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Effects of a fibrolytic enzyme and bacterial inoculants on the fermentation, chemical composition and aerobic stability of ensiled potato hash

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Abstract

The study was conducted to evaluate the effects of adding a fibrolytic enzyme in combination with bacterial inoculants on the fermentation, chemical composition and aerobic stability of ensiled potato hash (PH). Potato hash silage (PHS) was produced by mixing 800 g PH/kg and 200g wheat bran (WB)/kg. The mixture was ensiled with either no additive or enzyme Celluclast (low or high dose) or bacterial inoculants (Emsilage and Silosolve). These treatment combinations were produced: i) no additive (control); ii) Celluclast low dose (CLD); iii) Celluclast high dose (CHD); iv) Emsilage (EMS); v) CLD + EMS; vi) CHD + EMS; vii) Silosolve (SLS); viii) CLD + SLS; and ix) CHD + SLS. These treatments were ensiled in 81 x 1 L anaerobic jars for 90 days with nine replicates per treatment. Three samples per treatment were collected before ensiling and after 90 days' ensiling, were analysed for fermentation characteristics and chemical composition. In addition, samples of day 90 were subjected to an aerobic stability test, where they were exposed for five days. Enzyme addition reduced fibre, thus making more sugar available for fermentation. The combination of CHD and EMS reduced silage pH, thus preserving the silage compared with other treatment combinations. Enzyme addition (used at low and high dose), and bacterial inoculants improved fermentation. Enzyme addition improved the chemical composition, but impaired the aerobic stability of PHS. Further work to test these findings on animal performance is warranted.

Keywords: Deterioration, fibre, nutrients, pH, ruminant, water soluble carbohydrates
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Introduction

Agro-industrial by-products from food processing industries can be less expensive sources of nutrients for ruminant feeding because of their capacity to digest fibre-rich feedstuffs (Boucque & Fiems, 1988). This reduces the dependency of ruminant nutrition on mixed cereals as the major energy source, which is usually costly, since cereals may have to be imported, especially in times of shortage (Briedenhann, 2008). Potato hash, a food by-product that is derived from the production of snacks in South Africa, can benefit livestock production. It is a mixture of potato skins, starch, fats and yellow maize, containing 700 g starch/kg dry matter (DM), 11.2 MJ Metabolisable energy (ME)/kg, 105 g crude protein (CP) /kg DM, 369 g Neutral detergent fibre (NDF)/kg DM, and 162 g Acid detergent fibre (ADF) /kg DM (Nkosi *et al.*, 2010a). The production of PH is estimated at 50 tons per day, and if not effectively used, is dumped.

Ensiling can be regarded as an efficient way of preserving high moisture by-products if all essential principles are followed (Cao *et al*., 2009). Wilkinson (2005) indicated that a content of water soluble carbohydrates (WSCs), low buffering capacity, 250–400 g DM/kg, and adequate population of lactic acid bacteria (LAB) are requirements for effective fermentation of forages. Potato hash, like other potato by-products, contains low DM and WSCs (Nkosi *et al*., 2012a), which warrants the use of silage additives (McDonald *et al*., 2011). Nkosi *et al*. (2010b) produced silage successfully from PH by mixing with wheat bran (WB) as an absorbent and CP source or by producing a total mixed ration that contained 800 g/kg PH.

Aerobic stability, defined as the number of hours it takes silage temperature to rise 2 °C above ambient temperature, is important because of the consequential losses of nutrients, and the development of moulds, which could produce mycotoxins that pose health hazards to animals and humans (Driehuis & Oude

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Elferink, 2000). The extent of aerobic exposure can be indicated by the rate of CO_2 production, a rise in temperature and pH, and rapid growth of yeasts and moulds (Ashbell *et al*., 1991).

Well-preserved silages, particularly those inoculated with homofermentative LAB, can be prone to spoilage (Muck, 2010), because of lower production of VFAs that inhibit the growth of yeasts and moulds (Weinberg *et al*., 1993). However, the problem of aerobic stability can be solved with the use of *L. buchneri*, a heterolactic bacterium that converts moderate amounts of Lactic acid (LA) to Acetic acid (AA) under anaerobic conditions (Driehuis *et al*., 1999). Inoculants containing *L. buchneri* have improved the aerobic stability of various silages (Nkosi & Meeske, 2010).

