

Short communication: Effects of polyethylene glycol treatment on the chemical composition and *in vitro* dry matter degradability of *Searsia lancea* leaves

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Abstract

This study investigated the effects of incremental levels of polyethylene glycol (PEG) treatment on the chemical composition and *in vitro* dry matter degradability (IVDMD) of *Searsia lancea* leaves. Polyethylene glycol was sprayed onto *S. lancea* leaves at 0, 5, 10, 15, and 20 g/kg of dry matter (DM). Data were analysed using a one-way analysis of variance, while linear and quadratic responses were analysed using polynomial regression analysis. There were negative linear and quadratic effects on the crude protein (CP), neutral detergent fibre (NDF), total phenolic (TP), total tannin (TT), and condensed tannin (CT) concentrations in response to the incremental levels of PEG. Treatment with 20 g PEG/kg DM produced the lowest ether extract (EE), NDF, TP, TT, and CT concentrations. Magnesium showed a negative linear response to incremental levels of PEG, and treatment with 10 g PEG/kg DM produced the highest potassium, sodium, and sulphur concentrations. *In vitro* DM degradability at 36 hours showed a positive linear response to increasing levels of PEG, and 20 g PEG/kg DM resulted in the highest IVDMD. In conclusion, treating *S. lancea* leaves with incremental levels of PEG reduced the concentrations of CP, EE, NDF, TP, TT, and CT, while enhancing the IVDMD. Our results suggest that treatment with PEG at 20 g/kg may be most suitable for improving the nutritive value of *S. lancea*.

Keywords: browse species, nutritive value, ruminal fermentation, ruminants, tannins

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Ruminant production plays an important role in the economy and welfare of humans in southern Africa. Ruminants mainly rely on rangelands as their primary feed resource in this region; however, their productivity is often limited by the quality and quantity of feed available during the dry season (Ravhuhali *et al.*, 2022). Grasses available during the dry season usually contain high concentrations of fibre and low concentrations of essential nutrients such as protein, vitamins, minerals, and energy (Nyambali *et al.*, 2023). Researchers are therefore investigating other readily available feed resources to overcome such feed-related challenges. Browse species such as *Searsia lancea* have been identified as suitable feed resources for ruminants, especially during the dry season when the quality of natural pastures declines. *Searsia lancea* is an evergreen and drought-tolerant species (Gundidza *et al.*, 2008), and is abundantly distributed in the semi-arid rangelands of the North West Province of South Africa, especially

in the Ngaka Modiri Molema and Bojanala districts. Previous studies have reported that *S. lancea* is an excellent source of protein (10%–19%) and minerals (Mokoboki *et al.*, 2019; Matlabe *et al.*, 2022; Ravhuhali *et al.*, 2023), supporting its incorporation into ruminant diets. However, this species is also known to contain high concentrations of condensed tannins (CT) – with one study reporting a CT concentration of 124 g/kg dry matter (DM) – which may be harmful to animal performance (Hawu *et al.*, 2025). Tanniferous feeds are likely to reduce palatability, intake, rumen fermentation, and degradable protein, as tannins bind to proteins and other nutrients (Rojas Hernández *et al.*, 2015; Mahlake & Mnisi, 2020; Chuzaemi *et al.*, 2023). Furthermore, several studies have reported that tannin concentrations over 50 g/kg DM can be detrimental to ruminants, causing internal organ haemorrhages and abrasions, and even death (Mahlake & Mnisi, 2020; Kemboi *et al.*, 2023).

