Journal of Animal &Plant Sciences, 2016. Vol.28, Issue 3: 4488-4495
Publication date 1/05/2016, **http://www.m.elewa.org/JAPS**; *ISSN 2071-7024*

Effects of molasses levels and growing conditions on nutritive value and fermentation quality of *Opuntia* cladodes silage

K. Mokoboki[1,2], N. Sebola[1,2] and G. Matlabe[1]
[1]*Animal Science Programme, North West University, Mafikeng Campus, Mmabatho 2735, Private Bag x 2046, South Africa.*
[2]*Food Security and Safety Niche Area, School of Agricultural Sciences, Faculty of Agriculture, Science and Technology, North – West University, Mmabatho, Mafikeng 2735, South Africa.*

Corresponding author email:Hilda.mokoboki@nwu.ac.za
Keywords: cladode, silage, *opuntia*, molasses

1 ABSTRACT
The spineless *Opuntia ficus-indica* fruit (Prickly pear) industry in South Africa has increased in recent years and large quantities of cladodes are produced as a feed source for livestock. The objective of the study was to determine the effect of molasses levels and growing conditions on nutritive value and fermentation characteristics of *Opuntia* cladodes silage. The cladodes were removed by pruning from a Shaloom Farm outside Mahikeng in Burhmansdrift, North West Province. The following parameters were determined: dry matter, crude protein, acid detergent fibre, neutral detergent fibre, ether extract, pH, lactic acid and water-soluble carbohydrates. There were significant varietal, growing condition and molasses inclusion level interaction effects ($P<0.05$) on dry matter, moisture, crude protein, neutral detergent fibre, acid detergent fibre and fat contents. The inclusion of molasses (at 8%, 16% and 24%) into different types of prickly pear cladodes resulted in lower dry matter content as compared to without inclusion (0%) of molasses. The reason could be due to high moisture content produced during fermentation. Addition of molasses into prickly pear cladodes in this study resulted in lower acid detergent fibre; neutral detergent fibre and ether extract contents. The higher inclusion levels of molasses for variety *Roedtan* in both dry and irrigated lands resulted in a higher crude protein content. Lactic acid content from different cladode silages in this study varied from 46.5 to 100 g/kg DM. Animal performance testing to measure the digestibility and palatability of these two promising varieties is recommended to evaluate their effectiveness under practical feeding conditions.

2 INTRODUCTION
Opuntia cladodes (Figure 1) are used as livestock feed during drought period or food shortage in many arid and semi-arid regions (De Waal *et al.*, 2006). The spineless cactus pear fruit industry in South Africa has increased in recent years (Zeeman 2005; De Waal *et al.*, 2007). The industry is divided into a subsistence and commercial sector where the former is based on the naturalised prickly pear that is widely grown in the drier areas of South Africa. Moreover, a commercial sector is distributed in all provinces of the country. It is estimated that 1200 ha of prickly pear are planted and grown commercially in South Africa by commercial farmers. Spineless

Prickly pears are planted by commercial farmers for fruit production and fodder for livestock. Cladodes were planted vertically (upright) with one-third of the cladode below the soil surface. Plants were positioned with the flat side's orientated in-row and soil around the cladode firmed by mechanical means. *Roedtan* and *Van as* varieties were planted under dry land and irrigation condition. Large quantities of fresh cladodes are available when plants are pruned to improve fruit production and quality (Zeeman 2007) and this creates the prospect of utilizing the large quantities of plant material that are produced annually as a feed source for livestock (De Waal *et al*., 2007).

Figure 1: *Opuntia* cladodes

The feed intake of various types of leguminous forage is affected by many factors such as harvesting, physical and metabolic feedback and secondary compounds. Preservation method may affect these factors especially in reducing the secondary compounds, the anti-nutritional substances commonly present in legume forage. Ensiling protein rich foliage may have some constraints due to the low water soluble carbohydrates content, high buffering capacity and low dry matter content (McDonald *et al.,* 2002). Molasses is a good silage additive, because it is high in water-soluble carbohydrates content and reduced pH and ammonia levels in treated silages. Molasses can be a key ingredient for cost effective management of feeds and pastures. Although there is research available that evaluated molasses and legume species as supplements to *Opuntia* cladode, no specific data exists regarding the effect of molasses as the only supplementary source of energy to ruminant animals. There is very little information on studies to determine the effect of variety, growing condition and molasses inclusion levels on nutritive value and fermentation characteristics of *Opuntia* cladodes silage. Thus, the objective of this study was to determine the effect of molasses levels and growing condition on nutritive value and fermentation characteristics of *Opuntia* cladodes silage.

