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AUTHORS:
Gopolang Matlabe[1,2]
Hilda K. Mokoboki[2,3]
Amenda N. Sebola[4]
Cornelia K. Lebopa[2,3]
Khuliso E. Ravhuhali[2,3]
Onke Hawu[2]

AFFILIATIONS:
[1]Department of Agriculture, Environmental Affairs, Land Reform and Rural Development, Kimberley, South Africa
[2]Department of Animal Science, School of Agricultural Sciences, Faculty of Natural and Agricultural Sciences, North West University, Mahikeng, South Africa
[3]Food Security and Safety Niche Area, Faculty of Natural and Agricultural Sciences, North West University, Mahikeng, South Africa
[4]School of Agriculture and Life Sciences, Department of Agriculture and Animal Health, University of South Africa, Johannesburg, South Africa

CORRESPONDENCE TO:
Gopolang Matlabe

EMAIL:
Gmmatlabe@gmail.com

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Effects of browse legume species addition on nutritional composition, fermentation characteristics and aerobic stability of *Opuntia* cladodes silage

Forage legumes are commonly used as an absorbent additive in high-moisture silages. Thus this study was carried out to assess the nutritive value, fermentation characteristics and aerobic stability of *Opuntia*–legume browse mixed silages. Five browse legume species (*Leucaena leucocephala, Acacia mellifera, Searsia lancea, Prosopis velutina,* and *Grewia flava*) were mixed with *Opuntia* cladodes. The silage mixture was formulated at a ratio of 60 *Opuntia* cladodes: 40 leguminous browse species and ensiled in polythene bags and kept in a laboratory for 42 days to determine chemical composition and fermentation characteristics. Silage samples were also subjected to an aerobic stability test. One-way analysis of variance in a completely randomised design was used to analyse the data. The pH values for silages made from *Opuntia* cladodes with *L. leucocephala*, *A. mellifera* and *G. flava* were lower than 4.8, which is considered an indicator of good-quality silage. The water-soluble carbohydrates content of silages made with *Opuntia* cladodes and *S. lancea* and *G. flava* was within the range of 8–12 g/kg dry matter, which is sufficient for good fermentation. The highest CO_2 production, which signifies poor aerobic stability, was recorded for the control silage (*Opuntia*) compared to all *Opuntia*–legume mixed silage treatments. The addition of legume browse leaf-meal to *Opuntia* cladodes improved nutritive value, fermentation characteristics, and silage quality. Therefore, despite some limitations, *Opuntia*–legume browse silages, particularly *Opuntia*–*G. flava* and *Opuntia*–*L. leucocephala,* proved to be beneficial for livestock, as they meet the nutritional requirement of a ruminant.

Significance:

This study underlines the importance of co-ensiling *Opuntia* cladodes and high protein legume browse hay to offer an alternative feeding strategy for ruminant livestock and ensure sustainable provision of high-quality feed during dry periods.

Introduction

The problem associated with ruminant productivity in semi-arid areas is the scarcity of feed.[1] This scarcity is due to seasonal changes that influence feed quantity and quality. The cost of conventional feed is high and this negatively impacts on the reliability and sustainability of the industry due to increased mortality rates resulting in decreased production.[2] Therefore, alternative strategies to conserve forage material for utilisation during periods of feed scarcity, such as during droughts and winter, need further exploration.[3]

Opuntia is a genus of flowering plants in the cactus family (Family: Cactaceae) and is commonly known as the prickly pear. *Opuntia* species are found in most arid and semi-arid regions and have the capacity to grow in extremely harsh environments.[4] Consequently, the species can be considered as an alternative feed source for winter livestock during droughts. However, *Opuntia* cladodes (or flattened, photosynthetic shoots) have a high moisture content and are low in crude protein[5], which means that they are not easily ensiled. Utilisation of shrubs or trees as possible feeds for ruminant animals has become progressively more essential in several tropic and subtropic regions around the world.[6,7] Fodder trees and shrubs have high potential as feed sources for wildlife and domestic livestock as they can be incorporated successfully into various animal production systems as alternative feed resources for livestock.

