Ensiling quality of maize as influenced by the addition of wet distillers grains with soluble

R.M. Moyo[1], W.A. van Niekerk[1], A. Hassen[1#], R.J. Coertze[1], C.J.L du Toit[2] & B.S. Gemeda[1]

[1] Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa
[2] Department of Animal Science, Tshwane University of Technology, Pretoria 0001, South Africa

(Received 16 September 2014; Accepted 11 December 2015; First published online 9 February 2016)

Abstract

Wet distillers grains with soluble (WDGS) were blended with whole maize plants on an as-fed basis at 0%, 20%, 30%, and 40% and ensiled in 3 L bottles to evaluate the silage fermentation characteristics and ensiling quality in a complete randomized design. Each treatment was ensiled in 15 mini-silos and three bottles were opened on days 7, 21, 42 and 120. Each treatment was sampled for chemical composition and silage fermentation parameters. There was a steady decrease in dry matter (DM) concentration of silage with increasing WDGS inclusion level over time. Initial pH (at day 0) decreased with increasing level of WDGS inclusion, with 40% WDGS inclusion recording the lowest pH (3.6) at day 120. Lactic acid concentration was slightly lower for WDGS-blended silages compared with the control. In contrast, the acetic acid concentration for WDGS-blended silage increased across all treatments, suggesting a possible diminished effect of clostridium bacteria in the silage owing to a reduced pH. The acid detergent fibre (ADF), neutral detergent fibre (NDF) and IVDOM (*in vitro* digestible organic matter) did not differ at the time of ensiling among treatments. During post ensiling, ADF increased slightly over time for WDGS-blended treatments (at 120 days). The results from this study indicated that WDGS could be ensiled effectively with maize plants without compromising silage quality.

Keywords: Acetic acid, butyric acid, fermentation, lactic acid, preservation, silage
Corresponding author: Abubeker.Hassen@up.ac.za

Introduction

Expansion of ethanol production from maize has increased the availability of wet distillers grains with soluble (WDGS) in South Africa. Distillers grains are by-products produced after the extraction of ethanol from grains (Garcia & Kalscheur, 2004), and can be used as an alternative feed for ruminants. Distillers grains are available as wet or dry by-products. Drying increases the commodity price significantly. However, it allows better handling, transportation and longer shelf life, resulting in wider use (Loy, 2006). It is most advantageous for distillers to sell WDGS (30% - 50% DM) as this reduces the drying cost and increases production capacity. Although this reduces WDGS product costs significantly, the expense of water transportation and shorter shelf-life must be considered major constraints to farmers and require further investigation.

The transportation, storage costs and nutrient losses associated with the use of WDGS in livestock diets are greatly influenced by storage methods and farm handling (Loy *et al*., 2005). Ensiling WDGS alone is possible, but not recommended owing to its high moisture content (Kalscheur *et al*., 2003). Garcia & Kalscheur (2004) suggested the incorporation of WDGS in the ensiling of forages. WDGS have a pH of approximately 3, which would improve preservation and increase the nutritive value of silages (Garcia & Kalscheur, 2007; Mjoun *et al*., 2011). The inclusion of WDGS in silages results in a decreased initial pH during the ensiling process and reduced nutrient losses (Garcia & Kalscheur, 2007). McCullough *et al*. (1963) reported that distiller grains could be ensiled with forage crops such as maize and wheat to enhance preservation and improve the nutrient content of the silage. The objective of this study was to determine the ensiling quality and fermentation end-products of chopped maize plants (CMP) blended with WDGS.

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v46i1.3

Materials and Methods

The evaluation of the ensiling quality of CMP blended with wet WDGS was conducted at Hatfield Research Farm, University of Pretoria, South Africa. The experiment was carried out as a complete randomised block design. Pioneer Phb 3442 maize hybrid was planted in mid summer and harvested at the hard dough stage of growth. Wet distillers grains with soluble was supplied by a commercial ethanol plant in Ventersdorp, in the province of North West, South Africa, and stored at −4 ºC until ensiling. Whole maize plants were chopped at harvesting to between 10 and 20 mm with a silage harvester. Representative samples of the CMP and WDGS were collected at the point of ensiling for nutrient analysis (Table 1).

Table 1 Chemical composition of distillers grain and chopped whole maize plants used in the experiment (g/kg DM)

Ingredient	Nutrient composition					
	Dry matter	Crude protein	NDF	Fat	Calcium	Phosphorus
WDGS	251.2	334.1	519	155.1	2.9	8.5
CMP	350	84.8	532.9	80.9	4.1	2.6

NDF: neutral detergent fibre; WDGS: wet distillers grains with soluble; CMP: chopped maize plant.

