Original Research Article

Effect of molasses on nutritive value and *in-vitro* digestibility of elephant grass silage

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Abstract

Ensiling preserving a forage crop and its nutrients to feed later on as silage. In this study, elephant grass was ensiled with varying levels of molasses as an additive to evaluate its effect on the nutritive value and *in-vitro* digestibility of the silage. Four treatments of Pennisetum purpureum grass silage were produced with the inclusion of molasses at 0 (T1), 2.5 (T2), 5 (T3), and 7.5% (T4) inclusion levels. Elephant grass ensiled with higher levels of molasses inclusions (T3, and T4) had significantly higher (p < 0.05) dry matter, crude protein, nitrogen-free extract, and lactic acid than T1 and T2 silages. However, T1 and T2 silages had significantly higher (p < 0.05) crude fibre and ash contents. Furthermore, silage without molasses inclusion (T1) had significantly higher (p < 0.05) in most of the fibre fractions compared to other silages in this study. At 6 and 15 hours, there were no significant differences (p > 0.05) in the means of *in-vitro* gas production. However, gas production was significantly higher (p < 0.05) in silages with the inclusion of additives (T4, T3, and T2) at 9, 12, 18, 21, and 24 hours of incubation. Conspicuously, silages containing graded levels of molasses as an additive had significantly higher (p < 0.05) digestibility and organic matter digestibility than the silage without molasses. The methane gas and metabolisable contents were significantly higher (p < 0.05) in T3 and T4 silages than T1 and T2 silages. However, T3 had significantly the highest (p < 0.05) gas volume followed by T2, T4, and T1 silages. It can be concluded that our H1 hypothesis was confirmed and the inclusion of molasses as an additive in elephant grass silage resulted in improved dry matter, crude protein, lactic acid contents, reduced fibre fractions, and superior *in-vitro* digestibility values as well as relatively better gas production by the silage.

Keywords: additives; composition; conservation; dry matter; fermentation; fibre fraction; forages; gas production

INTRODUCTION

The constraint for profitable livestock production in developing countries is the uneven and insufficient supply of quality forage (Binuomote et al., 2019). The availability of green forage is mostly seasonal, only in the rainy season when plant growth is high. Seasonal forage scarcity can be considerably reduced by conserving the surplus forage during the high fodder availability period. Ensiling preserves forage crop and its nutrients to feed later to animals. According to Kung et al. (2000), the primary purpose of making silage is to maximise the preservation of original nutrients in the forage crop for feeding at a later date. Forage preservation in silage stabilises the fermentation process by decreasing the pH within the minimum fermentation period. es Low pH is usually accomplished through the fermentation

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of sugars in the crop to lactic acid by lactic acid bacteria, which decreases plant enzyme activity and prevents the proliferation of detrimental anaerobic microorganisms, especially clostridia, and enterobacteria (Yang et al., 2004). Among the non-grain grasses used in silage production, the elephant grass (Pennisetum purpureum) stands out as forage with excellent potential for dry matter production and a high amount of soluble carbohydrates (Santos et al., 2013). Elephant grass has low concentrations of fermentable carbohydrates and supplying additives can improve the quality of its silage (Iqbal et al., 2005). Molasses enriches the fresh material with carbohydrates and fill the gaseous pores. Thus the influx of oxygen into the silage is reduced. Using molasses as an additive can increase the amount of fermentation end products due to the fermentation of the available sugars (Yokota et al., 1992). Extensive research led to conserving Pennisetum purpureum as hay and silage for feeding ruminants using various additives in silage production. Molasses is a commonly used silage additive to provide readily available energy for lactic acid fermentation and it has positive effects on pH and lactic acid levels on grass silage. Hence, this study evaluated the effect of molasses as an additive on the chemical composition, in-vitro gas production, and dry matter digestibility of elephant grass silage.