Although bacterial inoculants have been reported to improve silage fermentation and aerobic stability, their effects on fibre degradation are not consistent because LAB cannot effectively use fibre as an energy source to produce LA. Most LAB have little or no ability to degrade plant cell walls (McDonald *et al*., 2011). Potato hash contains 369 g NDF/kg DM and 162 g ADF/kg DM (Nkosi *et al*., 2010a). When mixed with other fibrous sources, the fibre content may increase, which would render PH silage unsuitable for growing ruminants as a sole diet or for inclusion in diets at high levels.

It has been reported that fibrolytic enzyme application at ensiling has improved the fermentation and nutritive value of maize silage (Colombatto *et al*., 2003), Bermuda grass silage (Dean *et al*., 2005) and potato pulp (Okine *et al*., 2005). An improvement in silage aerobic stability due to *L. buchneri* and enzyme combination has been reported for high moisture maize silage (Taylor *et al*., 2000) and barley silage (Kung & Ranjit, 2001). However, Ebling (2002) reported that the addition of enzymes showed no further improvement in aerobic stability compared with the effect of *L. buchneri* alone in high moisture silage.

The objective of this study was therefore to determine the effects of addition of a fibrolytic enzyme and bacterial inoculants on the fermentation, chemical composition and aerobic stability of ensiled PH.

Materials and Methods

Potato hash was collected from Simba (336 Andre Greyvenstein Road, Isando, Gauteng, South Africa) and brought to Agricultural Research Council–Animal Production Institute (ARC–API) for chemical analysis and silage production. PHS was produced by mixing 800 g PH/kg with 200 g WB/kg. WB, a by-product from milling of wheat, is a cheap feed ingredient that contains high DM. It was used to improve the DM content during ensiling PH (Nkosi *et al*., 2010a). Celluclast® 1.5 L (Novozymes, Denmark), a fibrolytic enzyme, contains cellulose prepared from Trichoderma reesei and has a stated enzyme activity of 1500 NCU (novo cellulose units) ml^{-1}. Celluclast was applied at a rate of 1.1 L (1 L water mixed with 100 ml CLD, or 1.2 L (1 L water mixed with 200 ml CHD. These doses were used to treat 500 kg freshly mixed material. Silosolve™ AS 200 (Chr. Hansen Inc., Animal Health and Nutrition, Czech Republic), a heterofermentative LAB inoculant, contains *Lactobacillus plantarum* (DSM 16568 at 2.5 x 10^{10} cfu/g), *Enterococcus faecium* (DSM 22502/ NCIMB 11181 at 3.8 x 10^{10} cfu/g) and *Lactobacillus buchneri* (DSM 22501/CCM 1819 x 6.3 x 10^{10} cfu/g). Five grams of Silosolve were dissolved in 1 L distilled water and used to treat 500 kg freshly mixed material. Emsilage (Probiokashi (Pty) Ltd, Stellenbosch, South Africa), a heterofermentative LAB inoculant, contains *Lactobacillus plantarum, Lactobacillus fermentum* and *Lactobacillus lactis*. An amount of 250 ml of Emsilage was diluted in 6.75 L water, and 2 L of this solution was used to treat 500 kg of freshly mixed PH. The control (untreated) silage was sprayed with 2 L distilled water per 500 kg freshly mixed material to ensure the same amount of moisture as in the treated silage.

Nine treatment combinations were produced: i) no additive (control); ii) Celluclast low dose (CLD); iii) Celluclast high dose (CHD); iv) Emsilage (EMS); v) CLD + EMS; vi) CHD + EMS; vii) Silosolve (SLS); viii) CLD + SLS; and ix) CHD + SLS. The treatments were ensiled in 81 x 1.5 L anaerobic glass jars (J. Weck, GmBHu. Co., Wehr-Oflingen, Germany) with nine jars per treatment. Each jar was filled with approximately 850 g (wet weight) of fresh PH material without head space. The jars were stored in the laboratory at a temperature of 24–28 °C to allow fermentation to occur for 90 days. Three samples per treatment were collected before ensiling and analysed for pH, WSCs, DM, CP, Gross energy (GE), NDF and ADF. Samples of Day 90 were analysed for fermentation characteristics and chemical composition. In addition, samples of Day 90 were subjected to an aerobic stability test in which 250 g from each jar was loosely packed in an open plastic jar that was covered with two layers of cheesecloth and kept at 28 °C. Thermocouples (T-type copper constantan, 20-gauge wire) were placed in the geometric centre of the silage mass in each jar and in the room where the jars were stored to record temperature. The room temperature and temperature in each jar were recorded simultaneously at one-hour intervals using a CR7X data logger (Campbell Scientific, Logan, Utah) for five days. The number of hours recorded by the data logger was regarded as the time taken for the silage temperature to rise 2 °C above ambient temperature. CO_2 production, changes in PH and yeast and mould activity were determined after five days of aerobic exposure of silage using 2 L polyethylene terephthalate bottles according to a method described by Ashbell *et al*. (1991).