The CMP were blended at five levels of WDGS inclusion (0%, 10%, 20%, 30% and 40%) and ensiled in glass laboratory mini-silos (3 L canned bottles) on an as-fed basis (weight ratio). Each treatment was ensiled in 12 mini-silos. Ensiling was performed by filling and compacting as much treatment material into the bottle as possible to exclude air and create anaerobic conditions. Each bottle was tightly sealed with an airtight lid after ensiling. The mini-silos were then stored in a dark room at room temperature (20 - 25 ºC) until sub sampling began.

Samples representing zero fermentation (day 0) were collected directly post blending for chemical analysis. Triplicate mini-silos were prepared for each treatment and each sampling day. The mini-silos for each blend were opened at days 7, 21, 42 and 120 of ensiling to assess changes in nutrient composition and fermentation characteristics. Approximately one third of the material from each sampled mini silo was oven dried at 55 ºC for 48 h for nutrient analysis. Dried samples were ground to pass through a 1 mm particle size sieve using a Wiley mill, and analysed in triplicate for DM, nitrogen (N), acid detergent fibre (ADF), neutral detergent fibre (NDF) and *in vitro* organic matter digestibility (IVOMD). The remaining fraction of each sample was used to determine silage pH, volatile fatty acid (VFA), ammonia nitrogen (NH_3-N), lactic acid concentration and buffering capacity. Dry matter concentration was determined by taking approximately 1 g ground sample and drying at 105 ºC for 24 h (AOAC, 2002). Nitrogen was determined according to the Kjeldahl method (AOAC, 2002) by weighing out 0.15 g of ground silage material into 50 mL digestion tubes. The IVOMD was determined according to the three-stage Tilley & Terry (1963) method. The NDF and ADF concentrations of the silage were determined using the detergent system of fibre analysis, according to Robertson & Van Soest (1991).

Approximately 80 g of the wet silage samples were extracted with 320 mL distilled water into 500 mL extraction bottle containers. The extraction bottles were sealed tightly and shaken for six hours at 180 rpm with a horizontal shaker. The extract was filtered through four layers of cheesecloth to remove any plant matter, and the extract was transferred into four separated 25 mL plastic bottles labelled for buffering capacity, NH_3-N, VFA and lactic acid concentration. Silage pH was measured immediately after obtaining the extract with a Mettler Toledo electrode pH meter. Silage VFA concentrations were determined from the extract according to the procedure described by Webb (1994) using high performance liquid chromatography. The NH_3-N concentration was established according to the method of Broderick & Kang (1980) using the Technicon auto analyser. Lactic acid concentration was decided using the Barker-Summerson method, as described by Pryce (1969). The buffering capacity of the silage was ascertained from the fresh material using the procedure of Playne & McDonald (1966).

The general linear model (GLM) procedure of SAS (2008) was used to test statistical differences between treatments and sampling periods. Treatment means were compared using least square means and adjusted with Bonferroni's test for *P*-value significance (Samuels, 1989). Significance was declared at $P < 0.05$.

Results and Discussion

There were significant losses of silage DM content as the percentage of WDGS in the silage increased across all ensiling periods (Table 2). The percentage of DM of the control (0% WDGS) was 36.6% DM at time of ensiling. DM concentration decreased to 35.6%, 33.4%, 32.8% and 31.1% with the addition of 10%, 20%, 30% and 40% WDGS blends, respectively. Time of ensiling showed higher DM losses for treatments with 20% WDGS and above levels of inclusion compared with the control (0% WDGS) and 10% WDGS treatments. The differences in DM content between treatments were proportional to the amount of WDGS added.

Packing pressure could influence DM losses in silage. During the ensiling of high moisture plant material, soluble sugars, proteins and minerals are depleted because moisture is pressed out of the silage material during the compaction process and are lost as effluent (Muck, 1987). As the percentage WDGS added to maize increased, the moisture content increased, resulting in a reduced DM content of the silage and possible increased nutrient losses. These results are in agreement with work conducted by Muck (1988), who reported that increasing the inclusion level of WDGS in silage beyond 10% increased DM losses. Similarly, McDonald *et al*. (1991) reported DM depletion when maize silage was ensiled with wet distillers grains.

Silage N concentration increased significantly with increasing level of WDGS inclusion across all treatments. No significant effect was observed in silage N concentration as ensiling time advanced from 0 to 120 days, except in the control treatment (Table 2). Loses in N concentration were recorded throughout the ensiling period for 0% WDGS compared with other blended treatments. The increase in N concentration of silage with increasing level of WDGS inclusion could be explained by the higher N content of WDGS (53.5 g/kg DM) compared with the CMP (16.16 g/kg DM). The N content of the silage tended to decrease as the ensiling period increased in the control treatment (0% WDGS inclusion). This could have been owing to the occurrence of proteolysis as a result of bacterial activity (Kung & Shaver, 2001). The active proteolysis during the ensiling period might be owing to plant enzymes present in the silage (Bergen *et al*., 1994). An increase in N concentration of silage when ensiled with WDGS was also reported by Anderson *et al*. (2009) when WDGS was ensiled with soybean hulls. There is limited information in the literature on the effect of ensiling duration on N concentration of silage when WDGS are blended with various forages with which to compare the present results.

