Chemical composition and *in vitro* dry matter degradability of mistletoe (*Viscum verrucosum* (Harv.)) on *Vachellia nilotica* (L.) in North West Province of South Africa

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There is a dearth of information on the nutritive value of mistletoe (*Viscum verrucosum* (Harv)) as a potential feed source for ruminant animals in different growth environments. This study was conducted to determine the chemical composition and *in vitro* dry matter degradability (DMD) of mistletoe growing on *Vachellia nilotica* L., a common browse tree, at three growth sites (Lokaleng, Tsetse and Ramatlabama) in the North West Province of South Africa. The study found no site effects on neutral detergent fibre, acid detergent fibre, acid detergent lignin, organic matter and crude protein contents of mistletoe harvested from the three sites. Mistletoe plants harvested from Tsetse had a higher dry matter content (956.1 g/kg) than those from Lokaleng (927.3 g/kg) and Ramatlabama (855.3 g/kg). Mistletoe from Lokaleng had a higher potassium content (112.7 g/kg) than those from Ramatlabama (83.5 g/kg). Mistletoe from Tsetse had lower magnesium and sodium (5.31 g/kg and 0.89 g/kg, respectively) contents than those from Lokaleng (8.07 g/kg and 1.43 g/kg, respectively). Mistletoe from Ramatlabama had the highest iodine content (3.82 g/kg), followed by those from Lokaleng (0.510 g/kg) and the lowest levels were found in those from Tsetse (0.228 g/kg). The *in vitro* DMD at 2, 4, 8, 12, 24, 36, 48 and 72 hours of mistletoe plants from the three sites were the same ($p > 0.05$). Therefore, growth location influences chemical composition but not *in vitro* DMD of mistletoe plants, and site-specific recommendations for mineral supplementation can be made to resource-poor farmers as required.

Keywords: *Viscum verrucosum* (Harv), dry matter degradation, ruminants

Communal rangelands are the major sources of feeds for animals in the tropical parts of Southern Africa and grasses are the main forage sources for ruminants in arid and semi-arid areas (Ravhuhali et al. 2019). However, in the prolonged periods of the dry seasons, these areas experience seasonal fluctuations in forage quality and quantity characterized by high lignin and fibre contents, with protein being the major limiting nutrient (Mnisi and Mlambo 2017). In addition to the prolonged seasonal dry period, the development of human settlements and other infrastructure and erratic rainfall patterns have resulted in a shortage of forages with consequential negative effect on ruminant production and animal health, requiring interventions to evaluate alternatives that will optimize ruminant nutrition for pasture-based feeding systems. Browse trees are sources of protein and other nutrients in winter months and provide foliage for ruminants (Ndagurwa and Dube 2013). Hemi-parasitic plants such as mistletoe (*Viscum verrucosum*), an evergreen plant reported to have nutritional, therapeutic and anthelmintic properties, are known to grow on branches or trunks of various browse trees and can alternatively be harvested and fed to ruminants by resource-poor farmers (Madibela et al. 2000; Umucalılar et al. 2007).

According to Dean et al. (1994) as well as Pennings and Callaway (2002), mistletoe selectively parasitizes host species that are high in nitrogen content, which is not surprising because its common host, *Vachellia nilotica*, has high nitrogen content (Mnisi and Mlambo 2017). Nonetheless, there are limited studies on the nutritive value of mistletoe growing on various host plants and, in general, it has received lesser attention precisely

because, as aerial hemi-parasitic plant (Yoder 1999; Ntoukakis and Gimenze-Ibenze 2016), it causes little damage to commercial plants compared to root parasites. This study was, therefore, designed to determine the proximate constituents, mineral composition and *in vitro* DMD of mistletoe growing on *V. nilotica* trees at three growth sites.

Materials and methods

Description of harvesting site and sampling procedure

Mistletoe plants were harvested from three locations, Lokaleng (26º69'05'' S, 27º09'32'' E), Tsetse (25º73'14'' S, 25º67'02'' E) and Ramatlabama (25º38'57'' S, 25º34'29'' E) in the semi-arid region of North West Province, South Africa. These sites are used commonly by communal farmers as grazing lands for their livestock and they have open access to different kinds of domestic herbivores. Thus, the selection was based on the availability of *V. nilotica* as a host tree for mistletoe, as well as similar soil types and rainfall patterns. All the sites receive an average annual rainfall of 450 mm with ambient temperatures ranging between 3°C and 39°C. The vegetation type around these sites resembles that of a savannah biome, with a soil type varying from sandy loam to clay loam.

