

Fermentative attributes of wilted vs. unwilted *Digitaria eriantha* silage treated with or without molasses at ensiling

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Abstract

Silages made from directly cut or wilted *Digitaria eriantha* treated with or without molasses were evaluated to determine fermentative characteristics. The grass was harvested at the boot stage of growth and the material was ensiled in 1 kg mini-silos. Treatments were arranged in a factorial design of two moisture levels (directly cut or wilted) and two molasses addition (with or without molasses). Each treatment was ensiled in 12 separate bottles. The pH of molasses added silage declined faster than the pH of silage without molasses. The pH of unwilted silages continued to decline up to day 120 post ensiling compared to wilted treatments. Lactic acid concentration was higher for molasses added silage than those made without molasses at all ensiling times, and also higher for directly cut silage than those wilted prior to ensiling. In direct cut silages, acetic acid concentration was lower for molasses added silage than those made without molasses at all ensiling times. In both silages, with or without molasses, the acetic acid concentrations were significantly higher for unwilted silage than silage wilted prior to ensiling. The ammonia nitrogen (NH₃-N) as percentage of total nitrogen (N) was higher in unwilted silage than prior wilted silage without molasses, while in the presence of molasses the effect of wilting on NH₃-N as percentage of total N was not significant.

Keywords: Silage, fermentation process, moisture level, *Digitaria eriantha*, Smutsfinger grass

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Introduction

Digitaria eriantha (Smutsfinger) grass pastures have been established widely in many regions of southern Africa that are considered marginal for maize cropping (Schutte & Du Toit, 1992; Meeske *et al.*, 1999). The species is adapted to a wide range of climatic and soil conditions (Hardy *et al.*, 1990). The grass is a tufted, subtropical perennial and grazed by cattle and sheep. Farmers often make hay when surplus plant material is available (Meeske *et al.*, 1999). Variation in climatic conditions during summer limits hay production. The hay is often poor in quality and the nutritional value is inconsistent (Meeske *et al.*, 1999). On the other hand, foggage is subjected to a fire risk and must be utilised in the early to mid winter, because beyond this stage it will be underutilised because animals tend to select the new growth (Rethman, 1983). There is consequently a great need to maintain the supply of good quality roughage during the late winter and early summer through silage production.

The preservation of excess summer pasture in the form of silage is indispensable, and this allows mitigating the feed deficit that could be encountered during the critical (late winter and early summer) periods. The quality of the silage is, however, dependent on the quality of the crop at ensiling, type of fermentation, rate of pH decrease, moisture content of the crop and anaerobic conditions (Meeske *et al.*, 2000). The latter is in turn a reflection of the nutritive value and fermentation characteristics of these silages, which affects the preservation process and voluntary feed intake by the target animal (Wilkins *et al.*, 1971). The level of carbohydrate content varies with plant species, plant maturity, time of the day at cutting and with the amount of N applied to the crop (De Figueiredo, 2000). The objective of this study was to determine the influence of wilting practiced at ensiling, and molasses treatment on the fermentation process and potential nutritive value of *D. eriantha* silage for ruminants.

Materials and Methods

The study was conducted at the Potchefstroom Agricultural College, North West Province, South Africa (Altitude 1338 m above sea level, 26°42'13.07" S and 27°0.5'52.88" E). This location is an exclusively summer rainfall area with dry autumns and winters. The topsoil has a pH (in water) of 6.7

± 0.26 ; a pH (in KCl) of 5.7 ± 0.19 ; a P level of 60 ± 27.5 mg/kg; K of 53 ± 12.1 mg/kg; Ca of 494 ± 34.1 mg/kg; Mg of 359 ± 45.1 mg/kg and Na of 14 ± 2.8 mg/kg.

Digitaria eriantha cv. *eriantha* was established during February on a 0.8 ha plot under irrigation. During August of that same year the pasture was harvested and material removed. In October the pasture was top-dressed with 100 kg nitrogen (N)/ha (350 kg of LAN/ha) and again during January with 50 kg N/ha. The pasture received 25 mm water (rainfall and/or irrigation) weekly during the growing season.

Before cutting the plants for ensiling, 10 tufts were clipped of at a height of 10 cm above ground. The material was pooled and a sample was analysed for dry matter (DM) content. The third re-growth was harvested 31 days after the previous cut and chopped to a length of 12 ± 1 mm using a maize silage harvester. The material was then ensiled in mini-silos. Half of the material was ensiled directly after harvesting while the remaining half was wilted in the sun for 5 - 6 hours prior to ensiling. Both were ensiled with or without the addition of molasses (8 kg/ton DM). The treatments were: 1) Ensiled as directly-cut without molasses (T₁); 2) Ensiled directly-cut with molasses (T₂); 3) Wilted prior to ensiling and ensiled without molasses (T₃); 4) Wilted prior to ensiling and ensiled with molasses (T₄). Each treatment was ensiled in 12 glass bottles (1 L fruit jars). The bottles were filled and compacted in order to obtain anaerobic conditions. These mini-silos were then stored at room temperature (22 °C) for 0, 7, 21 or 120 days. Dry matter, water-soluble carbohydrate (WSC) concentration and buffering capacity of the fresh as well as the wilted grass sample were determined before ensiling. Thereafter, three mini-silos per treatment were opened on days 7, 21 and 120 post ensiling. One third of the material from each sample was oven-dried at 60 °C and analysed in duplicate for DM, organic matter (OM) and N content. The remaining fraction of each sample was stored in a freezer at -13 °C until it was analysed for pH and lactic acid, acetic acid and ammonia nitrogen (NH₃-N) concentration. About 40 g of a wet silage sample was extracted in a 1000 mL container to which 160 mL distilled water and saturated mercuric chloride were added as a preservative and stored overnight in cold storage at 7 °C. The extract was centrifuged for 6 h at 180 rpm and filtered through four layers of cheesecloth to remove any plant matter. The supernatant was then transferred to a 250 mL plastic bottle and stored in a freezer (-15 °C) for determination of pH, buffering capacity and lactic acid, acetic acid, N and NH₃-N concentrations. The pH was measured after allowing the samples to stand overnight at room temperature. Buffering capacity was determined following the procedure of Payne & McDonald (1966). Water-soluble carbohydrate was determined following the procedures of McDonald & Henderson (1964). The NH₃-N concentration was determined using a Technicon Auto Analyser II following the procedure of Davie (1989). Volatile fatty acid concentrations were determined from 2 mL of the silage extract following the procedure of Pryce (1969).

The general linear model (GLM) procedure of SAS (1989) was used to test statistical differences between the two factors and between sampling periods. Planned treatment comparison was made to examine the influence of wilting and molasses addition. Significance of differences between least squares means was determined using the Bonferroni's test (Samuels, 1989).

Results and Discussion

The WSC concentrations and buffering capacity were analysed on fresh material, while DM was analysed on both the fresh and wilted grass. The wilted grasses had a relatively high DM level (33 to 33.5%), a low WSC concentration (40 g/kg DM) and a low buffering capacity (125 meq/kg DM) compared to the unwilted material that contained 24% DM, 43 g WSC/kg DM and a buffering capacity of 132 meq/kg DM. The rate at which the pH drops is a function of the level of WSC and the epiphytic bacteria present on the crop prior to ensiling (Meeske *et al.*, 2000). If insufficient lactic acid bacteria are present on the crop and the readily available sugar concentration is low at ensiling this will result in a slow drop in pH. The pH should drop rapidly below 5 to prevent the growth of *Clostridium tyributyricum* (Meeske *et al.*, 2000). In the present study the pH of molasses added silage declined faster than the pH of silage without molasses. At day 7 post ensiling the addition of molasses reduced the pH of unwilted silage to 4.02 and to 4.33 for wilted silage. At day 120 post ensiling the untreated silage did not attain a comparable pH (4.2 - 4.5) to the molasses treated silages (Table 1). A rapid drop in pH is desirable from a preservation point of view, since this allows the fermentation to complete faster and preserves more nutrients. In the present study this was achieved through the provision of an extra energy source (sugar from molasses) to the lactic acid bacteria, which subsequently probably dominated the fermentation process (McDonald *et al.*, 1991; Kung & Shaver, 2001). The lactic acid

bacteria require WSC for metabolism. If the supply of WSC has been depleted before a sufficiently low pH has been achieved a stable product may not be attained (Sollenberger *et al.*, 2004). In contrast, the pH of the molasses, untreated silage showed little change between days 7 and 21, but remained significantly ($P < 0.05$) lower in wilted silage than the pH recorded on days 7 and 21 for unwilted silage. The slower rate of decrease in pH is more of a concern from a preservation point of view when the DM content of silage is low, while wilted silage (higher DM content) is more stable at a relatively higher pH. The present result is not in agreement with previous findings by Umaña *et al.* (1991), but in line with Van Niekerk *et al.* (2007), who found similar results in *Panicum maximum* silage. In the wilted silage, which contained 30% DM, the pH of silage made without molasses increased from day 7 to day 120 post ensiling. This is probably partly due to a lack of sufficient energy for optimal multiplication and growth of lactic acid bacteria during the anaerobic fermentation processes (Meeske *et al.*, 1999; 2000; Titterton & Bareeba, 2000; Kung & Shaver, 2001).

Table 1 Effect of wilting and molasses treatment on the pH of *Digitaria eriantha* silage at different stages post ensiling

Treatments	Fermentation time (days)			
	Day 0	Day 7	Day 21	Day 120
Ensiled directly without molasses (T ₁)	5.17 ₁ ^a	4.93 ₁ ^b	4.83 ₁ ^b	4.70 ₁ ^c
Ensiled directly with molasses (T ₂)	5.17 ₁ ^a	4.02 ₂ ^b	3.99 ₂ ^{bc}	3.90 ₂ ^c
Wilted prior to ensiling and ensiled without molasses (T ₃)	4.91 ₁ ^a	4.74 ₁ ^c	4.77 ₁ ^{bc}	4.88 ₁ ^{ab}
Wilted prior to ensiling and ensiled with molasses (T ₄)	4.91 ₁ ^a	4.33 ₂ ^b	4.31 ₂ ^b	4.33 ₂ ^b
Contrasts between wilted vs. non wilted treatments ³				
Direct cut silage vs. wilted silages				
T ₁ vs. T ₃	*	*	NS	*
T ₂ vs. T ₄	*	*	*	*

^{a,b,c,d} Values with different superscripts across a row differ significantly at $P < 0.05$

^{1,2} For each wilting state (direct cut vs. wilted), values with different subscripts (1, 2) within a column differ significantly at $P < 0.05$

³ * significant at $P < 0.05$; NS - non-significant at $P > 0.05$

At all post-ensiling times lactic acid concentration was higher for the molasses added silage than those without molasses, and also higher for unwilted silage than those wilted prior to ensiling (Table 2). This is in accordance with previous results on molasses (Singh & Pandita, 1984; Baytok *et al.*, 2005; Van Niekerk *et al.*, 2007), where the lactic acid concentration increased in molasses treated silage of various crops. The higher lactic acid values recorded in this study for either molasses added or unwilted silage compared to silage made without molasses or wilted prior to ensiling, respectively, agree with the corresponding lower pH values recorded for these treatments (Table 1). The reduction in lactic acid concentration for the wilted silage agrees with Vilela *et al.* (2001), who reported that wilting may reduce WSC, because of continued respiration of cells and consequently reduced lactic acid production. Except for unwilted silage with molasses, the lactic acid concentration did not differ between the post-ensiling sampling dates. In prior wilted silages, the lactic acid concentration of molasses added silage on day 7 post ensiling was twice as high as the value recorded for non-treated silage at days 21 and 120 post ensiling. This is partly due to restricted fermentation, a lack of sufficient energy for optimal multiplication and growth of lactic acid bacteria. The present result confirms previous findings on the benefit of molasses addition when ensiling tropical grasses because it stimulates maximum growth of lactic acid bacteria and consequently higher lactic acid concentrations in the silage (Baskay *et al.*, 1999; Aminah *et al.*, 2000; Bureenok *et al.*, 2005).

Table 2 Effect of wilting and molasses treatment on lactic acid concentration (g/kg DM) of *Digitaria eriantha* silage at different stages post ensiling

Treatments	Fermentation time (days)			
	Day 0	Day 7	Day 21	Day 120
Ensiled directly without molasses (T ₁)	1.41 ₁ ^b	24.47 ₂ ^a	24.52 ₂ ^a	25.22 ₂ ^a
Ensiled directly with molasses (T ₂)	1.41 ₁ ^d	53.47 ₁ ^c	64.24 ₁ ^a	58.97 ₁ ^b
Wilting prior to ensiling and ensiled without molasses (T ₃)	1.37 ₁ ^b	17.67 ₂ ^a	12.71 ₂ ^a	12.83 ₂ ^a
Wilting prior to ensiling and ensiled with molasses (T ₄)	1.37 ₁ ^b	24.08 ₁ ^a	22.86 ₁ ^a	24.14 ₁ ^a
Contrasts between wilted vs. non wilted treatments ³				
Direct cut silage vs. wilted silages				
T ₁ vs. T ₃	NS	*	*	*
T ₂ vs. T ₄	NS	*	*	*

^{a,b,c,d} Values with different superscripts across a row differ significantly at $P < 0.05$

^{1,2} For each moisture level (direct cut vs. wilted), values with different subscripts (1, 2) within a column differ significantly at $P < 0.05$

³ * significant at $P < 0.05$; NS - non-significant at $P < 0.05$

At all ensiling times acetic acid concentration in unwilted silages was lower in the molasses added silage than those without molasses (Table 3). However, on days 21 and 120 post ensiling a lower acetic acid concentration was recorded in wilted silages than in the silages treated with molasses. The lower acetic acid concentration in molasses treated silage agrees with previous results reported for *P. maximum* cv. Gatton (Van Niekerk *et al.*, 2007). Although acetic acid is the first acid that will be produced at the beginning of the anaerobic phase, higher concentrations of this acid are an indication of slower fermentation and this will reduce DM intake of the silage by animals (Erdman, 1993). Its accumulation also buffers against a decline in silage pH below 4.8 (Bates *et al.*, 1989). The deamination of amino acids is usually extensive, and consequently ammonia levels in acetate silage are higher than those found in lactate silage (McDonald *et al.*, 1991). In addition, a rapid development of lactic acid bacteria suppresses the growth of enterobacteriaceae (McDonald *et al.*, 1991). However, where their numbers are insufficient to produce sufficient lactic acid to lower the pH, enterobacteriaceae might dominate the fermentation process. The silage will then develop high levels of acetic acid, the main fermentation product of enterobacteriaceae (McDonald *et al.*, 1991). Compared to the control, the acetic acid concentration of prior wilted silage treated with molasses did not significantly differ between post-ensiling sampling dates. This is in contrast with a previous report by Chase (1988) who suggested a higher acetic acid production for molasses treated silage due to the fermentation of lactic acid into acetic acid by the action of hetero-fermentative bacteria. In both silages, those with or without molasses, the acetic acid concentrations were higher ($P < 0.05$) in directly cut silage than silage wilted prior to ensiling. This is in agreement with Kung & Shaver (2001) who reported a higher acetic acid concentration (>3 to 4% in DM) for extremely wet silage (<25% DM). This was probably due to the concomitantly higher incidence of clostridial spoilage (Chase, 1988). However, the acetic acid concentration of silage ensiled with molasses remained similar between days 7 and 120, but respectively higher and lower values of acetic acid concentrations were recorded on day 21 for non-treated silage, either wilted or not, prior to the ensiling process.