A representative 40 g (pre-ensiled and silage) sample was taken from each jar and mixed with 360 ml distilled water in a stomacher bag, homogenized and left at 10 °C for 24 hours (Suzuki & Lund, 1980). It was then homogenized for four minutes and filtered through a Whatman no. 4 filter paper (GIC Scientific, Midrand, South Africa). The pH was determined immediately with a pH meter (Thermo Orion Model 525, Thermo Fisher Scientific, Waltham, MA, USA). The filtrate was used to determine WSCs, LA, Volatile fatty acids (VFAs) and ammonia (NH_3-N).

The WSC fraction was determined by the phenol-sulphuric acid method of Dubois *et al*., (1956). LA was determined by the colometric method of Pryce (1969). The VFAs were analysed with a Varian 3300 flame ionization detector (FID) gas chromatograph (Varian Associates, Inc., Palo Alto, CA, USA) by the procedure of Suzuki & Lund (1980). Ammonia-N was determined according to Broderick & Kang (1980).

The DM of silage was determined by drying the samples at 60 °C to a constant mass and was corrected for loss of volatile fatty acids using the equation of Porter & Murray (2001), using the equation:

True DM= 19.96 + 0.9793 [0.987 (ODM60 – 0.260].

After drying, the samples were ground through a 1-mm screen (Wiley Mill, Standard Model 3, Arthur H. Thomas., Philadelphia, PA, USA) for chemical analysis.

NDF and ADF content were fractionated using heat-stable α-amylase (Sigma- Aldrich Co. LTD., Gillingham, UK, no. A-1278) with sodium sulphite according to Van Soest *et al*. (1991) using the Fibretech system equipment (Tecator Ltd, Thornbury, Bristol, UK). CP was estimated according to AOAC (1990 ID 976.05). GE was determined with a bomb calorimeter (MS-1000 modular calorimeter, Energy Instrumentation, 135 Knoppieslaagte, Centurion, South Africa). Enumeration of yeasts and moulds was done according to the procedure of IDF (1990).

Data on the effects of treatment combinations on fermentation, chemical composition and aerobic stability of the PH silage were analysed for a 3 x 3 factorial in a completely randomised design on a general linear model using Minitab Statistical Software Release 16 (Minitab, 2010). Tukey's test was used to compare the treatment means. The model was as follows:

$$Y_{ijk} = \mu + E_i + B_j + (E \times B)_{ij} + \varepsilon_{ijk}$$

Where: μ = overall mean
E_i = effect of the i^{th} enzyme inoculant
B_j = effect of the j^{th} bacterial inoculant
$(EB)_{ij}$ = effect of interaction between the i^{th} enzyme and j^{th} bacterial inoculants
ε_{ijk} = residual error

Results and Discussion

The chemical composition of pre-ensiled PH with or without WB is shown in Table 1.

Table 1 Chemical composition (g/kg dry matter) of pre-ensiled potato hash with or without wheat bran (n = 3)

Parameter	Potato hash	[a]Potato hash / wheat bran mixture
DM g/kg	188	352
CP	84.8	152
GE	13.9	16.3
NDF	500	449
ADF	146	139
WSC	3.35	76.0
pH	6.20	6.20

DM: dry matter; CP: crude protein; GE: gross energy; NDF: amylase-treated neutral detergent fibre; ADF: acid detergent fibre; WSCs: water soluble carbohydrates
[a]80% potato hash: 20% wheat bran

The DM of a crop at ensiling has a strong influence on the rate and extent of the resulting fermentation (McDonald *et al*., 2011). A low DM content with low sugar content increases the chances of clostridial fermentation and subsequent poor acceptance by animals (Fraser *et al*., 2000). DM content of 250–400 g/kg in forage is required for favourable fermentation (Wilkinson, 2005). The DM of PH (188 g DM/kg) was not ideal for effective fermentation and warranted the addition of WB, increasing the DM to 352 g/kg, which is within the required range. WSCs are regarded as essential substrates for the growth of LAB to enhance efficient fermentation (McDonald *et al*., 2011). Lunden-Petersen & Lindgren (1990) recommended 60–70 g WSC/kg DM for well-preserved silage. The addition of WB to PH at ensiling increased WSCs to 76 g WSC/kg DM, making more substrate available for LAB.

