# Effects of bacterial inoculants and an enzyme on the fermentation quality and aerobic stability of ensiled whole-crop sweet sorghum

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## Abstract

A study was conducted to evaluate the effects of bacterial inoculation and cellulase on the fermentation quality of ensiled whole-crop sweet sorghum (WCSS, *Sorghum bicolor* L. Moench). The WCSS (323 g dry matter (DM)/kg, 251 g water soluble carbohydrates (WSC)/kg DM, 43 g crude protein (CP)/kg DM and 439 g neutral detergent fibre (NDF)/kg DM) was ensiled with i) no additive (control); ii) *Lactobacillus buchneri* (LB); iii) *Lactobacillus plantarum* (LP); and iv) LB+E, a combination of LB and enzyme. These treatments were ensiled in 1 L anaerobic jars for 25 days. The jars were opened on days 3, 7 and 15 to determine pH, while those of day 25 were sampled to determine nutrient composition, fermentation characteristics and aerobic stability. Inoculation reduced pH, butyric acid and ammonia-N and increased lactic acid content in sweet sorghum silage compared with the control. The aerobic stability of WCSS was improved with LB, while it was reduced with the homofermentative LP treatment compared with the control. The LB+E reduced the fibre, but increased residual WSC of silage. The aerobic stability of LB+E silage was lower than LB treated silage. Using enzymes to increase the WSC content of crops that already have high levels of WSC may result in reduced aerobic stability of silage. Further work is needed to evaluate these effects on silage produced on farm scale and on animal production performance.

**Keywords**: Aerobic stability, enzyme, fermentation, inoculants, silage
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## Introduction

Sweet sorghum (*Sorghum bicolor* L. Moench) has sweet juicy stems and has been used predominantly to produce ethanol through solid-state fermentation (Bryan, 1990; Putman *et al*., 1991). Owing to the high potential for preserving sugar in the stalks of sweet sorghum, this forage can be an ideal energy source for ruminants and good-quality silage can be produced (Adewakun *et al*., 1989, Felix & Funso, 1994, Morris & McCormick, 1994). However, whole-crop sweet sorghum (WCSS) is rich in lignocellulosic fibres (Billa *et al*., 1997), which may negatively affect nutrient digestibility (Aydin *et al*., 1999). Consequently, silage additives (bacterial inoculants, enzymes, etc.) have been used to improve the ensiling process and nutrient utilization by ruminants (Muck, 2010).

Although bacterial inoculants improved silage fermentation quality, their effects on fibre degradation is not consistent because lactic acid bacteria (LAB) cannot effectively use fibre as an energy source to produce lactic acid. Consequently, the addition of enzymes to forage at ensiling has been reported to degrade silage cell wall and increase the availability of WSC that serve as substrate for LAB (McDonald *et al*., 1991; Spoelstra *et al*., 1992; Selmer-Olsen *et al*., 1993; Sheperd & Kung, 1996). Therefore, when LAB is combined with enzymes during ensiling, a stronger effect should be expected because more fermentable sugars will be released to produce more lactic acid in comparison with other fermentation products (Kung *et al*., 1991, Chen *et al*., 1994). In contrast, Stokes (1992) reported reduced enzyme activity in the presence of a LAB inoculant.

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Well-preserved and high-quality silages, particularly those inoculated with homofermentative LAB, can be more prone to spoilage than untreated silages (Muck, 2010). This can be attributed to the low production of acetic acid, which is well known to have an antifungal effect on aerobic micro-organisms (Weinberg *et al*., 1993). As a result, the problem of aerobic instability with homofermentative LAB inoculation can be solved with the use of *Lactobacillus buchneri* (LB), which has been reported to improve aerobic stability of silage in many studies (Taylor *et al*., 2002; Pedroso *et al*., 2008; Nkosi *et al*., 2009). In contrast, the heterofermentative pathway of LB can cause an increase in silage pH, and losses in energy and dry matter (DM) content (Oude Elferink *et al*., 2001). However, if aerobic stability is improved, the loss of nutrients incurred by the addition of LB may be moderate in comparison with what might have been lost at feed out through aerobic deterioration.

Although the fermentable substrate is usually not a limiting factor for the fermentation of sweet sorghum during ensiling, an increase in ethanol content caused by enzyme treatment has led to poor aerobic stability of silage (Spoelstra *et al*., 1992). Xing *et al.* (209) reported poor aerobic stability of sweet sorghum silage treated with a homofermentative LAB and an enzyme mixture at ensiling. It was therefore hypothesized that an enzyme and LB mixture will improve the aerobic stability of sweet sorghum silage. The present study therefore aimed to evaluate the effects of bacterial and enzyme inoculation on the fermentation and aerobic stability of ensiled WCSS.

