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Sensory and chemical evaluation of laboratory ensiled hybrid Napier grass prepared using *Lactobacillus plantarum* and propionic acid as additives#

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Abstract

The present study was undertaken to evaluate silage made from hybrid Napier grass (Pennisetum purpureum) ensiled in the laboratory using various additives with regard to sensory as well as chemical characteristics. Four different silages were prepared viz., grass ensiled without additives (GS) as control, and grass silage with Lactobacillus plantarum at 1 x 10^5 CFU per gram of fresh forage (GSL), grass silage with propionic acid at the rate 0.45 kg per ton of fodder (GSA), and grass silage with combination of Lactobacillus plantarum (1 x 10^5 CFU per gram of fresh forage) and propionic acid (0.45 kg per ton of fodder) (GSLA) in triplicates. One from each triplicate was opened on 21, 30 and 45 days of ensiling and was evaluated for sensory (colour, aroma, presence or absence of extraneous matter) and chemical (pH, lactic acid, proximate analysis and fibre fractions) characteristics. From the overall results, it could be concluded that hybrid Napier grass, ensiled for 21 days using L. plantarum was effective in preserving its nutritive value.

Keywords: *Laboratory ensiling, silage score card, hybrid Napier grass, Lactobacillus plantarum, propionic acid*

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Livestock plays an important role in Indian rural economy (Hosure *et al*., 2022) and maximisation of returns from dairy enterprises requires optimum utilisation of resources (George *et al*., 2022). Ensuring the availability of green fodder throughout year has been identified as a major constraint in economizing dairy cattle production in India (Kumar *et al*., 2015). Silage making is one of the economical methods of fodder preservation that could help to meet green fodder requirements during lean seasons (Stewart, 2011). Loss of nutrients during ensiling has been identified as a major challenge for effective silage production. Thus, strategies need to be optimized for preserving the nutritive value of forages while ensiling (Balehegn *et al*., 2022). Use of silage additives to modify fermentation process and to minimise nutrient losses from preserved fodder has been widely acknowledged.

Bacterial inoculum and organic acids can be used as silage additives. The technical and research innovations for identifying an ideal additive for silage preparation need to be explored. Quality of silage additives can be initially screened by laboratory ensiling of small quantity of fodder. Hence, the present study has been under taken to evaluate silage made from hybrid Napier grass ensiled in the laboratory prepared with or without additives.

Materials and methods

Laboratory ensiling

Laboratory ensiling of fodder grass was undertaken at Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy. Grass fodder ensiled with or without additives was evaluated for its physical and chemical characteristics and the silage adjudged to have highest nutritional quality was identified during the course of this study.

Hybrid Napier grass (*Pennisetum purpureum*) harvested at 45 days of maturity was procured from University Livestock Farm Fodder Research Development Scheme (ULF & FRDS), Mannuthy. *L. plantarum* and propionic acid (99 per cent pure) were used as silage additives for laboratory ensiling during the present study.

The chopped grass (2 to 3 cm length) was filled into a strong high-density polythene bag of size (45×60 cm) having a capacity to hold 2 to 6 kg of green fodder. Polythene bags used were clean, transparent and without any holes. During the study, grass fodder was ensiled without additives and with additives namely bacterial inoculum of *L. Plantarum* at a concentration of 1 x 10^5 CFU per gram of green fodder, propionic acid at the rate 0.45 kg per ton of green fodder, and a combination of both, as detailed in the Table 1. All four silages were prepared in three replicates. Ensiled mass was then gently and firmly squeezed by hand to expel air, and while compressing, the neck of the bag was twisted, turned over and tied with twine. Polythene bags containing chopped fodder were then inverted into another empty clean bag, which was also later closed and tied. The polythene bags were appropriately labelled showing details of date of packing and due date for opening. Sealed bags were then carefully placed in a plastic bucket to ensure protection from vermin and were stored at room temperature.

Table 1. Different types of grass silages prepared in laboratory

Sl. No	Details of silages prepared
1	Grass ensiled without additive (control) (GS)
2	Grass + bacterial inoculum (1 x 10^5 CFU per gram of fresh forage) (GSL)
3	Grass + propionic acid (0.45 kg per ton of fodder) (GSA)
4	Grass + bacterial inoculum (1 x 10^5 CFU per gram of fresh forage) + propionic acid (0.45 kg per ton of fodder) (GSLA)

One from each triplicate was opened on 21, 30 and 45 days of ensiling.