Fresh mistletoe plants growing on *V. nilotica* were randomly selected in a 100 m transect from the three sites. *Vachellia nilotica* was chosen as the host tree in these villages to allow proper replication since it is the most common host tree of mistletoe in the selected areas. From each site, ten samples (replicates) of mistletoe hosted by individual *V. nilotica* trees were harvested by hand, making a total of 30 samples, and placed in brown paper bags. The samples were oven-dried at 60ºC immediately after harvest until they reached constant weight and thereafter milled (Polymix PX-MFC 90 D) to pass through a 2 mm sieve and kept in labelled sample bottles pending nutritional assessments.

Chemical analyses

Milled mistletoe samples were analysed for dry matter, organic matter, neutral detergent fibre, acid detergent fibre, nitrogen and minerals. For dry matter determination, approximately 1 g of mistletoe sample was placed into pre-weighed crucibles and oven-dried at 105ºC for 24 h. Loss in weight was measured as moisture content and DM was calculated as the difference between initial sample weight and moisture weight (AOAC 2005; method no. 930.15). Organic matter content was determined by ashing the dried samples in a furnace set at 600ºC for 6 hours and OM content was calculated as the difference between DM and ash weight (AOAC 2005; method no. 924.05). Nitrogen content was determined following the standard macro-Kjedahl method (AOAC 2005, method no. 984.13) and was converted to crude protein by multiplying percentage of N content by 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by fluxing 0.45 – 0.5 g of mistletoe samples with neutral detergent and acid detergent solutions for an hour and one hour 15 minutes, respectively, using an ANKOM 2000 Fibre Analyser (ANKOM Technology, New York) according to Van Soest et al. (1991). A heat stable α-amylase enzyme was used for NDF analysis. Acid detergent lignin (ADL) content was determined by dissolving ADF residues in ANKOM F57 bags with 72% sulphuric acid. Mineral content was determined by using the dry ashing macro- and micro-minerals method following the guidelines provided by Agri-Laboratory Association of Southern Africa (AgriLASA 1998).

In vitro dry matter degradability

The *in vitro* DMD of mistletoe was determined using the ANKOM Daisy^{II} incubator according to ANKOM technology method number three for *in vitro* true digestibility. A cannulated Bonsmara donor cow, weighing approximately 550 kg, was used to collect rumen fluid in the morning prior to feeding. This animal was

cared for according to guidelines of the institutional and Federation of Animal Science Societies (FASS 2010) for animals in research and teaching. Ethical approval was obtained from North-West University Animal Research Ethics Committee: approval number NWU-00126-13-A9). The rumen fluid was collected into pre-warmed thermos flasks at Molelwane Research Farm (25.8560°S, 25.6403°E), where it was blended and strained through two layers of warm muslin cloth. Stained rumen fluid was held at 39ºC under a stream of carbon dioxide gas. Four Daisy^{II} jars containing 1600 mL of ANKOM buffer each and mistletoe samples sealed in ANKOM F57 filter bags were inoculated by adding 400 mL of rumen fluid to each digestion jar. The jars were regularly purged with CO_2 before being covered and placed in the incubation chamber. The ANKOM F57 filter bags were withdrawn at 2, 4, 8, 12, 24, 36, 48 and 72 hours after incubation. Withdrawn bags were washed with cold water for 15 minutes using ANKOM Fibre Analyser. Time 0-hour samples were not incubated but were washed the same way as the incubated samples, and thereafter oven-dried at 105ºC for 12 hours to determine *in vitro* ruminal DMD. Dry matter degradability parameters: *a*, *b*, and *c* were estimated using non-linear procedures of SAS (2010). Potential degradability (*PD*) was determined by the equation:

$PD = a + b$, whereas the effective degradability (*ED*) was calculated by the equation:

$ED = \frac{a+bc}{K+c}$, where *a* = rapidly degradable DM fraction (g/kg DM), *b* = slowly degradable DM fraction (g/kg DM), *c* = rate of degradation of the slowly degradable DM fraction '*b*' (%/hour), and K = rumen outflow rate (assumed to be 2%/h).

Statistical analyses

The data on proximate constituents, mineral composition and *in vitro* DMD of mistletoe were analyzed using the general linear model (GLM) procedure of SAS (2010), in a completely randomized design. The following linear statistical model was employed:

$$Y_{ij} = \mu + S_i + E_{ij}$$

Where *Yij* = dependent variable, μ = overall mean, S = ith effect of harvesting site, and E_{ij} = residual error, assumed to be independently distributed. For all statistical tests, significance was declared at $p < 0.05$. Least squares means (LSMEANS) were separated using the probability of difference option in the LSMEANS statement of SAS.

Results

The chemical composition of mistletoe plant is shown in Table 1. There were no significant differences in OM, CP, NDF, ADF and ADL contents of mistletoe plants from the three sites. Plants harvested from Tsetse had a higher DM content (956.1 g/kg) than those harvested from Lokaleng (927.3 g/kg) and Ramatlabama (855.3 g/kg), but were not significantly different ($p > 0.05$).