## Materials and Methods

Whole crop sweet sorghum (hybrid M81E, KS Breeder, Mississippi State University, USA) was harvested in Manhattan, Kansas, USA at dough stage using a harvester adjusted to 15 mm cutting length. The inoculants, *Lactobacillus buchneri* (LB, strain ATCC 4005) and *Lactobacillus plantarum* (LP, strain ATCC 14917), were obtained from the American Type Culture Collection (ATCC, 10801 University Blvd, Manassas, VA, USA). The cellulose enzyme (Cellic® Ctec2) that contained 87 - 95 filter paper units (FPU)/mL activity with optimum pH of 5 (Lu *et al*., 2011), was obtained from Novozymes, Franklinton, N.C., USA. The inoculants (either LB or LP) were prepared by mixing 0.2 g inoculant with 32 mL of distilled water and sprayed over a 16 kg fresh WCSS to obtain at least 2.5 x $10^5$ colony forming units of LAB/g fresh WCSS. The enzyme was prepared by mixing 0.2 g Cellic® CTec2 (enzyme complex consisting of cellulase, xylanase and beta-glucosidase activity) with 0.2 g LB, added to 32 mL distilled water and sprayed over 16 kg fresh WCSS. The application rates were done in accordance with the level of LAB and enzymes in the cultures as determined by the manufacturers. In order to add the same amount of moisture as in the treated WCSS, the control was treated with 32 mL of distilled water on 16 kg WCSS. The treatments were i) no inoculant (control), ii) LP, iii) LB and iv) LB+E, a combination of LB and cellulase enzyme. Triplicate samples (n = 3) from each treatment were collected and analysed for chemical composition before ensiling. The treatments were then ensiled in 48 x 1 L jars (12 jars/treatment), kept at room temperature of 24 - 28 °C. Each jar was filled with approximately 850 g (wet weight) chopped maize without headspace, and a packing density of 276 (± 0.628) kg DM/$m^3$ was obtained. Three jars per treatment were opened on d 3, 4, 15 and 25 of ensiling to determine pH, while analysis for chemical composition and fermentation characteristics were done only on samples at day 25.

Aerobic stability of silage was defined as the number of hours that it took for the silage temperature to rise 2 °C above that of the ambient temperature. Samples of day 25 were subjected to a 5 d aerobic test, where 500 g of sample from each jar was loosely packed in an open plastic jar that was covered with two layers of cheesecloth and kept at 28 °C. A temperature probe (ACR Smartbutton, ACR Systems Inc. Building 210-12960, 84 Avenue Surrey, BC, V3W 1K7, Canada) was placed in the geometric centre of the silage mass for each jar and also in the room where the jars were stored to record temperature. The room temperature and the temperature in each jar were monitored simultaneously at 1 h intervals for 5 d.

A representative 40 g silage sample was taken from each jar to determine the fermentation characteristics. The 40 g silage sample (n = 3) was mixed with 360 mL of distilled water in a stomacher bag, homogenized for 4 min and pH was determined immediately with a pH meter (Thermo Orion Model 525, Thermo Fisher Scientific, Waltham, MA, USA). It was then filtered through a Whatman No. 54 filter paper (Clifton, NJ, USA). The extract was used to determine pH, water-soluble carbohydrates (WSC), volatile fatty acids (VFAs), lactic acid (LA) and ammonia-N. The WSC were determined by the Dubois *et al*.'s (1956) phenol-sulphuric acid method and LA was determined by the Pryce's (1969) modified colorimetric method The VFAs were determined with a Varian 3300 FID Detector gas chromatograph (Varian Associates, Inc.,

Palo Alto, CA, USA) by the procedure of Suzuki & Lund (1980). Ammonia N was determined by distillation using a Buchi 342 apparatus and a Metrohm 655 Dosimat with an E526 titrator according to AOAC (ID 941.04, 1990).

