

Effects of silage sealing films on fermentation dynamics of ensiled maize and nutrient utilization by Damara rams

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Abstract

An experiment was conducted to evaluate the effects of two silage sealing films on whole crop maize (WCM) fermentation, aerobic stability and nutrient digestion by rams. Prior to ensiling (day 0), triplicate samples of the WCM were collected to determine dry matter, pH, water-soluble carbohydrates (WSC), lactic acid (LA) bacterial counts and nutrient concentration. A single bunker silo was divided into eight sections that were ensiled using either a standard polyethylene film (PE) or an orange oxygen barrier (OB) Silostop film for 180 days. After 180 days of ensiling, six silage samples were collected from each replicate. Three samples were used for nutrient analysis and the other three to determine aerobic stability. Sixteen four-year-old Damara rams with an average live weight of ± 54 kg were housed individually in wooden pens (2.2 m^2) to determine digestibility of nutrient in the silage. The digestibility study was conducted as a switchover design with four feeding phases. The silage was supplemented daily with 1% urea and fed to the rams ad libitum. Nutritional value of the silage was not ($P > 0.05$) affected by the ensiling films. The OB-ensiled maize had higher ($P < 0.05$) LA, lower pH, CO_2 production and yeast and mould populations, and lower WSC compared with the PE-ensiled maize. Dry matter intake, nutrient digestion and nitrogen balance of rams fed the silage were not affected ($P > 0.05$) by treatments. The OB film improved the fermentation and aerobic stability of WCM silage but did not influence its nutrient utilization by rams.

Keywords: cereals, digestibility, forage, rams, nitrogen, supplementation

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Introduction

Whole crop maize (*Zea mays*) is one of the most popular cereal crops for silage production because of high yield, low buffering capacity and a higher WSC concentration compared with other cereals (Densley *et al.*, 2001). In addition, maize silage is a major forage fibre source in ruminant production systems in South Africa (Meeske & Basson, 1998). However, maize silage has poor aerobic stability owing to a high concentration of LA and high residuals of WSC, which are the main sources of food for yeasts and other undesirable microorganisms (Moon, 1983; Canibe *et al.*, 2014). Achieving and maintaining anaerobic conditions when maize silage is stored in bunkers is critical to successful ensiling (Bernardes *et al.*, 2011). The preservation of maize silage usually takes place in bunker silos covered with low-density polyethylene film (PE), the principal function of the film being to seal the forage and allow anaerobic conditions to establish. Polyethylene film has been used for many years to seal bunker silos and drive-over piles because they are durable, not easily damaged by harsh environmental conditions, and are inexpensive compared with the alternatives (Bernardes *et al.*, 2011). However, the high oxygen permeability of PE films can contribute to a low quality of silage in the top layer of horizontal silos (Borreani *et al.*, 2007).

According to Bernardes *et al.* (2011), many farms in Brazil prefer horizontal silos with low construction costs and high work rates for filling and unloading. However, this design allows for large areas of the ensiled

material to be easily exposed to air. This causes the silage to be more prone to aerobic spoilage, especially in the upper layer and near the sides of silos (Bernardes *et al.*, 2011). This is also the case with many silage production farms in South Africa, since the use of PE films in maize silage production is common, and aerobic deterioration of the silage is apparent.

The permeability of plastic films to oxygen is a serious issue in silage production (Borreani *et al.*, 2007). For example, the PE sealing film has been reported to have higher oxygen permeability than the OB film (Borreani *et al.*, 2007; Dolci *et al.*, 2011). The ability of sealing films and procedures to prevent air from infiltrating the silage during storage and feeding phases has significant effects on the quality of silages and its stability against aerobic spoilage by microorganisms (Woolford, 1989; Wilkinson & Fenlon, 2013; Amaral *et al.*, 2014). If there is any ingress of oxygen into the silage material during the storage period, aerobic microorganisms, especially lactic utilizing yeast, degrade the LA, which is the preservation acid (Woolford, 1989; Holmes & Muck, 2007). A newly developed OB sealing film has reportedly produced similar results to the use of LAB silage inoculants under European conditions (Borreani *et al.*, 2007). Research on the ensiling of WCM using sealing films in South Africa is limited, and the effects of OB sealing films may vary due to different environmental conditions (Schmidt & Kung, 2010). The objectives of the present study were to determine the effect of OB and PE sealing films on whole maize silage fermentation characteristics and aerobic stability of maize silage, and to compare the nutrient utilization of maize silage produced using these films.