3 MATERIALS AND METHODS

3.1 Sample collection and preparation:

Two years old prickly pear cladodes from two varieties (*Roedtan* and *Van as*) were randomly harvested in August 2014, from both irrigated and non-irrigated plantations. The prickly pear cladodes were cut into small pieces using a sharp knife and let to dry at room temperature. Pieces of prickly pear cladodes were turned daily basis to prevent them from moulding. During the ensiling stage, additives (molasses and *Lactobacillus plantarum* inoculant) were added to increase the nutrients in the cladodes and then mixed according to the treatments. After cutting the cladodes into 20mm square pieces, it was divided into four equal portions for each of the two varieties under irrigation and dry land for application of the treatments: 0% molasses (T1), 8% molasses (T2), 16% molasses (T3) and 24% molasses (T4) on DM basis. Approximately, 1 kilogram of each mixture from every treatment was ensiled into plastic film bags (micro silos), tightly sealed with a vacuum sealer and stored at room temperature for four weeks. Immediately after opening the silos, the silage pH was measured using the silage extracts and a fresh silage sample was collected and kept at -20 ^{0}C pending laboratory analysis. A silage extract was obtained after mixing 20 g of fresh silage with 180 mL of distilled water for 30 s in a mixer. The extract was filtered, using two filter papers and the filtrate was kept to be used for lactic acid and water-soluble carbohydrates.

3.2 Chemical analysis: The silage dry matter was determined after being oven-dried for 72 h at 60 ^{0}C. The dried samples were ground to pass through a 1 mm screen. The samples were analyzed for ash (AOAC 1990; method no 924.05) and crude protein (CP) (AOAC 1990; method no 984.13. The acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined using method of the ANKOM 200 Fibre Analyser (ANKOM Technology Corporation, Fairport, NY, USA), described by Van Soest *et al.* (1991). Ether extract (EE) was determined using ANKOM XT10, Extractor 120V (ANKOM Technology Corporation, Fairport, NY, USA).

3.3 Fermentation characteristics: Determination of silage pH was done for both the fresh and ensiled samples. The measurement of pH was done using a digital pH meter. Water-soluble carbohydrate was determined using the phenol-sulphuric acid method by Dubois *et al.*, (1956) and lactic acid by the method of Barker and Summerson (1941).

3.4 Experimental design: The design for the study was 4 x 2 factorial arrangements in completely randomized design.

3.5 Statistical analyses: Nutritive value and fermentation characteristics data were analyzed using the general linear models (GLM) procedure of SAS (2008) for a 4 (molasses) × 2 (varieties) × 2 (growing ender) factorial treatment arrangement. The linear model employed was:

$$Y_{ijkl} = \mu + M_i + V_j + G_k + (D \times V)_{ij} + (D \times G)_{ik} + (V \times G)_{jk} + (D \times V \times G)_{ijk} + E_{ijkl},$$

Where Y_{ijkl} = observation of the dependent variable *ijkl*, μ = fixed effect of population mean for the variable,
M_i = effect of molasses (i = 4; 0%, 8%, 16% and 24%),
V_j = effect of varieties (j = 2; *Roedtan* and *Van as*),
G_k = effect of growing condition (k = 2; irrigated and dryland),
$(M \times V)_{ij}$ = effect of interaction between molasses level *i* and variety at level *j*,
$(M \times G)_{ik}$ = effect of interaction between molasses *i* and growing conditions *k*,
$(V \times G)_{jk}$ = effect of interaction between variety *j* and growing condition *k*,
$(M \times V \times G)_{ijk}$ = effect of interaction between molasses level *i*, variety *j* and growing condition *k*, and E_{ijk} = random error associated with observation *ijkl*. For all statistical tests, significance was declared at P ≤ 0.05.