The detrimental effects of the tannins in *S. lancea* should therefore be ameliorated to optimise its nutritive value and enhance its potential as a feed resource for ruminants, particularly when tannin levels exceed 50 g/kg DM. One potential strategy is to treat *S. lancea* leaves with polyethylene glycol (PEG). Although PEG is a non-nutritive synthetic polymer, it can facilitate the release of proteins from tannin-protein complexes and thereby mitigate the detrimental effects of tannins on rumen microbial fermentation and animal performance (Rojas Hernández *et al.*, 2015; Kemboi *et al.*, 2023). However, the effects of treating *S. lancea* with incremental levels of PEG have not been tested. It is important to determine the level of PEG required to neutralise these tannins, as this will ensure optimal binding between PEG and tannins while maximising the neutralisation of tannins. This study sought to determine the effects of incremental levels of PEG on the chemical composition and *in vitro* dry matter degradability (IVDMD) of *S. lancea* leaves. We hypothesised that incremental levels of PEG would decrease the tannin concentration and influence the chemical composition and IVDMD of *S. lancea*.

The study was approved by the North-West University (NWU) Animal Production Sciences Research Ethics Committee (NWU-00813-22-A5). *Searsia lancea* leaves were harvested at the NWU experimental farm in the North West Province of South Africa in the winter of 2023. The area (25°47'27" S; 25°37'18" E) is located at an altitude of 1290 m, with an average annual rainfall of 450 mm and temperature range of 11–38 °C. Five trees, which served as replicas, were randomly selected and 5 kg of leaves were hand harvested from a height of 1.5 m from each tree. The leaves were shade dried for 14 days to prevent nutrient loss before processing. Subsamples (500 g) of each sample were collected for the five treatment levels.

Polyethylene glycol (PEG (4000)) was sourced from Sigma-Aldrich (Gauteng, South Africa). Four solutions were prepared by dissolving 2.5, 5, 7.5, and 10 g of PEG in 500 mL of distilled water. Each solution was then sprayed onto 500 g of *S. lancea* leaves, providing incremental levels of 5, 10, 15, and 20 g PEG/kg DM. The untreated *S. lancea* leaves (500 g) were sprayed with 500 mL of distilled water only. The treated and untreated *S. lancea* samples were left to dry in a shed to allow the PEG to react with the tannins for 48 hours, whereafter they were ground in a Wiley mill and sieved through a 0.5 mm sieve before being stored in sealed containers until analysis.

The DM, ash, ether extract (EE), and crude protein (CP) contents were determined following the methods described by the Association of Official Analytical Chemists (2012), using methods 973.18, 973.18, 920.39, and 976.06, respectively. The neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) concentrations were determined as described by Van Soest *et al.* (1991). The Folin-Ciocalteu method was used to determine the total phenolic (TP) and total tannin (TT) concentrations, which were expressed as the grams of tannic acid equivalent per kilogram DM (g TAE/kg DM) (Makkar, 2003). The butanol-HCL method was used to determine the CT concentration, which was expressed as the grams of leucocyanidin equivalent per kilogram DM (g LE/kg DM) (Porter *et al.*, 1985). The mineral content was determined following AgriLASA (1998) guidelines. The IVDMD was determined by incubating the samples in ANKOM F57 filter bags in ANKOM Daisy II incubator jars containing Bonsmara cow rumen fluid following ANKOM Technology (2005) guidelines. The rumen fluid was obtained from a Bonsmara cow fed a diet of lucerne and blue buffalo grass hays.

Data were analysed using the Statistical Analysis System (SAS Institute, 2010) general linear model procedure in a completely randomised design. Linear and quadratic responses were analysed using polynomial regression analysis (IBM Corp., 2020). Significance was declared at $P < 0.05$.

There were negative linear effects on the CP ($R^2 = 0.956$; $P = 0.004$), NDF ($R^2 = 0.954$; $P = 0.004$), TP ($R^2 = 0.953$; $P = 0.004$), TT ($R^2 = 0.963$; $P = 0.003$), and CT ($R^2 = 0.989$; $P = 0.001$) concentrations in response to incremental levels of PEG (Table 1). There were also negative quadratic effects on the CP ($R^2 = 0.960$; $P = 0.040$), NDF ($R^2 = 0.993$; $P = 0.007$), TP ($R^2 = 0.976$; $P = 0.024$), TT

($R^2 = 0.973$; $P = 0.027$), and CT ($R^2 = 0.998$; $P = 0.002$) concentrations. The treatment of *S. lancea* with 20 g PEG/kg DM produced the lowest ($P < 0.05$) EE, NDF, TP, TT, and CT concentrations.