Materials and Methods

Maize (*Zea mays*) (Senkuil, Sensako, obtained in Brits, South Africa) was planted in November 2015 at ARC, Irene, South Africa, located at longitude 28° 13' S and latitude 25° 55' E at an altitude of 1524 m. The maize was harvested at the 50% milk line stage in mid February 2016 using a Feraboli 945 precision silage chopper (Fondata Nel, Cremona, Italy) to obtain a theoretical 5 mm chop length. The WCM was ensiled using two tractors for compaction in a concrete silage bunker (5.5 m width × 1.7 m height × 20.0 m length) and stored for 180 days.

The trial consisted of two experimental treatments, which were arranged in a randomized complete block design, namely i) a control in which 150 µm thick co-extruded black conventional PE plastic sealing film weighed down with used tyres all over the film was used; and ii) an OB film (Silostop, Bruno Rimini Ltd, Italy) of 45 µm thick co-extruded plastic sealing film. The OB film was constructed of two outer layers of polyethylene and a central layer of polyamide. Anti-ultraviolet close weave polypropylene nets were placed on top of the OB film to protect it from solar radiation and weighed down with 1-metre length Silostop gravel bags at the edges of the film to prevent the film and net from blowing off the silage.

The trial was conducted in one silage bunker (5.5 m width × 1.7 m height × 20.0 m length) to reduce possible variations that might result from differences in environmental conditions, bunker characteristics and silage compaction. The silage bunker was divided into eight equal parts (1.25 m each), with four replicates for each treatment. To avoid any movement of material and gases, the plastic films were overlapped at each end of the treatments. Samples were not collected within 2 m of the overlap (Figure 1).

At day 0 (pre-ensiling), triplicate samples of 2.5 kg per treatment were taken by hand from the middle of each replicate at a depth of 40 cm from top surface of the compacted material in the bunker. The samples were analysed for chemical composition: dry matter (DM), acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP), ether extract (EE), and gross energy (GE). Water-soluble carbohydrates, pH and yeast counts were also determined. The coring holes were refilled with chopped material, and the locations that were sampled were recorded to ensure that later sampling took place within 50 cm of previous sampling sites. At day 180, the same sampling procedures were applied as on day 0 to determine chemical composition with pH, LA and yeast and moulds also measured. Three-hundred-gram samples, which were sub-sampled from the 2.5 kg sample of day 180, were also subjected to an aerobic stability test. These samples were packed loosely in an open plastic jar, which 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 also in the room where the jars were stored. Carbon dioxide production was determined as described by Ashbell *et al.* (1991) and pH and numbers of colony forming units (cfu) of yeasts and moulds were determined after the five-day exposure.

