**	**25**	**NS**
Population density ('000 plants·ha^{-1}) (P)								
Sorghum	*Lablab*							
150*	0	76.6	305.9	477.5	666	798	574	139.3
112.5	37.5	88.6	323.3	492.8	670	801	596	147.1
75	75	106.5	322.9	503.4	680	828	655	160.9
37.5	112.5	114.5	356.8	523.1	659	794	604	142.9
0	150	188.8	406.1	514.8	561	775	566	128.0
150	150	112.5	342.1	513.5	660	798	603	145.9
LSD (P = 0.05)		**8.5**	**15.7**	**24.8**	**12**	**17**	**30**	**9.3**
S × P interaction								
BMR	*Lablab*							
150	0	73	301	485	690	813	608	138.5
112.5	37.5	82	332	514	690	808	633	145.5
75	75	103	321	516	700	855	720	173.8
37.5	112.5	114	348	524	680	803	623	150.5
0	150	188	391	490	590	788	568	138.3
150	150	116	346	521	670	805	623	148.8
BDS	Lablab							
150	0	81	311	470	650	783	540	140.0
112.5	37.5	95	315	471	650	795	560	148.8
75	75	111	325	491	660	800	590	148.0
37.5	112.5	115	365	523	640	785	585	135.3
0	150	189	421	540	530	763	565	117.8
150	150	109	338	506	650	790	583	143.0
LSD (P = 0.05)		**NS**	**NS**	**NS**	**21.3**	**NS**	**45.8**	**17.8**

A higher (65% - 70% of total acids) lactic acid fraction is indicative of a good fermentation, and it results in lowest dry matter and energy losses from the crop. However, high (>30 - 40 $g \cdot kg^{-1}$ DM) acetic acid values will reduce the overall dry matter and energy recovery from the silage mixture [15]. Therefore, lactic acid formation is preferred over acetic acid production. However, lactic acid production is governed by the nature of fermentation and the balance between homolactic or heterolactic fermentation processes [16] with heterolactic fermentation producing a weaker (lactic) acid than the homolactic process. The ratio of lactic: acetic acid decreased with the increased lablab proportion in the silage. There was no significant difference in lactic: acetic acid ratio between Brown mid rib and Brachytic dwarf sorghum mixtures.

Bolsen [17] reported that forage sorghum had normally higher acetic acid concentration, which was further increased by the addition of lablab to the mixture. It was suggested that a 3:1 lactic: acetic acid ratio is a good indicator of fermentation. Results from this study showed that lactic: acetic acid ratio was decreased as lablab content increased in the mixture, and it was 3.6 to 1.9 in Brown mid-rib-lablab mixture and 3.9 to 1.1 in Brachytic dwarf sorghum-lablab. This could be attributed to sorghum which is normally low in acetic acid (12.8 to 16.6 $g \cdot kg^{-1}$ DM). Adding lablab to sorghum would potentially increase acetic acid concentration to a level (31.5 $g \cdot kg^{-1}$ DM) that could affect palatability and dry matter intake. This will result in a decrease in dry matter intake by cattle [14] and present a disadvantage of adding lablab to the silage mixture. Anil *et al.* [18] showed that silage made from the maize-soybean mixtures contained greater lactic and acetic acid concentrations than that of sole maize. Increase in lactic acid concentration when cereal was ensiled in mixture with other legumes. Therefore, options, methods, and possibilities for mixing forage sorghum with lablab bean in a silage mixture should be explored further during subsequent studies.

5. Conclusion

The findings of this study concluded that nutritive and fermentation profile was significantly influenced by the addition of lablab in sorghum silage mixture. Lablab can be ensiled with Brown mid rib and Brachytic dwarf sorghum genotypes, and they produce greater crude protein and acceptable fermentation in all mixtures. However, a greater benefit in nutritive value was observed when lablab was between 75 and 112.5 seeding ratios of the mixture. In addition, increased crude protein concentration in the silage could potentially reduce crude protein supplementation to cattle and possibly reduce N excretion to the environment.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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