Evaluation and selection of ensiled grass fodder

The ensiled materials were evaluated for their physical and chemical characteristics at 21st, 30th and 45thday of ensiling. A score card was devised for evaluating the physical and chemical characteristics of silages obtained from laboratory ensiling. Total score of silage was calculated by taking the sum of assigned scores.

Physical characteristics of silage

Silage odour was measured using score card devised. The sweet aroma, pungent and putrid odours were assigned +1, -1, and -2 scores, respectively.

Silage colour was evaluated using the colour scores table for silages developed by Tahuk *et al*. (2021). The natural green or yellowish green colour was given score of +2, dark green or yellowish colour was awarded +1 and brown to black colour was given score of -1, respectively.

Presence or absence of extraneous material as fungus or worms were observed and evaluated using score card. The absence of extraneous material was given a +1 score and their presence was given a -1 score, respectively.

Chemical characteristics of silage

Silage pH was evaluated using the score card. The pH of silage was estimated by method described by Australian Fodder Industry Association Laboratory Methods Manual (2011).

Silage samples collected at the end of ensiling period were used for the estimation of lactic acid (g/100g fresh silage) following the procedure given by Wilson and Wilkins (1972), using a spectrophotometer (Avi Scientific India, Thane, Mumbai).

The proximate composition, fibre fraction and acid insoluble ash were analysed by AOAC (2016). The toluene dry matter (DM) of ensiled material was calculated using formula given by Haigh (1979).

Toluene DM = 0.95 Oven DM + 3.3

Results and discussion

Sensory characteristics of silages

The sensory and chemical characteristics of various silages at 21st, 30th and 45th day of ensiling are presented in Tables 2, 3 and 4, respectively. GS had an undesirable pungent odour at 21st, 30th and 45th day of sampling. A dark green colour was observed on 2st day of ensiling while brown to black colour was recorded on 30th and 45th day of ensiling. Fungal growth was observed on the 45th day of ensiling. GSL produced a desirable sweet aroma in all samples collected at 21st, 30th and 45th day of ensiling. A yellowish green colour was observed at the 21st day of ensiling but the colour changed to dark green towards the 30th and 45th day of ensiling. Presence of fungus or worms was not observed while using *L. plantarum* as additive during various periods of ensiling. GSAhad an undesirable pungent odour and dark green colour at all three periods of sampling. The silage material was devoid of the presence of any fungus or worms at 21st, 30th and 45th day of sampling. GSLA had a sweet aroma at 21st and 30th day of ensiling while it had an undesirable pungent odour at the 45th day of sampling. A dark green colour was observed at each ensiling period and the ensiled materials were devoid of the presence of fungus or worms. Based on the results, GSL was found to be better in preserving the nutritive value of grass fodder. Similar observations were made by Obrien *et al.* (2008), who noted that the excellent quality silages had fresh texture and should not contain any fungal organisms in it. In a similar study, Randa *et al.* (2018) observed a light yellow to greenish colour and an acidic pungent smell for Napier grass ensiled for 45 days. They opined that good silage should possess moderately sweet aroma and this observation was similar to the present study. Habib *et al.* (2022) found that the colour of Napier grass silage as straw yellowish with a sweet fruity aroma and his observation was in collaboration with present the present study.

Table 2. Sensory and chemical characteristics of silages at 21st day of ensiling

Sl. No		Silage characteristics	Score obtained for silages			
			GS	GSL	GSA	GSLA
1		pH	5.6	4.3	5.2	4.4
	a	Less than 4.2 (+++)	0	0	0	0
	b	4.2-4.5(++)	0	+2	0	+2
	c	4.5-4.8(+)	0	0	0	0
	d	More than 4.8 (-)	-1	0	-1	0
2		Odour				
	a	Sweet Aroma (+)	0	+1	0	+1
	b	Pungent (-)	-1	0	-1	0
	c	Putrid (- -)	0	0	0	0
3		Appearance (colour)				
	a	Natural green or yellowish green (++)	0	+2	0	0
	b	Dark green or brownish green (+)	+1	0	+1	+1
	c	Brown to black (-)	0	0	0	0
4		Presence or Absence of extraneous matter Fungus or Worms (-/+)	+1	+1	+1	+1
		Total score	0	+6	0	5