Data on the fermentation characteristics of PH after 90 days of ensiling are presented on Table 2. The pH, WSC, LA, AA, and PA were significantly influenced by interaction between the bacterial inoculant and the enzyme. It is documented (McDonald *et al*., 2011) that one of the most important factors affecting silage quality is the rate of decrease in pH of the plant material being preserved. A pH range of 3.7–4.2 is generally considered beneficial for forage preservation (Kung & Shaver, 2001). That of the present study was less than 3.5, an indication of well-preserved silage.

Table 2 Effects of enzyme and bacterial inoculants on the fermentation characteristics of potato hash after 90 days of ensiling (n = 3)

Treatment	Enzyme	Bact. Inoc.	DM (g/kg)	pH	WSC (g/kg DM)	LA (g/kg DM)	AA (g/kg DM)	PA (g/kg DM)	BA (g/kg DM)	NH_3-N (g/kg TN)
1	0	0	321	3.51^{a}	12.7^{e}	66.4^{c}	6.35^{ef}	1.35^{b}	0.13	4.32
2	CLD	0	317	3.36^{de}	16.7^{bcd}	86.7^{a}	6.82^{de}	0.90^{d}	0.32	3.74
3	CHD	0	327	3.37^{ef}	19.5^{a}	84.3^{a}	7.44^{bc}	0.88^{d}	0.00	3.65
4	0	EMS	327	3.34^{f}	9.00^{f}	86.2^{a}	7.15^{cd}	1.08^{c}	0.25	3.93
5	CLD	EMS	381	3.42^{c}	15.6^{d}	77.6^{b}	6.02^{f}	0.79^{e}	0.00	3.36
6	CHD	EMS	325	3.34^{f}	17.5^{bc}	61.5^{d}	6.12^{f}	0.69^{f}	0.00	3.71
7	0	SLS	356	3.45^{b}	12.1^{e}	61.9^{cd}	9.64^{a}	1.68^{a}	0.28	4.42
8	CLD	SLS	373	3.37^{d}	16.2^{cd}	63.2^{cd}	7.70^{b}	1.70^{a}	0.20	4.43
9	CHD	SLS	362	3.37^{d}	18.0^{ab}	60.8^{d}	7.91^{b}	1.67^{a}	0.13	4.43
SEM			819.5	0.000178	0.97	7.95	0.10	0.001	0.02	0.06
Enzyme means										
0			334	3.46^{a}	11.3^{c}	63.2^{b}	8.24^{a}	1.37^{a}	0.22^{a}	3.93^{b}
CLD			357	3.36^{b}	16.2^{b}	75.8^{a}	6.85^{b}	1.13^{b}	0.17^{ab}	3.89^{b}
CHD			345	3.35^{b}	18.3^{a}	77.1^{a}	6.64^{b}	1.08^{c}	0.04^{b}	4.26^{a}
SEM			819.5	0.000178	0.97	7.95	0.10	0.001	0.02	0.06
Bacterial inoculant means										
0			321^{b}	3.40^{a}	15.1	79.1^{a}	7.39^{b}	1.04^{b}	0.15	3.75^{b}
EMS			351^{ab}	3.37^{b}	15.3	75.1^{b}	6.43^{c}	0.85^{c}	0.08	3.94^{b}
SLS			364^{a}	3.39^{a}	15.5	62.0^{c}	7.90^{a}	1.68^{a}	0.20	4.39^{a}
SEM			819.5	0.000178	0.97	7.95	0.10	0.001	0.02	0.06
Significance										
Bact. Inoc. (B)			**	**	NS	**	**	**	NS	**
Enzyme (E)			NS	**	**	**	**	**	*	**
ExB			NS	**	**	**	**	**	NS	NS

