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Determination of the Effectiveness of Microbial Inoculants in Improving the Quality of Sorghum bicolor X Sorghum Sudanese Silage

Mikail Yeniçeri[1], Ayşe Gül Filik[2] , Hakan Kır[3] , Gökhan Filik[2]

[1]Agricultural of Biotechnology, Garduate School of Natural and Applied Sciences, Kırşehir Ahi Evran University, Kırşehir, Türkiye
[2]Department of Agricultural Biotechnology, Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Türkiye
[3]Department of Field Crops, , Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Türkiye

Abstract

This study was conducted to determine the effects of adding five different commercial inoculants to Sorghum BicolorXSorghum Sudanese silage on silage quality, microbial development, and aerobic stability. The study consisted of a control group (C) and five inoculant groups (SSSILD, SSSILAP, SSLAC, SSSILAL, SSMIC), prepared in 2 kg vacuum-sealed packages with eight replicates. The prepared silage samples were subjected to fermentation under laboratory conditions, and physical, chemical, microbiological, and aerobic stability analyses were performed after 90 days. Chemical analyses revealed that inoculant addition significantly reduced the pH levels of the silages (P<0.01), with the lowest pH value (4.55±0.00) observed in SSSILAP. The highest crude protein content (10.20±0.00%) was found in SSMIC, while the highest water-soluble carbohydrate (WSC) value (16.15°Brix) was recorded in Control group. Although no statistical differences were observed in color parameters among the groups, inoculant applications positively influenced color stability in terms of ΔE* and hue (h) values (P>0.05). Microbiological analyses showed the highest lactic acid bacteria population (11.00 log5 cfu/g) in SSMIC, with the lowest yeast and mold development. This supports the positive effect of inoculants on aerobic stability. Regarding nutritional value parameters, SSSILAL exhibited the highest digestible dry matter (60.16%), SSMIC had the highest total digestible nutrients (59.02%), Control, SSSILAP and SSMIC showed the highest metabolic energy (2.13 Mcal/kg), and SSSILAP and SSMIC had the highest net energy for lactation (NEL: 1.41 Mcal/kg). In conclusion, inoculant applications significantly improved silage quality, enhanced fermentation efficiency by lowering pH levels, suppressed yeast and mold formation, and improved aerobic stability. SSSILAL and SSMIC outperformed the others, particularly in terms of chemical composition, energy value, and microbiological quality.

Key Words: *Sorghum Bicolor X Sorghum Sudanese Plant, Silage Quality, Microbial Inoculant, Fermentation, Aerobic Stability, Lactic Acid Bacteria, Color Stability, Energy Value, Feed Quality*

Introduction

Silage is an effective preservation method that allows forage crops to be stored for extended periods through fermentation under anaerobic conditions and is used as a fundamental feed source in animal husbandry. High-quality silage production increases agricultural efficiency by reducing animal feeding costs and contributes to environmental sustainability (McDonald et al., 1991). Sorghum bicolor X Sorghum sudanese hybrids are increasingly utilized for silage due to their high biomass yield, drought tolerance, and adaptability to diverse climates (Bean et al., 2013). These hybrids are particularly valued for their ability to produce substantial dry matter yields under water-limited conditions, making them a viable alternative to traditional silage crops like maize in arid and semi-arid regions (Getachew et al., 2016). Studies have shown that Sorghum bicolor X Sorghum sudanese hybrids exhibit favorable nutritional profiles for silage, with moderate crude protein content and high digestible dry matter, enhancing their suitability for ruminant diets (Sánchez-Duarte et al., 2019). For instance, research by Marsalis et al. (2010) demonstrated that these hybrids, when harvested at the soft dough stage, achieve optimal fermentable carbohydrate levels, improving silage quality and aerobic stability. However, their high fiber content and variable carbohydrate levels can complicate fermentation, necessitating the use of microbial inoculants to enhance silage quality (Kung et al., 2003). Lactic acid bacteria (LAB) produce lactic acid by utilizing easily fermentable carbohydrates in plant material, lowering the silage pH, reducing nutrient losses, and inhibiting the growth of undesirable microorganisms (e.g., molds and yeasts) (Kung et al., 2003). Homofermentative bacteria (e.g., Lactobacillus plantarum, Pediococcus acidilactici) enhance aerobic stability through rapid acid production, while heterofermentative bacteria (e.g., *Lactobacillus buchneri*) are effective in preventing aerobic spoilage (Filya, 2004; Muck, 2010). Enzyme-containing inoculants (e.g., cellulase, xylanase) break down plant cell walls, providing more fermentable substrates for LAB and increasing silage digestibility (Özduven et al., 2017). Inoculants combining LAB and enzymes have been reported to reduce NDF and ADF contents and improve organic matter digestibility (Özduven et al., 2017). Similarly, Özduven et al. (2009) reported that LAB inoculants improved fermentation parameters and aerobic stability in corn silage. This study aims to investigate the effects