Table 1 Chemical composition of *Searsia lancea* leaves after treatment with polyethylene glycol

	Polyethylene glycol (g/kg DM)					SE	P-value	
	0	5	10	15	20		Linear	Quadratic
DM (g/kg)	993.8 ^a	989.1 ^a	983.9 ^b	980.3 ^b	967.0 ^c	1.73	0.008	0.026
Ash (g/kg DM)	99.2	96.2	98.6	94.4	94.5	2.43	0.113	0.377
EE (g/kg DM)	35.5 ^a	33.9 ^{ab}	31.8 ^{bc}	30.6 ^c	28.9 ^d	0.87	0.001	0.004
CP (g/kg DM)	103.1 ^a	101.0 ^b	99.2 ^c	95.3 ^d	95.0 ^d	0.29	0.004	0.040
NDF (g/kg DM)	406.7 ^a	401.1 ^a	394.3 ^a	391.7 ^b	389.3 ^b	1.94	0.004	0.007
ADF (g/kg DM)	305.0	302.7	303.2	305.3	304.7	2.12	0.656	0.591
ADL (g/kg DM)	115.4	114.6	115.1	115.3	115.2	1.38	0.807	0.754
TP ^{TAE} (g/kg DM)	19.5 ^a	14.0 ^b	12.3 ^c	10.0 ^d	7.4 ^e	0.07	0.004	0.024
TT ^{TAE} (g/kg DM)	9.4 ^a	5.8 ^b	5.0 ^c	2.8 ^d	1.1 ^e	0.08	0.003	0.027
CT ^{LE} (g/kg DM)	122.4 ^a	116.6 ^b	111.5 ^c	104.9 ^d	96.3 ^e	1.37	0.001	0.002

^{a-e} Means in the same row with different superscripts differ ($P < 0.05$). DM: dry matter, EE: ether extract, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, TP: total phenolics, TT: total tannins, CT: condensed tannins, SE: standard error. ^{TAE} Expressed as grams tannic acid equivalent/kg DM. ^{LE} Expressed as grams leucocyanidin equivalent/kg DM.

The utilisation of *S. lancea* as ruminant feed is limited by the presence of antinutritional factors such as tannins and phenolics (Ravhuhali *et al.*, 2020), and in this study, *S. lancea* leaves were found to contain 122.4 g CT/kg DM. The CT concentration thus exceeded the 50 g/kg DM considered detrimental in ruminant forages, as it can reduce feed palatability, impair DM degradability, and consequently depress animal performance (Hawu *et al.*, 2022). Polyethylene glycol prevents the binding of tannins to proteins and other nutrients to form complexes (Xie *et al.*, 2021), and this study tested the potential of PEG to improve the nutritional value of *S. lancea*.

The treatment of *S. lancea* with PEG linearly and quadratically reduced the concentrations of tannins and phenolics, demonstrating the efficiency of PEG in managing these compounds. These results align with those of Dentinho *et al.* (2018), who reported lower TP, TT, and CT concentrations in the leaves and soft stems of *Cistus ladanifer* L. Furthermore, Matabane *et al.* (2022) reported a decrease in the TP concentration in *Camellia sinensis* in response to incremental levels of PEG.