Hypotheses

H1: The inclusion of varying levels of molasses as additives significantly affects the chemical composition, *in-vitro* gas production, and dry matter digestibility of elephant grass silage.

H0: The inclusion of varying levels of molasses as additives does not affect the chemical composition, *in-vitro* gas production, and dry matter digestibility of elephant grass silage.

MATERIALS AND METHODS

Experimental site and design

The study was carried out at the Animal Science section of the Teaching and Research Farm of Osun State University, College of Agriculture, Ejigbo Campus, Osun State. The experiment was arranged in a Completely Randomised Design (CRD) with four replicate plots. There were four treatments: T1 – Control (no molasses), T2 – 2.5% molasses, T3 – 5% molasses, and T4 – 7.5% molasses.

Silage production

The elephant grass was harvested at 4 weeks of age, allowed to wilt, chopped into chips of approximately 3 cm length, and weighed. Molasses were added at 0, 2.5, 5, and 7.5% inclusion levels, thoroughly mixed and consolidated then stacked and compressed into bucket silos and sealed for about 3 weeks.

Chemical analysis

Chemical analysis of elephant grass silage was done by taking randomly, a 250 g sample from each bag and then it was dried in a hot air oven at 65–70 °C and stored for further analysis. Three oven-dried samples were ground through a 1 mm sieve and analysed for proximate analysis (AOAC 2008) while fibre contents such as acid detergent fibre, neutral detergent fibre, acid detergent lignin, cellulose, and hemicellulose contents were determined using the procedure of Van Soest et al. (1991).

In-vitro dry matter digestibility

The silage samples were analysed for *in-vitro* DM digestibility according to the method of Tilley and Terry (1963). Rumen contents collected from a healthy goat were squeezed through four layers of cheesecloth and kept in a water bath at 39 °C until incubation. Representative samples of the mixtures (2.5 g DM) were taken in a separate bottle having 0.05 litres of rumen liquor and 0.2 litres of buffer solution (Buffer solution: KCl 0.57 g/L, MgSO₄. 7H₂O 0.12 g/L, NaCl 0.47 g/L, CaCl2 0.04 g/L, Na₂HPO₄.12H₂O 9.30 g/L, NaHCO₃ 9.80 g/L, Cysteine 0.25 g/L (Elmenofy et al., 2012, Tilley and Terry, 1963). The bottles were kept in a water bath at 39°C. The samples were run for *in-vitro* dry matter digestibility at 6, 9, 12, 15, 18, 21, and 24 hours of incubation.

The *in-vitro* DM digestibility was determined by using the following formula:

In-vitro DM digestibility = <u>Initial weight-final weight</u> Initial weight

Data analysis

The data collected were subjected to a one-way analysis of variance procedure of the General Linear Model (SAS 2008). The means were separated using the Duncan New Multiple Range Test.

RESULTS

There were significant differences in the chemical composition of the elephant grass ensiled with varying levels of molasses (Table 1). Elephant grass ensiled with higher levels of molasses inclusions (T3, and T4) had significantly higher (p < 0.05) dry matter, crude protein, nitrogen-free extract, and lactic acid than T1 and T2 silages. However, T1 and T2 silages had significantly higher (p < 0.05) crude fibre and ash contents.