of different microbial inoculants (SSSILD, SSSILAP, SSLAC, SSSILAL, SSMIC) on the physical, chemical, microbiological, and aerobic stability properties of Sorghum bicolor x Sorghum sudanese silage, optimizing silage quality and nutritional value. The study seeks to contribute to the more effective use of alternative crops like Sorghum bicolor X Sorghum sudanese in sustainable livestock systems.

Materials and Methods

In this study, Sorghum-Sudan Grass Hybrid (Sorghum Bicolor X Sorghum Sudanese) harvested from the research field of Kırşehir Ahi Evran University's Department of Field Crops (Latitude: 39.1286°N, Longitude: 34.1078°E) was used as the primary silage material. The Sorghum Bicolor X Sorghum Sudanese was harvested at the dough stage, chopped to approximately 2.0 cm using a silage machine, and 1000 g of plant material was placed into 2 kg plastic bags. Control group (Control-SS) and five inoculant groups Sorghum Bicolor X Sorghum Sudanese+*Lactobacillus plantarum*, *Enterococcus faecium* bacteria and cellulase, pentosanase, amylase enzymes (SILAID, Global Nutritech Biotechnology LLC, USA), Sorghum Bicolor x Sorghum Sudanese+*L.plantarum, Pediococcus acidilactici, P. pentosaceus, Propionibacteria acidipropionici* and xylanase, β-glucanase enzymes (SILAP Timac Agro, USA), Sorghum Bicolor X Sorghum Sudanese+*Lactobacillus plantarum* only (LAC, Centro Sperimentale Del Latte, Italy), Sorghum Bicolor X Sorghum Sudanese+*L. plantarum, P. acidilactici, P. pentosaceus, P. acidipropionici* and xylanase, β-glucanase, cellulase, amylase enzymes (SILAL, Alltech, UK), Sorghum Bicolor X Sorghum Sudanese+*Lactobacillus brevis, Enterococcus faecium, Bacillus subtilis, Pediococcus acidilactici* and alpha–amylase (*A. oryzae*), cellulase, hemicellulase (*A. niger*) enzymes (MIC, Cuprem®, USA) were treated with inoculants at a concentration of 1×10^5 cfu/g, sprayed homogeneously onto the fresh material After inoculation, the air inside the bags was vacuumed using a vacuum device (Packtech PT-VKM-CPRO). Following vacuum sealing, a total of 30 silage samples (eight replicates per group) were incubated in laboratory conditions at 18.5±2°C in a dark environment for 90 days. At the end of the fermentation process, eight parallel samples from each group were taken, and physical, chemical, microbiological, and statistical analyses were conducted. Dry matter (DM), crude protein (CP), and ash contents of silage samples were analyzed according to AOAC (1998) methods. Organic matter (OM) content was calculated using the formula [%OM = 100 - %ash]. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyses were performed following the methods described by Van Soest et al. (1991), using amylase and sodium sulfite, with results expressed including residual ash. Additionally, NDF and ADF contents were corrected for residual ash and reported as NDFom and ADFom, respectively. Hemicellulose content was calculated as [Hemicellulose = NDF - ADF] as defined by Van Soest et al. (1991). Ether extract (EE) content was determined using the ANKOM XT15 Extraction System based on AOCS (2005) protocols. The pH values of silages were measured following Chen et al. (1994), and total soluble solids (TSM) contents were measured according to Singh et al. (2020). Total digestible nutrients (TDN), digestible crude protein (DCP), and metabolizable energy values were calculated using formulas reported by Filik (2020). Color measurements of silages were conducted using a Konica-Minolta CR-410 colorimeter after opening the silage packages, recording L*, a*, and b* values from three different regions of each sample. Chroma (C*, saturation index) and hue angle (h°) were calculated from a* and b* values following AMSA (2012) methods. Lactic acid bacteria, yeast, and mold counts in silage samples were determined using the method described by Seale et al. (1990). Carbon dioxide (CO_2) and pH values on the fifth day after opening the silage packages were measured according to the procedure reported by Ashbell et al. (1991). Relative feed value (RFV) and relative forage quality (RFQ) of silages were calculated using formulas reported by Kılıç and Abdiwali (2016) and Filik (2020). These analyses were conducted at the Animal Biotechnology Laboratory and the Enzyme and Microbial Biotechnology Laboratory of Kırşehir Ahi Evran University's Faculty of Agriculture, Department of Agricultural Biotechnology. Statistical analyses were performed using the SAS (2001) statistical package program. The General Linear Model (PROC GLM) procedure was applied based on a randomized complete block design, and linear relationships between experimental groups were evaluated using orthogonal polynomial contrast analysis. Differences between groups were determined using Duncan's Multiple Comparison Test (Genç & Soysal, 2018).