There were no significant effects of WDGS inclusion level in maize silage on the NH_3-N concentration except at the 40% inclusion rate, which was lower compared with other treatments (Table 2). Ensiling period showed a significant effect on NH_3-N concentration. The NH_3-N concentration of all treatments increased as the ensiling period increased. All treatments had increased NH_3-N concentration at day 120 post ensiling. The lower NH_3-N concentration at the 40% WDGS treatment throughout the ensiling period might have been because of the possible presence of increased sulphuric acid concentration in the silage. It is well documented that sulphuric acid inhibits the activities and proliferation of fermentative bacteria responsible for proteolysis (Garcia & Kalscheur, 2007). In addition to the sulphuric acid content, proteolysis could have been reduced because of plant-degrading enzymes and undesirable clostridial micro-organisms (Seglar, 2003). The final NH_3-N concentrations of all treatments in the present study ranged between 35.4 g/kg N and 48.6 g/kg N, which is lower than figures reported by Kung & Shaver (2001) and Selgar (2003) for maize silage. This is an indication that the fermentation process was near optimal, with less proteolysis associated with clostridial activity. This was accompanied by a reduced loss of N concentration during the ensiling period for all the treatments. Theoretically, high amounts of NH_3-N in silage should not have a negative effect on animal performance if the total dietary N fractions are in balance (Kung & Shaver, 2001) as they do not alter palatability and therefore intake. However, high levels of NH_3-N in silages usually correlate with high butyric acid and amine concentrations, which are undesirable to ruminants owing to the negative effect on palatability and intake of the silage (Seglar, 2003).

There was a significant decrease in silage pH with an increase in ensiling duration across all treatments. As the proportion of WDGS in the silage increased, there was a reduction in silage pH except for day 21 of sampling (Table 2) with the 40% WDGS treatment yielding the lowest pH after day 42 of ensiling. The results presented in Table 2 are in agreement with those recorded by Kung & Stokes (2005), who reported pH values ranging between 3.7 and 4.2 when WDGS was included in silage. A rapid decrease in pH to below 5 is essential to prevent clostridia bacterial growth, ensuring higher-quality silage (Kung & Stokes, 2005). The pH for the 0% and 10% WDGS treatments did not continue to decrease past day 21 of ensiling. However, the 20%, 30% and 40% WDGS treatments continued to decrease over time. All silage treatments had a pH below 4 by day 21 of ensiling. These results are consistent with those reported in several studies in which WDGS were ensiled with forages (Schneider *et al*., 1995; Garcia & Kalscheur 2007; Anderson *et al*., 2009). A high rate of fermentation of silage material is desirable as indicated by a rapid decrease in pH ensuring a faster preservation of nutrients (McDonald *et al*., 1991).

Table 2 Mean (± SEM) nutrient composition of maize ensiled at inclusion of five levels of wet distillers grains with soluble

