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Influence of Additives and Fermentation Periods on Silage Characteristics, Chemical Composition, and In Situ Digestion Kinetics of Jambo Silage and Its Fodder in Nili Buffalo Bulls

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Abstract: Jambo grass (*Sorghum bicolor* × *Sorghum sudanese*) harvested 50 days after sowing was ensiled in laboratory silos using 2 additives, cane molasses and ground corn grains, each at the rate of 2%, 4%, and 6% levels for 30, 35, and 40 days at room temperature (28 °C). Data regarding incubation days were pooled. The pH and lactic acid concentration in jambo grass silage were not affected by the additive type, level, or fermentation period. Dry matter (DM) content of jambo silage was significantly ($P < 0.05$) affected by additive type; however, additive levels and fermentation periods did not affect its DM contents. Crude protein content of jambo grass was not affected by ensiling time, additive type, or level. The true protein contents of jambo grass silage were significantly ($P < 0.05$) higher with molasses than with ground corn. Neutral detergent fiber (NDF) and hemicellulose content of jambo grass were significantly ($P < 0.05$) affected by additives and their levels, but fermentation periods did not influence the NDF contents of jambo grass silage. Cellulose, acid detergent fiber, and acid detergent lignin contents of jambo silage were not affected by additive type, level, or fermentation period. Therefore, jambo grass ensiled with 2% molasses for 30 days was selected for in situ digestion kinetics in bulls. Dry matter and NDF degradabilities of jambo grass were significantly higher than those of its silage at 48 h of ruminal incubation. Ruminal lag time, and rate and extent of DM and NDF degradation were similar for jambo grass and its silage. The results of this study implied that jambo grass ensiled with 2% molasses for 30 days has similar nutritive value to that of its forage in bulls.

Key Words: Jambo silage, additive, digestion kinetics, buffalo bull

Introduction

Irregular and inadequate supply of quality forage is the most critical constraint for profitable livestock production in developing countries. In south Asia, the rapidly growing human need for food has limited the area under fodder cultivation. Low per acre fodder yield coupled with fodder scarcity periods has further deteriorated fodder availability (1). Ensiling of multi-cut high yielding fodders during the fodder availability period could bridge the gap between supply and demand of fodder in the region.

The most preferred crop for ensiling is maize (2); however, jambo grass (*Sorghum bicolor* × *Sorghum sudanese*) is a highly nutritious multi-cut hybrid fodder and could be ensiled to meet the fodder needs during lean forage availability periods (1). For better preservation, the fodder must have high concentrations of fermentable

carbohydrates, low buffering capacity, relatively low dry matter (DM) content (20%-30%), and adequate lactic acid bacteria. However, some non-leguminous fodders harvested at full maturity contain low concentrations of non-structural carbohydrate and thus various additives like molasses (2) and ground grains (3) were used as a source of nonstructural carbohydrate during ensilage to increase the lag phase in silage.

There is little information available about the effect of fermentable carbohydrates and fermentation periods on chemical composition and ruminal digestion kinetics of jambo grass and its silage in buffaloes. Therefore, the objective of the present project was to establish a suitable additive, its level, and duration of fermentation for ensilage of jambo grass and to compare the digestion kinetics of jambo grass and its silage in ruminal cannulated buffalo bulls.

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Materials and Methods

Fodder

Jambo grass seed was procured from Imperial Chemicals Industry (ICI) Pakistan Limited and was sown in the fields adjacent to the Animal Nutrition Research Center, University of Agriculture, Faisalabad, Pakistan. Jambo grass was harvested at 50 days after sowing and samples were analyzed for DM yield and nutrient concentration. The fodder was chopped in a locally manufactured chopper. Samples were dried at 55 °C and ground to particle size of 2 mm through a Wiley mill. These samples were analyzed for DM, nitrogen (N) content, and total ash using the methods described by AOAC (4), and neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and acid detergent lignin (ADL) by the methods reported by Van Soest et al. (5).