Results and Discussion

SSSILD had the highest DM content (941.00 g/kg), while SSMIC showed the lowest value (937.15 g/kg). The differences were significant ($p=0.0060$). No significant difference was found in OM and ash ratios ($p=0.1404$), indicating that the mineral content of the silages was similar. SSMIC had the highest CP (10.20%), while SSSILD showed the lowest value (8.87%). There were significant differences in terms of protein content ($p=0.0002$). SSMIC had the highest EE (4.95%), while SSSILAL showed the lowest value (3.86%) ($p<0.0001$). SSSILD has the highest CF (28.91%), ADF (38.74%) and NDF (65.82%) values, indicating higher fiber content. SSSILAL has the lowest ADF (36.90%) and ADFom (29.55%) values, indicating lower lignification and better digestibility. Square and cubic effects for CP, EE, ADF and NDF are significant ($p<0.05$), indicating that the effects of additives on nutrient content are not linear (Table 1). Fiber content (ADF, NDF) in the document aligns with Öten et al. (2024), where ADF ranged from 30.74–35% and NDF from 57.86–62%. The document's SSSILD (ADF: 38.74%, NDF: 65.82%) is higher, indicating lower digestibility, while SSSILAL's lower values (ADF: 36.90%, NDF:63.30%) match BMR cultivars' improved digestibility.