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Variables (%)	T 1	T2	Т3	T4	p-value
Dry matter	72.03 ^b	72.10 ^b	73.440ª	73.80 ^a	0.0142
Crude protein	10.20 ^b	10.86^{b}	12.27^{a}	12.88^{a}	0.0122
Crude fibre	26.80ª	26.32 ^a	26.00 ^{ab}	25.14 ^b	0.0220
Ether extract	2.30	2.93	2.23	2.30	0.2692
Ash	12.25^{a}	12.76ª	11.33 ^b	12.17^{ab}	0.0281
Nitrogen free extract	21.91°	21.98°	24.09 ^a	22.87^{b}	0.0049
Fibre fractions					
Acid detergent fibre	48.96ª	47.26 ^b	46.10 ^c	46.95°	0.0089
Neutral detergent fibre	67.80ª	65.55°	65.00 ^c	66.15 ^b	0.0039
Acid detergent lignin	22.80^{a}	22.90 ^{ab}	22.39 ^c	23.15 ^{ab}	0.0384
Cellulose content	26.92ª	24.22 ^b	23.00 ^c	21.92^{d}	0.0004
Hemicellulose	17.72^{a}	14.17 ^b	13.22°	11.85^{d}	<0.0001
Lactic acid (mmol/100 ml)	4.30 ^b	5.80 ^{ab}	6.51 ^{ab}	8.43ª	0.0137
рН	3.38	3.37	3.52	4.17	0.7095

Table 1. Chemical composition of elephant grass ensilage with molasses

Table 2. In-vitro gas production of elephant grass silage

Variables	T 1	Т2	Т3	T4	p-value
6hrs	13.67	14.00	17.33	16.67	0.2403
9hrs	21.00^{b}	23.33 ^{ab}	24.33 ^{ab}	28.00ª	0.0376
12hrs	25.00^{b}	28.00^{ab}	31.67 ^{ab}	34.00ª	0.0285
15hrs	27.00	28.67	34.00	35.67	0.1387
18hrs	27.67 ^c	30.33 ^{bc}	36.00 ^{ab}	38.00ª	0.0266
21hrs	29.67 ^b	33.00 ^{ab}	38.33ª	38.67ª	0.0499
24hrs	32.67 ^b	34.00 ^{ab}	40.33ª	38.67 ^{ab}	0.0469
Methane gas (CH_4) (ml/g)	18.33 ^b	20.67^{b}	26.33ª	25.33ª	0.0071
Carbon dioxide (CO ₂) (ml/g)	13.67	15.67	15.33	14.00	0.1023
Gas volume (ml/g)	18.67 ^b	20.00 ^{ab}	26.33ª	24.67 ^{ab}	0.0269
Digestibility (%)	51.70 ^c	47.60^{d}	58.33 ^b	61.41ª	<0.0001
Metabolisable Energy (MJ/kg)	7.20^{d}	7.34 ^b	8.48ª	8.23ª	< 0.0001
Organic Matter Digestibility (%)	48.32 ^d	51.48°	$55.57^{ m b}$	57.43ª	<0.0001

Means with different superscripts are significantly different (p < 0.05)

Furthermore, silage without molasses inclusion (T1) had significantly higher (p < 0.05) most of the fibre fractions compared to other silages in this study indicating that the inclusion of molasses as additives reduced the fibre fractions of silages which might be attributed to the effect of anaerobic fermentation during the ensiling process. At 6, 9, 12, and 15 hours, there were no significant differences (p > 0.05) in the means of in vitro gas production (Table 2). However, gas production was significantly higher (p < 0.05) in silages with the inclusion of additives (T4, T3, and T2) at 18, 21, and 24 hours of incubation. Conspicuously, silages containing graded levels of molasses as an additive had significantly higher (p < 0.05) digestibility and organic matter digestibility than without molasses. The methane gas and metabolisable contents were significantly higher (p < 0.05) in T3 and T4 silages than

in T1 and T2 silages. However, T3 had significantly the highest (p < 0.05) gas volume followed by T2, T4 and T1 silages.

DISCUSSION

The dry matter (DM) in this study was greater than the values reported by Alabi et al. (2019). Crude fibre (CF) decreased with the increased inclusion of molasses in the silage. The CP obtained in the study was above the critical value recommended for small ruminants (NRC, 2001). The ADF was similar to the values reported by Pirmohammadi et al. (2006). However, the Neutral Detergent Fibre fell within the minimum and maximum range of 54.1–79.9% reported by Egbunogie and Bamikole et al. (2014). Acid Detergent Lignin (ADL) was lower than the 35% reported by Ogunjobi et al. (2014) and did not differ from the values range of 27–59%

reported by Egbunogie and Bamikole et al. (2014). The cellulose content (CC) values in this study were lower than the 45.60% reported by Tangendjaja et al. (1990). The hemicellulose content (HC) was similar to the value of 20.78% reported by Ogunjobi et al. (2014). Quality fermentation is the way to remove odours, prevent spoilage, and protect nutrients in silage feeds. Protection is based on high acidity depending on lactic acid formation (Isnandar et al., 2010). The amount and composition of organic acids such as lactic acid formed during silage fermentation determines fermentation quality, and lactic acid is one of the main preservative organic acids in silage (Filya, 2001). The lactic acid content in this study fell within 7.64%-12.33% as reported by Seydoşoğlu (2017) depending on the maturity period. Ergün et al. (2013) reported that the optimum pH range for the milk acid bacteria growing in the acid medium was 3.8-4.2 and that bacteria causing spoilage and decomposition did not live in silage with a value in this pH range. The increase in *in-vitro* dry matter digestibility and in-vitro organic matter digestibility with increasing protein levels indicates the potential to improve the feed efficiency of low-quality forages (Bargo et al., 2003). Cellulolytic activity is highly dependent on the type and amount of substrate in the form of fiber. If the fiber content of the feed is high, the level of cellulase enzyme activity will increase, and conversely. The high cellulolytic activity is caused by the ability of microbes to digest cellulose quickly (Qori'ah et al., 2016). The differences in the nutrient contents and *in-vitro* digestibility parameters might be attributed to the differences in the ages of grasses at harvest, ecological zones, the process of anaerobic fermentation during silage production, and molasses inclusion.

CONCLUSION

It can be concluded that the inclusion of molasses as an additive in elephant grass silage led to improved dry matter, crude protein, lactic acid contents, reduced fibre fractions and superior *in-vitro* digestibility values as well as relatively better gas production of the silage.

CONFLICT OF INTEREST

The authors declared no conflicts of interest concerning research, authorship, and publication of this article.

ETHICAL COMPLIANCE

The authors have followed ethical standards in conducting the research and preparing the manuscript.

REFERENCES

- Alabi B.O., Lamidi A.A., Oyediran P.J., Omoloye H.O. (2019): Quality evaluation and acceptability of silage combinations of maize cobs, *Moringa oleifera* leaves and cassava peels and its effects on blood parameters of West African Dwarf bucks. CPQ Nutrition 3:01–12.
- AOAC (Association of Official Analytical Chemists), (2008): Official method of analysis of the AOAC (W. Horwitz, Editor) Eighteenth Edition. Washington D.C, AOAC.
- Bargo F., Mullar L.D., Kolver E.S., Delahoy J.E. (2003): Invited review: Production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science 86: 1–42.
- Binuomote R.T., Bamigboye F.O., Amuda A. J., Ayebogan G.M (2019): Silage quality and preference of Elephant grass (*Pennisetum purpureum*) with varying levels of poultry droppings and cassava peel by West African Dwarf goats. International Journal of Scientific Research in Science Engineering and Technology 16: 2395–1990.
- Egbunogie B.O., Bamikole M.A. (2014): Nutrient content variability in three forms of elephant grass (*Pennisetum purpureum*) preservation. Nigerian Journal of Agriculture and Food Environment 10: 48–52.
- Elmenofy E.K., Bassiouni M.I., Belal M.B., Gaafar H.M.A., Abdel-Raouf E.M., Mahmoud, S.A. (2012): Improving the nutritive value of ensiled green rice straw 2 *in-vitro* gas production. Nature and Science 10: 86–91.
- Ergün A., Tuncer Ş.D., Çolpan İ., Yalçın S., Yıldız G., Küçükersan M.K., Küçükersan S., Şehu A., Saçaklı, P. (2013): Feed, Feed Hygiene and Technology. – Ankara University, Faculty of Veterinary Medicine, Extended 5th Edition, Ankara, Turkey, 448 p. (In Turkish).
- Filya İ. (2001): Silage Technology. Uludağ University, Faculty of Agriculture, Department of Animal Science, Bursa, Turkey. (In Turkish).
- Iqbal S., Bhatti S.A., Mahr-un-Nisa., Sarwar M. (2005): Influence of varying levels of organic green culture and enzose on silage characteristics of mott grass and its digestion kinetics in Nili-Ravi buffalo bulls. International Journal of Agricultural Biology 7: 1011–1014.
- Isnandar U.R., Chuzaemi S., Sutariningsih E., Yusiati L.M. (2010): The role of lactic acid bacteria on silage duration process and rumen content silage quality. The 5th International Seminar on Tropical Animal Production, Community Empowerment