$^{a-f}$ Means in the same column within the same section with different superscripts differ significantly (P <0.05). CLD: Celluclast low dose; CHD: Celluclast high dose; EMS: Emsilage; SLS: Silosolve; Bact. Inoc: bacterial inoculant; DM: dry matter; TN: total nitrogen; WSCs: water soluble carbohydrates; LA: lactic acid; AA: acetic acid; PA: propionic acid; BA: butyric acid; NH_3-N: ammonia nitrogen; NS: not significant; *P <0.05; **P <0.01

The addition of Celluclast reduced (P <0.05) silage pH compared with other treatments. This is consistent with other reports (Nkosi *et al*., 2012b) in which silage pH was reduced with the addition of enzyme to forage (peas, ryegrass, wheat, sweet sorghum) at ensiling. However, the level of enzyme addition (CLD vs CHD) did not differ (P >0.05) in the reduction of silage pH. The reduction in pH due to Emsilage addition is consistent with results from another study (Pedroso *et al*., 2008) and could be attributed to the higher LA content observed in this silage (Table 2), typical of a homofermentative LAB inoculant pathway (Pahlow *et al*., 2003).

The addition of Celluclast resulted in higher (P <0.05) residual WSC content of PH silage, indicative that more fibre fractions were degraded by enzyme addition to increase sugar content of ensiled PH. These results are consistent with those of other studies (Nadeau *et al*., 2000) where enzymes were reported to increase WSC of ensiled forages. Increasing the level of enzyme application (CHD) increased (P <0.05) the residual WSC content of PHS compared with CLD and the control treatments.

The increased (P <0.05) LA observed in the EMS treatment (86.2 g LA/kg DM) was higher than the 67.3 g LA/kg DM observed by Okine *et al*. (2005). This could be attributed to the differences in inoculant activities in the various studies. Increasing Celluclast application did not have a superior effect on LA production compared with the low dose, but the overall enzyme addition increased LA production compared with the control, consistent with findings of Chamberlain & Robertson (1992).

The production of AA during silage production is a result of a heterofermentative pathway that leads to a reduction in production of aerobic microbes and increase in silage pH (McDonald *et al*., 2011). However, an increase in silage AA production leads to the inhibition of spoilage microorganisms because of its antifungal characteristics, thereby promoting aerobic stability (Danner *et al*., 2003). A concentration of more than 3 g AA/kg DM is enough to stabilize silage during aerobic exposure (Weissbach, 1996). The AA content in the current study ranged between 6.02 and 9.64 g AA/kg DM, which is higher than the AA content reported in the study by Ozduven *et al*. (2010). These variations could be attributed to the differences in chemical composition of the ensiled material (McDonald *et al*., 2011).

The treatment combinations involving Silosolve, that is. SLS + no enzyme, SLS + CLD, and SLS + CHD, had increased (P <0.05) AA content compared with other treatments. However, combinations of enzyme and EMS, that is, CLD + EMS and CHD + EMS, had reduced (P <0.05) AA concentration compared with other treatment combinations. Silosolve contains *L. buchneri* and therefore an increase in AA was expected (Ranjit *et al*., 2002). The reduced AA with Emsilage or enzyme addition is typical of homofermentative LAB, which have often reduced the aerobic stability of silages because of lower concentrations of VFAs, which inhibit the growth of yeasts and moulds (Muck & Kung, 1997). Enzyme alone and EMS reduced (P <0.05) silage PA, contributing to poor aerobic stability (Muck & Kung, 1997).

Silage Butyric acid (BA) content indicates that it has undergone a clostridial type of fermentation, which results in the loss of energy (McDonald *et al.*, 2011). An amount of <0.1 g BA/kg DM is typically found in well-preserved silage (Kung & Shaver, 2001). Enzyme addition reduced (P <0.05) BA content compared with control, which is consistent with the findings of Adogla-Bessa *et al*., (1999). Increasing the application level of CHD reduced (P <0.05) silage BA concentration compared with CLD and the control. The increased (P <0.05) BA concentration in control silage is associated with the increased pH in this silage, typical of clostridial fermentation silages (McDonald *et al*., 2011).

The NH_3-N content in silage reflects the degree of protein degradation (Wilkinson, 2005). Well-preserved silages should contain less than 100 g NH_3-N/kg TN (McDonald *et al*., 2011). The concentration of NH_3-N in the present study was less than 5 g NH_3-N/kg T. CHD reduced (P <0.05) silage NH_3-N and pH compared with control, indicating that less proteolysis has occurred in these silages. Silosolve contains *L. buchneri* and its inoculation increased (P <0.05) silage NH_3-N and pH compared with control, indicating that proteolysis occurred with this treatment. This study contradicts the results from other studies (Kung *et al*., 2007), which reported no differences in NH_3-N concentration between *L. buchneri* control silages. Further research could be carried out to investigate these contradictions.

The chemical composition of PHS produced without or with a fibrolytic enzyme and bacterial inoculants is presented in Table 3. The increased (P <0.05) CP concentrations in CLD and CHD compared with control were consistent with findings from Bermuda grass silages (Dean *et al*., 2005) and perennial ryegrass silage (Rodriguez *et al*., 2001). The increase in CP concentration in the CLD treatment was due to reduced NH_3-N content in this treatment (Table 2). The increased (P <0.05) GE concentration in EMS silage could be attributed to the lower BA (Table 2) concentration in this silage.

The reduced (P <0.05) fibre content with CLD and CHD silages compared with control is consistent with previous observations on enzyme-treated Bermuda grass silage (Dean *et al*., 2005), wheat silage (Adogla-Bessa *et al*., 1999), maize silage (Colombatto *et al*., 2004, Donmez *et al*., 2003), and orchard grass and alfalfa silages (Nadeau *et al*., 2000), perennial ryegrass silage (Rodriguez *et al*., 2001) and whole-plant sweet sorghum (Nkosi *et al*., 2012a). The reduction in fibre content (NDF and ADF) with Emsilage and

Silosolve could be attributed to partial hydrolysis of hemicelluloses (Muck & Kung, 1997), and is consistent with the results of Nkosi *et al*. (2011).

Table 3 Effects of a fibrolytic enzyme and bacterial inoculants on the chemical composition of ensiled potato hash (n = 3)

Treatment	Enzyme	Bact. Inoc.	CP (g/kg DM)	GE (MJ/kg DM	NDF (g/kg DM)	ADF (g/kg DM)
1	0	0	141^{d}	16.9^{b}	443^{a}	151^{a}
2	CLD	0	148^{b}	17.0^{b}	343^{c}	111^{f}
3	CHD	0	151^{a}	17.1^{b}	311^{d}	109^{f}
4	0	EMS	143^{c}	17.6^{a}	348^{c}	131^{d}
5	CLD	EMS	147^{b}	17.5^{a}	420^{b}	145^{c}
6	CHD	EMS	131^{e}	16.0^{c}	419^{b}	118^{e}
7	0	SLS	123^{f}	15.0^{d}	356^{c}	120^{e}
8	CLD	SLS	121^{g}	14.6^{e}	454^{a}	148^{ab}
9	CHD	SLS	120^{g}	13.7^{f}	412^{b}	148^{ab}
SEM			0.93	0.03	89.0	3.25
Enzyme means						
0			135^{b}	16.5^{a}	406^{a}	133^{a}
CLD			139^{a}	16.4^{a}	406^{a}	135^{a}
CHD			134^{c}	15.6^{b}	357^{b}	125^{b}
SEM			0.93	0.03	89.0	3.25
Bacterial inoculant means						
0			147^{a}	17.0^{a}	436^{a}	143^{a}
EMS			140^{b}	17.0^{a}	396^{b}	137^{b}
SLS			121^{c}	14.4^{b}	337^{c}	113^{c}
SEM			0.93	0.025	89.0	3.25
Significance						
Bact. Inoc. (B)			**	**	**	**
Enzyme (E)			**	**	**	**
ExB			**	**	**	**

[a-f] Means in the same column within a section with different superscripts differ significantly (P <0.05). CLD: Celluclast low dose; CHD: Celluclast high dose; EMS: Emsilage; SLS: Silosolve; Bact. Inoc.: Bacterial inoculant; DM: Dry matter; CP: Crude protein; GE: Gross energy; NDF: Neutral detergent fibre; ADF: Acid detergent fibre. **P <0.01