Preparation of laboratory silos

Jambo grass chopped with a locally manufactured chopper was ensiled in laboratory silos (45 × 30 × 30 cm) using 2 additives, cane molasses and ground corn grains, each at the rate of 2%, 4%, and 6% levels for 30, 35, and 40 days at room temperature (28 °C). The data regarding fermentation period of 30, 35, and 40 days were pooled and presented as a single figure. After opening the laboratory silos, pH and lactic acid contents were determined immediately (6). These samples were also analyzed for DM, N, true protein (TP), and total ash using the methods described by AOAC (4), and NDF, ADF, hemicellulose, cellulose and ADL by the methods reported by Van Soest et al. (5).

In situ trial

Jambo grass ensiled with 2% molasses for 30 days was chosen and ensiled in bunker silos for in situ digestion kinetics study. Eight ruminally cannulated Nili buffalo (*Bubalus bubalis*) bulls were blocked in 2 groups according to their body weights. The buffalo bulls were housed on a concrete floor in separate pens. Ten days were given as an adaptation period to the diet at the start of the experiment, followed by 4 days of incubation for the in situ nylon bags. Ruminally cannulated buffalo bulls were fed the same diet (jambo grass or its silage) as a sole diet at the rate of 2% of their body weights as they were being incubated in their rumen to avoid the effects of diet on the ruminal fermentation (7).

The jambo grass and its silage were ground to 2 mm through a Wiley mill. Daily consumption of jambo grass was 6.75 kg DM by the buffalo bulls. Nylon bags measuring 10 × 23 cm, with an average pore size of 50 µm, were used. Each bag contained a 10 g sample of jambo grass and its silage (DM basis) separately. The bags were closed and tied with nylon fishing line and were exposed to ruminal fermentation for 1, 2, 4, 6, 10, 16, 24, 36, 48, and 96 h. For each time point, there were 3 bags for each sample. Two bags were used to determine DM and NDF digestion, while the third was kept as a blank. All these bags were soaked in distilled water (39 °C) for 15 min just before placing them into the rumen (8). These bags were placed in the rumen in reverse sequence and all bags were removed at the same time to reduce variation associated with the washing procedure (9). After removal from the rumen, these bags were washed in running tap water until the rinse was clear. The bags were then dried in a forced air oven at 55 °C. After equilibration, the bags were weighed back and residues were transferred to 100 ml beakers for NDF analysis. Digestion coefficient of DM and NDF were calculated at 48 h of incubation. Rate of disappearance, lag time, and extent of digestion of DM and NDF of jambo grass and its silage were determined by the methods described by Sarwar et al. (10).

Statistical analysis

The data on each parameter (lag time, rate and extent of digestion of DM and NDF) were analyzed according to completely randomized design. In the case of significant differences ($P < 0.05$), the means were subjected to pairwise comparison using Duncan's multiple range test (11).

Results

Chemical composition of jambo grass

The chemical analysis revealed that jambo grass harvested at 50 days of age had 18.0% DM, 11.0% CP, and 75.2% NDF, while ADL, cellulose and ash contents were 39.7%, 35.4%, and 8.59%, respectively (Table 1).

Characteristics of jambo grass silage

The pH of jambo grass silage was not affected by the additive type or level (Table 2). The same response was noted for fermentation period. Maximum (3.98) and minimum (3.78) pH was recorded at 2% ground corn

Table 1. Chemical composition of jambo grass¹ on dry matter basis.

Parameters	Composition (%)
Dry matter	18.0 ± 2.12
Crude protein	11.0 ± 1.02
Neutral detergent fiber	75.2 ± 3.25
Acid detergent fiber	39.7 ± 2.02
Acid detergent lignin	4.30 ± 1.01
Hemicellulose	35.5 ± 3.11
Cellulose	35.4 ± 4.35
Ash	8.59 ± 1.08

¹Jambo grass was harvested at 50 days of age

after 30 days of fermentation and 6% corn after 40 days of fermentation, respectively. In the case of molasses, maximum and minimum pH was 3.76 and 3.50 at 2% molasses after 30 days of fermentation and 6% molasses after 40 days of fermentation, respectively. Lactic acid concentration also remained unaltered by additive type and level (Table 2).

Chemical composition of jambo grass silage

DM contents of jambo silage were significantly ($P < 0.05$) affected by additive type. It was higher when jambo grass was ensiled with molasses than with corn grain; however, additive levels, fermentation periods, and interactions did not affect DM contents of jambo grass during ensiling (Table 2).

