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EFFECTS OF DIFFERENT SILAGE PRESERVATIVES ON SILAGE QUALITY OF *Pennesitum purpureum* HARVESTED AT DIFFERENT HARVESTING PERIODS

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ABSTRACT: *The study was conducted to determine the effects of preservatives on the chemical composition of elephant grass (P. purpureum Bana cv.) harvested from N-fertilized and unfertilized treatments at different periods (3, 6 and 9 months). The plants were grown on 1st November 2008 and harvested every 3 months until July 2009. The grass was chopped and a 500 g sample obtained and was mixed with 4% molasses, 4% molasses+0.25% urea and 2.5% dicalcium phosphate, respectively with plain silage as a control. The samples were ensiled with respective preservative in duplicates and were analyzed for pH and proximate after 30 days of ensiling. Molasses added silage had a higher (P<0.05) DM at 3 months on both N-fertilized and unfertilized treatments, whereas molasses added silage prepared from unfertilized treatment harvested at 3 months of growth, had lowest (P<0.05) pH and was highly (P<0.05) digestible but digestibility declined as the plant matured.*

ORIGINAL ARTICLE

Key words: *Elephant grass, Harvesting periods, Silage preservatives, Silage quality.*

INTRODUCTION

Elephant grass is tall growing perennial grass which is indigenous to tropical and subtropical climates. Since *Pennesitum purpureum Bana cv.* yield high biomass it can be used for silage production which will ensure sufficient availability of feed on farm throughout the year. Nisa et al. (2008) stated that uneven and insufficient supply of quality forage is the most critical constraint for profitable livestock production in developing countries. Ensiling is the process of preserving a forage crop and its nutrients to feed later on. According to Kung et al. (2000), the primary purpose of making silage is to maximize the preservation of original nutrients in the forage crop for feeding at a later date.

Botswana is one of the countries that are susceptible to drought and this shows the need to address shortage of feed during drought periods. During summer the quantity and nutritive composition of grasses is high while in winter the quality decline and availability is scanty. Higashiyama and Hirata (2006) emphasized that during the dry season herbage quality declines and it forms the main diet of ruminants in the semi-arid grasslands in the tropics for several months of the year. Therefore, preserving feed can help to sustain livestock industry during drought and dry seasons.

Seglar (2003) confirmed that quality of silage is a major concern, especially in dairy farming and that cows should be fed the highest quality ensiled forages possible for maximum milk production. This indicates that silage quality is important to dairy profitability. Yunus et al. (2000) stated that the quality of silage made from tropical herbages are generally of low fermentation quality as silage do not contain large amount of lactic acid but considerable acetic acid. Elephant grass silage has a low fermentation quality leading to reduced intake and digestibility. Masturi (2004) confirmed that good quality silage requires production of lactic acid to rapidly reduce the pH fermentation which requires sufficient fermentable carbohydrates. The faster the fermentation is completed, the more nutrients will be retained in the silage. Kung (2000) reported that a quick reduction in silage pH will help to limit the breakdown of protein in the silo by inactivating plant proteases. In addition, a rapid decrease in pH will inhibit the growth of undesirable anaerobic microorganisms such as enterobacteria and clostridia. Preservatives can be used to improve silage quality. Therefore, the purpose of this study is to determine the effects of harvesting stage and additives on quality of elephant grass silage.

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MATERIALS AND METHODS

Elephant grass Bana cv. was harvested at different periods: 3, 6 and 9 months, respectively from 5 plots of N-fertilized and unfertilized treatments. The whole plants were chopped at length of 2 cm which is a suitable length that allows firmer packaging, easy handling and have less separation of coarse and fine material. Even distribution of this material facilitated good packing. The length of forage material that Mühlbach (2001) used to make silage was 2.5 cm pieces and was opened after 45 days. Chopped grass of 500 g was thoroughly mixed with preservatives and staffed in the temporary plastic bag silo. This experiment had 4 treatments where different preservatives were added in order to determine effects of different silage preservatives on quality of elephant grass silage. Treatments included control (no preservative added), molasses 4%, urea (0.25%) + molasses 4% and 2.5% of dicalcium phosphate and were done in duplicates. The material was physically and effectively compressed with hands to remove excess air to create anaerobic environment. These silages were opened after 30 days, which was adequate enough to the fermentation phases of silage. Jurgens (2002) indicated that normal fermentation process lasts for 21 days and that there will not be any change unless air is allowed in since silage can stay unspoilt for a long time under anaerobic conditions. The samples were taken for laboratory analysis for pH and proximate analysis. Silages were opened for laboratory analysis on 2nd March 2009, 30th May 2009 and 30th August 2009, respectively. Ensiled elephant grass was analyzed for pH by weighing 20 g of silage which was placed in a blender jar then diluted with 200 g of deionized distilled water and blended for 30 seconds in a high-speed blender. The diluted samples were filtered through four layers of cheese cloth, and pH measured immediately with a pH meter (Contreras-Govea et al. 2009).

Split-plot in Completely Randomized Block Design model was used in analysis. Analysis of Variance was performed on data collected using General Linear Model (PROC GLM) procedure of (SAS 2000-2003). Means were tested for significance using Duncan Multiple Range Test.

RESULTS

Effects of different preservatives on chemical composition of *P. purpureum* harvested on N-fertilized and unfertilized treatments harvested at 3 months

The preservatives resulted in silages with different pH (Table 1). The pH level of molasses added silages from N-fertilized and unfertilized treatment were the lowest (P<0.05) followed by that of plain silage which was also lower (P<0.05) than pH of silage added urea+ molasses and dicalcium phosphate which had similar pH. Molasses added silage had a pH of 4.61 while for plain silage it was 5.57, urea+ molasses was 6.44 and dicalcium phosphate was 6.02 prepared grass harvested at 3 months from unfertilized treatment while on N-fertilized treatment it was 4.78, 5.58, 6.7 and 5.67, respectively.

The dry matter (DM) level of plain silage was similar to that of urea+molasses and dicalcium phosphate added silages (Table 1). The DM level of these three silages were higher (P<0.05) than that of molasses added silage from both N-fertilized and unfertilized treatments. In this study, preservatives did not lead to change in cell wall content of silages, as neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of silages were numerically different. Acid detergent lignin of plain and silage added molasses were similar but lower (P<0.05) than that of urea+ molasses and dicalcium phosphate that were similar on unfertilized treatment. The *in vitro* true dry matter digestibility (INVTDMD) of plain, molasses and urea+ molasses silage was the same but higher (P<0.05) than that of dicalcium phosphate added silage.

Effects of different preservatives on chemical composition of *P. purpureum* harvested at 6 months period

Different silage preservatives had different effects on the chemical composition of silage of *P. purpureum* prepared from six months harvested grass. Molasses resulted in lower (P<0.05) pH on both N-fertilized and unfertilized treatments, when compared to pH of plain, silage added urea + molasses and dicalcium phosphate that had similar pH (Table 2). The DM of silage added dicalcium phosphate was the highest (P<0.05) than that of other silages on unfertilized treatment, while it was similar on N-fertilized treatment. The NDF of plain and dicalcium phosphate silages was the same but higher (P<0.05) than that of molasses and urea+ molasses on both N-fertilized and unfertilized treatments. Acid detergent fibre of plain silage was higher (P<0.05) than that of other silages with preservatives but was similar to that of dicalcium phosphate on N-fertilized treatment. Table 2 shows that acid detergent lignin (ADL) and INVTDMD of all silages from N-fertilized and unfertilized treatments harvested at different periods were the same.

Effects of different preservatives on chemical composition of *P. purpureum* harvested after nine months harvesting period

Data of silage prepared from nine month aged *P. purpureum* and mixed with different preservatives are presented in Table 3. Molasses silage made from N-fertilized and unfertilized treatments had lowest (P<0.05) pH. The pH of silage added molasses was 4.71 while plain silage, urea+ molasses silage and dicalcium phosphate added silage had a pH of 7.35, 7.53 and 7.58, respectively on unfertilized treatment, while it was 6.3, 7.3, 7.2 and 7.65 respectively, on N-fertilized treatment.

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The DM and INVTDMD of different silages prepared from unfertilized and N-fertilized treatment were similar. Neutral detergent fibre of molasses and urea+ molasses silage were the same but lower (P<0.05) than that from silage added dicalcium phosphate while NDF of plain was similar to that of all preservatives on unfertilized treatment. On N-fertilized treatment, NDF of plain silage was higher (P<0.05) than that of molasses added silage but numerically higher than other silages which were similar to molasses silage. The ADF and ADL of the silages with different preservatives from unfertilized treatment were similar. The ADF of urea+ molasses silage from N-fertilized treatment was lower (P<0.05) than that of plain and dicalcium phosphate silage but similar to ADF of molasses added silage prepared from N-fertilized treatment. The ADL content of plain, molasses and dicalcium phosphate silages were numerically different but ADL of silage added urea+ molasses was lower (P<0.05) than that of plain silage.

DISCUSSION

Effects of silage preservatives on pH

The present results showed that different preservatives had different effects on the acidity of the silage prepared from both N-fertilized and unfertilized treatments harvested at different periods. This is in line with Yunus et al. (2000) who explained that high pH level of plain silage could be due to the fact that elephant grass contains low level of water soluble and fermentable carbohydrate. The pH level increased with increasing period of harvesting. Seglar (2003) observed that, maturity had effect on the quality of silage, as grasses often do not completely ferment to decrease pH into a desirable range because not enough substrate is available to complete fermentation. Kunkle et al. (2009) confirmed that forages that are too high in DM may not ensile well and this could be the reason for high silage pH at 6 and 9 months elephant grass silage. At plant maturity, non-structural sugar becomes structural and this reduces fermentable sugar of the silage; hence high pH on plants harvested at 6 and 9 months. Kunkle et al. (2009) observed that though mature grass is chopped, it does not easily pack and compress resulting in trapping air that hinders proper fermentation.

The pH of silage prepared from 3 months age grass suggests that the plain silage was dominated by acetic acid. Schroeder (2004) observed that acetic acid-producing bacteria ferment soluble carbohydrates and produce acetic acid which leads to silage pH decreasing from about 6.0 to 5.0. This pH will not alter rumen environment as most of rumen microbes strive under neutral pH (Russell and Wilson 1996). Acetic acid production is one of the desirable organic acids because ruminants can utilize it as a source of energy. It is produced when cellulolytic and hemicellulolytic bacteria degrade the cell wall material.

In the present study, molasses led to a lower (P<0.05) pH compared to other preservatives that were used. The pH readings of silage prepared from *P. purpureum* harvested from unfertilized treatment at 3, 6 and 9 months were 4.61, 4.66 and 4.71, respectively. Silage prepared from N-fertilized treatment with molasses also had lowest (P<0.05) pH of 4.78, 4.44 and 6.3 harvested at 3, 6 and 9 months, respectively. This is in line with Yunus et al. (2000) who reported a significant variation between molasses and urea silage as molasses reduced (P<0.05) silage pH. Homofermentative bacteria (lactic-acid bacteria) convert water-soluble carbohydrate to lactic acid and proper silage with proper lactic acid production in grasses has pH around 4.2. Previous study of Yokota et al. (1998) reported a pH of 3.85 on elephant grass silage mixed with molasses which contained the highest amount of lactic acid. Molasses is a source of readily available energy, thus sugars which have helped in rapid fermentation of elephant grass as under anaerobic condition lactic acid bacteria ferment sugars and produce organic acids (lactic acid) which lower the pH to about 4.2. Schroeder (2004) observed that when the silage pH drops below 5.6, acetic acid-producing bacteria begin to decline in numbers, while lactic acid-producing bacteria begin to thrive and rapidly reduce the pH. Furthermore, Schroeder (2004) reported that quality silage is achieved when lactic acid is the predominant acid produced, as it is the most efficient fermentation acid which will lead to rapid decline of the pH of the silage. Seglar (2003) observed that the faster the fermentation is completed, the more nutrients are retained in the silage. When silage is consumed, it lowers the rumen pH which affects rumen microbiota. According to Russell and Wilson (1996), ruminant animals depend on cellulolytic ruminal bacteria to digest cellulose, but these bacteria cannot resist the low ruminal pH that modern feeding practices can create. Since the cellulolytic bacteria cannot grow on cellobiose at low pH, pH sensitivity is a general aspect of growth and not just a limitation of the cellulases *per se*. The rumen will be dominated by lactic acid utilizing microbes such as *Megasphaera elsdenii.*

Previous study of Counotte et al. (1983) reported that *Megasphaera elsdenii* convert more than 80% of the DL-lactate fermented to volatile fatty acids (VFA). Bergman (1990) indicated that propionate was removed by the liver but was largely converted to glucose. Propionate is converted to succinyl-coenzyme A, which enter tricarboxylic acid cycle and is converted to malate, when malate is transported to the cytosol where it is converted to oxaloacetate. During this process, malate releases energy yielding molecule Nicotinamide Adenine Dehydroxynade (NADH) that enter Electron Transport Chain (ETC) in mitochondrion to produce three moles of Adenosine Triphosphate (ATP). Oxaloacetate is then converted to phosphoenolpyruvate in gluconeogenesis which will help to yield net of 34 moles of ATP.

Accumulation of lactic acid in the rumen may lead to lactic acidosis. Therefore, the silage containing high concentrations of lactic acid and easily fermentable sugars may be harmful to the ruminant, causing lactic acidosis and digestive disorders. According to Seglar (2003), it is crucial that lactic dominated silage is fed to cattle that need more energy such as dairy cows to ensure that they rapidly utilize lactic acid produced to produce milk.

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Table 1 - Effects of preservatives on Silage prepared from *P. purpureum* harvested at three months at Notwane Farm

Parameters / Treatments	pH			DM			NDF			ADF			ADL			INVTDMD		
	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM
Plain silage	5.57^{by}	5.58^{by}	0.20	14.44^{bz}	18.21^{by}	1.29	69.36^{ay}	68.51^{ay}	5.85	38.22$^{a\,y}$	35.71^{ay}	1.76	3.27^{by}	3.96^{ay}	0.43	74.39^{ay}	66.8^{ay}	5.91
Silage+ molasses	4.61^{cy}	4.78^{cy}	0.13	19.35^{az}	24.21^{ay}	0.82	68.84^{ay}	65.32^{ay}	3.93	37.01^{ay}	35.96^{ay}	1.45	3.28^{by}	3.95^{ay}	0.41	70.79^{aby}	61.68^{az}	2.78
Silage + urea + molasses	6.44^{ay}	6.7^{ay}	0.64	14.90^{by}	16.92^{by}	2.04	72.74$^{a\,y}$	67.36^{ay}	4.86	35.67^{ay}	36.48^{ay}	1.92	4.56^{ay}	4.34^{ay}	0.62	77.04^{ay}	65.51^{az}	4.12
$DiCaPO_4$	6.02b^{ay}	5.67^{by}	0.23	14.48^{by}	15.9^{by}	1.64	71.74^{ay}	66.88^{ay}	4.65	35.37^{ay}	34.30^{ay}	2.13	4.57^{ay}	4.49^{ay}	0.51	61.86^{by}	65.66^{ay}	5.35
SEM	0.20	0.13		1.29	0.82		5.85	3.93		1.76	1.45		0.43	0.41		5.91	2.78	

^{abc}Means on the same column with different superscripts are significantly (P< 0.05) different; ^{yz}Means of the same row with different superscripts are significantly (P<0.05) different. Unfert=unfertilized plot, fert= N-fertilized plot. SEM=Standard error of the mean. $DiCaPO_4$=dicalcium phosphate added silage, DM=Dry matter, NDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, INVTDMD=In Vitro True Dry matter digestibility.

Table 2 - Effects of preservatives on Silage prepared from *P. purpureum* harvested at six months of growth at Notwane Farm

Parameters / Treatments	pH			DM			NDF			ADF			ADL			INVTDMD		
	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM
Plain silage	6.66^{ay}	6.06^{ay}	4.05	71.73^{by}	68.03^{ay}	0.84	74.55^{ay}	73.73^{aby}	1.38	51.77^{ay}	50.4^{ay}	1.51	10.77^{ay}	13.33^{ay}	1.96	33.24^{ay}	33.24^{ay}	8.11
Silage+molasses	4.66^{by}	4.44^{by}	0.89	72.55^{by}	73.51^{ay}	0.16	70.65^{by}	71.66^{by}	1.48	47.11^{by}	47.64^{by}	1.59	8.67^{ay}	11.79^{ay}	2.12	41.47$^{a\,y}$	41.47^{ay}	7.38
Urea+Molasses silage	7.29^{ay}	6.96^{ay}	1.63	71.40^{by}	71.8^{ay}	0.38	70.73^{by}	70.61^{by}	0.96	45.98$^{b\,y}$	44.83^{by}	1.21	9.84^{ay}	9.56^{ay}	1.23	36.82^{ay}	36.82^{ay}	7.09
Silage+ $DiCaPO_4$	6.01^{ay}	6.57^{ay}	0.87	74.32^{ay}	71.74^{ay}	0.85	74.44^{ay}	74.35^{ay}	1.11	47.57^{by}	49.06^{ay}	0.57	9.54^{ay}	9.11^{ay}	1.03	44.31^{ay}	44.31^{ay}	9.00
SEM	0.64	0.23		4.86	1.64		4.86	4.65		1.92	2.13		0.62	0.51		4.12	5.35	

^{abc}Means on the same column with different superscripts are significantly (P<0.05) different. ^{yz}Means of the same row with different superscripts are significantly (P<0.05) different. Unfert=unfertilized treatment, fert= N-fertilized treatment. SEM=Standard error of the mean. $DiCaPO_4$=dicalcium phosphate added silage, DM=Dry matter, NDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, INVTDMD= *In vitro* True Dry matter digestibility

Table 3 - Effects of preservatives on Silage prepared from *P. purpureum* harvested at nine months of growth at Notwane Farm

Parameters / Treatments	pH			DM			NDF			ADF			ADL			INVTDMD		
	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM	Unfert	Fert	SEM
Plain silage	7.35^{az}	7.3^{ay}	0.87	69.33$^{a\,y}$	69.89^{ay}	0.84	79.07^{aby}	79.88^{ay}	1.10	56.78^{az}	57.13^{aby}	0.56	13.66^{ay}	14.5^{ay}	1.03	40.43^{ay}	40.6^{ay}	8.69
Silage+molasses	4.71^{by}	6.3^{ay}	0.63	68.11^{ay}	67.56^{ay}	2.04	77.26^{by}	73.70^{by}	0.85	56.15^{ay}	55.49^{aby}	1.14	13.91^{ay}	12.32^{aby}	1.40	46.66^{ay}	47.96^{ay}	4.32
Urea+ molasses	7.53^{ay}	7.2^{az}	0.72	65.50^{ay}	67.84^{ay}	2.72	77.20^{by}	76.82^{aby}	3.44	54.64$^{a\,y}$	53.20^{ay}	1.54	14.21^{ay}	8.86^{ay}	1.32	43.25^{ay}	45.8^{ay}	2.80
$DiCaPO_4$	7.58^{ay}	7.65^{ay}	0.57	68.29^{ay}	66.91^{ay}	1.88	80.16^{ay}	78.37^{aby}	1.23	78.63^{ay}	57.39^{ay}	1.00	16.53^{ay}	10.31^{abz}	2.85	38.20^{ay}	40.32^{ay}	4.32
SEM	4.05	0.89		0.84	0.16		1.38	1.48		1.51	1.59		1.96	2.12		8.11	7.38	

^{abc}Means on the same column with different superscripts are significantly (P< 0.05) different. ^{yz}Means of the same row with different superscripts are significantly (P<0.05) different . Unfert=unfertilized treatment, fert= N-fertilized treatment. SEM=Standard error of the mean. $DiCaPO_4$=Dicalcium phosphate added silage, DM=Dry matter, NDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, DMD=Dry matter digestibility

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Beauchemin (2007) observed that absorption of VFA from the rumen occurs passively through papillae, which are finger-like projections located on the rumen wall. The papillae increase gradually in length when cows are fed a close-up diet or a lactation diet that contains more grain than the dry cow diet. Increased surface area and absorptive capacity of the rumen protects the cow from accumulation of VFA in the rumen which is the main driver of rumen pH depression.

The pH level for urea+molasses added silage in this study was numerically higher than dicalcium phosphate pH but was higher ($P<0.05$) than other silages on both N-fertilized and unfertilized treatments. The level of this silage was neutral and will not change rumen environment. Orthophosphates and non-protein nitrogen (urea) are buffering agents (Seglar, 2003). Yunus et al. (2000) indicated that urea decreases the fermentation quality of the silage by raising the pH.

Effects of silage preservatives on dry matter, cell wall components and dry matter digestibility of elephant grass at different stages of growth

Molasses added silage prepared from both N-fertilized and unfertilized treatments had high ($P<0.05$) DM. This finding is in agreement with Yunus et al. (2000) who observed that molasses increases the DM content of the silage. Yokota et al. (1998) also observed that the DM of Napier grass was 8.62% while the DM of silage added molasses was 13.44%. This could have been due to the fact that cellulolytic microbes could not strive under acidic condition resulting in no microbes reducing DM. Table 1 shows that DM of plain, urea+molasses and dicalcium-phosphate added silages were similar. Yokota et al. (1998) observed that the inclusion of urea-molasses increases the DM percentage.

Preservatives led to numerical reduction of NDF and ADF at different harvesting periods on both N-fertilized and control treatment, except for dicalcium phosphate. Masturi (2004) reported that inclusion of legumes on dwarf Napier silage led to reduction of NDF from 69.1% to 61.6%. In the present study, study urea+ molasses decreased ($P<0.05$) NDF from 74.55% of plain silage to 70.73% and 79.07 to 77.2% at 6 and 9 months harvesting periods, respectively on unfertilized treatment. In agreement with current result, Masturi (2004) further reported that inclusion of legume on the dwarf Napier grass silage reduced ADF and ADL content. The INVTDMD of silage with preservatives prepared from grass harvested after 6 and 9 months periods was higher than for plain silage. This could be due to low NDF, ADF and ADL on these silages. Yunus et al. (2000) reported that addition of molasses and urea+molasses to elephant grass prepared from grass harvested at different heights, led to reduction of NDF, ADF and ADL while the INVTDMD increased when compared to plain silage.

CONCLUSION

It can be concluded that different silage preservatives have different effects on the chemical composition of elephant grass silage prepared from the grass harvested at different periods. N-fertilized treatment had no effects on the quality of the silage. Silage prepared from elephant grass harvested at three months from unfertilized treatment, with molasses as a preservative had a lowest pH. It also had a higher INVTDMD while its cell wall contents were low, indicating that the animal will get more nutrients in a day since the silage is digestible. So, *P. purpureum* silage with molasses as a preservative will be ideal for maximum production of lactic acid and preservation of nutrients in feeding ruminants.

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