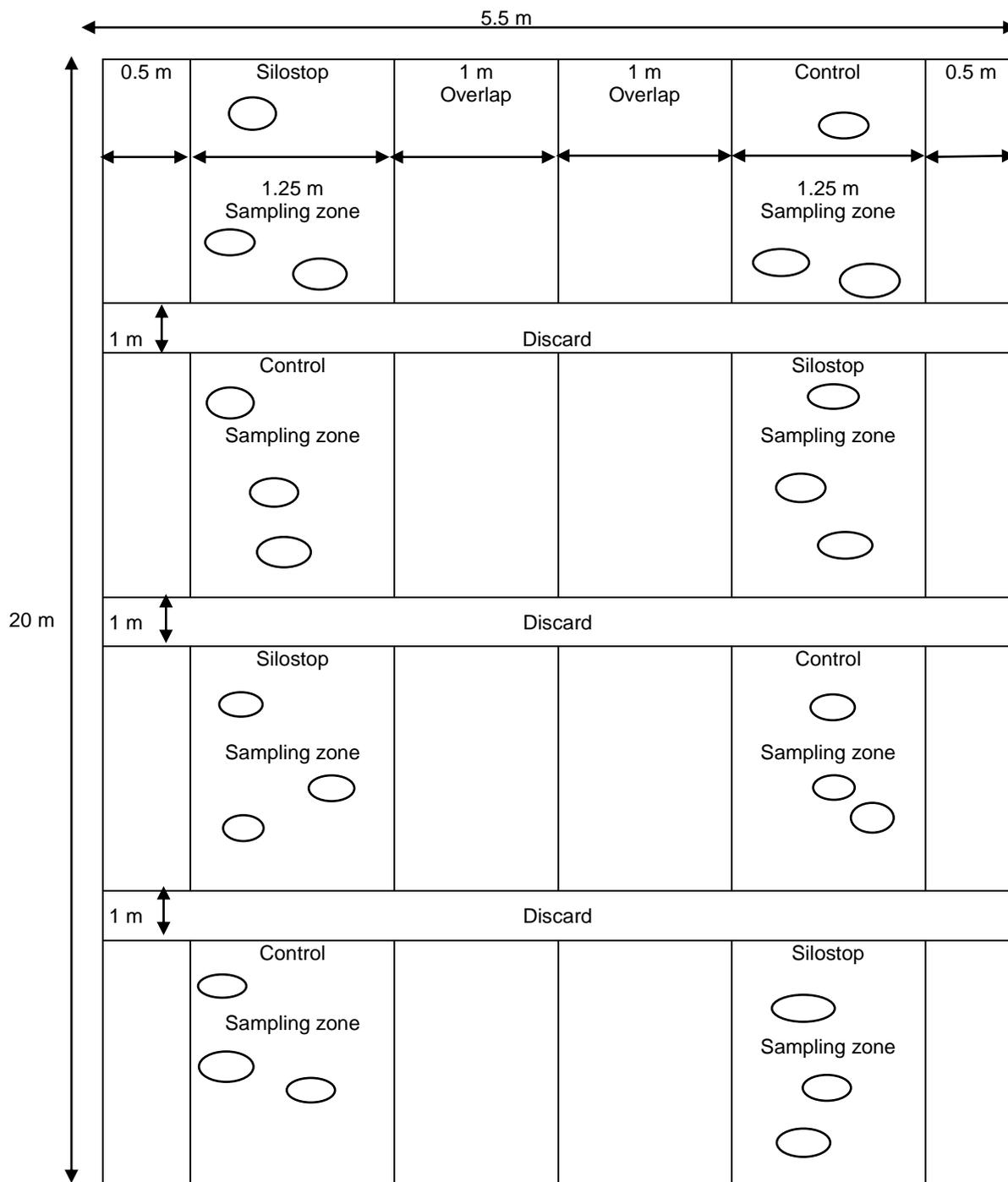


Figure 1 Overlay of the silo bunker and sampling points

After 180 days of ensiling the WCM, the top 40 cm of the silage in bunkers was harvested and fed to Damara rams to determine nutrient digestibility. This study design consisted of four phases, with each phase lasting 21 days. Each phase had 14 days of adaptation and seven days of urine and faecal collection. All animals were treated according to the regulations of the Animal Ethics Committee of the ARC-API (APIEC14/011). Sixteen 4-year-old Damara rams were stratified by bodyweight (± 54 kg live weight) and allocated to treatments. In addition, the rams were treated against internal parasites with Valbazen® (Pfizer South Africa, 85 Bute Lane, Sandton, Gauteng, South Africa), and external parasites using Deadline (Bayer Pty Ltd, 27 Wrench road, Isando, Gauteng, South Africa). Rams were housed in individual metabolic crates (1.2 m length x 0.74 m width x 0.92 m height) in an insulated well-ventilated barn. Each crate was fitted with

an individual feeder and waterer. The average ambient temperature during the trial ranged from 24 to 28 °C. The silages made using the PE and OB films were fed to the rams in a switchover design (i.e. rams that were fed OB silage in the first phase were fed the control silage at the next phase, and vice versa, until four phases had been completed).