Parameter	Ensiling duration (days)	Treatment					SEM
		0% WDGS	10% WDGS	20% WDGS	30% WDGS	40% WDGS	
Dry matter (g/kg)	0	$366^{a}{}_{1}$	$356^{a}{}_{1}$	$334^{a}{}_{2}$	$328^{a}{}_{2}$	$311^{a}{}_{3}$	± 0.133
	7	$352^{bc}{}_{1}$	$321^{b}{}_{2}$	$311^{b}{}_{2}$	$298^{b}{}_{3}$	$288^{b}{}_{3}$	± 0.133
	21	$350^{c}{}_{1}$	$319^{b}{}_{2}$	$309^{b}{}_{2}$	$293^{b}{}_{3}$	$272^{c}{}_{4}$	± 0.133
	42	$361^{ab}{}_{1}$	$322^{b}{}_{2}$	$298^{c}{}_{3}$	$286^{bc}{}_{4}$	$270^{c}{}_{5}$	± 0.133
	120	$360^{ab}{}_{1}$	$309^{b}{}_{2}$	$292^{c}{}_{3}$	$275^{c}{}_{4}$	$266^{c}{}_{4}$	± 0.133
	SEM	± 0.252	± 0.252	± 0.252	± 0.252	± 0.252	
Buffering capacity	0	$141.2^{a}{}_{1}$	$142.3^{a}{}_{1}$	$146.0^{a}{}_{1}$	$146.5^{a}{}_{1}$	$147.5^{a}{}_{1}$	± 0.702
(meq/100 g DMD)	7	$53.4^{b}{}_{4}$	$80.9^{b}{}_{2}$	$70.8^{b}{}_{3}$	$81.0^{b}{}_{2}$	$94.9^{b}{}_{1}$	± 0.702
	21	$53.2^{b}{}_{1,2}$	$59.5^{c}{}_{1}$	$58.0^{c}{}_{1}$	$51.8^{c}{}_{2}$	$50.5^{c}{}_{2}$	± 0.702
	42	$49.7^{b}{}_{1}$	$48.0^{d}{}_{1}$	$44.5^{d}{}_{1}$	$42.8^{d}{}_{1}$	$46.76^{c}{}_{1}$	± 0.702
	120	$41.2^{c}{}_{1}$	$34.2^{d}{}_{2}$	$31.1^{e}{}_{2,3}$	$27.9^{e}{}_{3}$	$34.2^{d}{}_{2}$	± 0.702
	SEM	± 1.571	± 1.571	± 1.571	± 1.571	± 1.571	
pH	0	$5.89^{a}{}_{1}$	$5.39^{a}{}_{2}$	$5.20^{a}{}_{3}$	$5.22^{a}{}_{3}$	$4.89^{a}{}_{4}$	± 0.006
	7	$3.92^{b}{}_{3}$	$4.14^{b}{}_{2}$	$4.24^{b}{}_{1}$	$4.28^{b}{}_{1}$	$4.24^{b}{}_{1}$	± 0.006
	21	$3.81^{c}{}_{3}$	$3.89^{c}{}_{2}$	$3.87^{c}{}_{2}$	$3.89^{b}{}_{2}$	$3.98^{c}{}_{1}$	± 0.006
	42	$3.78^{c}{}_{2}$	$3.80^{d}{}_{1}$	$3.84^{c}{}_{1}$	$3.78^{d}{}_{2}$	$3.65^{d}{}_{3}$	± 0.006
	120	$3.77^{c}{}_{1}$	$3.79^{d}{}_{1}$	$3.73^{d}{}_{2}$	$3.71^{e}{}_{2}$	$3.62^{d}{}_{3}$	± 0.006
	SEM*	± 0.014	± 0.014	± 0.014	± 0.014	± 0.014	
Nitrogen	0	$16.16^{a}{}_{5}$	$23.62^{ab}{}_{4}$	$26.88^{ab}{}_{3}$	$32.40^{ab}{}_{2}$	$39.60^{ab}{}_{1}$	± 0.166
(g/kg DM)	7	$15.04^{ab}{}_{4}$	$22.08^{b}{}_{3}$	$25.28^{b}{}_{3}$	$30.88^{b}{}_{2}$	$36.48^{c}{}_{1}$	± 0.166
	21	$15.04^{ab}{}_{5}$	$20.64^{b}{}_{4}$	$25.768^{b}{}_{3}$	$30.40^{b}{}_{2}$	$38.32^{abc}{}_{1}$	± 0.166
	42	$14.72^{b}{}_{5}$	$21.12^{b}{}_{4}$	$27.04^{b}{}_{3}$	$30.88^{b}{}_{2}$	$39.20^{ab}{}_{1}$	± 0.166
	120	$13.92^{b}{}_{5}$	$25.44^{a}{}_{4}$	$30.40^{a}{}_{3}$	$34.48^{a}{}_{2}$	$40.40^{a}{}_{1}$	± 0.166
	SEM	± 0.371	± 0.371	± 0.371	± 0.371	± 0.371	
Ammonia-N	0	$1.62^{d}{}_{1}$	$1.70^{d}{}_{1}$	$1.63^{d}{}_{1}$	$1.89^{d}{}_{1}$	$1.28^{e}{}_{2}$	± 0.025
(g/kg N)	7	$22.05^{c}{}_{1}$	$24.72^{c}{}_{1}$	$21.98^{c}{}_{1}$	$21.77^{c}{}_{1}$	$14.72^{d}{}_{2}$	± 0.025
	21	$30.14^{b}{}_{1}$	$33.57^{b}{}_{1}$	$33.20^{b}{}_{1}$	$33.40^{b}{}_{1}$	$23.00^{c}{}_{2}$	± 0.025
	42	$30.36^{b}{}_{2}$	$33.63^{b}{}_{1,2}$	$36.35^{b}{}_{1}$	$37.59^{b}{}_{1}$	$29.54^{b}{}_{2}$	± 0.025
	120	$35.36^{a}{}_{2}$	$44.77^{a}{}_{1}$	$47.95^{a}{}_{1}$	$48.56^{a}{}_{1}$	$36.21^{a}{}_{2}$	± 0.025
	SEM	± 0.055	± 0.055	± 0.055	± 0.055	± 0.055	

^{a,b,c,d,e} Means with different superscript across the column for each parameter are significantly (P <0.05) different.
^{1,2,3,4,5} Means with different subscript across the rows for each parameter are significantly (P <0.05) different.
WDGS: wet distillers grains with soluble; SEM: sum errors of the mean.