Compared to the fresh grass, total N concentration has declined by day 7, and this was apparent regardless of molasses treatment or the wilting practice. In the wilted treatments N concentration has further declined between days 7 and 120 (Table 4). There are conflicting data in the literature regarding the effect of molasses on N concentration of silage. Some researchers reported that the addition of molasses to silage increased the N concentration of silages (Lattema *et al.*, 1985; Kennedy, 1990; Baytok *et al.*, 2005), others did not observe an effect (Spoelstra *et al.*, 1990; O'Kiely 1992; Van Niekerk *et al.*, 2007) while some reported a decrease in the N concentration of silages (Moore & Kennedy, 1994). In this study molasses

addition had no significant effect on the total N concentration of silages at all ensiling times, but the N concentration of wilted fresh material and silage (on day 7) was higher compared to the directly ensiled fresh material. Van Niekerk *et al.* (2007) also found higher N concentrations in wilted *P. maximum* silage compared to unwilted silage treated with molasses.

Table 3 Effect of wilting and molasses treatment on acetic acid concentration (g/kg DM) of *Digitaria eriantha* silage at different stages post ensiling

Treatments	Fermentation time (days)			
	Day 0	Day 7	Day 21	Day 120
Ensiled directly without molasses (T ₁)	5.28 ₁ ^c	17.72 ₁ ^b	23.71 ₁ ^a	19.42 ₁ ^b
Ensiled directly with molasses (T ₂)	5.28 ₁ ^b	10.31 ₂ ^a	11.89 ₂ ^a	11.58 ₂ ^a
Wilted prior to ensiling and ensiled without molasses (T ₃)	6.19 ₁ ^c	8.76 ₁ ^{bc}	11.43 ₁ ^b	16.80 ₁ ^a
Wilted prior to ensiling and ensiled with molasses (T ₄)	6.19 ₁ ^a	6.35 ₁ ^a	5.08 ₂ ^a	5.34 ₂ ^a
Contrasts between wilted vs. non wilted treatments ³				
Direct cut silage vs. wilted silages				
T ₁ vs. T ₃	NS	*	*	NS
T ₂ vs. T ₄	NS	*	*	*

^{a,b,c,d} Values with different superscripts across a row differ significantly at P < 0.05

^{1,2} For each moisture level (direct cut vs. wilted), values with different subscripts (1, 2) within a column differ significantly at P < 0.05

³ * significant at P < 0.05; NS - non-significant at P < 0.05

Table 4 Effect of wilting and molasses treatment on total nitrogen concentration (g/kg DM) of *Digitaria eriantha* silage at different stages post ensiling

Treatments	Fermentation time (days)			
	Day 0	Day 7	Day 21	Day 120
Ensiled directly without molasses (T ₁)	31.82 ₁ ^a	28.85 ₁ ^b	29.89 ₁ ^{ab}	29.72 ₁ ^{ab}
Ensiled directly with molasses (T ₂)	31.82 ₁ ^a	27.17 ₁ ^b	28.85 ₁ ^b	27.81 ₁ ^b
Wilted prior to ensiling and ensiled without molasses (T ₃)	36.88 ₁ ^a	31.02 ₁ ^b	29.09 ₁ ^{bs}	28.30 ₁ ^c
Wilted prior to ensiling and ensiled with molasses (T ₄)	36.88 ₁ ^a	32.56 ₁ ^b	29.04 ₁ ^c	27.66 ₁ ^c
Contrasts between wilted vs. non wilted treatments ³				
Direct cut silage vs. wilted silages				
T ₁ vs. T ₃	*	NS	NS	NS
T ₂ vs. T ₄	*	*	NS	NS

^{a,b,c,d} Values with different superscripts across a row differ significantly at P < 0.05

^{1,2} For each moisture level (direct cut vs. wilted), values with different subscripts (1, 2) within a column differ significantly at P < 0.05

³ * significant at P < 0.05; NS - non-significant at P > 0.05

Table 5 Effect of pre-ensiling moisture level and molasses treatment on ammonia nitrogen (NH₃-N) concentration (g/kg N) of *Digitaria eriantha* silage at different stages post ensiling

Treatments	Fermentation time (days)			
	Day 0	Day 7	Day 21	Day 120
Ensiled directly without molasses (T ₁)	44.94 ₁ ^b	59.71 ₁ ^b	59.09 ₁ ^b	87.31 ₁ ^a
Ensiled directly with molasses (T ₂)	44.94 ₁ ^a	30.30 ₂ ^{ab}	27.76 ₂ ^b	27.12 ₂ ^b
Wilted prior to ensiling and ensiled without molasses (T ₃)	34.97 ₁ ^a	43.67 ₁ ^a	41.01 ₁ ^a	39.31 ₁ ^a
Wilted prior to ensiling and ensiled with molasses (T ₄)	34.97 ₁ ^{ab}	31.60 ₁ ^{ab}	42.28 ₁ ^a	26.32 ₁ ^b
Contrasts between wilted vs. non wilted treatments ³				
Direct cut silage vs. wilted silages				
T ₁ vs. T ₃	NS	*	*	*
T ₂ vs. T ₄	NS	NS	NS	NS

^{a,b,c,d} Values with different superscripts across a row differ significantly at P < 0.05

^{1,2} For each moisture level (direct cut vs. wilted), values with different subscripts (1, 2) within a column differ significantly at P < 0.05

³ * significant at P < 0.05; NS - non-significant at P < 0.05

Although the extent of proteolysis during ensiling is influenced by DM content, pH and temperature (McDonald *et al.*, 1991), the rate of decrease of pH is more important in determining the extent of proteolysis. If the pH is slow to fall, more protein will be broken down. However, once a pH of 4.3 has been reached, further proteolysis would be negligible (McDonald *et al.*, 1991). The NH₃-N concentration on day 7 post ensiling did not differ compared to the value recorded for the fresh grass (Table 5). This is unexpected considering the endogenous proteolysis and aerobic fermentation that will occur at the initial phase and during the feeding stage of the ensiling process, and loss which might be associated with the effluent (Mühlbach, 2000; Oude Elferink *et al.*, 2000; Schroeder, 2004). In all silages, the NH₃-N concentrations did not differ between days 7 and 21 post ensiling, but on day 120 post ensiling the NH₃-N levels increased in directly cut silage made without molasses and decreased in prewilted treatments with molasses compared to day 21 post ensiling. This agrees with Kung & Shaver (2001), who reported higher concentrations of ammonia in wetter silages because of the potential for clostridial fermentation. Baytok *et al.* (2005) reported numerically higher NH₃-N concentrations in silage treated with molasses compared to the control. This is due to increased fermentation in the molasses-treated group as a result of a higher WSC of molasses. At all ensiling times in the present study the NH₃-N concentrations were higher in directly cut silage than in the prewilted silage without molasses, while in the presence of molasses the effect of wilting on NH₃-N was not significant. The lower silage NH₃-N concentration in wilted material agrees with Bates *et al.* (1989) and Vilela *et al.* (2000), who found lower ammonia concentration in wilted silage compared to directly ensiled forage. This is because the hydrolysis of starch during wilting can boost the supply of sugar for fermentation (Kaiser *et al.*, 2000) other than the reduction in buffering capacity (Sollenberger *et al.*, 2004) and a relatively high tolerance of lactic acid bacteria to low moisture conditions (Woolford, 1984). In contrast, in a review Mühlbach (2000) quoted increased NH₃-N levels in wilted forage silage compared to unwilted silage produced from millet, elephant grass or their mixtures with cassava. Prolonged wilting also resulted in poor fermentation due to proteolysis by endogenous enzymes, which can be reflected in lower true protein proportions in the forage and, consequently, higher NH₃-N proportions in the silage (Mühlbach, 2000).

Conclusion

Molasses treatment at ensiling resulted in improved fermentation and preservation of direct cut and wilted *D. eriantha*. Wilting of *D. eriantha* prior to ensiling reduced proteolysis in silage when no molasses was added.

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