The DM of the pre-ensiled mixtures and that of the silages were determined by drying the samples at 60 °C until a constant mass was achieved, and was corrected for loss of volatiles using Weissbach & Strubelt's (2008) equation. After drying, the samples were ground through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) for chemical analysis. The neutral detergent fibre (NDF) was analysed using Van Soest *et al*.'s (1991) method with a heat stable α-amylase and sodium sulphate was added. The acid detergent fibre (ADF) was analysed using a Fibertec System 1010 (FOSS Analytical AB, Sweden) by boiling samples in an acidic solution followed by filtration (Van Soest *et al*., 1991). Separate samples were used for ADF and aNDF analysis and both included residual ash. Crude protein (CP) (ID 968.06) and ether extract (EE) (ID 963.15) were determined according to the procedure, while the gross energy (GE) was determined with an adiabatic bomb calorimeter (IKA C7000, Staufen, Germany).

Data were organized as a completely randomized design (CRD) with replicates nested within treatments. Therefore, a one-way analysis of variance (ANOVA) with Genstat (2005) was used to test for the differences among treatments using sample error as experimental error. Means of significant treatment effects ($P$ <0.05) were compared using the Student`s t-LSD (least significant difference) at a 5% significant level (Snedecor & Cochran, 1980). The statistical model used was: $Y_{ij} = \mu + t_i + ß_j + \varepsilon_{ij}$
where $Y_{ij}$ is the individual observations of the i-th treatment and the j-th replicate, $\mu$ is the overall mean, $t_i$ is the effect of the i-th treatment, $\beta_j$ is the effect of the j-th replicate, and $\varepsilon_{ij}$ is the residual error.

## Results and Discussion

The high sugar content in WCSS makes it an ideal forage for silage making. High residual sugar content in silage can serve as a dietary energy supplement for ruminants but may render the silage susceptible to aerobic deterioration during the feeding phase (Weinberg *et al*., 1993). Bacterial inoculants are added to forage at ensiling in order to stimulate lactic acid (LA) fermentation by accelerating the decrease in pH, and thus improving silage preservation (McDonald *et al*., 2002). If low numbers of sufficient lactic acid bacteria (LAB) are present in the crop at ensiling, a slow rate of pH decrease will occur. Results for the chemical composition of pre-ensiled WCSS are shown in Table 1. When the silage DM content is lower than 300 g/kg while having a high content of WSC, chances for ethanol production during fermentation are high (McDonald *et al*., 2002), which may reduce silage intake (Wilkins *et al*., 1971). The DM content of freshly chopped WCSS was 323 g DM/kg, ideal for ensiling.

**Table 1** Chemical composition (g/kg DM unless stated otherwise) of pre-ensiled whole-crop sweet sorghum (n = 3)

| Parameter | |
|---|---|
| Dry matter (DM), g/kg | 323 ± 1.62 |
| pH | 6.5 ± 1.36 |
| Water-soluble carbohydrate | 251 ± 0.51 |
| Ash | 42 ± 0.27 |
| Gross energy (MJ/kg DM) | 20.6 ± 1.32 |
| Crude protein | 43 ± 0.35 |
| Ether extract | 13 ± 1.74 |
| aNDF | 439 ± 1.65 |
| ADF | 282 ± 0.92 |

aNDF: amylase treated neutral detergent fibre; ADF: acid detergent fibre.

A pH range of 3.7 - 4.2 is generally considered beneficial for whole-crop cereal preservation (Kung & Shaver, 2001) and that of our study was less than 3.9, indicative of well-preserved silage. Inoculation reduced ($P$ <0.05) the pH of sweet sorghum silage compared with the control, which is in agreement with previous studies (Fellner *et al*., 2001; Nkosi *et al*., 2009) who reported reduced pH in inoculated maize silage compared with the control. The pH of the inoculated WCSS was below 4.0 as of d 3 of ensiling while that of the control took 7 d to get below 4.0 (Figure 1). However, our present study is in contrast with the work of Xing *et al.* (2009), who reported a lack of effect on pH with bacterial inoculation to sweet sorghum straw at ensiling. According to these researchers, this could be attributed to the sufficient amount of WSC contained in sweet sorghum straws, which can easily produce a rapid drop in silage pH without the use of additives.

Water-soluble carbohydrates are regarded as essential substrates for the growth of LAB for proper fermentation (McDonald *et al*., 1991). Sweet sorghum stalks have been reported to contain 250 - 350 g WSC/kg DM (Griffiths *et al*., 2004). The WSC content in our WCSS prior to ensiling was 251 g WSC/kg DM, more than sufficient for LAB (McDonald *et al*., 2002). However, after 25 d of ensiling, the LB+E treatment had higher ($P$ <0.05) residual WSC compared with the other treatments, consistent with other workers (Stokes, 1992; Masuko *et al*., 1996). This reflects that the WSC was not fermented completely by the LB+E treatment (Schmidt *et al*., 1997) and this treatment degraded NDF to WSC. The LP treated silage had lower residual WSC among the treatments, suggesting a more intensive use of sugars for LA production, consistent to Pedroso *et al*. (2008) when sugarcane silage was inoculated with LP.

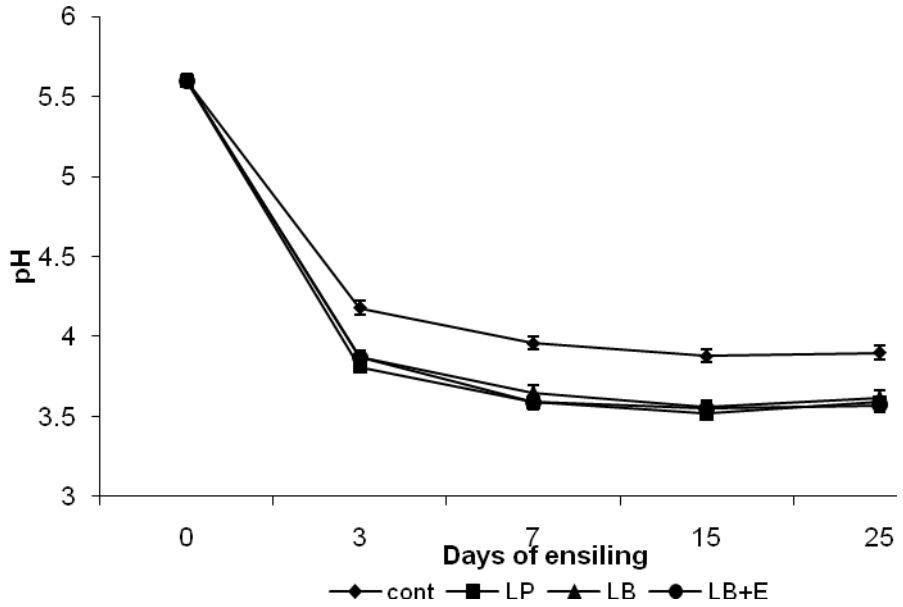


**Figure 1** Effects of bacterial inoculation on the pH of whole-crop sweet sorghum during 25 days of ensiling.

A high-quality silage is likely to be achieved when LA is the predominant acid produced, and good quality silage should contain LA concentration in the range of 40 to 120 g/kg DM (McDonald *et al*., 1991). The LA content in silage of our study was within this recommended range, an indication of well-fermented silage. However, the LA concentration recorded in our study was lower than 160 g LA/kg DM reported in sweet sorghum silage (Xing *et al*., 2009) but consistent with that reported by Podkowka & Podkowka (2011). It has been reported that homofermentative LAB or LAB+enzyme inoculation has positive effects on the silage fermentation by increasing LA compared with the control (Kung *et al*., 1991, Chen *et al.*, 1994). As expected, inoculation increased ($P$ <0.05) the concentration of LA compared with the control.

**Table 2** Effects of bacterial inoculation and enzyme on the nutrient composition, fermentation characteristics and aerobic stability of whole-crop sweet sorghum after 25 days of ensiling (n = 3)

| | Treatments | | | | SEM | *P* |
|---|---|---|---|---|---|---|
| | Control | LP | LB | LB+E | | |
| Nutrient composition (g/kg DM unless stated otherwise) | | | | | | |
| Dry matter | 308 | 314 | 305 | 310 | 8.17 | 0.144 |
| Ash | 43.4$^{b}$ | 42.2$^{b}$ | 46.2$^{a}$ | 43.9$^{b}$ | 0.65 | 0.014 |
| Crude protein | 39$^{c}$ | 41.8$^{a}$ | 41.4$^{a}$ | 40$^{b}$ | 0.28 | 0.001 |
| GE (MJ/kg DM) | 18.7$^{d}$ | 19.2$^{a}$ | 18.9$^{c}$ | 19.0$^{b}$ | 0.02 | 0.001 |
| Ether extract | 16.3$^{a}$ | 15.1$^{b}$ | 15.0$^{b}$ | 14.2$^{c}$ | 0.18 | 0.001 |
| aNDF | 489$^{a}$ | 438$^{c}$ | 471$^{b}$ | 441$^{c}$ | 3.37 | 0.001 |
| ADF | 331$^{a}$ | 297$^{c}$ | 314$^{b}$ | 295$^{c}$ | 3.01 | 0.001 |
| Fermentation characteristics (g/kg DM unless stated otherwise) | | | | | | |
| pH | 3.90$^{a}$ | 3.59$^{bc}$ | 3.62$^{b}$ | 3.57$^{c}$ | 0.004 | 0.001 |
| WSC | 116.4$^{c}$ | 101.8$^{d}$ | 152.4$^{b}$ | 193.5$^{a}$ | 13.8 | 0.001 |
| LA | 81.2$^{c}$ | 127.3$^{a}$ | 95.3$^{b}$ | 94.7$^{b}$ | 6.82 | 0.012 |
| AA | 25.2$^{c}$ | 7.4$^{d}$ | 41.4$^{a}$ | 32.6$^{b}$ | 3.42 | 0.001 |
| PA | 0.019 | 0.021 | 0.018 | 0.019 | 0.071 | 0.0154 |
| BA | 0.121$^{a}$ | 0.062$^{b}$ | 0.069$^{b}$ | 0.067$^{b}$ | 0.084 | 0.015 |
| $NH_3$-N (g/kg TN) | 52.2$^{a}$ | 41.4$^{b}$ | 400.1$^{b}$ | 39.3$^{b}$ | 2.62 | 0.001 |
| Aerobic stability | | | | | | |
| No. of hours | 53$^{b}$ | 46$^{c}$ | 72$^{a}$ | 55$^{b}$ | 2.82 | 0.001 |
| pH after aerobic stability test | 4.92$^{b}$ | 5.4$^{a}$ | 4.23$^{c}$ | 4.05$^{d}$ | 0.019 | 0.001 |

[a-d] Means with different superscripts in a row differ significantly ($P$ <0.05).
GE - gross energy; aNDF - amylase treated neutral detergent fibre; ADF - acid detergent fibre; WSC - water-soluble carbohydrate; LA - lactic acid; AA - acetic acid; PA - propionic acid; BA - butyric acid; $NH_3$-N - ammonia nitrogen.

Some studies have reported a reduced concentration of LA while increasing acetic acid (AA) concentrations with LB inoculation to maize silage compared with the control (Muck, 2010). However, the AA concentrations in silages in our study were generally low compared with the LA concentration (Table 2), indicative of good preservation quality of the silage (McDonald *et al*., 1991). This agrees with Mari *et al*. (2009) who reported increased LA and AA in corn silage inoculated with LB compared with the control.

The inoculation of LB and LB+E resulted in an increased ($P$ <0.05) concentration of AA compared with the other treatments. Kung *et al.* (2007) reported increased AA in high moisture maize that was treated with LB+E, and LB treatment was also reported to increase AA in other studies (Pedroso *et al*., 2008; Nkosi *et al*., 2009), consistent with the present study. However, the AA concentration of the LB+E treatment in our present study is higher than 20 g AA/kg DM, reported from sweet sorghum silage that was inoculated with a combination of inoculant and enzyme (Xing *et al*., 2009). This might be attributed to the fact that the latter study combined an enzyme with a homofermentative LAB, while our present study combined an enzyme with LB, a heterofermentative LAB, known to increase AA.

Ammonia-N in silage reflects the degree of protein degradation, and extensive proteolysis adversely affects the utilization of N by ruminants (Wilkinson, 2005). Well-preserved silages should contain less than 100 g ammonia-N/kg TN (McDonald *et al*., 2002) but this can be high in forages that are high in CP content such as legumes. Our sweet sorghum silage had ammonia-N concentrations that were lower than this value, which is indicative of well-preserved silage. It has been reported that inoculation reduced proteolysis during ensiling and resulted in improved efficiency of silage protein utilization and reduced N losses (McDonald *et al*., 2002). According to McDonald *et al*. (1991), this effect arose as a result of pH reduction with

inoculation, which inhibits protein degradation in silages. This supports our findings, since inoculation reduced ($P$ <0.05) both the pH and ammonia-N production compared with the control.

Silage from cereal crops is fed usually to high-producing animals as a source of energy. It is well established that inoculation of LAB to forage at ensiling reduces the concentration of butyric acid (BA) in silage (McDonald *et al*., 1991). A higher ($P$ <0.05) concentration of BA occurred in the control, leading to a reduced energy content of the silage compared with the other silages. A concentration of <0.1 g BA/kg DM is typically found in well-preserved silage (Kung & Shaver, 2001) and our WCSS had values lower than this threshold, an indication of a well-preserved silage. In addition, BA is associated with a clostridial type of fermentation and usually associated with high-moisture silages (McDonald *et al*, 1991) and the WCSS had 323 g DM at pre-ensiling. The GE recorded in the WCSS in our study is in agreement with that of Lema *et al.* (2000), who recorded 20.09 MJ/kg in sweet sorghum silage. Margan *et al*. (1994) and Tine *et al*. (2001) reported 18 GE MJ/kg DM in maize silage, which is comparable with that of WCSS in our study.

The content of CP in our WCSS is lower than those reported in sweet sorghum silage (Morris & McCormick, 1994, Di Marco *et al*., 2009, Xing *et al*., 2009), which ranged between 77 and 95 g CP/kg DM. This difference could be attributed to different hybrids, soil conditions and harvest stage between the two studies (Lema *et al*., 2000). However, Lema *et al*. (2000) reported <46 g CP/kg in silage produced from various sweet sorghums, which is consistent with our present study. This CP content is too low to support animal production and may need protein supplementation to improve animal performance.

It has been reported that the effects of LAB inoculants on fibre degradation are not consistent because LAB cannot effectively use fibre as an energy source to produce lactic acid (Muck, 2010). Faber *et al*. (1989) attributed the lack of response with LAB inoculation to a lower environmental temperature that inhibited hemicellulose degradation. The LB+E and LP reduced ($P$ <0.05) the fibre content of silage compared with the other treatments, consistent with other studies (Nadeau *et al*., 2000). In contrast, some reports showed that LAB+enzyme inoculants (Meeske *et al*., 1993; Kung *et al*., 2007) and LAB (Faber *et al*., 1989) did not affect cell wall contents in silages.

The effect of acetic acid on the control of yeasts to improve silage aerobic stability was proposed by Weinberg *et al*. (1993). According to these researchers, increasing AA concentrations in silage inhibited spoilage micro-organisms and promoted aerobic stability. According to previous research (Taylor *et al*., 2002, Pedroso *et al*., 2008, Nkosi *et al.*, 2009) inoculation with LB typically results in acetic acid concentrations ranging from 36 to 50 g/kg DM, suitable for controlling yeast during aerobic exposure of silage. The acetic acid concentration of 40 g/kg DM in the LB and LB+E treated WCSS was enough to control yeast. In contrast, Xing *et al*. (2009) reported no effects on the aerobic stability of sweet sorghum silage when either LAB or enzyme was used. This can be attributed to the fact that they used a homofermentative LAB inoculant, which is well known to reduce aerobic stability of silage (Nkosi *et al*., 2010). When exposed to air for five days, the LB silage had a higher aerobic stability as indicated by the higher ($P$ <0.05) numbers of hours it remained stable together with its lower pH value (Table 2) compared with the other treatments. According to the evidence that silages of higher LA concentrations or those with more residual sugar contents are less stable when exposed to air (Weinberg *et al*. 1993), the aerobic stability of WCSS in our study was reduced ($P$ <0.05) with LP inoculation compared with other treatments, supporting other studies (e.g. Nkosi *et al*., 2010). This is also evident from Table 2 where a higher increase in pH was observed with LP inoculation after 5 d of aerobic exposure. The aerobic stability of WCSS that was treated with LB+E lasted for 55 h, similar to the control and better than the LP treated silage. Inoculation of LB to sugarcane at ensiling resulted in a 78 h aerobic stability (Pedroso *et al*., 2008), which is comparable with the 72 h aerobic stability obtained in the LB treated WCSS in our study.

## Conclusions

It was concluded that the WCSS produced in the present study was of good quality in terms of the fermentation characteristics. The application of LAB inoculant treatments LP, LB and LB+E resulted in reduced pH, BA and ammonia-N and increased LA content in sweet sorghum silage compared with the control. The WSC content of sweet sorghum prior to ensiling was high and WSC did not limit preservation in any of the treatments. The aerobic stability of silage was reduced with the homofermentative LP treatment compared with the control. The most noticeable effect of LB+E treatment was the reduction of fibre but increased residual WSC. The aerobic stability of LB+E silage was lower than LB treated silage. Using enzymes to increase the WSC content of crops that already have high levels of WSC may result in reduced

aerobic stability of silage. Further work to evaluate these effects on silage produced on farm scale and on animal production performance is needed.

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