Data on the aerobic stability of PHS treated without or with additives (fibrolytic enzyme and bacterial inoculants) are presented in Table 4. Aerobic deterioration of silage is a complex process and is usually initiated by aerobic yeasts that use residual WSCs or LA for their metabolism. After exposure to air for five days, silages treated with Silosolve (SLS, CLD + SLS, and CHD + SLS) showed improved (P <0.05) aerobic stability, as indicated by the increased (P <0.05) number of hours they remained stable, their low pH, and their reduced (P <0.05) CO_2 and yeast and mould population compared with other silages. These results are consistent with those of Nkosi *et al*. (2012b), who reported improved aerobic stability with a heterofermentative LAB + enzyme addition in sweet sorghum silage. Addition of enzyme, alone or in combination with Emsilage, impaired the aerobic stability of silage, as indicated by higher (P <0.05) pH, CO_2 production, and yeast and mould population counts, and reduced (P <0.05) the number of hours compared with silages treated with Silosolve LAB inoculant. These results were inconsistent with those of Chen *et al*. (1994), who reported reduced aerobic stability owing to enzyme-inoculant addition to maize silage.

Table 4 Effects of a fibrolytic enzyme and bacterial inoculants on the aerobic stability of potato hash silage (n = 3)

Treatment	Enzyme	Bact. Inoc.	pH	CO_2 (g/kg DM)	Hrs	Yeast & Moulds
1	0	0	7.07^{c}	15.5^{e}	63.5^{d}	$3x10^{5bc}$
2	CLD	0	8.14^{b}	29.3^{b}	57.9^{e}	$3x10^{7a}$
3	CHD	0	8.90^{a}	31.5^{a}	55.1^{e}	$3x10^{7a}$
4	0	EMS	8.23^{b}	18.9^{d}	66.1^{cd}	$3x10^{5bc}$
5	CLD	EMS	7.30^{c}	11.0^{g}	76.2^{b}	$7x10^{5b}$
6	CHD	EMS	7.44^{c}	26.5^{c}	66.5^{c}	$3x10^{7a}$
7	0	SLS	5.31^{d}	8.43^{h}	88.7^{a}	$2x10^{c}$
8	CLD	SLS	7.27^{c}	11.0^{g}	89.0^{a}	$2x10^{c}$
9	CHD	SLS	7.12^{c}	12.9^{f}	88.2^{a}	$4x10^{c}$
SEM			0.08	0.78	2.87	$7x10^{10}$
Enzyme means						
0			7.46^{b}	15.3^{c}	80.2^{a}	$1x10^{7b}$
CLD			6.62^{c}	17.7^{b}	74.4^{b}	$2x10^{7a}$
CHD			8.19^{a}	22.0^{a}	62.6^{c}	$2x10^{5c}$
SEM			0.08	0.78	2.87	$7x10^{10}$
Bacterial inoculant means						
0			7.44	29.1^{a}	58.9^{c}	$1x10^{5c}$
EMS			7.30	12.8^{b}	77.0^{b}	$1x10^{7b}$
SLS			7.44	13.1^{b}	81.3^{a}	$2x10^{7a}$
SEM			0.08	0.78	2.87	$7x10^{10}$
Significance						
Enzyme (E)			**	**	**	**
Bact. Inoc.(B)			NS	**	**	**
ExB			**	**	**	**

[a-f] Means in the same column within a section with different superscripts differ significantly (P <0.05).CLD: Celluclast low dose; CHD: Celluclast high dose; EMS: Emsilage; SLS: Silosolve; Bact. Inoc.: Bacterial inoculant; DM: Dry matter; CO_2: Carbon dioxide; Hrs: Hours; NS: not significant; **P <0.01

Conclusions

Silages treated with a high dose of the enzyme had lower fibre content than the untreated silages, indicating effective fibre degradation. However, enzyme inoculation, used at low and high doses, increased the WSC content. Thus, it provided more substrate for the LAB, increased LA, and reduced the pH of PHS. Although enzyme addition improved fermentation, this resulted in silages of low aerobic stability owing to increased residual sugar content. Inoculation with LAB inoculants improved the fermentation process. However, this effect was more prominent for the homofermentative LAB inoculant, which is typical of these inoculants. Further work needs to be done to elucidate the effects of these silage additives on animal performance.

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Authors' contributions

TFM, BDN and JJB were in charge of project design and writing of the manuscript. TFM and TL were in charge of project implementation. All co-authors participated in results, statistics and interpretation of the study.

Conflict of interest declaration

We wish to confirm that there are no known conflict of interest associated with the publication of this manuscript and there has been no significant financial support for this work that could have influenced its outcome. We also confirm that this manuscript has been read and approved by all authors and that the order of authors listed in the manuscript has been approved by all of us.

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