and Tropical Animal Industry, October 19–22, Yogyakarta, Indonesia, pp. 243–249.

- Kung J., Robinson L.J.R., Pesek J.D. (2000): Microbial populations, fermentation end-products and aerobic stability of corn silage treated with ammonia or a propionic acid-based preservative. Journal of Dairy Science 83: 1479–1486.
- NRC (National Research Council) (2001): Nutrient requirements of dairy cattle. 7th ed. The National Academies Press, Washington, D.C.
- Ogunjobi J.A., Adu B.W., Jayeola O.B. (2014): Growth performance of captive-bred juvenile male grasscutters (*Tyonomys swinderianus* Temminck 1827) fed two common grasses in Nigeria. International Journal of Agricultural Science 4: 11926.
- Pirmohammadi R., Rouzbehan Y., Reza Y.K., Zahedif A.M. (2006): Chemical composition, digestibility and in situ degradability of dried and ensiled apple pomace and maize silage. Small Ruminant Research 66: 150–155.
- Qori'ah A, Surono S., Sutrisno S. 2016: Sintesis protein mikroba dan aktivitas selulolitik akibat penambahan level zeolit sumber nitrogen slow release pada glukosa murni secara *in vitro*. Jurnal Ilmu-Ilmu Peternakan 26: 1–7. http://dx.doi. org/10.21776/ub.jiip.2016.026.02.1
- Santos R.J.C., Lira M.A., Guim A., Santos M.V.F., Dubeux junior J.C.B., Mello A.C.L. (2013): Elephant grass clones for silage production. Scientia Agricola 70: 6–11.
- SAS Institute (2008): SAS user's guide. Statistic, version 9.0. Statistic Institute Inc. Cary, North Carolina, USA. 1028 p.

- Seydoşoğlu S. (2017): Investigation of the effects of different sowing times of different varieties on second crop maize silage yield and quality. – PhD Thesis, Institute of Natural and Applied Sciences University of Dicle, Diyarbakır, Turkey, 201 p. (In Turkish).
- Tangendjaja B., Rahardjo Y.C., Lowry J.B. (1990): Leucaena leaf meal in the diet of growing rabbits: evaluation and effect of a low-mimosine treatment. Animal Feed Science Technology 29: 63–72.
- Tilley J.M.A., Terry R.A. (1963): A two-stage technique for in-vitro digestion of forage crops. Journal of British Grassland Society 18: 104–111.
- Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides about animal nutrition. Journal of Dairy Science 74: 3583–3597.
- Yang W.Z., Beauchemin K.A., Vedres D.D., Ghorbani G.R., Colombatto D., Morgavi D.P. (2004): Effect of direct-fed microbial supplementation on ruminal acidosis, digestibility and bacteria protein synthesis in continuous culture. Animal Feed Science and Technology 114: 179–193.
- Yokota H., Okojima T., Oshima M. (1992): Nutritive value of napier grass (*Pennisetum purpureum* Schum) silage ensiled with molasses by goats. Asian Australasian Journal of Animal Science 5: 33–37.

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