Table 1: Proximate constituents (g/kg DM, unless otherwise stated) of mistletoe *(Viscum verrucosum)* growing on *Vachellia nilotica* harvested from three sites

	Proximate constituents					
Sites	DM (g/kg)	OM	CP	NDF	ADF	ADL
Lokaleng	927.3[a]	821.6	123.4	488.5	316.2	210.4
Tsetse	956.1[b]	859.8	110.3	518.3	340.1	198.2
Ramatlabama	855.3[a]	823.7	130.0	563.50	367.8	247.2
SEM	23.89	13.43	8.63	48.42	32.12	21.22

[a,b]In a column, means with common superscripts do not differ ($p > 0.05$).

[1]Proximate constituents: DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin.

The macro-mineral content of mistletoe harvested from three sites is shown in Table 2. In all three sites, there were no site differences ($p > 0.05$) in mistletoe plants in terms of calcium, phosphorus and chloride content. Mistletoe plants harvested from Lokaleng had higher potassium content (112.7 g/kg) than those harvested from Ramatlabama (83.5 g/kg). However, mistletoe plants harvested from Tsetse had comparable ($p < 0.05$) potassium content to those harvested from both Lokaleng and Ramatlama. Mistletoe plants harvested from Lokaleng had higher magnesium and sodium content (8.07 g/kg and 1.43 g/kg, respectively) than those harvested from Tsetse (5.31 g/kg and 0.89 g/kg, respectively). There were no significant differences in mistletoe plants harvested from Ramatlabama compared to those harvested from both Tsetse and Lokaleng in terms of magnesium and sodium content.

The micro-mineral concentration of mistletoe plants growing on *V. nilotica* harvested from three sites is presented in Table 3. There were no differences ($p > 0.05$) in manganese, cobalt, copper, iron and zinc contents of mistletoe harvested from the three sites. Mistletoe harvested from Ramatlabama had the highest iodine content (3.82 g/kg), followed by plants harvested from Lokaleng (0.510 g/kg) and the lowest levels were plants harvested from Tsetse (0.228 g/kg).

The *in vitro* DMD of mistletoe plants harvested from three sites is shown in Table 4. There were no significant differences in mistletoe plants in terms of DMD at 2, 4, 8, 12, 24, 36, 48 and 72 hours.

The *in vitro* DMD parameters of mistletoe *(V. verrucosum)* harvested from three sites are given in Table 5. There were no site effects ($p > 0.05$) on *a*, *b*, *c*, *PD* and *ED* of mistletoe plants.

Table 2: Macro-mineral concentration (g/kg DM) of mistletoe *(Viscum verrucosum)* growing on *Vachellia nilotica* harvested from three growth sites

	Macro-minerals (g/kg DM)					
Sites	Calcium	Phosphorus	Potassium	Magnesium	Sodium	Chloride
Lokaleng	79.46	1.73	112.7^{b}	8.07^{b}	1.43^{b}	76.75
Tsetse	69.92	1.58	96.0^{ab}	5.31^{a}	0.89^{a}	72.33
Ramatlabama	79.30	1.62	83.5^{a}	6.29^{ab}	1.41^{ab}	59.91
SEM	5.97	0.21	5.73	0.55	0.14	9.96

^{a,b}In a column, means with common superscripts do not differ ($p > 0.05$).

Table 3: Micro-mineral content (g/kg DM) of mistletoe *(Viscum verrucosum)* growing on *Vachellia nilotica* harvested from three growth sites

	Micro-minerals (g/kg DM)					
Sites	Iodine	Manganese	Cobalt	Copper	Iron	Zinc
Lokaleng	0.510^{b}	0.049	0.001	0.030	0.423	0.013
Tsetse	0.228^{a}	0.049	0.001	0.036	0.420	0.018
Ramatlabama	3.820^{c}	0.050	0.002	0.036	0.463	0.017
SEM	0.039	0.0074	0.0003	0.003	0.0533	0.0028

^{a,b,c}In a column, means with common superscripts do not differ ($p > 0.05$).