Table 1. Nutrient Content of Sorghum bicolor X Sorghum Sudanese silage

Parameters[5]		DM[1-4]	OM[2]	Ash[2]	CP[2]	EE[2]	CF[2]	ADF[2]	ADFom[3]	NDF[2]	NDFom[3]
Control		939.55^{BAC} ±0.15	92.68^{a} ±0.06	7.33^{A} ±0.06	9.97^{A} ±0.05	4.43^{B} ±0.00	28.10^{B} ±0.14	37.44^{C} ±0.02	30.11^{BC} ±0.05	62.94^{B} ±0.44	55.62^{B} ±0.38
SSSILD		941.00^{a} ±0.10	92.46^{A} ±0.00	7.55^{A} ±0.00	8.87^{c} ±0.00	3.94^{c} ±0.02	28.91^{A} ±0.11	38.74^{A} ±0.21	31.20^{A} ±0.21	65.82^{A} ±0.62	58.28^{A} ±0.61
SSSILAP		938.30^{DC} ±0.60	92.59^{A} ±0.09	7.42^{A} ±0.08	10.01^{a} ±0.17	4.51^{b} ±0.02	28.20^{b} ±0.22	38.09^{b} ±0.19	30.67^{bA} ±0.27	63.56^{B} ±0.54	56.14^{B} ±0.63
SSLAC		939.25^{bC} ±0.15	92.43^{A} ±0.01	7.58^{A} ±0.00	9.45^{B} ±0.11	4.37^{b} ±0.08	28.79^{A} ±0.16	38.23^{B} ±0.15	30.66^{BA} ±0.15	63.66^{B} ±0.33	56.09^{B} ±0.33
SSSILAL		940.05^{BA} ±0.55	92.65^{A} ±0.00	7.35^{A} ±0.00	9.27^{b} ±0.01	3.86^{C} ±0.04	27.96^{B} ±0.02	36.90^{d} ±0.03	29.55^{d} ±0.03	63.30^{B} ±0.00	55.95^{B} ±0.00
SSMIC		937.15^{D} ±0.55	92.44^{A} ±0.13	7.57^{A} ±0.14	10.20^{A} ±0.00	4.95^{A} ±0.04	28.43^{BA} ±0.04	37.56^{C} ±0.04	30.00^{DC} ±0.10	62.06^{B} ±0.96	54.50^{B} ±0.82
SED		0.1683	0.0287	0.0287	0.0350	0.0173	0.0551	0.0535	0.0641	0.2300	0.2174
P values		0.0060	0.1404	0.1404	0.0002	<.0001	0.0124	0.0006	0.0029	0.0389	0.0326
Effects ¥	L	0.0987	0.0964	0.0964	0.3380	0.0862	0.0644	0.0257	0.1652	0.9681	0.7711
	Q	0.5665	0.6848	0.6848	0.0200	0.0055	0.4365	0.0045	0.0128	0.0490	0.0436
	C	0.0055	0.0883	0.0883	<.0001	<.0001	0.0035	0.0033	0.0235	0.0246	0.0278

1 g/kg natural material, 2 (%) of dry matter, 3 ADFom=ADF ash, NDFom=NDF ash; 4 DM: In Air Dry Matter (g/kg); OM: Organic Matter (%); Ash (%); CP: Crude Protein (%); EE: Ether Extract (%); CF: Crude Fibre (%); ADF: Acid Detergent Fibre (%) and NDF: Neutral Detergent Fibre (%). a,b,c,d .Means within the same column with no common superscript differ. significantly (P<0.01). ¥ L: linear; Q: quadratic; C: cubic effects. SED: Standard error of the difference between 2 means.

SSSILAL had the highest NFE value (51.56%), while SSMIC showed the lowest value (48.87%) (p<0.0001). SSSILAL had the highest NFC (16.23%), while SSSILD showed the lowest value (13.83%). However, this difference was not statistically significant (p=0.2149). SSSILD had the highest TC (79.65%), while SSMIC showed the lowest value (77.30%) (p=0.0001). Control, SSSILAP and SSMIC had the highest energy values (DE: 2.60 Mcal/kg, ME: 2.13 Mcal/kg), while SSSILD showed the lowest energy values (DE: 2.54 Mcal/kg, ME: 2.08 Mcal/kg). NEL, NEM and NEG values also followed a similar trend. The square and cubic effects on energy values are significant (p<0.05), indicating that the effects of additives on energy content are complex (Table 2). Öten et al. (2024) reported digestible energy (DE) and metabolizable energy (ME) improvements with molasses, with DE values around 2.5–2.7 Mcal/kg, closely matching the document's range (2.54–2.60 Mcal/kg). The document's high-energy silages (Control, SSSILAP, SSMIC) align with these findings.

Table 2. Energy values of Sorghum bicolor X Sorghum Sudanese silage

Parameters[1, 2, 3]		NFE	NFC[1]	TC[1]	DE	ME	NE_L	NE_M	NE_G
Control		50.17^{C} ±0.15	15.35^{BA} ±0.45	78.29^{CB} ±0.01	2.60^{A} ±0.01	2.13^{A} ±0.00	1.32^{A} ±0.00	1.27^{A} ±0.00	0.70^{A} ±0.00
SSSILD		50.74^{b} ±0.08	13.83^{B} ±0.59	79.65^{a} ±0.02	2.54^{c} ±0.00	2.08^{c} ±0.00	1.29^{c} ±0.00	1.23^{c} ±0.00	0.66^{c} ±0.00
SSSILAP		49.87^{c} ±0.07	14.52^{bA} ±0.26	78.07^{C} ±0.28	2.60^{A} ±0.01	2.13^{A} ±0.00	1.33^{A} ±0.01	1.27^{A} ±0.01	0.71^{A} ±0.01
SSLAC		49.82^{c} ±0.14	14.95^{BA} ±0.30	78.61^{B} ±0.02	2.57^{b} ±0.00	2.11^{b} ±0.00	1.31^{b} ±0.01	1.25^{B} ±0.00	0.69^{b} ±0.00
SSSILAL		51.56^{a} ±0.07	16.23^{A} ±0.04	79.52^{A} ±0.05	2.56^{B} ±0.00	2.10^{B} ±0.00	1.30^{B} ±0.00	1.25^{B} ±0.00	0.68^{B} ±0.00
SSMIC		48.87^{D} ±0.13	15.24^{ba} ±1.13	77.30^{D} ±0.17	2.60^{A} ±0.00	2.13^{A} ±0.00	1.33^{A} ±0.00	1.28^{A} ±0.00	0.71^{A} ±0.00
Effects ¥	SED	0.0454	0.2356	0.0554	0.0014	0.0014	0.0011	0.0016	0.0011
	P values	<.0001	0.2149	0.0001	<.0001	0.0004	0.0004	0.0011	0.0001
	L	0.0073	0.8455	0.3471	0.0688	0.3794	0.4680	0.3153	1.0000
	Q	0.0371	0.1413	0.0224	0.0123	0.0123	0.1340	0.0498	0.0134
	C	0.0040	0.3746	0.0002	<.0001	<.0001	<.0001	0.0003	<.0001

[1](%) of dry matter;[2] Data represent the mean of four applications of each treatment; [3] NFE: nitrogen-free extract (g/kg); NFC: non-fibre carbohydrates (g/kg) and TC: total carbohydrates (g/kg), DE: digestible energy (Mcal/kg); ME: Metabolizable energy (ME Mcal/kg), NEL : net energylactation (Mcal/kg), NEM: net energy-maintenance (Mcal/kg), NEG : net energy-gain (Mcal/kg). a,b,c Means within the same column without common superscript are significantly different (P<0.01). ¥ L: linear; Q: quadratic; C: cubic effects. SED: Standard error of the difference between 2 means.

SSSILAL had the highest DDM (60.16%), while SSSILD had the lowest value (58.72%) (p=0.0006). SSMIC had the highest DMI (1.93%), while SSSILD had the lowest value (1.83%) (p=0.0444). SSMIC had the highest TDN (59.02%), while SSSILD had the lowest value (57.61%) (p=0.0003). SSMIC had the highest RFV (89.43) and RFQ (92.81) values, while SSSILD showed the lowest values (RFV: 83.00, RFQ: 85.40). Square and cubic effects were significant for DDM, TDN, RFV and RFQ (p<0.05) (Table 3). Öten et al. (2024) reported RFV values of 63.78–81.53 for Sorghum x Sudan grass hybrids, lower than the document's range (83.00–89.43). The document's SSMIC (RFV: 89.43) exceeds these values, likely due to lower ADF/NDF and higher CP. DDM and DMI values in the document are comparable to high-quality silages reported by Aguiar et al. (2006), where Sudan grass silage matched ruminant nutritional standards.

The control group had the highest temperature (20.28°C), while SSSILD showed the lowest temperature (16.18°C). The temperature differences are statistically significant (p<0.0001). Lower temperature may indicate better preservation of silage. SSSILAP had the lowest pH value (4.55), while the control group showed the highest

pH (4.68). Lower pH means better lactic acid fermentation and silage stability. SSLAC had the highest WSC value (17.40), indicating more fermentable carbohydrates. SSSILAL had the lowest WSC (15.23). SSSILAL has the highest L* (56.33, lighter color) and ΔE (59.77, total color difference) values, which may indicate that the color change is more pronounced. SSSILD has the highest a* (4.08, redness), while b* (yellowishness) and C* (chroma) values are generally similar. Although there are some significant differences in color parameters (e.g. p=0.0341 for h), no major differences are observed overall. Linear (L), quadratic (Q) and cubic (C) effects for temperature and pH are significant (p<0.0001). However, these effects are less pronounced in color parameters (Table 4). A study by Salman and Budak (2015) evaluated Sorghum x Sudan grass hybrids (e.g., Nutri Honey, Aneto) and reported pH values for silages ranging from 4.2 to 4.8, aligning with the document's range (4.55–4.68). The lower pH in SSSILAP (4.55) is consistent with high-quality silage, as pH below 4.6 is optimal for lactic acid fermentation.

Table 3. Forage quality of Sorghum bicolor X Sorghum Sudanese silage

Parameters[1, 2, 3]		DDM	DMI	TDN	RFV	RFQ
Control		59.74^{b} ±0.01	1.91^{a} ±0.02	58.81^{a} ±0.06	88.30^{a} ±0.64	91.16^{ba} ±0.75
SSSILD		58.72^{d} ±0.17	1.83^{b} ±0.02	57.61^{c} ±0.01	83.00^{b} ±1.02	85.40^{c} ±0.83
SSSILAP		59.23^{c} ±0.14	1.89^{a} ±0.01	58.85^{a} ±0.19	86.71^{a} ±0.95	90.36^{ba} ±1.08
SSLAC		59.12^{c} ±0.12	1.89^{a} ±0.01	58.23^{b} ±0.13	86.40^{a} ±0.62	89.24^{b} ±0.66
SSSILAL		60.16^{a} ±0.02	1.90^{a} ±0.00	58.09^{b} ±0.02	88.42^{a} ±0.02	89.54^{ba} ±0.03
SSMIC		59.64^{b} ±0.03	1.93^{a} ±0.03	59.02^{a} ±0.00	89.43^{a} ±1.44	92.81^{a} ±1.44
	SED	0.0422	0.0066	0.0395	0.3646	0.3687
	P values	0.0006	0.0444	0.0003	0.0204	0.0142
Effects ¥	L	0.0268	1.0000	0.2922	0.6369	0.8495
	Q	0.0046	0.0498	0.0259	0.0313	0.0424
	C	0.0035	0.0338	<.0001	0.0171	0.0060

[1] (%) of dry matter; [2] Data represent the mean of four applications of each treatment; [3] DDM: digestible dry matter (%); DMI: dry matter intake (live weight: LW, %); TDN: total digestible nutrients (%); RFV: relative feed value and RFQ: relative forage quality. a,b,c Means within the same column without common superscript are significantly different (P<0.01). ¥ L: linear; Q: quadratic; C: cubic effects. SED: Standard error of the difference between 2 means.

Table 4. Quality and color of Sorghum bicolor X Sorghum Sudanese silage

Parameters[1]		°C±SEM	pH	WSC (°Brix)	*L**	*a**	*b**	*ΔE**	*h*	*C**
Control		20.28^{a} ±0.16	4.68^{a} ±0.02	16.15^{ba} ±0.06	52.02^{bA} ±1.68	2.98^{b} ±0.15	17.91^{A} ±0.69	55.10^{A} ±1.81	80.56^{a} ±0.37	18.16^{A} ±0.70
SSSILD		16.18^{d} ±0.14	4.59^{bc} ±0.01	16.35^{a} ±0.26	49.22^{B} ±1.09	4.08^{A} ±0.38	17.94^{A} ±0.30	52.55^{A} ±1.12	77.22^{b} ±1.04	18.40^{A} ±0.35
SSSILAP		16.58^{c} ±0.09	4.55^{c} ±0.00	16.58^{ba} ±0.19	51.25^{ba} ±2.65	3.90^{Ba} ±0.26	18.51^{a} ±1.02	54.64^{a} ±2.82	78.09^{ba} ±0.54	18.92^{a} ±1.03
SSLAC		17.10^{b} ±0.04	4.59^{bc} ±0.01	17.40^{a} ±0.57	51.72^{ba} ±1.17	3.68^{ba} ±0.40	18.23^{a} ±0.59	54.97^{a} ±1.30	78.64^{ba} ±1.04	18.61^{a} ±0.62
SSSILAL		16.92^{cb} ±0.22	4.66^{a} ±0.01	15.23^{b} ±0.08	56.33^{a} ±3.11	3.22^{Ba} ±0.21	19.71^{A} ±1.06	59.77^{a} ±3.27	80.63^{a} ±0.88	19.97^{a} ±1.04
SSMIC		17.05^{b} ±0.06	4.64^{ba} ±0.03	15.45^{b} ±0.76	53.98^{ba} ±1.99	3.15^{Ba} ±0.26	19.04^{a} ±0.91	57.33^{a} ±2.15	80.54^{a} ±0.96	19.31^{a} ±0.89
SED		0.0544	0.0071	0.1732	0.8508	0.1184	0.3295	0.9054	0.3455	0.3307
P Value		<.0001	0.0003	0.0142	0.2778	0.0780	0.5923	0.3204	0.0341	0.6502
Effects ¥	L	<.0001	0.0008	0.2462	0.9044	0.1546	0.6762	0.8660	0.2130	0.6125
	Q	<.0001	0.0008	0.5439	0.4416	0.0344	0.8511	0.5233	0.0335	0.7348
	C	<.0001	0.5705	0.3891	0.5007	0.3508	0.7032	0.5274	0.2462	0.7644

[1] °C: Celsius degree; WSC: water soluble carbohydrate value (Brix degree 0 - 25°); L: Lightness; a*: Redness; b*: Yellowness; ΔE*: The total color difference; h: hue angle and C*: Chroma or saturation. a,b,c Means within the same column without common superscript are significantly different (p<0.01). ¥ L: linear; Q: quadratic; C: cubic effects. SED: Standard error of the difference between 2 means.*

DM (After Aerobic Stability), SSLAC had the highest DM (55.54%), while SSMIC showed the lowest value (48.91%) (p<0.0001). SSLAC had the lowest pH (4.36), while SSSILD showed the highest value (4.54) (p<0.0001). Low pH is advantageous in terms of aerobic stability. There is no significant difference in CO_2 production (p=0.9669), which may indicate that aerobic degradation was similar in all silages. SSMIC had the highest LAB count (11.00 $Log10^5$ cfu/g) before aerobic stability, while LAB counts were low in other silages (1.00-2.50 $Log10^5$ cfu/g). However, this difference is only marginally significant (p=0.0527). Linear, quadratic and cubic effects for DM and pH are significant (p<0.05). Öten et al. (2024) reported pH values of 3.99–4.5 post-aerobic exposure, aligning with the document's range (4.36–4.54). SSLAC's low pH (4.36) matches high-stability silages enhanced by molasses.

The low pH in SSLAC (4.36) indicates effective preservation, Likely due to high organic acid content (e.g., acetic acid), which inhibits spoilage organisms. This aligns with findings by Kung et al. (2003), who reported that rapid pH decline in silages treated with homofermentative LAB enhances preservation by limiting the growth of yeasts and molds. Similarly, Contreras-Govea et al. (2013) observed that sorghum silages with pH below 4.4, driven by increased lactic and acetic acid production, exhibited superior fermentation quality. The higher pH in SSSILD