The silage diet fed to the rams was supplemented with 0.8% of urea. The amount of feed offered was always 10% greater than the amount that was consumed the previous day to ensure ad libitum intake, and water was freely available. Feed refusals were weighed daily before the morning feeding and DM intake was calculated from the total feed andorts. The rams were adapted to the experimental diets and metabolism crates for 14 days, followed by a seven-day urine and faeces collection period. Rams were fitted with leather harnesses and canvass bags that were attached to the back of each ram three days before the collection period. Urine was collected with a funnel fitted under the cages using 10 L buckets that contained 100 ml of 100 g sulphuric acid/kg solution, and a 10% aliquot was composited daily for each ram and frozen for N and energy analyses. Faeces were collected daily, recorded, and dried at 65 °C in a forced-air oven for 72 hours. A 10% of daily collection of dried faeces was composited for each ram and frozen for analyses. Apparent digestion coefficients were calculated from quantitative feed and faecal data collected during the experiment.

The DM of pre-ensiled maize and of silages was determined by drying the samples at 60 °C until a constant mass was achieved and was corrected for loss of volatiles using the equation of Weissbach & Strubelt (2008). After drying, the samples were ground through a 1-mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) to determine CP and GE. Crude protein was determined according to the procedure of AOAC (1990) (ID 968.06) and aNDF, ADF, and ADL were determined according to the procedures of Van Soest *et al.* (1991). The aNDF was determined using heat stable α -amylase (Sigma-Aldrich Co. Ltd., Gillingham, UK, no. A-1278) with sodium sulfite, and the ADF was determined using the Fibretec System equipment (Tecator Ltd., Thornbury, Bristol, UK). Separate samples were used to determine ADF and aNDF and both included residual ash. Gross energy in diets and faeces was determined with a bomb calorimeter (MC-1000, Energy Instrumentation, 135 Knoppieslaagte, Centurion, South Africa). Analysis of N in the diets, faeces and urine samples was done according to AOAC (ID 968.06, 1990).

A representative 40 g sub-sampled from original sample of the pre-ensiled maize and that of silage samples were taken from each treatment to determine the fermentation characteristics. Each 40 g silage sample ($n = 3$ per replicate) was mixed with 360 ml of distilled water in a stomacher bag, homogenized for 4 min for immediate pH determination with a pH meter (Thermo Orion Model 525, Thermo Fisher Scientific, Waltham, MA, USA). The sample was then filtered through Whatman no. 54 filter paper (G.I.C. Scientific, Midrand, Gauteng, South Africa). The extract was used to determine WSC and LA. The WSC was determined with the phenol-sulphuric acid method according to Dubois *et al.* (1956). The LA was determined with the modified colorimetric method of Pryce (1969).

The experiment was designed as a randomized complete block design with two treatments (PE and OB sealing films) that were replicated four times. Analysis of variance (ANOVA) was performed to test the difference between two treatment factors (PE and OB sealing films) to determine the fermentation characteristics, chemical composition, aerobic stability and nutrient digestion of WCM silage by rams (Snedecor & Cochran, 1967). The data were acceptably normal. Treatment means were compared using Fisher's protected t-test least significant difference (LSD) at the 5% level of significance (Snedecor & Cochran, 1980). Data on the fermentation characteristics, chemical composition and aerobic stability of silage were analysed with the model:

$$Y_{ij} = \mu + t_i + \beta_j + \varepsilon_{ij}$$

Where: Y_{ij} = an individual observation,
 μ = the overall mean,
 t_i = the effect of the i^{th} treatment (PE or OB),
 β_j = the effect of the j^{th} replicate, and
 ε_{ij} = the residual error.