The initial buffering capacities of all treatments were similar, ranging from 141.2 meq/100 g DMD to 147.5 meq/100 g DMD for the 0% and 40% WDGS treatments, respectively. Kung & Stokes (2005) reported a buffering capacity of whole plant maize lower than 200 meq/100 g DMD at the time of ensiling. This corresponds with the results reported in the present study. The drastic decrease in buffering capacity by day 7 across all treatments was a result of silage micro-organisms fermenting water soluble carbohydrates to produce lactic acid, which was responsible for a reduction in silage pH (Horvey, 2003). The decrease in silage pH as ensiling duration increased corresponded with a decrease in the buffering capacity of the silage. The slower reduction in silage pH with increasing inclusion level of WDGS when compared with the control treatment (0% WDGS) in Table 2 could be explained by the higher buffering capacity of silage as the level of

WDGS increased. This could be owing to an increase in the protein content as the level of WDGS inclusion increased, which extends the onset of the fermentation process during ensiling (Horvey, 2003).

The level of WDGS inclusion did not increase the acetic acid concentration of silage significantly except at 120 days post-ensiling. Time of ensiling had a significant effect on silage acetic acid concentration compared with day 0 (Table 3). The proportions of acetic acid produced during silage fermentation depend on crop maturity, moisture content and epiphytic bacteria populations of the harvested crop (Selgar, 2003). Relatively small amounts of acetic acid are produced by anaerobic hetero-fermentative bacteria during early ensiling (Selgar, 2003). The slow increase in acetic acid concentration reported in the present study could be explained by the low initial pH of WDGS, which inhibited the proliferation of hetero-fermentative bacteria responsible for acetic acid production during the early ensiling phases (Garcia & Kalscheur, 2007).

A low pH at time of ensiling inhibits homo-fermentative bacteria and allows for hetero-fermentative bacterial proliferation, which is responsible for acetic acid production during the early phases of ensiling (Garcia & Kalscheur, 2004). These authors reported that blending WDGS with other feeds resulted in fermentation patterns that differ from the traditional lactic acid fermentation patterns towards more acetic acid production. This is in agreement with the results reported for the present study in which acetic acid concentration was higher in maize silage treatments blended with WDGS compared with the control treatment at day 120 of ensiling. In the present study, the high concentration of crude protein indicated by the increased N content (Table 2) owing to the inclusion of WDGS could have resulted in higher concentrations of acetic acid production, as suggested by Kung & Shaver (2001).

At the time of ensiling, propionic acid was present at low concentrations and did not differ (P >0.05) among treatments. These findings are in agreement with work done by Anderson *et al*. (2009) for soybean hull silage blended with WDGS. However, there was an increase (P <0.05) in propionic acid concentration over time for the 0%, 10%, 20% and 30% WDGS treatments, but the rate of increment was relatively low. The 40% WDGS treatment remained unchanged throughout the ensiling period and recorded the lowest propionic acid concentration (0.33 g/kg DM) post ensiling. In well-preserved silages, propionic acid is produced in lower levels during fermentation, which aids in maintaining aerobic stability (Kung & Stokes, 2005). These authors reported that silages should contain very low concentrations of propionic acid (<0.2% to 0.3%), which was within the range of the present study.

The lactic acid concentration of the silage did not differ (P >0.05) among treatments prior to ensiling (day 0), but increased drastically at different rates from day 7. Lactic acid concentration was the highest for the control treatment at day 7, which could have been as a result of sudden proliferation of *Lactobacillus* spp., resulting in an increase in the production of lactic acid (Kung & Shaver, 2001). The lower initial pH of WDGS (pH 3.2) used in the present study could have inhibited the proliferation of homo-fermentative bacteria responsible for lactic acid production (Selgar, 2003). Significant increases in lactic acid occurred between day 7 and 21 for all treatments, but no further increases were recorded for the 20% and 30% WDGS treatments until day 120. In previous silage trials (Selgar, 2003; Garcia & Kalscheur, 2007), the peak lactic acid concentrations were reached by day 21 of ensiling. This is consistent with results presented in Table 3 for the 20% and 30% WDGS inclusion treatments. An early peak in lactic acid is beneficial as it results in a rapid decrease in pH and assists in preserving the silage and reducing nutrient losses (Selgar, 2003). In the present study, the control treatment produced the highest concentration of lactic acid at day 120 of ensiling. The reduced proportion of WCM with an increasing level of WDGS inclusion in the silage may have had an effect on the lactic acid production owing to the reduced availability of fermentative substrate (water-soluble carbohydrates) contributed by maize. Similar results were reported by Garcia & Kalscheur, (2004) when ensiling WDGS with maize.