Studies have revealed that protein supplementation enhances the nutritional value of *Opuntia*-based diets offered to ruminants to improve their productivity.[8] Browse legumes are generally higher in nitrogen composition[9,10] but with variable digestibility rates depending on the composition of the bioactive compounds[11,12]. Co-ensiling *Opuntia* cladodes and high-protein legume browse hay offers an alternative feeding strategy to ensure sustainable provision of high-quality feed throughout the year, including during winter and dry periods.[13] Therefore, the current study was carried out to assess the nutritive value, fermentation characteristics and aerobic stability of *Opuntia*–legume browse mixed silages.

Materials and methods

Study site

This experiment was conducted at the Agricultural Research Farm of North West University (Mafikeng Campus), which is located approximately 7 km from Mmabatho (latitude: 25°47'27''S and longitude 25°37'18''E) in Mahikeng region in the North West Province of South Africa. The study area is categorised as semi-arid with an average annual rainfall of 300 mm. The area of Mahikeng is characterised by fairly high temperatures in summer (27–38 °C) and cold in winter (11–18 °C). The area is composed of a mixture of bushveld and grassveld under dry Highveld grassland vegetation.[14]

Sample collection

Fresh *Opuntia* cladodes (Roedtan variety) were cut on the Shalom farm close to Mahikeng town in the North West Province. The *Opuntia* cladodes collected were cut into smaller pieces (20–30 mm) with a sharp machete (Lasher-302). *Senegalia mellifera*, *Searsia lancea*, *Prosopis velutina* and *Grewia flava* leaves were harvested from Mahikeng, North West Province and *Leucaena leucocephala* leaves were collected from the Nkangala District, Dr James Moroka local municipality, Mpumalanga Province, South Africa. The harvested area in Mpumalanga Province consists of a flat landscape with loamy-sandy soil. The average temperature varies between 25 °C in summer and 12 °C in winter. Annual rainfall ranges from 400 mm to 800 mm. The area receives rainfall mainly in summer.

After collection, the harvested leaves were air-dried on a plastic sheet in a shaded area for 7 days, then ground into various leaf-meals using a Wiley mill (Philadelphia, PA, USA) to pass through a 1-mm sieve.

Sample preparation

Each of the prepared leaf-meals was then mixed with fresh cut *Opuntia* cladodes prior to ensilage. The silage mixture comprised 60% fresh *Opuntia* cladodes and 40% browse legume leaf-meal on a weight/weight basis and was ensiled in polythene bags. In addition, 2% of molasses (Kalori 3000) was applied to the mixture in order to enhance silage fermentation. For each treatment, five replicates were ensiled. The silage was incubated in a rodent-free laboratory room for 42 days, after which a representative sample was drawn from all the treatments for pH analysis. The representative samples from every treatment were also ground using a Wiley mill (Philadelphia, PA, USA) to pass through a 3-mm sieve and were further analysed for silage nutritional composition.

Nutritional composition and fermentation characteristics analyses

The dry matter (DM) content of the silages was determined after drying samples in an oven at 105 °C to a constant mass. Organic matter was determined by igniting the samples at 600 °C overnight. The nitrogen (N) composition of the samples was determined using the technique of the Association of Official Analytical Chemists.[15] Acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were evaluated according to the procedure of Van Soest et al.[16] The atomic absorption spectrophotometer (AAS-Buck 205) was used to analyse the mineral composition (calcium (Ca) and magnesium (Mg)).[17] Phosphorus composition of the silages was assessed calorimetrically.[17] The determination of pH was done using a digital pH meter (Hanna HI-2211). Water-soluble carbohydrate (WSC) content was determined by the phenol-sulfuric acid technique.[18] Lactic acid in the silage was determined as described by the Lepper methodology.[19] The formula applied to estimate total digestible nutrients (TDN) was 82.38 – (0.7515 x ADF) as given by Bath and Marble[20]. Estimated potential daily dry matter intake (DMI) was calculated according to Mertens[21] using the following formula:

$$DMI = \frac{1.2 \times bodyweight}{NDF\%}, \qquad \text{Equation 1}$$

where

$$NDF\% = \frac{(W3-(W1 \times C1)) \times 100}{W2}, \qquad \text{Equation 2}$$

and W1 is the bag tare weight, W2 is the sample weight, W3 is the final bag weight and C is the correction factor.

Aerobic stability determination

An aerobic stability test was performed on silage samples on day 42 by drawing a representative 500-g silage sample from all the polythene bags and placing them into plastic jars, covering the jars using a double layer of cheesecloth and storing them at an ambient temperature of about 28 °C. Production of carbon dioxide (CO_2)[22], pH and yeast and moulds was determined after the 7-day aerobic exposure as described by the International Dairy Federation[23] technique.

Statistical analysis

All data generated were analysed by an analysis of variance using the general linear model procedure of SAS[24] for a completely randomised design. The following model was used:

$$Yij = \mu + Di\ (i = 6) + Eij, \qquad \text{Equation 3}$$

where Yij = response variable ij; μ = overall mean; Di = dietary treatment effect (i=6; control, *L. leucocephala*, *A. mellifera, S. lancea, P. velutina* and *G. flava*); and Eij = random error.

For parameters with significant difference, multiple comparisons of treatment means were conducted by applying the probability of difference (pdiff) alternative of the general linear model procedures of SAS.[24] Correlation (linear regression) was used to determine the degree of association amongst the variables:

$$Y = aX + b, \qquad \text{Equation 4}$$

where Y is the dependent variable, a is constant, X is the independent variable and b is the intercept.

Results

Nutritional composition before ensiling

The results of the nutritional composition analyses of cladodes and leaves from *L. leucocephala*, *A. mellifera*, *S. lancea*, *G. flava* and *P. velutina* are presented in Table 1. The DM content of various legume browse species ranged from 955.23 g/kg DM to 972.94 g/kg DM. *L. leucocephala* and *A. mellifera* had similar ($p > 0.05$) DM contents compared to that of *P. velutina*. Between the species, crude protein content from legume browse species ranged from 39.13 g/kg DM in *Opuntia* to 364.70 g/kg DM in *G. flava*. *P. velutina* had the highest neutral (483.67 g/kg DM) and acid detergent fibre (347.20 g/kg DM) than any other legume browse species. *S. lancea* had the highest acid detergent lignin (253.70 g/kg DM) as compared to *Opuntia* (88.63 g/kg DM).

Nutritional composition after ensiling

Data on nutritional composition of the six silages are displayed in Table 2. The results indicate that *Opuntia–L. leucocephala* (OLL; 421.77 g/kg, 932.67 g/kg DM) and *Opuntia–A. mellifera* (OAM; 404.00 g/kg, 927.00 g/kg DM) silages had higher dry matter and organic matter, respectively, than other silages. The crude protein content was significantly different ($p < 0.05$), being highest in *Opuntia–G. flava* (OGF) and lowest in *Opuntia* alone. Ether extracts of the silages also differed significantly ($p < 0.05$), with the lowest ether extract level observed in OAM and the highest in *Opuntia* alone (control). There were significant differences in ash contents of the silages. The highest ash content was recorded for *Opuntia–P. velutina* (OPV; 147.73 g/kg DM) followed by OLL (106.69 g/kg DM), and the lowest were from OLL (67.33 g/kg DM) and OAM (73.00 g/kg DM). The highest NDF and ADF contents were recorded for *Opuntia* alone and the lowest for OAM. Calcium contents of the silages were significantly different ($p < 0.05$), with the lowest recorded for OPV and the highest for *Opuntia* alone. The highest phosphorus level was recorded for OGF while the lowest level was for *Opuntia* alone.

The highest total digestible nutrient content was recorded for *Opuntia–S. lancea* (OSL), followed by OAM and OLL, and the lowest was recorded for *Opuntia* alone (Figure 1).

Table 1: Nutritional composition (g/kg dry matter) of *Opuntia* and legume browse species

Species	Dry matter	Crude protein	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin
Opuntia	957.5[b]	39.1[f]	297.1[d]	181.3[e]	88.6[f]
Leucaena leucocephala	972.9[a]	275.1[b]	236.0[f]	178.7[e]	192.6[b]
Acacia mellifera	971.3[a]	170.2[c]	424.6[b]	245.4[d]	106.6[e]
Searsia lancea	966.7[ab]	98.8[f]	386.8[c]	340.6[b]	253.7[a]
Prosopis velutina	955.2[c]	137.7[d]	483.7[a]	347.2[a]	176.0[c]
Grewia flava	963.9[abc]	364.7[a]	255.5[e]	248.2[c]	157.8[d]
p-value	0.0124	<0.0001	<0.0001	<0.0001	<0.0001
Standard error	3.29	0.28	1.37	0.84	0.32

Means within the same column with different superscript letters differ significantly ($p<0.05$).

Table 2: Nutritional composition (g/kg dry matter, unless otherwise stated) of the silages used in the experiment

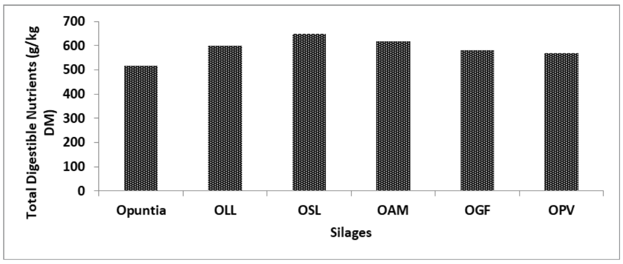
Species	Dry matter	Organic matter	Ash	Crude protein	Ether extract	Neutral detergent fibre	Acid detergent fibre	Ca%	P%	Mg%
Opuntia	251.3[c]	852.3[e]	124.7[b]	50.0[f]	85.5[a]	624.5[a]	406.6[a]	3.83[a]	0.25[f]	1.19[a]
Opuntia–Leucaena leucocephala	421.8[a]	932.7[a]	67.3[e]	106.7[b]	24.2[d]	544.2[d]	296.5[d]	1.67[c]	0.99[b]	0.89[c]
Opuntia–Acacia mellifera	404.0[a]	927.0[a]	73.0[e]	64.4[e]	13.6[e]	533.7[e]	272.3[e]	1.39[d]	0.87[c]	0.86[c]
Opuntia–Searsia lancea	310.3[b]	887.6[c]	112.4[c]	92.5[d]	44.1[b]	567.3[b]	234.6[f]	0.98[e]	0.67[e]	0.69[e]
Opuntia–Prosopis velutina	291.1[bc]	875.3[d]	147.7[a]	100.5[c]	37.4[c]	558.8[c]	337.3[b]	0.93[e]	0.77[d]	0.76[e]
Opuntia–Grewia flava	281.1[bc]	897.8[b]	102.2[d]	134.5[a]	48.4[b]	518.6[f]	321.7[c]	1.84[b]	1.11[a]	1.04[b]
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Standard error	1.40	2.07	2.07	1.93	1.98	0.08	0.16	0.03	0.02	0.01

Means within the same column with different superscript letters differ significantly ($p<0.05$).

Table 3: Fermentation characteristics and aerobic stability of the silages used in the experiment

Parameter	Silages							
	Opuntia	***Opuntia–Leucaena leucocephala***	***Opuntia–Acacia mellifera***	***Opuntia–Searsia lancea***	***Opuntia–Prosopis velutina***	***Opuntia–Grewia flava***	***p*-value**	**Standard error**
Fermentation characteristics								
pH	6.59[a]	4.66[de]	4.34[e]	5.48[c]	5.97[b]	4.75[d]	<0.0001	0.117
Lactic acid (g/kg dry matter)	0.23[d]	15.07[a]	12.00[b]	2.43[c]	0.50[d]	2.50[c]	<0.0001	0.612
Water-soluble carbohydrates (g/kg dry matter)	7.49[d]	11.28[a]	11.12[ab]	8.66[e]	8.39[c]	10.79[b]	<0.0001	0.115
Yeast and moulds (log cfu/g)	6.03[a]	2.73[c]	1.33[d]	1.73[d]	3.83[b]	1.00[d]	<0.0001	3.075
Aerobic stability								
pH	8.82[a]	6.71[d]	6.17[e]	7.21[c]	7.80[b]	6.17[e]	<0.0001	0.129
CO_2 (g/kg dry matter)	32.28[a]	2.38[c]	9.00[bc]	11.57[b]	5.37[bc]	1.92[c]	<0.0001	2.933

Means within the same column with different superscript letters differ significantly ($p<0.05$).



OLL, Opuntia–Leucaena leucocephala*; OSL,* Opuntia–Searsia lancea*; OAM,* Opuntia–Acacia mellifera*; OGF,* Opuntia–Grewia flava*; OPV,* Opuntia–Prosopis velutina

Figure 1: Effect of silages on total digestible nutrients (g/kg dry matter).

Fermentation characteristics and aerobic stability

The silages had significant differences in pH, lactic acid, WSC, and yeast and moulds (Table 3). The pH levels varied significantly, with the highest pH recorded for *Opuntia* alone and the lowest for OAM. The highest WSCs were recorded in OLL, OAM and OGF silages compared to OPV, OSL and the control (*Opuntia* cladodes alone). Addition of browse legume leaf-meal into *Opuntia* cladodes resulted in improved lactic acid composition of the resultant silages. The pH level of silages subjected to the aerobic stability test varied significantly, with the highest pH level recorded in *Opuntia* alone and the lowest in OGF and OAM. CO_2 production was highest in *Opuntia* ensiled alone than in any of the experimental silages.

Negative relationships were observed between DMI and NDF, and DMI and Ca composition of silages, whereas positive relationships were observed between DMI and crude protein, and DMI and WSC of the silages (Table 4).

Discussion

The nutritive value of forages for livestock feeding depends on the balance between the nutritive components of the plants, the digestibility of such nutrients and the quality of the nutrient ingested by the animal.[25] In order to minimise the cost and the time involved in *in vivo* forage evaluation, laboratory procedures have been developed. According to Ligouri et al.[26], *Opuntia* have very low dry matter content. However, the results from the current study showed that *Opuntia* silage had higher dry matter content than the 90.7 g/kg reported by Inácio et al.[27] for *Opuntia* as the average. The increase in the dry matter content of the silages is associated with the addition of browse species.[28] This suggests that incorporation of legume browse and *Opuntia* cladodes can result in production of silage with moderate ruminal degradability and without laxative effects, and ultimately improve animal production. *Opuntia* cladodes ensiled alone had lower crude protein composition but was within the range of 3.66–8.08% DM observed by Inácio et al.[27] and Mciteka[29]. As a general rule, browse species such as *L. leucocephala*, *A. mellifera*, *P. velutina* and *G. flava* which have crude protein within the range 11–13% are capable of supplying adequate protein for maintenance and growth.[6,7]

The silage formulated using *G. flava* with *Opuntia* (OGF) legume leaves had the highest crude protein (134.47 g/kg DM) of all the silages and also had higher than the average for cereal silages (110 g/kg DM).[30] According to the current findings, the lowest organic matter (852.27 g/kg DM) was observed for *Opuntia* silage (control). These findings are similar to the organic matter content of different *Opuntia* cladode varieties reported in the literature.[31,32] *Opuntia*, *L. leucocephala* and *G. flava* had low neutral detergent fibre contents which fell within the range of 20–35% reported by Nsubuga et al.[33] for a variety of browse species, and these species will be more digestible than *A. mellifera*, *S. lancea* and *P. velutina* which have more than 35% NDF. Silage pH ranged between 4.34 and 6.59. The pH values for silages made from *Opuntia* cladodes with *L. leucocephala*, *A. mellifera* and *G. flava* were lower than 4.8, which, according to Cürek and Özen[13], is considered an indicator of good quality silage. The lower the pH level, the higher are the soluble sugars in the silage ingredients.[34] The pH values of the *Opuntia*–legume browse mixed silages were higher than those reported by Nogueira et al.[35] of 3.8 and 4.2. The legumes generally have resistance to pH reduction in silages due to their high buffering capacity mainly from the presence of cations.

Water-soluble carbohydrates are considered vital for growth of lactic acid bacteria for enhanced fermentation.[36] The WSC contents of all silages except *Opuntia* alone were within the range of 8–12 g/kg DM, and thus sufficient for good fermentation.[37] The concentrations of lactic acid in silages in the current study were lower than those observed by Cürek and Özen[13] and da Silva Brito et al.[38] who obtained *Opuntia* silages with average lactic acid contents of 25.90 g/kg and 9.03 g/kg DM, respectively. Achieving an increase in the content of lactic acid is the most reliable indicator for the success of a microbial additive in improving silage quality. The addition of legume browse (except *P. velutina*) to *Opuntia* at ensiling increased ($p<0.05$) the lactic acid concentration. Yeasts are the main silage degrading microorganisms, especially after aerobic exposure of the material, promoting marked losses of DM throughout the ensiling process. These microorganisms cannot grow in the silages used in the current study, as the pH is above 3.5, because they can use lactic acid as a substrate when in anaerobiosis, thus increasing the pH in the silages, which may favour the emergence of undesirable fermentations.[39]

The greater CO_2 concentration recorded in *Opuntia* (control) ensiled alone indicates microbial metabolic activity, which may prompt silage temperature to rise, leading to deterioration of silage quality.[22,40] The aerobic stability of the *Opuntia* silage was affected by browse legume addition. Although the addition of browse legumes to *Opuntia* silage resulted in higher residual sugar and lactic acid compared to the control, the differences in these fermentation indices affected the aerobic stability of the silage. The relationship between NDF, crude protein and WSC versus DMI was established. The relationship between NDF content and DMI was negative because this component is less digestible than non-fibrous components such as protein. In the current investigation, the regression coefficient value was 0.99 between NDF and DMI, which is

Table 4: Regression equations predicting dry matter intake from chemical composition and fermentation+characteristics of silages

Factor	Y-variable	Formula	R^2	*p*-value
Crude protein (CP)	Dry matter intake	Y = 0.142CP + 84.109	0.5015	0.0100
Neutral detergent fibre (NDF)	Dry matter intake	Y = -0.165NDF + 189.296	0.9964	<0.0001
Acid detergent fibre (ADF)	Dry matter intake	Y = -0.057ADF + 114.871	0.3026	0.0639
Ca	Dry matter intake	Y = -3.716Ca + 103.719	0.4229	0.0220
Mg	Dry matter intake	Y = -10.771Mg + 106.869	0.1087	0.2952
Water-soluble carbohydrate (WSC)	Dry matter intake	Y = 3.197WSC + 66.379	0.7315	0.0004
Lactic acid (LA)	Dry matter intake	Y = -6.781LA + 135.993	0.2006	0.1442
Total digestible nutrients (TDN)	Dry matter intake	Y = 0.757TDN + 52.461	0.3026	0.0639

higher than the range reported by Coleman and Moore[41]. Water-soluble carbohydrates are, however, often associated with dry matter intake.[42]

Conclusion and recommendations

The addition of browse legume species had an effect on the nutritional composition, fermentation characteristics and aerobic stability of *Opuntia* cladode silage. Ensiling *Opuntia*-*G. flava* and *Opuntia*–*L. leucocephala* can make good-quality silage and small-scale farmers can use these silages as feed for ruminant livestock during dry seasons. Further studies are needed to explore the influence of bacterial inoculants and fibrolytic enzymes on fermentation characteristics and quality of *Opuntia*–legume silage and also to determine *Opuntia*–legume silage effect on animal performance. Further *in vivo* trials are required to assess the productivity of animals fed these silages.

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Competing interests

We have no competing interests to declare.

Authors' contributions

G.M., H.K.M., C.K.L. and A.N.S.: Conceptualisation; methodology. G.M.: Data collection, sample analysis, data curation. H.K.M., O.H. and K.E.R.: Data analysis. H.K.M., C.K.L., A.N.S., K.E.R.: Validation. G.M., K.E.R., O.H. and H.K.M.: Writing – the initial draft, writing – revisions. H.K.M., C.K.L. and A.N.S.: Student supervision. H.K.M.: Project leadership. All authors read and approved the manuscript.

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