Table 3. Sensory and chemical characteristics at 30th day of ensiling

Sl. No		Silage characteristics	Scores obtained for silages			
			GS	GSL	GSA	GSLA
1		pH	5.7	4.4	5.4	4.4
	a	Less than 4.2 (+++)	0	0	0	0
	b	4.2-4.5(++)	0	+2	0	+2
	c	4.5-4.8(+)	0	0	0	0
	d	More than 4.8 (-)	-1	0	-1	0
2		Odour				
	a	Sweet Aroma (+)	0	+1	0	+1
	b	Pungent (-)	-1	0	-1	0
	c	Putrid (- -)	0	0	0	0
3		Appearance (colour)				
	a	Natural green or yellowish green (++)	0	0	0	0
	b	Dark green or brownish green (+)	0	+1	+1	+1
	c	Brown to black (-)	-1	0	0	0
4		Presence or Absence of extraneous matter-Fungus or Worms (-/+)	+1	+1	+1	+1
		Total score	-2	+5	0	+5

Silage pH

The pH value of GS ranged from 5.6 to 5.9. However, the pH of GSL silage at 21st, 30th and 45th day of ensiling were found to be 4.3, 4.4, and 4.6 respectively. The pH of GSA ranged from 5.2 to 5.8; while that of GSLA ranged from 4.4 to 4.9. Kung *et al.* (2018) stated that pH values of good quality silage ranged between 4.3 and 4.7. Johnson *et al.* (2005) observed that the pH of the silages prepared with and without bacterial inoculum were 3.94 and 4.21. These observations were in accordance with the present study.

Chemical composition of silages

The chemical composition of various silages at 21st, 30th and 45th day of ensiling are presented in Tables 5, 6 and 7, respectively. At different periods of ensiling, GS had the DM content of 23.49 to 24.52 per cent, while GSL had 24.26 to 25.36 per cent DM. Similarly, the GSA and GSLA had the DM content of 24.17 to 24.31 and 23.25 to 23.70 per cent, respectively.

Table 4. Sensory and chemical characteristics at 45th day of ensiling

Sl. No		Silage characteristics	Scores obtained for silages			
			GS	GSL	GSA	GSLA
1		pH	5.9	4.6	5.8	4.9
	a	Less than 4.2 (+++)	0	0	0	0
	b	4.2-4.5(++)	0	0	0	0
	c	4.5-4.8(+)	0	+1	0	0
	d	More than 4.8 (-)	-1	0	-1	-1
2		Odour				
	a	Sweet Aroma (+)	0	+1	0	0
	b	Pungent (-)	-1	0	-1	-1
	c	Putrid (- -)	0	0	0	0
3		Appearance (colour)				
	a	Natural green or yellowish green (++)	0	0	0	0
	b	Dark green or brownish green (+)	0	+1	+1	+1
	c	Brown to black (-)	-1	0	0	0
4		Presence or Absence of extraneous matter-Fungus or Worms (-/+)	-1	+1	+1	+1
		Total score	-4	+4	0	0

Similar observations were made by Oliveira *et al.* (2017), who noted that the DM content of silages made from tropical grasses ranged between 24.85 to 31.80 per cent.

After laboratory ensiling, GS had the CP content of 6.40 to 6.90 per cent and GSL had CP content of 9.72 to 9.73 per cent. The CP content ranged from 8.37 to 8.60 per cent, in GSA whereas GSLA had CP content of 8.45 to 8.88 per cent, respectively. In accordance with the values observed in this study, Yammuen *et al.* (2020) observed that *L. plantarum* as additive had resulted in silage having a CP content of 6.80 per cent. Similarly, Xiong *et al.* (2022) reported that when *L. plantarum* inoculated hybrid Napier silage had a CP content of 8.20 per cent.

During lab enisling, all the four silage combinations had a CF content that ranged between 28.43 to 29.63 per cent. GS had the EE content of 1.72 to 1.75 per cent, while GSL had 2.10 to 2.98 per cent EE. Similarly, the EE content in GSA and GSLA were 1.88 to 2.26 and 2.28 to 3.23 per cent, respectively. In accordance with the results of present study, Srisaikham (2022) observed that CF content and EE content were 26.85 and 2.48 per cent, respectively in Napier grass ensiled for 21 days.

After laboratory ensiling, GS had the NDF content of 66.62 to 66.72 per cent and GSL silage had NDF content of 59.62 to 60.65 per cent. The NDF content ranged between 59.49 to 63.62 per cent, in GSA whereas GSLA had NDF content of 62.68 to 63.65 per cent, respectively. The ADF content of GS and GSL silages were ranged from 49.07 to 49.43 per cent. The GSA silage had ADF content ranging from 50.38 to 50.42 while that of GSLA was 54.44 to 55.38 per cent respectively.

Similarly, Mbuthia and Gachuiri (2003) reported that ensiling had resulted in a reduction of NDF value from 52 to 61.30 per cent in Napier grass silage and Bureenok *et al*. (2012) reported that the NDF content ranged between 62.30 to 72.60 per cent when grass was preserved with Lactic Acid Bacterial (LAB) inoculum.

Lactic acid concentration **(g/100g fresh silage)**

The lactic acid concentration was found to be in the range of 0.11 to 0.22 for GS. For GSL silage at 21st, 30th and 45th day of sampling the lactic acid concentrations were 5.47, 4.22 and 2.39 respectively (Table 8). The lactic acid concentration of GSA ranged between 0.13 to 0.51 and the values for GSLA

Table 5. Chemical composition of various silages at 21st day of ensiling

Parameters	Nutritive values (%, on DM basis)				p-value
	GS	GSL	GSA	GSLA	
Dry Matter	23.49^{c} ± 0.08	25.36^{a} ± 0.21	24.17^{b} ± 0.03	23.25^{c} ± 0.06	<0.001**
Crude Protein	6.90^{c} ± 0.02	9.72^{a} ± 0.07	8.37^{b} ± 0.03	8.45^{b} ± 0.11	<0.001**
Crude Fibre	28.43^{b} ± 0.06	28.62^{b} ± 0.10	28.61^{b} ± 0.06	29.58^{a} ± 0.10	<0.001**
Ether Extract	1.75^{c} ± 0.04	2.98^{a} ± 0.03	1.88^{b} ± 0.03	2.94^{a} ± 0.02	<0.001**
Total Ash	10.73^{a} ± 0.09	10.71^{a} ± 0.06	10.40^{b} ± 0.04	10.09^{c} ± 0.08	<0.001**
NFE	52.19^{a} ± 0.15	47.97^{d} ± 0.07	50.74^{b} ± 0.08	48.94^{c} ± 0.10	<0.001**
NDF	66.67^{a} ± 0.05	60.44^{c} ± 0.08	59.49^{d} ± 0.07	62.68^{b} ± 0.06	<0.001**
ADF	49.10^{d} ± 0.07	49.33^{c} ± 0.05	50.42^{b} ± 0.04	54.44^{a} ± 0.07	<0.001**

** Significantly different with in the row

Table 6. Chemical composition of various silages at 30th day of ensiling

Parameters	Nutritive values (%, on DM basis)				p-value
	GS	GSL	GSA	GSLA	
Dry Matter	24.52^{b} ± 0.21	25.31^{a} ± 0.15	24.31^{b} ± 0.09	23.58^{c} ± 0.07	<0.001**
Crude Protein	6.41^{d} ± 0.04	9.73^{a} ± 0.01	8.60^{c} ± 0.08	8.80^{b} ± 0.003	<0.001**
Crude Fibre	28.48^{b} ± 0.11	28.46^{b} ± 0.04	28.67^{b} ± 0.08	29.52^{a} ± 0.09	<0.001**
Ether Extract	1.74^{c} ± 0.07	2.10^{b} ± 0.07	1.99^{b} ± 0.03	2.28^{a} ± 0.04	<0.001**
Total Ash	10.61^{a} ± 0.04	10.41^{b} ± 0.08	10.45^{b} ± 0.03	10.30^{b} ± 0.09	<0.001**
NFE	52.76^{a} ± 0.14	49.30^{d} ± 0.10	50.29^{b} ± 0.16	49.10^{c} ± 0.10	<0.001**
NDF	66.62^{a} ± 0.06	59.62^{c} ± 0.06	59.57^{c} ± 0.09	63.62^{b} ± 0.11	<0.001**
ADF	49.07^{d} ± 0.07	49.43^{c} ± 0.09	50.38^{b} ± 0.04	54.50^{a} ± 0.07	<0.001**

** Significantly different with in the row

Table 7. Chemical composition of various silages at 45th day of ensiling

Parameters	Nutritive values (%, on DM basis)				p-value
	GS	GSL	GSA	GSLA	
Dry Matter	24.26^{a} ± 0.21	24.36^{a} ± 0.10	24.13^{a} ± 0.09	23.70^{b} ± 0.23	<0.001**
Crude Protein	6.40^{d} ± 0.24	9.73^{a} ± 0.15	8.54^{c} ± 0.08	8.88^{b} ± 0.06	<0.001**
Crude Fibre	28.55^{b} ± 0.08	28.62^{b} ± 0.05	28.81^{b} ± 0.09	29.63^{a} ± 0.10	<0.001**
Ether Extract	1.72^{c} ± 0.01	2.11^{b} ± 0.10	2.26^{b} ± 0.23	3.23^{a} ± 0.17	<0.001**
Total Ash	10.59 ± 0.09	10.41± 0.01	10.27 ± 0.18	10.30± 0.08	0.194^{ns}
NFE	52.74^{a} ± 0.12	49.13^{c} ± 0.12	50.12^{b} ± 0.25	47.96^{d} ± 0.06	<0.001**
NDF	66.72^{a} ± 0.03	60.65^{c} ± 0.05	63.62^{b} ± 0.08	63.65^{b} ± 0.08	<0.001**
ADF	49.16^{c} ± 0.05	49.36^{c} ± 0.09	50.40^{b} ± 0.08	55.38^{a} ± 0.11	<0.001**

** Significantly different with in the row, ns- non significant

Table 8. Lactic acid concentration (g/100g fresh silage) of various silages at different periods of ensiling

Ensiling Period	Different combinations of silage				p– value
	GS	GSL	GSA	GSLA	
21 Days	0.20^{d} ± 0.001	5.47^{a} ± 0.015	0.51^{c} ± 0.002	4.07^{b} ± 0.030	<0.001**
30 Days	0.17^{d} ± 0.002	4.22^{a} ± 0.030	0.33^{c} ± 0.002	2.36^{b} ± 0.005	<0.001**
45 Days	0.11^{c} ± 0.003	2.39^{a} ± 0.035	0.13^{c} ± 0.004	1.98^{b} ± 0.008	<0.001**

** Significantly different with in the row

ranged from 1.98 to 4.07. Similarly, Boonkoed *et al.* (2018) reported that lactic acid concentration of Napier silage was 4.45 per cent of total volatile fatty acid. Based on the total of various grass silages, it could be concluded that use of additives had improved the sensory attributes and chemical characteristics of silage. Hence GSL at 21st day of ensiling was adjudged to be the best quality silage.

Conclusion

Based on the results of laboratory ensiling, it was found that the Hybrid Napier grass ensiled with *L. plantarum* as additive at the rate of 1 x 10^5 CFU per gram of fresh forage had superiorly preserved the nutritive value of green grass than other three groups. *L. plantarum* helps in rapid reduction of silage pH and increased lactic acid production that enhances the keeping quality of ensiled material. Hence, use of *L. plantarum* had resulted in production of superior quality silage with acceptable acidic pH, greenish yellow colour, desirable aromatic smell, and higher lactic acid concentration. Observations made in the current study also suggested that combination of additives (*L. plantarum* and propionic acid) also resulted in production of good quality silage. However, use of propionic acid alone could not result in production of good quality silage. From the overall results, it could be concluded that hybrid Napier grass, ensiled for 21 days using *L. plantarum* was effective in preserving its nutritive value,

Conflict of interest

The authors declare that they have no conflict of interest.

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