The silage ADF, NDF and IVDOM concentrations were measured at days 0 and 120 of ensiling (Table 4). Total ADF, NDF and IVDOM concentrations at the time of ensiling did not differ (P >0.05) between treatments. The NDF and IVDOM concentration did not change over time for all treatments, except for the NDF level of the 20% WDGS treatment. These results are contrary to reports by Seglar (2003), who reported a slight increase in NDF concentration of silage as the ensiling duration increased owing to a reduction of the WSC content of the silage. The ADF concentration increased over time across all treatments. The high levels of NDF in the maize plants and WDGS ensured that the concentration was consistent between treatments at the time of ensiling. *In vitro* digestible organic matter ranged from 78.2% to 81.4% across all treatments at the day of ensiling and decreased slightly (P >0.05) at day 120 of ensiling ranging, from 76.6% to 77.8% for the 0% and 40% WDGS treatments, respectively.

Table 3 Mean values (± SEM) of lactic acid, acetic acid and propionic acid concentration (g/kg DM) for maize ensiled at five levels of wet distillers grains with soluble inclusion

Parameters	Time (days)	Treatment					SEM
		0% WDGS	10% WDGS	20% WDGS	30% WDGS	40% WDGS	
Lactic acid (g/kg DM)	0	$0.03^{d}{}_{4}$	$0.29^{d}{}_{3}$	$0.44^{d}{}_{2}$	$0.24^{d}{}_{3}$	$0.90^{d}{}_{1}$	± 0.186
	7	$18.52^{c}{}_{1}$	$12.75^{c}{}_{2}$	$10.40^{c}{}_{3}$	$8.74^{c}{}_{3,4}$	$7.77^{c}{}_{4}$	± 0.186
	21	$22.34^{b}{}_{1}$	$21.32^{b}{}_{1}$	$21.47^{a}{}_{1}$	$21.50^{a}{}_{1}$	$18.54^{b}{}_{2}$	± 0.186
	42	$23.11^{b}{}_{1}$	$23.42^{a}{}_{1}$	$19.56^{b}{}_{2}$	$19.73^{b}{}_{2}$	$22.67^{a}{}_{1}$	± 0.186
	120	$24.90^{a}{}_{1}$	$22.29^{ab}{}_{2}$	$21.67^{a}{}_{2}$	$21.90^{a}{}_{2}$	$21.32^{a}{}_{2}$	± 0.186
	SEM	± 0.415	± 0.415	± 0.415	± 0.415	± 0.415	
Acetic acid (g/kg DM)	0	0.65^{c}	1.16^{d}	1.02^{c}	1.05^{d}	0.79^{d}	± 0.315
	7	$8.01^{b}{}_{1}$	$5.87^{c}{}_{2}$	$5.05^{b}{}_{2}$	$5.08^{c}{}_{2}$	$3.72^{c}{}_{2}$	± 0.315
	21	$10.81^{ab}{}_{1}$	$7.89^{bc}{}_{2}$	$7.28^{b}{}_{2}$	$6.03^{c}{}_{2}$	$7.15^{b}{}_{2}$	± 0.315
	42	$11.68^{ab}{}_{1}$	$9.07^{b}{}_{2}$	$7.81^{b}{}_{2}$	$9.57^{b}{}_{2}$	$9.83^{b}{}_{2}$	± 0.315
	120	$12.48^{a}{}_{2}$	$17.10^{a}{}_{1,2}$	$20.03^{a}{}_{1}$	$19.51^{a}{}_{1}$	$24.53^{a}{}_{1}$	± 0.315
	SEM	± 0.704	± 0.704	± 0.704	± 0.704	± 0.704	
Propionic acid (g/kg DM)	0	0.06^{c}	0.13^{b}	0.11^{d}	0.12^{b}	0.13	± 0.259
	7	$1.39^{a}{}_{1}$	$0.43^{b}{}_{2,3}$	$0.64^{c}{}_{2}$	$0.34^{b}{}_{2,3}$	0.133	± 0.259
	21	$1.33^{a}{}_{1}$	$0.84^{a}{}_{2}$	$1.12^{b}{}_{1,2}$	$0.84^{a}{}_{2}$	0.193	± 0.259
	42	$0.94^{b}{}_{1}$	$0.84^{a}{}_{1}$	$0.98^{bc}{}_{1}$	$1.03^{a}{}_{1}$	0.182	± 0.259
	120	$1.07^{ab}{}_{2}$	$1.02^{a}{}_{2}$	$1.76^{a}{}_{1}$	$1.18^{a}{}_{2}$	0.333	± 0.259
	SEM*	± 0.579	± 0.579	± 0.579	± 0.579	± 0.579	

^{a,b,c,d,e} Means with different superscript across the column for each parameter are significantly (P <0.05) different.
^{1,2,3,4,5} Means with different subscript across the rows for each parameter are significantly (P <0.05) different.
WDGS: wet distillers grains with soluble. SEM: sum errors of the mean.