4 RESULTS

4.1 Nutritive value: The 3-way interaction term 'molasses × variety × growing condition' did (P<0.05) affect all nutritive value parameters analyzed in the present study (Table 1). There was a significant (P<0.05) Variety × growing condition for dry matter (P<0.05), acid and neutral detergent fibre (P<0.05), crude protein and ether extract (P<0.05) (Table 1). Molasses and variety significantly affected all nutritive value parameters. Growing condition did (P>0.05) not affect ether extract (Table 1).

Table 1: Statistical significance (P values) of the effects of the main factors and their interactions on dry matter, crude protein, acid detergent fibre, neutral detergent fibre and ether extract

	Effect of treatment			Interaction			
Parameter	**Molasses**	**Variety**	**Growing condition**	**M x V**	**M x G**	**V x G**	**M x V x G**
Dry matter	*	*	*	*	*	*	*
Crude protein	*	*	*	*	*	*	*
Acid detergent fibre	*	*	*	*	*	*	*
Neutral detergent fibre	*	*	*	*	*	*	*
Ether extract	*	*	NS	*	*	*	*

* P<0.05; NS Non significance

There were significant varietal, growing condition and molasses inclusion level interaction effects (P<0.05) on dry matter, crude protein, acid detergent fibre, neutral detergent fibre and fat contents (Table 2). Among the interactions that were investigated in this study, the ensiled *Van as* variety grown under irrigation without molasses inclusion had the highest dry matter content as compared to any other variety tested under the same or various growing conditions with the same or different molasses inclusion levels (Table 2). According to Table 2 results, the average CP composition of different *Opuntia* silages evaluated in this investigation was 6.5% on DM basis and varied significantly (P<0.05) from 5.3% (*Roedtan*-irrigation with no molasses inclusion) to 7.3% (*Roedtan*-dry land with 24% molasses inclusion level).

Table 2 : Nutritive value of *Opuntia* cladode silage under dry land and irrigated growth.

		Dry land		Irrigation	
Parameters	**Molasses**	***Roedtan***	***Van as***	***Roedtan***	***Van as***
Dry matter (%)	0	91.3^{aB}	91.7^{aA}	90.2^{aB}	92.5^{aA}
	8	87.7^{cA}	85.7^{cB}	88.1^{cA}	84.4^{dB}
	16	88.6^{bA}	85.5^{dB}	87.5^{dA}	85.7^{cB}
	24	87.1^{dA}	85.9^{bB}	89.8^{bA}	87.9^{bB}
Crude protein (% DM)	0	6.86^{cA}	6.06^{Cb}	5.25^{dB}	6.05^{dA}
	8	6.64^{dB}	6.66^{cB}	5.64^{cB}	6.57^{cA}
	16	6.89^{bB}	6.92^{aB}	5.81^{bB}	7.15^{aA}
	24	7.35^{aA}	6.94^{aA}	6.12^{aB}	7.22^{aA}
Acid detergent fibre	0	21.3^{aA}	19.8^{aB}	10.9^{aA}	17.4^{aB}
	8	10.3^{cA}	9.58^{cB}	8.21^{bB}	10.2^{bA}
	16	9.21^{bA}	7.55^{cB}	8.24^{bA}	7.14^{dB}
	24	9.67^{cA}	7.31^{dB}	7.08^{cB}	8.52^{cA}
Neutral detergent fibre	0	25.9^{a}	24.8^{a}	18.1^{a}	21.6^{a}
	8	13.4^{b}	14.8^{b}	11.3^{c}	13.0^{b}
	16	11.7^{d}	10.5^{d}	9.07^{d}	9.80^{d}
	24	12.0^{c}	10.7^{c}	11.6^{b}	12.0^{c}
Ether extract (% DM)	0	6.64^{dB}	6.06^{dB}	5.25^{dB}	6.05^{dA}
	8	7.35^{aA}	6.94^{aA}	6.12^{aB}	7.22^{aA}
	16	6.89^{bB}	6.92^{Ba}	5.81^{bB}	7.15^{aA}
	24	6.86^{cA}	6.66^{cB}	5.64^{cB}	6.57^{cA}

^{abcd}Means within the same column with different lowercase superscripts differ (P<0.05);
^{AB}Means within the same row with different uppercase superscripts differ (P<0.05);

The results shown in Table 2 indicate that the ADF contents of the various *Opuntia* silages used in this investigation varied significantly (P< 0.05) from 7.08% (*Roedtan*-irrigation with 24% molasses inclusion level) to 21.3% (*Roedtan*-dry land with 0% molasses inclusion level) with an average of 10.8% on DM basis. The NDF content varied by approximately 185% between the silage with the highest (*Roedtan*-dry land with 0% molasses inclusion level) and the silage with the lowest (*Roedtan*-irrigation with 24%) molasses inclusion level (Table 2). The fat content varied by approximately 185.2% between silage with the highest (*Van as*-irrigated with 8% molasses inclusion level) and the silage with the lowest (*Roedtan*-irrigation with 16% molasses inclusion level). The fat contents of various silages evaluated in this investigation varied significantly (P<0.05) (Table 2).There were significant varietal, growing condition and molasses inclusion level interaction effects (P< 0.05) on pH, water-soluble carbohydrates and lactic acid (Table 3). Within the interactions investigated in this study, the ensiled *Roedtan* variety grown under irrigation without molasses inclusion had the lowest pH content as compared to *Van as* variety tested under the dry land condition with the 24% molasses inclusion level. The results shown in Table 3 indicated that the pH level of the silages increased (P<0.05) with increase in molasses inclusion level.

Table 3: pH, water-soluble carbohydrates and lactic acids content (g/kg DM) of Prickly pear cladode silage under dry land and irrigation at different levels of molasses inclusion.

		Dry land		Irrigation	
Parameters	**Molasses**	***Roedtan***	***Van as***	***Roedtan***	***Van as***
pH	0	3.08^{dB}	3.23^{bA}	3.68^{bB}	3.81^{bA}
	8	3.37^{cA}	3.11^{cB}	3.24^{dB}	3.52^{cA}
	16	3.57^{bA}	3.15^{bcB}	3.57^{cB}	3.75^{bA}
	24	3.94^{aA}	3.83^{aB}	3.87^{aB}	3.98^{aA}
Water soluble carbohydrates g/kg DM	0	24.5^{dB}	88.5^{dA}	14.0^{dB}	15.5^{dA}
	8	41.0^{cB}	90.0^{cA}	29.0^{cB}	41.5^{cA}
	16	57.0^{bB}	91.0^{bA}	31.0^{bB}	51.0^{bA}
	24	91.5^{aB}	147.0^{aA}	38.0^{aB}	60.0^{aA}
Lactic acid g/kg DM	0	56.5^{dA}	46.5^{cB}	68.5^{dA}	65.5^{dA}
	8	65.5^{cA}	49.5^{cB}	75.5^{cA}	74.5^{cA}
	16	74.5^{bA}	68.5^{bB}	90.5^{bA}	82.5^{bB}
	24	87.5^{aB}	98.5^{aA}	100.5^{aA}	99.5^{aA}

[a,b,c,d]Means within the same column with different lowercase superscripts differ (P<0.05)
[A,B]Means within the same row with different uppercase superscripts vary (P<0.05)

5 DISCUSSION

5.1 Nutritive value: The inclusion of molasses (at 8%, 16% and 24%) into different types of prickly pear cladodes resulted in lower dry matter (84.4% to 89.8%) content as compared to without inclusion (0%) (90.2 – 92.5%) of molasses. The reason could be due to high moisture content produced during fermentation (Schroeder 2004; Kunkle *et al.*, 2006). The reason for the lower dry matter content could also be the method used for determination of the dry matter content of different prickly pear cladodes silages. *Opuntia* cladode silage cannot be determined accurately by oven drying, as some of the fermentation products are lost such as volatile fatty acids (Dewar and McDonald 1961). If the dry matter content is determined by oven drying, it will underestimate the value between 0.2% and 0.1% (De Brouwer *et al.*, 1991). Addition of molasses into prickly pear cladodes in this study resulted in lower acid detergent fibre, neutral detergent fibre and ether extract contents. The acid detergent fibre values are important because they relate to the ability of an animal to digest the forage. The neutral detergent fibre values are important because they reflect the amount of forage the animal can consume. The results from the study regarding the fibre content were within the ranges reported by Snyman *et al.*, (1990) and Mciteka (2008). During ensilage, hydrolysis of hemicellulose could occur and types of LAB can ferment pentoses to lactic acid and acetic acid (McDonald *et al.*, 2002). Because of this fermentation, the prickly pear cladode silage in this study resulted into lower neutral detergent fibre. The lower contents of both acid and neutral detergent fibre could result into increases in digestibility of the silage and dry matter intake as there is a negative correlation to both ADF and NDF with digestibility and intake. Different types of prickly pear cladode silages had average ADF and NDF contents of 10.78% and 14.39% that are higher 8.07% and 6.48% found by Curek and Ozen (2001). There are other factors, which could be responsible for this difference such as climate, age of cladodes and varieties. Statistically, the neutral detergent fibre content showed significant differences between different inclusion levels of molasses. Addition of increasing levels of molasses had a significant effect on reducing the neutral detergent percentages, which is in agreement with the findings of Shahsavan (2009). Addition of 16 % molasses causes a significant decrease on neutral detergent fibre percentage. Alikhali *et al.* (2005) concluded that molasses alone has a significant effect on reducing both acid and neutral detergent fibre content. The results were in agreement of the findings of the study in which addition of molasses resulted in a reduction of acid detergent fibre content. The higher inclusion levels of molasses for variety *Roedtan* in both dry and irrigated lands resulted in a higher crude protein content. This finding is in agreement with the findings of other researchers who reported that adding urea and molasses increases the amount of crude protein (Mehtap *et al.*, 2007; Alikhani *et al.*, 2005). The fat content of the feed is the unstable part (Van der Merwe and Smith, 1991). With inclusion of molasses the fat content of different prickly pear cladode silages were higher than in those without inclusion of molasses. Alikhani *et al* (2005) reported similar findings that addition of more molasses reduces the fat content in the silo, which could be contributed to the low amounts of fat in molasses itself, and its diluting effect. However, the EE content of different prickly pear cladode silages for both dry and irrigated lands were low to cause problems during ensiling. The ether extract contents of different prickly pear cladode silages were in agreement with the 2.33% reported by Curek and Ozen (2001).

5.2 Fermentation quality: Silage with a high pH values falling within the range of 5.0 to 7.0 results into poorly preserved silage (McDonald *et al.*, 2002). Clostridia microbes prefer growing at pH values of 7.0 to 7.4 (McDonald *et al.*, 2002). The pH values of both varieties with 24% molasses inclusion and 16% and 0% inclusion level of molasses under dry land were within the recommended pH values (above 3.5 but less than 5.0) for a good quality and well preserved silage. The water-soluble carbohydrates content in the 24% inclusion level of molasses were higher than in the 0%, 8% and 16% inclusion levels. The soluble carbohydrates content in *Roedtan* and *Van as* varieties were within the range reported by Grobler *et al.* (2010). Molasses increases the water soluble carbohydrates (Shahsavan, 2009). Lactic acid content from different cladode silages in this study varied from 46.5 to 100 g/kg DM, and this range was higher than the 25.9 and 32 g/kg DM from young cladode silages and old cladode silages reported by Curek and Ozen (2001).

6 CONCLUSION

There were significant varietal, molasses inclusion level and growing condition effects on fermentation characteristics of prickly pear cladode silage. The results show that there was a change of the amount of dry matter (from 92.5 % to 84.4 %) and water soluble carbohydrates (from 14.4 g/kg DM to 147.0 g/kg DM). If significantly decreased NDF (from 25.9 % to 9.07 %) changed ADF (from 21.3 % to 7.08 %), changed pH (from 3.08 to 3.98). Results clearly indicate that molasses could be effectively used in Prickly pear cladodes.

7 RECOMMENDATION

The inclusion of dry hay at ensiling is needed for further study as the moisture content increased after addition of molasses. Animal performance testing to measure the digestibility and palatability of these two promising varieties is recommended to evaluate their effectiveness under practical feeding conditions. There is a need to evaluate volatile fatty acids from ensiled prickly pears.

8 REFERENCES

Alikhali, M., A. Asadi, Q. Qorbani, and N. Sadeqi (2005). The effect of molasses, urea and bacteria inoculation on the chemical component and dry matter degradability of sunflower silos. Agriculture Science and Techniques and Natural Resources Magazine, Issue 3, p11.

AOAC, 1990. Official methods of analysis. Vol. 1. 15th ed. AOAC, Arlington VA.

Barker, S. B. and W. H. Summerson (1941). The colorimetric determination of lactic acid in

biological material. J. Biol Chem. 138: 535-554.

Curek, M., and N. Ozen (2001). Feed value of Cactus and cactus silage. Turk. J. Vet Anim Sci. 28: 633-639.

De Brouwer, C.H.M., H. J. Van der Merwe and L. D. Snyman (1991). A laboratory study of the composition and fermentation of various crop silages. S. African J. Anim Sci. 1:21.

De Waal, H.O., D.C. Zeeman and W.J Combrinc (2006). Wet faeces produced by sheep fed dried spineless cactus pear cladodes in balanced diets. S. African J. Anim Sci. 36:10-13.

De Waal, H.O., H. Fouche and J. Potgieter (2007). Turksvye as voerbron vir herkouers. Opuntia – the Cinderella feed source? Bonsmara SA 2007, pp. 48-53.

Dewar, W.A., P. McDonald (1961). Determination of dry matter in silage by distillation with toluent J Sci Food and Agric. 12: 790-795.

Dubois, M., K.A. Glies, J.K. Hamilton J. K, P.A. Rebes, and F. Smith (1956). Colorimetric method for determination of sugars and related substances. Anal Chem. 28:35-356.

Grobler, S.M., K. Dearlove, and M.M. Scholtz. (2010). Palatability of *Opuntia* varieties available in South Africa for dryland sheep. S. African J. Anim Sci. 40 (1):495-498.

Kunkle, W.E., and C.G. Chambliss (2002). Florida forage handbook. Inst. Of food and Agric. Sci. University of Florida, Gainesville.

Kunkle, W.E., C.G. Chambliss, A.T. Adesogan, and M.B. Adjei. (2006). Silage harvesting, storage and feeding. Florida forage handbook. Univ of Florida.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh, and C.A. Morgan (2002). Animal Nutrition. 6th Ed. Pearson Prentice Hall, England.

Mciteka, H. (2008). Fermentation Characteristics and Nutritional Value of *Opuntia ficus-indica Var. Fusicaulis* Cladode Silage, MSc Thesis. University of the Free State, Bloemfontein, RSA.

Mehtap, G., D. Murat, and C. Sibel (2007). Effect of urea plus molasses supplementation to sorghum silage on the quality, in vitro organic matter digestibility and metabolic energy contents. J Biol Sci 7(2): 401-404.

Nefzaoui, A., and H. Ben Salem (1996). Nutritive value of diets based on spineless cactus (*Opuntia ficus-indica var. inermis*) and Atriplex (*Atriplex nummularia*). In: Native and Exotic Fodder Shrubs in Arid and Semi- Arid Zones, Regional Training Workshop, Tunisia.

Schoeder, J.W. (2004). Silage fermentation and preservation. NDSU. www.ag.ndsu.ed.

Shahsavan, A., (2009). A study on the effects of enzymes and molasses on the nutritional value of reed silage in Sistan silos. MA thesis of Animal Feed. Agriculture Faculty of Zabol University, p 100.

Snyman, L.D., H.J. Van der Merwe, and H.J. Van Schalkwyk (1990). Effect of formalin preservation on the fermentation characteristics, chemical composition and protein properties of maize silage. S Afr J Anim Sci. 20:3

Van Man, N. and H. Wiktorsson (2006). The effect of molasses o quality, feed intake and digestibility by heifers of silage made from cassava tops. Dept. of Anim. Nutr., UAF, Sweden.

Van der Merwe, F.J., and W.A. Smith (1991). Dierevoeding, Ani. Sci. (Pty) Ltd.

Zeeman, D.C. (2005). Evaluation of sun-dried *Opuntia ficus indica var. Algerian* cladodes in sheep diets. Thesis. Dept. of Anim., Wildlife and Grassland Sciences, Univ. of Free State.