Table 4: *In vitro* dry matter degradability (g/kg) of mistletoe *(Viscum verrucosum)* plants harvested from three growth sites

	Hours of incubation							
Sites	2	4	8	12	24	36	48	72
Lokaleng	266.5	281.6	328.1	367.5	425.5	474.4	446.7	516.9
Tsetse	272.8	309.5	356.6	362.7	424.7	462.8	476.0	511.6
Ramatlabama	235.5	261.9	321.2	320.6	421.2	449.0	457.2	510.6
SEM	15.84	20.29	22.52	26.16	23.05	17.85	22.48	33.74

Table 5: *In vitro* dry matter degradability parameters (g/kg DM) of mistletoe *(Viscum verrucosum)* harvested from three growth sites

	DM degradability parameters				
Site	*a*	*b*	*c*	*PD*	*ED*
Lokaleng	206.7	222.8	0.061	429.5	373.2
Ramatlabama	218.9	228.4	0.051	447.3	374.9
Tsetse	197.5	237.1	0.064	434.6	376.4
SEM	15.7	19.3	0.01	22.3	15.9

DM degradability parameters: *a* = rapidly degradable DM fraction (g/kg DM), *b* = slowly degradable DM fraction (g/kg DM); *c* = rate of degradation of the slowly degradable DM fraction '*b*' (%/hour); *PD* = potential degradability; *ED* = effective degradability.

Discussion

Ruminant animals found in tropical areas have low productivity due to low availability of nutrient-rich feedstuffs. These animals usually feed on poor quality pastures as a result of prolonged dry periods. To meet their nutritional requirements, these animals resort to browse trees, such as the *Vachellia* species, which are reported to be potential protein and other nutrient sources (Smith et al. 2005; Mnisi and Mlambo 2017). In addition to browsing on these trees, goats have a tendency to consume mistletoe which parasitizes most browse trees. Results from this study indicate that mistletoe can be harvested and used as a potential protein (110.3 – 130 g/kg DM) source for ruminants during the dry season. However, for animals in high production stages, supplementation would still be required to meet the protein requirements as recommended by NRC (2001). There was a lack of significant differences in OM, CP, NDF, ADF and ADL on all three sites, which could be due to the close proximity and similar environmental conditions of the sites. The lack of variation in CP content was not in agreement with the findings of Madibela et al. (2000), who reported a significant site effect for this parameter. Another reason for the lack of site effects could be the ability of the mistletoe to photosynthesize and cater for its own nutrient requirements despite different growth environments.

The fibre components (NDF and ADF) as well as the lignin content were not influenced by growing site, which for the ADF was in agreement with the observations of Madibela et al. (2004) who found no significant difference in ADF content of mistletoe plant on

Vachellia tree harvested from different locations. Nonetheless, the ADF and ADL values reported in this study were higher than those reported by Madibela et al. (2004), which could be attributed to different stages of maturation and growth environments. However, the high fibre and lignin content were not surprising as, according to Pope et al. (2006), mistletoe is largely composed of stems which have a higher proportion of more recalcitrant vascular bundles (xylem and other sclerenchyma cells) than leaves.

Minerals such as potassium, magnesium, sodium and iodine were influenced by different growing sites, with mistletoe plants from Lokaleng having the highest concentration of potassium, magnesium and sodium whereas those from Ramatlabama had the highest concentration of iodine. The potassium (83.4 – 112.7 g/kg DM) and magnesium (5.31 – 8.07 g/kg DM) concentrations reported in this study were higher than the recommended levels reported by CSIRO (2007) for ruminant animals, suggesting that precautions should be taken into consideration before feeding mistletoe in order to avoid toxicities. The high levels of magnesium could be attributed to the fact that this element is essential for photosynthesis as a building block of chlorophyll, which makes the plant evergreen. The no-site effect on phosphorus, chloride, cobalt, iron and zinc contents in mistletoe was supported by Madibela et al. (2000) who reported no significant differences in contents of these elements.

Species, plant parts and location are some of the interrelated factors influencing forage degradability and vary from one region to another. Rittner and Reed (1992) stated that browse plants in the tropical zones have higher fibre content than those in humid regions, which might, in turn, influence the feed value of certain species. However, in this study, mistletoe from different sites had the same *in vitro* DMD at all the withdrawal periods. There were also no site influences on *in vitro* DMD parameters (*a*, *b*, *c*, *PD* and *ED*), which can be explained by the work of Bruinenberg et al. (2004) and Keim et al. (2013), who reported that a lack of variation in the chemical composition, especially the fibre components, would most likely result in no differences on degradability values. Belachew et al. (2013) also reported that different tree species can have differences in degradation parameters; however, in this study, only *V. nilotica* was used as the host plant. Findings from this study will have wider applications, not only in semi-arid areas of the world, but also in other rangeland ecosystems where host–parasite interactions are commonplace and may influence the nutritive value of the plants.

Conclusion

This study showed that different growth environments affect the dry matter and some mineral content of mistletoe. It also revealed that mistletoe from various rangelands can serve as a potential protein source for ruminants in dry seasons. No-site differences were observed on *in vitro* DMD of mistletoe. Therefore in this geographical area, growth location influences chemical composition but not *in vitro* DMD of mistletoe plants and site-specific recommendations for mineral supplementation can be made to resource-poor farmers as required.

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