(4.54) suggests reduced stability, possibly due to lower acid production or consumption by aerobic microbes, consistent with Muck (2010), who noted that inadequate acid accumulation can lead to secondary fermentation and reduced aerobic stability. The lack of significant CO_2 differences implies that all silages experienced comparable aerobic microbial activity post-exposure, despite differences in pH and DM. This could indicate that CO_2 production is less sensitive to additive effects in this study, as supported by Ashbell et al. (1991), who found that CO_2 levels in silages may not always correlate with microbial inoculant efficacy due to variable aerobic deterioration rates. Weinberg and Muck (1996) further suggest that CO_2 production is influenced by residual fermentable substrates, which may be similar across treatments in this case. LAB Count: SSMIC's high LAB count (11.00 Log10 cfu/g) reflects strong fermentation, likely driven by homofermentative or heterofermentative LAB inoculants. Filya (2004) reported that high LAB populations in inoculated silages enhance lactic acid production, improving fermentation efficiency. However, the low DM (48.91%) in SSMIC suggests that LAB activity may have consumed DM during aerobic exposure, reducing stability, as noted by McAllister et al. (2018), who found that excessive LAB activity in sorghum silages can lead to DM losses under aerobic conditions. Other treatments (e.g., SSLAC) with lower LAB counts maintained higher DM, indicating better aerobic stability, consistent with Contreras-Govea et al. (2013), who emphasized that balanced LAB populations optimize both fermentation and aerobic stability in sorghum silages.

Table 5. Effects of Sorghum bicolor X Sorghum Sudanese silages

Parameters[5]	DM after Aerobic Stability	pH_2	CO_2	Yeast After Aerobic Stability (Log 10^5 Cfu/g)	Lactic Acid Bacteria Before Aerobic Stability (Log10^5 cfu/g)	Lactic Acid Bacteria (Anaerobic) (Log10^5 cfu/g)	Molt After Aerobic Stability
Control	49.36^{cb} ±0.41	4.48^{b} ±0.01	2.20^{a} ±0.31	-	1.00^{B} ±	-	-
SSSILD	49.15^{C} ±0.01	4.54^{a} ±0.01	2.08^{a} ±0.07	-	1.00^{B} ±	-	-
SSSILAP	50.05^{B} ±0.03	4.40^{c} ±0.02	2.08^{a} ±0.07	-	1.50^{B} ±0.50	-	-
SSLAC	55.54^{A} ±0.34	4.36^{d} ±0.00	2.02^{a} ±0.13	-	2.50^{B} ±0.50	-	-
SSSILAL	49.43^{cb} ±0.23	4.48^{b} ±0.01	2.14^{a} ±0.13	-	1.00^{B} ±	-	-
SSMIC	48.91^{c} ±0.08	4.46^{b} ±0.02	2.14^{a} ±0.13	-	11.00^{A} ±2.00	-	-
SED	0.0969	0.0049	0.0458	-	0.7905	-	-
P values	<.0001	<.0001	0.9669	-	0.0527	-	-
Effects ¥ L	<.0001	<.0001	0.4644	-	0.6328	-	-
Q	<.0001	0.0006	0.8447	-	0.8287	-	-
C	0.0042	<.0001	0.8033	-	1.0000	-	-

CO_2: Amount of Carbon Dioxide; pH: After Aerobic Stability pH. Means within the same column without common superscript are significantly different (P<0.01). SED: Standard error of the difference between two means. L: Linear; Q: Quadratic; C: Cubic effects.

Conclusion

The quality of Sorghum bicolor X Sorghum Sudanese silages varies significantly depending on the LAB inoculant applied. Especially SSLAC and SSMIC (homofermantative) groups showed superior performance compared to other groups in terms of silage quality, nutritional values and microbiology. Both high energy content and good feed quality parameters in these groups support the preference of these inoculants in practice. In conclusion, this study shows that different LAB inoculants can significantly improve the properties of Sorghum bicolor X Sorghum Sudanese Silage silages. This can provide important contributions to farmers in terms of reducing feed costs and increasing animal health and performance. Future research should focus on optimizing inoculant combinations, different plant species, and regional climate conditions. Additionally, more comprehensive studies on the long-term effects and economic feasibility of inoculants could support the widespread adoption of this technology at a commercial scale. Such studies will contribute to developing innovative and environmentally friendly solutions in global feed production.

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