Crude protein contents of jambo grass were not affected by the additive type, level, or fermentation periods (Table 2). The TP content of jambo grass silage was significantly ($P < 0.05$) higher when it was ensiled with molasses compared with ground corn. Levels of additive, fermentation periods, and interactions did not affect the TP of jambo grass during ensiling. The highest (5.74%) TP was observed when jambo grass was ensiled with 4% molasses for 30 days followed by 5.50% for jambo grass ensiled with 2% molasses for 30 days, and was the lowest (4.50%) TP for jambo grass ensiled with 2% ground corn grain for the same fermentation period (Table 2).

NDF of jambo grass was significantly ($P < 0.05$) affected by additives and their levels, but fermentation periods did not influence the NDF contents of jambo grass silage (Table 2). The highest NDF content (71.53%) was observed when jambo grass was ensiled with 2% molasses for 30 days followed by 71.8% and 71.6% for jambo grass ensiled with 6% and 4% molasses for the same period, while the lowest NDF (68.3%) was observed for jambo grass ensiled with 6% corn grain for 30 days. Hemicellulose contents of jambo grass were significantly ($P < 0.05$) affected by additives and their levels but the effect of fermentation periods was not significant (Table 2). Hemicellulose was the highest for jambo grass ensiled with 2% molasses for 30 days and the lowest for jambo grass ensiled with 6% corn grain for 40 days.

Table 2. Influence of different additives on silage characteristics and chemical composition (DM basis) of jambo grass silage.

Parameters (%)	Corn			Molasses			SE	Main Effects		Interaction
	2%	4%	6%	2%	4%	6%		A	B	AB
pH	3.83	3.80	3.78	3.67	3.65	3.60	0.12	NS	NS	NS
Lactic Acid	3.93	3.92	3.91	4.00	4.02	4.04	0.21	NS	NS	NS
Dry Matter	17.17	16.9	16.43	18.27	17.53	17.6	0.14	+	NS	NS
Crude Protein	9.93	9.80	9.79	9.79	9.94	9.90	0.21	NS	NS	NS
True Protein	5.80	5.04	4.15	5.45	5.50	5.40	0.31	+	NS	NS
Neutral Detergent Fiber	70.9	70.5	69.43	71.53	71.37	71.23	0.25	+	NS	NS
Acid Detergent Fiber	42.57	42.4	42.47	42.33	42.8	42.8	0.08	NS	NS	NS
Hemicellulose	28.33	28.1	25.97	29.20	28.57	28.43	0.09	+	+	NS
Cellulose	38.90	38.8	38.9	38.73	39.2	39.6	0.52	NS	NS	NS
Acid Detergent Lignin	3.66	3.63	3.58	3.62	3.62	3.55	0.41	NS	NS	NS
Ash	9.48	9.53	9.58	9.50	9.55	6.78	0.52	NS	NS	NS

NS: Non-significant, +: Significant ($P < 0.05$), A: Additives, B: Additive levels, AB: interaction

Digestion kinetics of jambo grass and its silage

Comparative in situ DM and NDF digestion kinetics of jambo grass and its silage are given in Table 3. Ruminal DM and NDF degradabilities of jambo grass were significantly higher than those of its silage at 48 h of ruminal incubation. Ruminal lag time, rate, and extent of DM and NDF digestion were similar for jambo grass and its silage.

Discussion

The chemical analysis revealed that jambo grass had a good nutrient profile (Table 1) due to its harvest at the appropriate age (50 days). As the grass age increases, the nutrient content decreases due to lignification. Higher NDF content might be due to lignification phenomena. Thus jambo grass harvested at 50 days of age was used for laboratory ensiling and in situ digestion kinetic studies.

In contrast to the present findings, McDonald et al. (12) reported that addition of molasses at the time of ensiling produced more lactic acid. Addition of additives like corn and molasses improved the fermentable carbohydrate contents of silage that provided a suitable environment for lactic acid bacteria to lower the final pH

of the silage (2). Man and Wiktorsson (13) reported significantly lower pH values of grass silage when molasses was added before ensilage. In the present study, similar pH and lactic acid contents of jambo grass ensiled with molasses or ground corn grains may be attributed to their similar rate of hydrolysis of fermentable carbohydrates from both the additives.

The higher DM of jambo grass ensiled with molasses might be attributed to the fact that the use of molasses as a silage additive prevented DM loss during ensiling (13) because of the early decline in pH and silage stability (14).

The unaltered CP contents of jambo grass by the additive type, level, and fermentation periods might be attributed to well preserved jambo grass silage and the process of proteolysis by microbes and plant proteolytic enzymes was checked in all treatments because of its low pH. Khorasani et al. (15) and Bolsen et al. (2) reported that addition of fermentable carbohydrates could prevent the loss of nutrients in ensiling material due to early stabilization of the medium. However, in contrast to the present findings, Man and Wiktorsson (13) reported reduced CP content when grass was ensiled with additives. The reduction in CP content was attributed to the extensive proteolysis during the ensiling process (16). The higher TP content of jambo grass silage ensiled with

Table 3. Dry matter and NDF digestion kinetics of jambo grass¹ and its silage.²

Parameters	Jambo grass	Jambo grass silage	SE
Dry matter			
Digestibility ³ (%)	64.4 ^a	58.7 ^b	0.91
Lag (hour)	1.35	1.46	0.31
Rate of degradation (% hour ⁻¹)	3.69	3.63	0.56
Extent ⁴ (%)	70.5	70.2	0.91
Neutral detergent fiber			
Digestibility ³ (%)	62.8 ^a	59.0 ^b	0.95
Lag, hour	1.76	1.88	0.47
Rate of degradation (% hour ⁻¹)	3.44	3.31	0.38
Extent ⁴ (%)	67.5	67.2	1.10

Mean values with in row bearing different superscripts differ significantly (P < 0.05)

¹Harvested at 50th day; ²ensiled with 2% molasses for 30 days

³Digestibility was determined at 48 h of ruminal incubation

⁴Extent of digestion was determined at 96 h of ruminal incubation.

molasses compared with ground corn may be because of the fact that during the ensiling process there was extensive proteolysis resulting in higher NPN concentration and lower TP contents of silage (16). Fairbairn et al. (17) also reported that NPN, ammonia nitrogen ($\text{NH}_3\text{-N}$), free $\text{NH}_3\text{-N}$, and peptide N increased during fermentation. High DM and better anaerobiosis inhibited microbial proliferation especially *clostridia* spp., which explained the low $\text{NH}_3\text{-N}$ and high TP in well preserved silages (18). In the present study, the better TP content of jambo grass ensiled with molasses indicated better preservation and less proteolytic activity due to early pH decline.

The significant change in hemicellulose contents of jambo grass by additives and their levels is in line with the findings published by Selmer-Olsen et al. (19), who reported a decrease in hemicellulose content during ensiling of perennial and Italian grasses. Similar findings have also been reported by others (20-22) and they attributed this to the hydrolysis of hemicellulose due to microbial fermentation. Unaltered cellulose and ash content in the present study are in concordance with Khorasani et al. (15), who reported similar cellulose and ADF contents of well-preserved grass silages indicating less loss of nutrients during ensiling.

In the present study, hydrolysis of fermentable carbohydrates from both additives was probably similar; however, the preservation of jambo grass silage was

better when molasses was used rather than ground corn grains. The additive level and fermentation periods did not affect the silage quality. Therefore, jambo grass ensiled with 2% molasses for 30 days was selected for in situ digestion kinetics.

The reason for the better digestion kinetics of jambo grass was the presence of higher readily degradable carbohydrate contents than that of its silage (23). During the ensiling process there had been loss of readily degradable carbohydrate contents by lactic acid producing bacteria (16). The findings of the present study were contrary to the results published by Nadeau et al. (24), who reported increased degradabilities of orchard grass during the ensiling process due to fermentative decomposition of the cell wall component of fodder.

In conclusion, the results from this study imply that preservation of jambo grass was better with molasses than with ground corn grain. The additive level and fermentation periods did not affect the silage quality. Ruminal DM and NDF degradabilities of jambo grass were significantly higher than that of its silage. Lag time, rate, and extent of DM and NDF degradation remained similar for jambo grass and its silage. However, further research is warranted involving larger numbers of animals to examine the influence of jambo grass ensiled with 2% molasses for 30 days on productive and reproductive traits of Nili buffaloes.

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