Table 4 Mean values (± SEM) of neutral detergent fibre, acid detergent fibre and in vitro digestible matter (g/kg DM) concentrations for maize ensiled at five levels of wet distillers grains with soluble inclusion

Parameter	Time (days)	Treatment					SEM
		0% WDGS	10% WDGS	20% WDGS	30% WDGS	40% WDGS	
NDF (g/kg DM)	0	507.6	519.4	519.2^{a}	526.4	521.6	± 0.288
	120	481.6	493.52	$473.1^{b}{}_{2}$	512.2	522.3_{1}	± 0.288
	SEM*	± 0.643	± 0.643	± 0.643	± 0.643	± 0.643	
ADF (g/kg DM)	0	238.6	236.7	224.9^{b}	226.4^{b}	223.4^{b}	± 0.220
	120	250.7	255.5	$265.7^{a}{}_{1,2}$	$278.9^{a}{}_{1}$	$279.1^{a}{}_{1}$	± 0.220
	SEM*	± 0.491	± 0.491	± 0.491	± 0.491	± 0.491	
IVDOM (g/kg DM)	0	782.3	786.8	790.7	795.6	813.9	± 0.373
	120	766.5	766.7	767.5	776.7	777.5	± 0.373
	SEM*	± 0.835	± 0.835	± 0.835	± 0.835	± 0.835	

^{a,b,c,d,e} Means with different superscript across the column for each parameter are significantly (P <0.05) different.
^{1,2,3,4,5} Means with different subscript across the rows for each parameter are significantly (P <0.05) different.
NDF: neutral detergent fibre; ADF: acid detergent fibre; IVDOM: *in vitro* digestible organic matter; WDGS: wet distillers grains with soluble; SEM: sum errors of the mean.

The inclusion of WDGS in the silage had no effect on its digestibility (Table 4), which is in agreement with work by Schingoethe *et al*. (2002). The IVDOM content for the control post ensiling was higher than that reported by Ferreira & Mertens (2005) for maize ensiled at various chopping lengths. The increase in the ADF fraction for WDGS blends, especially above 20% inclusion, might be because of the utilization of readily digestible NDF present in WDGS (Schingoethe, 2004) by fermentative bacteria as an energy source rather than water-soluble carbohydrates. Kalscheur *et al*. (2003) ensiled a blend of WDGS and chopped whole plant maize at ratios of 25 : 75 and 50 : 50 (WDGS : whole plant maize), for 120 days and recorded ADF concentration as 24% and 20%, respectively. The ADF concentrations presented in this study were higher than those recorded by Kalscheur *et al*. (2003) post ensiling.

Conclusion

Blending wet distillers grains with soluble and whole plant maize at various inclusion levels did not affect silage preservation negatively. Inclusion of WDGS in maize silage at 10%, 20%, 30% and 40% levels on an as-fed basis resulted in an increased N and acetic acid content. Lactic acid production decreased with an increase in WDGS inclusion, but the lower initial pH of the WDGS ensured sufficient preservation of the silage. The WDGS at 40% inclusion preserved well, despite fermentation patterns that followed acetic acid production. The high acetic acid content in silages containing WDGS is cause for concern and requires further research to determine how to encourage typical lactic acid fermentation patterns through the use of inoculants and other additives.

Authors' contributions

RMM collected data as part of his MSc (Agric.) study project, conducted the experimental work, did the literature , and prepared the first draft of the document. WAVN funded the project, was involved in planning the study, supervised the MSc student, interpreted the results, and edited the prepared manuscript. AH was involved in planning the study, co-supervised the MSc student, was involved in data analysis and interpreted the results, prepared the manuscript, and acted as corresponding author. RJC assisted in planning the study and conducted the statistical analyses. CJLDT assisted with interpreting the results and editing the manuscript. BSG assisted with layout and gathering additional literature used in the manuscripts.

References

AOAC, 2002. Official methods of analysis (19th ed.). Association of Official Analytical Chemists, Inc., Washington, D.C., USA.

Anderson, J.L., Kalscheur, K.F., Garcia, A.D., Schingoethe, D.J. & Hippen, A.R., 2009. Ensiling characteristics of wet distiller's grains mixed with soybean hulls and evaluation of the feeding value for growing Holstein heifers. J. Anim. Sci. 87, 2113-2123.