In this study, the CP content ranged from 95 to 103.1 g/kg DM, which is above the minimum of 80 g/kg DM required for optimal rumen function (Orskov, 1982). Although PEG effectively prevents CTs from binding to proteins, and thus increases the availability of CP (Xie *et al.*, 2021), a decrease in the CP content following PEG application was found in this study. While this was unexpected, these findings concur with those of Dentinho *et al.* (2018) and Tshiambara *et al.* (2024), who observed a decrease in the CP content of the leaves and soft stems of *C. ladanifer* L. and the pods of *Prosopis juliflora* with the addition of PEG. Polyethylene glycol has been reported to have solvent properties (Hoffmann, 2022), which can solubilise proteins, making them more susceptible to degradation during the extraction process. Furthermore, it can possibly leach lipids from the leaves, thus reducing the EE content.

In contrast with the findings of Dentinho *et al.* (2018), incremental levels of PEG decreased the NDF content in this study, which is consistent with the findings of Matabane *et al.* (2022). Yisehak *et al.* (2014) suggested that PEG disrupts tannin-NDF complexes, making NDF more susceptible to degradation by microbial enzymes. The decrease in the NDF content is crucial for ruminant productivity, as it can result in a higher feed intake.

Magnesium ($R^2 = 893$; $P = 0.015$) showed a negative linear response to incremental levels of PEG (Table 2): $y = 2.080(\pm 0.024) - 0.010(\pm 0.002)x$. Treatment of *S. lancea* with 10 g PEG/kg DM resulted in the highest ($P < 0.05$) potassium, sodium, and sulphur concentrations. Minerals are important for supporting balanced growth, enzyme activity, and immune functions, which, in turn, impact the health status and productive and reproductive performance of ruminants (Byrne & Murphy, 2022). Tannins

associate with minerals to form complexes (Bhatta *et al.*, 2002), and the ability of PEG to disrupt these complexes may have exposed magnesium to leaching, leading to the observed decrease in the magnesium content. This could also allow for the absorption of magnesium from the leaves by ruminants. The mineral concentrations obtained in this study are similar to the findings of Ravhuhali *et al.* (2023) for the same species. These values meet the thresholds for the minimum requirements for potassium (5 g/kg DM), calcium (1.8 g/kg DM), magnesium (1 g/kg DM), copper (5 mg/kg DM), and manganese (20 mg/kg DM) for the maintenance of livestock (Meissner, 2000). The concentrations of phosphorus, sodium, and zinc did not meet the minimum requirements of 1.6, 0.4, and 0.02 g/kg DM, respectively, and this may limit livestock productivity if the concentrations of these minerals are not corrected or balanced (Meissner, 2000).

Table 2 Mineral concentrations in *Searsia lancea* leaves after treatment with polyethylene glycol

	Polyethylene glycol (g/kg DM)					SE	Significance	
	0	5	10	15	20		Linear	Quadratic
Macro minerals (g/kg DM unless otherwise indicated)								
Potassium	14.7 ^b	14.4 ^c	15.2 ^a	14.3 ^c	14.3 ^c	0.10	0.539	0.715
Phosphorus	1.1 ^a	1.0 ^b	1.1 ^a	1.0 ^b	1.0 ^b	0.01	0.308	0.308
Calcium	9.1 ^a	8.6 ^b	9.0 ^a	8.6 ^b	8.6 ^b	0.10	0.250	0.585
Magnesium	2.1 ^a	2.0 ^b	2.0 ^b	1.9 ^c	1.9 ^c	0.02	0.015	0.082
Sodium (mg/kg DM)	41.2 ^b	38.4 ^b	52.0 ^a	36.4 ^b	42.4 ^{ab}	3.45	0.987	0.934
Sulphur	1.2	1.2	1.3	1.2	1.1	0.02	0.450	0.229
Micro minerals (mg/kg DM)								
Iron	145.8 ^a	128.3 ^b	133.8 ^{ab}	145.0 ^a	140.7 ^{ab}	4.00	0.827	0.656
Copper	6.2 ^a	5.9 ^c	6.0 ^b	5.9 ^c	5.9 ^c	0.04	1.000	1.000
Zinc	16.0	15.7	15.9	16.1	15.8	0.22	0.137	0.371
Manganese	34.6 ^a	32.4 ^b	34.2 ^a	32.6 ^b	32.3 ^b	0.26	0.248	0.592
Boron	26.1 ^a	24.7 ^b	25.7 ^a	24.0 ^c	24.3 ^{bc}	0.18	0.143	0.424

^{a-c} Means in the same row with different superscripts differ ($P < 0.05$). SE: standard error.

Polyethylene glycol treatment was used with the aim of mitigating the detrimental effects of CT on the degradation of substrates in the rumen. Treatment with PEG linearly increased the IVDMD at 12, 24, 36, and 72 hours, demonstrating the capacity of PEG to deactivate CT and improve rumen microbial fermentation (Table 3). The IVDMD at 36 hours ($R^2 = 0.918$; $P = 0.010$) showed a positive linear response to increasing levels of PEG: $y = 355.140(\pm 3.843) + 1.820(\pm 0.314)x$.

Table 3 *In vitro* DM degradability (g/kg DM) of *Searsia lancea* after treatment with polyethylene glycol

	Polyethylene glycol (g/kg DM)					SE	Significance	
	0	5	10	15	20		Linear	Quadratic
6 hours	234.1	230.2	234.9	238.1	242.9	4.47	0.068	0.078
12 hours	260.2 ^d	307.4 ^c	325.9 ^b	330.9 ^{ab}	338.4 ^a	2.76	0.035	0.025
24 hours	264.2 ^d	323.1 ^c	356.9 ^b	360.2 ^b	372.7 ^a	2.96	0.029	0.019
36 hours	277.8 ^d	357.6 ^c	377.4 ^b	384.4 ^{ab}	389.7 ^a	2.95	0.010	0.081
48 hours	296.3 ^d	388.3 ^c	401.6 ^b	409.6 ^b	423.0 ^a	2.94	0.061	0.075
72 hours	312.9 ^e	401.5 ^d	425.0 ^c	438.6 ^b	454.3 ^a	3.04	0.034	0.038

^{a-e} Means in the same row with different superscripts differ ($P < 0.05$). DM: dry matter, SE: standard error.

Treatment with 20 g PEG/kg DM produced the highest IVDMD ($P < 0.05$) at all hours of withdrawal. Our findings concur with those of Knowles *et al.* (2017), who observed increased DM degradation of tanniferous legumes with higher PEG inclusion. The inhibitory effects of tannins were ameliorated by PEG, allowing increased proteolysis and fibre degradation, thereby improving the IVDMD (Knowles *et al.*, 2017; Fagundes *et al.*, 2020).

In conclusion, treating *S. lancea* leaves with incremental PEG levels reduced the CP, EE, NDF, TP, TT, and CT contents while enhancing IVDMD. These findings indicate that PEG efficiently alleviates the detrimental effects of tannins on *in vitro* ruminal fermentation, enhancing the nutritional value of *S. lancea*. Although the results suggest that the inclusion of PEG at 20 g/kg DM may be suitable for improving the nutritive value of *S. lancea*, the practical application of these results is limited, as the CT concentration remained greater than 50 g/kg DM. This indicates that this forage could still be detrimental to ruminant performance, and the effects of higher PEG levels need to be explored. Furthermore, an *in vivo* study is needed to determine whether these treatments would improve ruminant productivity.

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Authors' contributions

O.H., O.M., and N.S. collected the data for this study. O.H. and N.S. conducted the statistical analyses. O.H., O.M., N.S., and K.E.R. collaborated in interpretation of the results, and wrote the initial draft of this manuscript. O.H., H.K.M., C.K.L., and K.E.R. developed the original hypotheses, designed the experiments, collaborated in interpreting the results, and finalised the manuscript. All authors have read and approved the finalised manuscript.

Conflict of interest declaration

The authors have no conflicts of interest to declare.

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