Data on nutrient digestion by sheep were analysed with the model:

$$Y_{ijkl} = \mu + \gamma_i + \pi_j + \tau_{k(ij)} + \xi_{l(i)} + \delta_j \lambda_{l(ij)} + \varepsilon_{ijkl}$$

Where: Y_{ijkl} = an individual observation,
 μ = the overall mean,
 γ_i = effect of the i^{th} sequence,

π_j = effect of the j^{th} period,

$\tau_{k(ij)}$ = effect of the k^{th} treatment nested within sequence and period,

$\xi_{k(i)}$ = random effect of the i^{th} ram nested within sequence,

$\delta_j \lambda_{m(ij)}$ = the first-order carryover effect due to treatment where m designates the treatment which was applied to the i^{th} ram in the previous period, and

δ_j = an indicator variable which = 0 if period = 1 and = 1 if period > 1.

Results and Discussion

Data on the chemical composition of freshly chopped WCM at pre-ensiling are shown in Table 1. The DM content of forages at ensiling has a strong influence on the rate and extent of the resulting fermentation. When silage DM content is less than 300 g/kg, conditions for clostridial bacterial activity are favourable, resulting in high DM losses and silage of low nutritional value (McDonald *et al.*, 2010). In this study, the DM of pre-ensiled WCM was 316 g DM/kg (Table 1), suitable for ensiling. The chemical composition of the freshly chopped WCM indicates that it was harvested at an early stage of maturity, as indicated by its DM of 316 g/kg, comparable with that reported by Nkosi *et al.* (2009).

Table 1 Chemical composition of freshly chopped whole crop maize before ensiling (n = 12)

Characteristic	Mean \pm SE
DM (g/kg)	316 \pm 0.25
pH	6.4 \pm 0.28
WSC g/kg DM	119.5 \pm 0.85
CP g/kg DM	69.6 \pm 0.32
GE MJ/kg DM	15.6 \pm 0.09
EE g/kg DM	1.8 \pm 0.88
ADF g/kg DM	253.8 \pm 0.48
aNDF g/kg DM	501.5 \pm 1.42
Yeast log ₁₀ cfu/g	5.75 \pm 0.64

DM: dry matter, WSC: water-soluble carbohydrates, CP: crude protein, GE: gross energy, EE: ether extract, ADF: acid detergent fibre, aNDF: amylase treated neutral detergent fibre, cfu: colony forming units

Data on the fermentation characteristics and nutrient composition of maize silage after 180 days of ensiling are shown in Table 2. The terminal pH of the maize silage was similar ($P > 0.05$) among the treatments. The WSC and the yeast counts were higher ($P < 0.05$) in the maize silage produced with PE compared with OB film. However, LA production was higher ($P < 0.05$) in the OB film compared with the PE film. The nutrient composition of the maize silage did not ($P > 0.05$) differ between the two films. It is well documented that the pH of silage is one of the most important factors that affect silage preservation (McDonald *et al.*, 2010). A pH range of 3.7 - 4.2 is generally considered beneficial for the preservation of cereal forages (Kung & Shaver, 2001) and that of the present study was less than 3.8, indicative of well-preserved silage. The treatment did not affect the terminal silage pH, which is common in maize silage, since maize have enough WSC and low buffering capacity (Meeske, 2005).

Water-soluble carbohydrates are regarded as essential substrates for growth of LAB for proper fermentation (McDonald *et al.*, 1991). The WSC concentration of WCM at ensiling in the present study was 120 g WSC/kg DM, which is more than the minimum level of 60 g/kg that is required for efficient fermentation (Lunden-Petersen & Lindgren, 1990). However, the maize silage produced with OB film had reduced ($P < 0.05$) residual WSC compared with that for silage produced with PE film. It can be assumed that the low oxygen permeability of the OB created suitable conditions for maximum growth of LAB, which resulted to more WSC being consumed by LAB under the OB film compared with the control.

Lactic acid is the strongest of all silage acids and its high concentration will decrease the pH more effectively than other volatile fatty acids (McDonald *et al.*, 2010). In the present study, more LA was produced in the silage sealed with the OB film, which is consistent with low residuals of WSC ($P < 0.05$) (Table 4). This indicates that the OB film created an anaerobic environment that is suitable for the growth of LAB to convert more WSC to LA (Borreani *et al.*, 2007).

Table 2 Fermentation characteristics and nutrient composition of whole crop maize silage collected at the top 40 cm after 180 days of ensiling sealed with either standard polyethylene film or an orange oxygen barrier Silostop film (n = 12)

Fermentation characteristics	Treatments ¹		SE	P-value
	PE	OB		
pH	3.8	3.7	± 0.08	0.317
WSC g/kg	15.9	11.4	± 0.31	0.002
Lactic acid g/kg	66.6	73.6	± 1.12	0.022
Yeast log ₁₀ cfu/g	5.8	3.8	± 1.72	0.046
<i>Nutrient composition</i>				
DM g/kg	303.8	363.2	± 8.53	0.016
CP g/kg	80.8	81.7	± 2.30	0.379
GE MJ/kg	16.1	15.9	± 0.98	0.246
EE g/kg	2.3	2.3	± 0.19	0.147
aNDF g/kg	457.0	436.0	± 0.95	0.048
ADF g/kg	323.0	244.1	± 3.18	0.017

¹ PE: polyethylene sealing film, OB: orange oxygen barrier Silostop sealing film
DM; dry matter; WSC, water soluble carbohydrates; CP, crude protein; GE, gross energy; EE, ether extract; ADF, acid detergent fibre; aNDF, amylase treated neutral detergent fibre

Lower concentrations of aNDF in silages, compared with the original herbage concentrations, reflect the extent of breakdown primarily of hemicellulose during ensiling. This breakdown yields additional substrate for the fermentation process. Hemicellulose can also be degraded through hydrolysis by organic acids that are produced during fermentation or that can be applied with silage additives (McDonald *et al.*, 1991). The fibre fractions of the WCM were reduced with ensiling (see Tables 1 and 2), which reflects the degradation of fibre to release sugar to be consumed by LAB. However, differences between treatments were not significant post ensiling.

Data on the aerobic stability of maize silage produced with either PE or OB films are shown in Table 3. The pH, CO₂ production, and populations of yeast and moulds were lower ($P < 0.05$) in the maize silage produced with OB film compared with those produced with PE film. In a warm climate, such as that of South Africa, WCM silage is susceptible to accelerated aerobic deterioration. Aerobic yeasts that metabolize LA to carbon dioxide and water (producing heat) are most active between 20 °C and 30 °C (Ashbell *et al.*, 2002). Silage that has spoiled is undesirable owing to an increased risk of proliferation of potentially pathogenic or undesirable microorganisms that negatively affect animal performance (Driehuis *et al.*, 1999; Trevisi *et al.*, 2003).

The high pH of the silage that was produced using PE film compared with the silage that was produced with the OB film (Table 3) was probably caused by high yeast populations, which were present in greater numbers owing to the high permeability of the PE sealing film to oxygen (Dolci *et al.*, 2011). High yeast concentrations in silage are known to initiate and accelerate its aerobic deterioration (McDonald *et al.*, 1991; Driehuis *et al.*, 2001). Several studies (Borreani *et al.*, 2007; Muck & Holmes, 2009) have demonstrated the consequences of high concentrations of oxygen in silage because of the high permeability of the sealing film to oxygen, resulting in DM and nutrient losses and accumulations of pathogens. Dolci *et al.* (2011) compared maize silage produced with either OB or PE film, and reported reduced silage aerobic stability in silage produced under PE film compared with that produced under OB film. The results of the present study are thus consistent with the previous results of Dolci *et al.* (2011). Production of CO₂ from silage during the aerobic stability test is an indication of the levels of yeasts and moulds in the silage (Woolford, 1989; Ashbell *et al.*, 1991). According to Weinberg *et al.* (1993), silages with higher residual of WSC concentrations are less stable when exposed to air. Here, WCM silage that was produced with PE film had a higher residual WSC concentration; hence its aerobic stability was impaired deemed compared with that produced with OB film. Silages with yeast concentrations greater than 10⁵ cfu/g are prone to deteriorate

on exposure to air (McDonald *et al.*, 1991). In the present study use of the PE sealing film led to a yeast population of 10^5 cfu/g.

Table 3 Effects of standard polyethylene film or an orange oxygen barrier Silostop sealing film on the aerobic stability of maize silage after five days' aerobic exposure (n = 12)

Indicators of aerobic stability	Treatments ¹		SE	P-value
	PE	OB		
DM g/kg	218.0	273.0	± 1.79	0.001
pH	6.8	5.8	± 0.13	0.010
CO ₂ g/kg	58.5	38.2	± 3.33	0.023
Yeast & moulds log ₁₀ cfu/g	8.43	4.21	± 0.84	0.008

¹ PE: polyethylene sealing film, OB: orange oxygen barrier Silostop sealing film
DM: dry matter; CO₂, carbon dioxide

Data on intake, nutrient digestion and N retention in rams fed maize silage are shown in Tables 4 and 5, respectively. No significance ($P > 0.05$) differences occurred in the intake, nutrient digestion and N retention in rams fed maize silage that was produced with using either PE or OB sealing film.

Table 4 Effects of sealing ensiled chopped whole crop maize with a standard polyethylene film or an orange oxygen barrier Silostop film on daily feed intake and apparent nutrient digestibility by Damara rams

Intake	Treatments ¹		SE	P-value
	PE	OB		
DM	1444.0	1089.0	± 0.62	0.289
CP	121.2	130.3	± 0.17	0.295
GE MJ/day	191.3	182.0	± 0.17	0.350
EE	59.8	54.6	± 0.22	0.911
NDF	507.0	507.3	± 0.17	0.837
ADF	278.6	305.6	± 0.17	0.204
<i>Apparent digestibility (g/g)</i>				
DM	701.1	673.7	± 0.17	0.285
CP	647.5	625.4	± 0.17	0.966
ADF	586.6	605.9	± 0.17	0.157
NDF	585.0	549.3	± 0.17	0.488

¹ PE: polyethylene sealing film, OB: orange oxygen barrier Silostop sealing film
DM: dry matter, CP: crude protein, GE: gross energy EE: ether extract, ADF: acid detergent fibre NDF: neutral detergent fibre

It is generally known that maize silage has a low CP concentration. Hence, dietary supplementation of CP should be done to meet the daily CP requirements of ruminants fed maize silage. In this present study, 0.8% of urea was added to the daily ration of the rams, based on the daily intake of each ram. However, silage treatments did not differ in the intake, DM, CP, NDF and ADF digestion and N utilization by rams (Table 4 and 5). This might be attributed to the similar nutrient composition of the two silages.

Table 5 Effects of sealing ensiled chopped whole crop maize with a standard polyethylene film or an orange oxygen barrier Silostop film on nitrogen intake and retention in Damara rams fed whole crop maize silage

Components of nitrogen (N) flux	Treatments ¹		SE	P-value
	PE	OB		
N intake, g/d	19.70	21.60	± 0.160	0.294
Faecal N, g/day	6.27	6.87	± 0.166	0.587
Urinary N, g/day	4.45	4.42	± 0.168	0.466
N retention, g/day	8.99	10.35	± 0.170	0.193
N retention, %	46.4	48.2	± 0.160	0.394

¹ PE: polyethylene sealing film, OB: orange oxygen barrier Silostop sealing film

Conclusion

Fermentation characteristics and aerobic stability of maize silage were improved with the use of the OB sealing film compared with the PE sealing film. However, these effects did not lead to improved nutrient concentration, or digestibility and N balance in rams fed the two silages. Thus, use of the OB sealing film could improve the efficiency of maize silage production compared with the use of PE sealing film under South African conditions. The cost effectiveness of using OB film for the production of maize silage under South African conditions should be determined.

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Authors' Contributions

BDN, RM, IMM, CJLT, NWN and KAN worked on the original report and data analysis. BDN, RM, IMM and NWN worked on the study design, execution and data collection. NWN, BDN and IMM worked on the manuscript preparation.

Conflict of Interest Declaration

There is no conflict of interest associated with this manuscript.

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