Bergen, W.G., Cash, E.H. & Henderson, H.E., 1994. Changes in nitrogenous compounds of the whole corn plants during ensiling and subsequent effects on dry matter intake in sheep. J. Anim. Sci. 39, 629-637.

Broderick, G.A. & Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci. 63, 64-75.

Erdman, R., 1992. Silage fermentation characteristics affecting feed intake. Proceedings of National Silage Production Conference. NRAES. Syracuse, New York, USA.

Ferreira, G. & Mertens, D.R., 2005. Chemical and physical characteristics of corn silages and their effects on *in vitro* disappearance. J. Dairy Sci. 88, 4414-4425.

Garcia, A.D. & Kalscheur, K.F., 2004. Ensiling wet distillers grains with other feeds. Cooperative Extension Services. South Dakota, USA.

Garcia, A.D. & Kalscheur, K.F., 2007. Storage of wet distillers grains. Proceedings of Tri-State dairy Nutrition Conference, Fort Wayne, In., USA. pp. 111-118.

Horvey, R., 2003. Making good silage. Ag-Info Centre. Alberta Agriculture and Rural Development. www.agric.gov.ab.ac

Kalscheur, K.F., Garcia, A.D., Hippen, A.R. & Schingoethe, D.J., 2003. Fermentation characteristics of ensiling wet corn distillers' grains in combination with corn silage. J. Dairy Sci. 86 (suppl.1), 211.

Kung, L. & Shaver, R., 2001. Interpretation and use of silage fermentation analysis reports. Forage Research Centre, Madison, Wisconsin, USA.

Kung, L. & Stokes, M.L., 2005. Analyzing silages for fermentation end products. University of Delaware, Delaware, USA.

Loy, D.D., Strohbelm, D.R. & Martin, R.E., 2005. Factors affecting the economics of corn co-products in cattle feed. Ethanol co-products for cattle. Iowa State University Extension Services, Iowa, USA.

Loy, D.D., 2006. Reducing energy costs in ethanol production through improved on-farm storage methods. Iowa Energy Center, Iowa. USA.

McCullough, M.E., Sisk, L.R. & Sell, O.E., 1963. Influence of corn distillers dried grains with solubles on the feeding value of wheat silage. J. Dairy Sci. 46, 43-45.

McDonald, P., Henderson, A.R. & Heron, S.J.E., 1991. The Biochemistry of Silage. Chalcombe Publications, Marlow, UK.

Mjoun, K., Kalscheur, K.F. & Garcia, A.D., 2011. Fermentation characteristics and aerobic stability of wet corn distillers grains with soluble ensiled in combination with whole plant corn. J. Sci. Food Agric. 91, 1336-1340.

Muck, R.E., 1987. Dry matter level effects on alfalfa silage quality. 1. Nitrogen transformations. Trans. Am. Soc. Agric. 30, 7-14.
Muck, R.E., 1988. Factors influencing silage quality and their implications for management. J. Dairy Sci. 71, 2992-3002.
Playne, M.J. & McDonald, P.M., 1966. The buffering constituents of herbage and silage. J. Sci. Food Agric. 17, 264-268.
Pryce, J.D., 1969. A modification of the Barker-Summerson method for the determination of lactic acid. Analyst 94, 1151-1152.
Robertson, J.B. & Van Soest, P.J., 1981. The detergent system of analysis and its application to human foods. In: The Analysis of Dietary Fibre in Food. Eds: James, P.T. & Theander, O., Marcel Dekker, New York, USA. pp. 123-158.
Samuels, M.L., 1989. Statistics for the Life Sciences. Collier MacMillan, London, UK.
SAS, 2008. SAS/STAT® 9.2 User's Guide. SAS Institute Inc, Cary, North Carolina, USA.
Schingoethe, D.J., Kalscheur, K.F. & Garcia, A.D., 2002. Distillers grains for dairy cattle. Cooperative Extension Services, South Dakota State University, South Dakota, USA.
Schingoethe, D.J., 2004. Distillers grains for dairy cattle. South Dakota State University. Extension Service Extension Extra, South Dakota, USA.
Schneider, R. M., Harrison, J.H. & Loney, K.A., 1995. The effects of bacterial inoculants, beet pulp, and propionic acid on ensiled wet brewer's grains. J. Dairy Sci. 78, 1096-1105.
Seglar, B., 2003. Fermentation analysis and silage quality testing. Global Agronomy and Nutritional Sciences. Proceedings of the Minnesota Dairy Health Conference. College of Veterinary Medicine, University of Minnesota, Minneapolis, USA.
Tilley, J.M. & Terry, R.A., 1963. A two stage technique for the *in vitro* digestion of forage crops. J. Br. Grassl. Soc. 18, 104-111.
Webb, E.C., 1994. Synthesis of long chain fatty acids in ruminants and their effects on meat quality. PhD thesis, Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa.