NUTRITIONAL COMPOSITION OF *Arceuthobium vaginatum* subsp. *vaginatum* AND *A. globosum* subsp. *grandicaule* AND THEIR EFFECT ON *IN VITRO* RUMINAL FERMENTATION KINETICS †

[COMPOSICIÓN NUTRICIONAL DE *Arceuthobium vaginatum* subsp. *vaginatum* Y *A. globosum* subsp. *grandicaule* Y SU EFECTO EN LA CINÉTICA DE FERMENTACIÓN RUMINAL *IN VITRO*]

Maria Mitsi Nalleli Becerril-Gil [1], Agustín Olmedo-Juárez [2], Angel Rolando Endara-Agramont [1] and Julieta Gertrudis Estrada-Flores[1*]

[1]Instituto de Ciencias Agropecuarias y Rurales (ICAR). Universidad Autónoma del Estado de México (UAEM). Campus UAEM El Cerrillo, C.P. 50090, Toluca, Estado de México, México. Email: jgestradaf@uaemex.mx
[2] Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad (CENID). Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Km. 15.5 Carretera Federal Cuernavaca-Cuautla No. 8534, Col. Progreso, Jiutepec, Morelos. C.P. 62574 Morelos, México.
**Corresponding author*

SUMMARY

Background: *Arceuthobium vaginatum* subsp. *vaginatum* (BM; black mistletoe) and *Arceuthobium globosum* subsp. *grandicaule* (YM; yellow mistletoe), are two parasitic plant species abundant in the forests of northern and central Mexico and Central America, affect 43% of the *P. hartwegii* tree population in the Nevado de Toluca Flora and Fauna Protection Area (NTFFPA), including mistletoe as a complementary feed in sheep can reduce the environmental impact generated by these pests to the forest and also reduce the purchase of feed for livestock. **Objective**: To evaluate the chemical composition, phenolic content and *in vitro* fermentation kinetics of two mistletoe species (M) *Arceuthobium vaginatum* subsp. *vaginatum* (BM; black mistletoe) and *A. globosum* subsp. *grandicaule* (YM; yellow mistletoe), in four age categories (AC) of *Pinus hartwegii* (AC: small sapling, large sapling, juvenile and adult) collected in the Nevado de Toluca Flora and Fauna Protection Area (NTFFPA). **Methodology**: The chemical composition (dry matter DM; neutral detergent fiber NDF; acid detergent fiber ADF and crude protein CP), phenolic content (total phenols TP; total tannins TT and condensed tannins, CT), *in vitro* fermentation kinetics parameters and *in vitro* digestibility were analysed. The experimental design used was completely randomized design with 2x4 factorial arrangement. **Results**: DM content was different between M ($P< 0.05$), the highest was found in BM. The NDF and ADF content was different between M, ranging from 364.45-467.43 g/kg DM. No differences ($P>0.05$) were observed in CP which averaged 62.08 g/kg DM. The TP, TT and CT content was different between M ($P<0.05$), the highest content was in YM with no effect observed in AC. B-gas production (mL of gas) presented differences between M and AC sampled ($P<0.05$). The gas production rate c on average was 0.042. Lag time was different between M ($P<0.05$). The *in vitro* digestibility of dry matter and organic matter were different between M ($P<0.05$). **Implications**: The results reported here serve as a tool for decision making on its possible inclusion as a forage addition to a diet in sheep feeding. **Conclusions:** The chemical composition and *in vitro* digestibility was different between M and the AC, contain secondary metabolites such as total phenols and condensed tannins and have an impact on *in vitro* fermentation.
Keywords: chemical composition; condensed tannins; *in vitro* fermentation; *Arceuthobium*; dwarf mistletoe.

RESUMEN

Antecedentes: *Arceuthobium vaginatum* subsp. *vaginatum* (MN; muérdago negro) y *Arceuthobium globosum* subsp. *grandicaule* (MA; muérdago amarillo), son dos especies de plantas parásitas abundantes en los bosques del norte y centro de México y Centroamérica, afectan al 43% de la población arbórea de *P. hartwegii* en el Área de Protección de Flora y Fauna Nevado de Toluca (APFFNT), incluir el muérdago como alimento complementario en ovinos puede reducir el impacto ambiental generado por estas plagas al bosque y además disminuir la compra de alimento para el ganado. **Objetivo**: Evaluar la composición química, el contenido fenólico y la cinética de fermentación ruminal *in*

† *Submitted February 2, 2023 – Accepted June 22, 2023.* *http://doi.org/10.56369/tsaes.4765*

ISSN: 1870-0462.
ORCID = M.M.N. Becerril-Gil, http://orcid.org/0000-0001-5417-9569; **A. Olmedo-Juárez**, http://orcid.org/0000-0001-5499-7449.; **A.R. Endara-Agramont**, http://orcid.org/0000-0001-8413-6551.; J.**G Estrada-Flores**, http://orcid.org/0000-0002-3376-5128

vitro de dos especies de muérdago (M) *Arceuthobium vaginatum* subsp. *vaginatum* (MN; muérdago negro) y *A. globosum* subsp. *grandicaule* (MA; muérdago amarillo), en cuatro categorías de edad (AC) de *Pinus hartwegii* (CA: brinzal pequeño, brinzal grande, juvenil y adulto) colectadas en el Área de Protección de Flora y Fauna Nevado de Toluca (APFFNT). **Metodología**: Se analizó la composición química (materia seca MS; fibra detergente neutro NDF; fibra detergente ácido ADF y proteína cruda PC), contenido fenólico (fenoles totales TP; taninos totales TT y taninos condensados, TC), parámetros cinéticos de fermentación *in vitro* y digestibilidad *in vitro*. El diseño experimental utilizado fue completamente aleatorizado con arreglo factorial 2x4. **Resultados**: El contenido de MS fue diferente entre M (P< 0.05), el mayor se encontró en BM. El contenido de NDF y ADF fue diferente entre M, oscilando entre 364.45-467.43 g/kg MS. No se observaron diferencias (P>0.05) en la PC, cuyo promedio fue de 62.08 g/kg MS. El contenido de TP, TT y CT fue diferente entre M (P<0.05), el contenido más alto fue en YM sin observarse efecto en AC. La producción de gas B (mL de gas) presentó diferencias entre M y AC muestreados (P<0.05). La tasa de producción de gas c en promedio fue de 0.042. El tiempo lag fue diferente entre M (P<0.05). DivDM fue diferente entre M (P<0.05). **Implicaciones**: Los resultados aquí reportados sirven como herramienta para la toma de decisiones sobre su posible inclusión como forraje adicionado a una dieta en la alimentación ovina. **Conclusiones**: La composición química y digestibilidad ruminal *in vitro* fue diferente entre M y el AC, contienen metabolitos secundarios como fenoles totales y taninos condensados y tienen un impacto en la fermentación *in vitro*.

Palabras clave: composición química; taninos condensados; fermentación *in vitro*; *Arceuthobium*; muérdago enano.

INTRODUCTION

Arceuthobium vaginatum subsp. *vaginatum* (BM; black mistletoe) and *Arceuthobium globosum* subsp. *grandicaule* (YM; yellow mistletoe) known as "mistletoe", are two parasitic plant species abundant in the forests of northern and central Mexico and Central America. They belong to the Santalaceae family and the *Arceuthobium* genus. They are hemiparasitic shrubs whose shoots are mainly composed of vascular tissue and cellulose. The establishment of both species occurs through a system of haustoria attached to the tree parenchyma, from which the mistletoe obtains nutrients, water and secondary compounds necessary for growth and reproduction (Hawksworth and Wiens, 1996; Queijeiro-Bolaños and Cano-Santana, 2018). *A. vaginatum* and *A. globosum* affect 43% of the *P. hartwegii* tree population in the Nevado de Toluca Flora and Fauna Protection Area (NTFFPA) (Sáenz-Romero *et al*., 2020; Endara-Agramont *et al*., 2022). Manual pruning is an alternative in the control of both species of mistletoe, as it reduces the degree of infestation of the trees (Sotero-García *et al*., 2018), in its different age categories; therefore, its use in animal feed can be a sustainable alternative on farms in high mountain areas, so it is important to know its nutritional characteristics in the different age categories of affected trees, which in addition, by including mistletoe as a complementary feed can reduce the environmental impact generated by these pests to the forest and also reduce the purchase of feed for livestock. Its use in sheep feed (Jibril *et al*., 2020) and its medicinal uses of the secondary metabolites (Shah, 2017) have been reported. The content of total phenolics and condensed tannins in forages can improve nutrient utilisation due to their antibacterial and antifungal properties as previously reported in some mistletoe species such as *A. oxycedri* and *A. americanum* (Zaidi *et al.,* 2006; Pernitsky *et al.,* 2011; PKB, 2021a, 20021b), furthermore condensed tannins possess the ability to bind proteins that promote the passage of protein into the duodenum where it is absorbed (Giridhar *et al.*, 2018; Lagrange *et al*., 2021; Menci *et al*., 2021), inhibit the secretion of microbial enzymes that digest the cell wall of forages (Zhang *et al*., 2021) and thereby modify the total digestibility of the ration (Vázquez-Carrillo *et al*., 2020; Huang *et al*., 2021). The addition of condensed tannins to ruminant diets provides some environmental benefits by reducing ammonia emissions (Menci *et al*., 2021). There are few studies describing the nutritional characteristics of both mistletoe species, nor their relationship with the age of the host on which they are established. Therefore, the aim of this study was to evaluate the nutritional composition of the mistletoe species *A. globosum* subsp. *grandicaule* and *A. vaginatum* subsp. *vaginatum* by analysing the chemical composition, phenols, total tannins, condensed tannins, *in vitro* ruminal fermentation kinetics and digestibility of dry matter, organic matter and neutral detergent fibre to assess their effect on the feeding of sheep on NTFFPA farms.

MATERIAL AND METHODS

Sample collection

The collection of mistletoe species was carried out in the NTFFPA, located in the Central and Neovolcanic Axis, Transmexican Neovolcanic Belt (SEMARNAT, 2022), with a total area of 53,590 hectares (19°07'07" N, 099°46'53" W). The climate is temperate sub-humid with rainfall from May to October, the mean annual temperature is 4.0°C (range: 8.7 to -0.7°C). The average annual rainfall is 1,215.9 mm (SMN, 2022). Black and yellow mistletoe species (BM and YM, respectively) were collected in June-August 2021, the predominant phenological stages were fruiting and seed dispersal (Queijeiro-Bolaños *et al*., 2014). Collection was carried out at twelve sampling sites within the NTFFPA where the parasitic plant was found, were selected according to the accessibility of the infested areas. Four age categories (AC) of *P. hartwegii* trees were collected at each sampling site; small sapling (≥ 30cm < 1.5 m in height), large sapling (≥ 1.5 m in height < 2.5 diameter at breast height or DBH), juveniles (≥ 2.5 < 7.5 DBH) and adults (≥ 7.5 DBH). A total of 105 mistletoe samples were collected, of which 54 were yellow mistletoe and 51 were black mistletoe. Of yellow mistletoe, 9 were collected from small saplings, 14 from large saplings, 16 from juvenile trees and 15 from adult trees. For black mistletoe, 6 were collected from small saplings, 10 from large saplings, 14 from juvenile trees and 21 from adult trees. Dry matter (DM) was estimated in a botanical dryer at 50° C for 72 hours to avoid denaturation of secondary metabolites (Makkar, 2003a). They were then ground to 1-2 mm in a Cyclon 2000 and Pulvex mill, for subsequent proximate chemical analysis, phenolic composition, and *in vitro* gas production. The standardised methodology described by AOAC (2023) was used to determine organic matter (OM) and crude protein (CP) content. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were analysed according to Van Soest *et al*. (1991) using ANKOM-57 micro bags on the Ankom200 fibre analyser (ANKOM, 2023). Total phenols (TP) and total tannins (TT) content were analysed by the Folin-Ciocalteu method based on Makkar *et al*. (2003a), with a 50 mg sample. The concentration of condensed tannins (CT) was analysed by the technique described by Makkar (2003a) based on Porter *et al*. (1986), with a sample of 10 mg. For the analysis of these variables, each sample was analysed in triplicate. The measurement of *in vitro* gas production was performed according to Theodorou *et al*. (1994). The reading of gas volume produced was recorded after 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, 28, 36, 36, 44, 52, 60, 72, 84 and 96 hours of incubation, using a Lutron pressure transducer. The data were fitted to the model to analyse the fermentation kinetics (France *et al*. 2000):

$$G = A \times (1 \ 〚\text{^}(-e - c(t - L))〛)$$

where G= total cumulative gas production over time (mL);
A= is the asymptotic gas production (mL);
c= *in vitro* gas production rate (/h) from the slowly fermentable fraction of the feed (b) (fermentable fraction of NDF);
T= *Lag* time (h) and
L= is the time *Lag* for the onset of NDF fermentation (h).

In vitro digestibility of dry matter (IvDMD), organic matter (IvOMD) and neutral detergent fibre (IvNDFD) were estimated by the method proposed by Pell and Schofield (1993).

Experimental design and data analysis

Chemical composition, total phenols content, *in vitro* gas production and digestibility results were analysed by ANOVA using a completely randomized design with 2x4 factorial arrangement (Mosley et al., 2017) considering as factors the two mistletoe species (M) (BM and YM), the four age categories of sampled trees (AC) (small sapling, large sapling, juveniles and adults) and their interactions (M*AC), a total of 8 treatments were evaluated whose mathematical expression was:

$$Yijk = \mu + \text{Mi} + \text{AC j} + (\text{M} * \text{AC})\text{ij} + \text{e ijk}$$

Where: μ=overall mean of the treatments
Mi =effect of the i-th mistletoe species (i=2).
AC j =effect of the j-th tree age category (j=4).
(M * AC) ij = effect of the interaction between mistletoe species and tree age category.
e ijk = experimental error.

Additionally, the means among treatments were compared with Tukey's test at a significance level of 0.05. The statistical package used was SAS (2006).

RESULTS

The chemical composition of yellow and black mistletoe collected at different tree ages categories is shown in Table 1. Significant differences (P< 0.05) in dry matter content were observed between mistletoe species and age category (P< 0.05). Dry matter content in black mistletoe was higher (P< 0.05) at all age category compared to yellow mistletoe collected at different age category. There is a trend towards increased plant matter content in mistletoes establishing in mature trees compared to saplings. Significant differences (P< 0.05) were detected in organic matter (OM) content between mistletoe species. The NDF and ADF values were different between mistletoe species (P< 0.0001); the highest fibre content was observed in black mistletoe at all age category. No significant differences (P> 0.05) were found in CP content between mistletoe species and age category of the sampled trees.

The results of phenolic composition (total phenolics, total tannins and condensed tannins) of yellow and black mistletoe at different tree age categories are shown in Table 2. Significant differences (P<0.05) were observed in total phenolics content between mistletoe species; the highest values were found in yellow mistletoe. No significant differences (P>0.05) were observed in age category.

Table 1. Proximal chemical composition (g/kg) of yellow and black mistletoe collected from four different age categories of *P. hartwegii*.

Variable	Mistletoe species	Age category				P value			
		Small sapling	Large sapling	Juvenile	Adult	SEM	M	AC	M*AC
DM	Yellow	352.39 ^{B}	351.30 ^{B}	351.83 ^{B}	385.90 ^{A}	14.80	<0.0001	0.0048	0.2897
	Black	412.62 ^{A}	401.62 ^{A}	406.46 ^{A}	417.01 ^{A}				
OM	Yellow	925.58 ^{AB}	928.05 ^{AB}	932.12 ^{AB}	922.24 ^{B}	10.85	<0.0001	0.9569	0.5885
	Black	949.59 ^{AB}	948.20 ^{AB}	948.83^{AB}	953.31 ^{A}				
NDF	Yellow	364.45 ^{B}	391.37 ^{B}	399.73 ^{B}	409.23 ^{B}	12.62	<0.0001	0.1466	0.0097
	Black	467.43 ^{A}	464.53 ^{A}	459.72 ^{A}	459.46 ^{A}				
ADF	Yellow	270.18 ^{B}	292.93 ^{B}	291.52 ^{B}	300.66 ^{B}	9.20	<0.0001	0.1391	0.0345
	Black	337.05 ^{A}	334.91 ^{A}	331.17 ^{A}	333.22 ^{A}				
CP	Yellow	65.33	66.12	62.08	62.94	5.78	0.1002	0.9206	0.8851
	Black	56.88	63.63	60.25	58.55				

For each variable, mean with different capital letter between columns and rows indicate significant differences by Tukey's test (P<0.05). Yellow (*A. globosum*) and Black (*A. vaginatum*). M: mistletoe species, AC: age category, SEM: standard error of the mean, DM: dry matter, OM: organic matter, NDF: neutral detergent fibre, ADF: acid detergent fibre, CP: crude protein.

Table 2. Total phenolics, total tannins and condensed tannins content (%) of yellow mistletoe and black mistletoe collected from *P. hartwegii* of four different age categories.

Variable	Mistletoe specie	Age Category				SEM	P value		
		Small sapling	Large sapling	Juvenile	Adult		M	AC	M*AC
TP	Yellow	6.93 ^{A}	6.74 ^{AB}	6.68 ^{ABC}	6.75 ^{AB}	0.43	<0.0001	0.9556	0.888
	Black	5.59 ^{C}	5.86 ^{ABC}	5.66 ^{BC}	5.70 ^{BC}				
TT	Yellow	6.16 ^{A}	6.07 ^{A}	5.97 ^{AB}	6.06 ^{A}	0.46	<0.0001	0.5076	0.5314
	Black	4.65 ^{C}	5.34 ^{ABC}	4.82 ^{BC}	5.25 ^{ABC}				
CT	Yellow	1.44 ^{A}	1.35 ^{AB}	1.32 ^{AB}	1.31 ^{AB}	0.13	<0.0001	0.6898	0.8766
	Black	1.14 ^{AB}	1.10 ^{AB}	1.15 ^{AB}	1.06 ^{B}				

For each variable, averages with different capital letter columns and rows indicate significant differences by Tukey's test (P<0.05). Yellow (*A. globosum*) and Black (*A. vaginatum*). M: mistletoe species, AC: age category, SEM: standard error of the mean, TP: Total Phenols tannic acid equivalent, TT: Total Tannins tannic acid equivalent and CT: Condensed Tannins leucocyanidin equivalent.

Significant differences (P<0.05) were found between mistletoe species in total tannins content; total tannins content was higher in yellow mistletoe. Significant differences (P<0.05) were observed between mistletoe species for condensed tannins content, with the highest values found in yellow mistletoe.

Table 3 shows the *in vitro* fermentation parameters of yellow and black mistletoe calculated using the equation proposed by France *et al*. (2000); digestibility of both mistletoe species in the four age categories are also indicated. Significant differences (P<0.05) were observed in B between mistletoe species and age categories of trees sampled. A higher gas production was observed in the yellow mistletoe, and in general the small saplings and large saplings had the highest values. No significant differences were found in c *in vitro* gas production rate (P>0.05), on average 0.041 for yellow mistletoe and 0.043 for black mistletoe. Lag time was significantly affected by mistletoe species (P<0.05), the longest fermentation time was observed in yellow mistletoe (mean 9.1 h).

Significant differences (P<0.05) were detected in *in vitro* dry matter digestibility and *in vitro* organic matter digestibility between mistletoe species; it was higher in yellow mistletoe in small saplings (479.73 g/kg DM). There were no significant differences (P>0.05) on *in vitro* neutral detergent fiber digestibility between species.

Table 3. Gas production B (mL gas), *in vitro* gas production rate c (/h), Lag time (h), digestibility of dry matter, organic matter and neutral detergent fibre of yellow mistletoe (*A. globosum*) and black mistletoe (*A. vaginatum*) collected from *P. hartwegii* of four different age categories.

Variable	Mistletoe specie	Age Category				SEM	P value		
		Small sapling	Large sapling	Juvenile	Adult		M	AC	M*AC
B	Yellow	84.06 AB	85.50 A	81.20 AB	72.05 BC	4.86	<0.0001	<0.0001	0.7352
	Black	76.03 AB	73.09 BC	73.74 ABC	63.68 C				
c	Yellow	0.040	0.044	0.038	0.044	0.01	0.5869	0.465	0.3891
	Black	0.056	0.040	0.038	0.040				
Lag	Yellow	11.24 A	9.01 AB	7.77 AB	8.39 AB	1.62	0.0071	0.2256	0.398
	Black	6.96 B	7.84 AB	6.93 B	6.52 B				
IvDMD	Yellow	479.73 A	464.10 AB	453.68 AB	429.89 B	14.06	0.0056	0.166	0.0095
	Black	430.89 B	437.64 B	441.82 AB	443.40 B				
IvOMD	Yellow	465.75 A	450.13 AB	439.70 AB	415.91 B	14.06	0.0056	0.1002	0.0095
	Black	416.92 B	423.65 B	427.85 AB	429.42 AB				
IvNDFD	Yellow	134.45	175.00	144.77	140.85	31.44	0.3445	0.2996	0.7465
	Black	169.94	178.29	138.32	163.57				

For each variable, averages with different capital letter columns and rows indicate significant differences by Tukey's test (P<0.05). Yellow (*A. globosum*) and Black (*A. vaginatum*) M: mistletoe species, AC: age category, SEM: Standard error of the mean, B: Fermentable fraction of NDF (mL gas), c: In vitro gas production rate (/h), Lag: NDF fermentation onset time (h), IvDMD: *In vitro* dry matter digestibility (g/kg DM), IvOMD: *In vitro* organic matter digestibility (g/kg DM), IvNDFD: *In vitro* neutral detergent fiber digestibility (g/kg DM).

DISCUSSION

The nutritional composition of *Arceuthobium* spp. has been little studied. Due to the ecological impact of this plant, research on these species has focused on the distribution in coniferous forests. Regarding their chemical composition, the NDF and ADF content of both mistletoe species is due to maturity, because the samples were collected at the seed dispersal and fruiting stage. There are some reports on the chemical composition of this unconventional forage such as in *Viscum rotundifolium*, *Viscum album* and *V. verrucosum* (Ramantsi *et al*., 2019; Jibril *et al*., 2020; Atalay, 2020), but compared to other such species proposed for animal feed, such as *Tagetes lucida*, *Tithonia tubiformis* (Díaz-Medina *et al*., 2021), *Millettia ferroginea* and *Cordia africana* (Sisay *et al*., 2018), they are similar in NDF content. Mistletoe represents a forage resource, where the dry matter content is different (P<0.05) according to the age of the tree in which it is established, in adult trees yellow mistletoe and all ages of black mistletoe can obtain a higher content of plant material, with a higher proportion of fermentable structural carbohydrates in the black mistletoe species compared to the yellow mistletoe species. No significant differences (P>0.05) were found in the protein content of the two mistletoe species, the protein content being due to their photosynthetic capacity (Costa-Santos *et al*., 2021) as well as the translocation of nutrients from the host to the hemiparasitic plant (Monteiro *et al*., 2022). The crude protein content of both mistletoe species in the different ages of trees sampled was lower than that reported for shrubs in Ethiopia, such as *Sesbania somalensis* and *Acacia bussei* (Gebeyew *et al.*, 2020). The protein intake of these species can meet the requirement for adult sheep in maintenance (68 g/d) according to AFRC (1993) and can be an alternative forage in sheep feeding. The total phenols, total tannins and condensed tannins content were similar (P>0.05) in the different tree age categories of *P. hartwegii* and different between mistletoe species (P<0.05), due to the interspecific relationship that exists between them, where the dispersal of yellow mistletoe is greater than black mistletoe due to the low tree density conditions that allow greater illumination which favours the growth of yellow mistletoe (Queijeiro-Bolaños *et al*., 2014). In addition, the highest content of secondary metabolites in the yellow mistletoe species indicates a better adaptation as a defence mechanism against attacks by microorganisms and herbivores that allow their survival within the ecosystem (Lázaro-González *et al*., 2018). The content of total phenols, total tannins and condensed tannins are not as high as in some forage legumes (Huang *et al.*, 2021), but show a significant effect on total gas production, dry matter digestibility, organic matter and NDF (Table 3). This is due to the content of other secondary metabolites such as volatile oils, which although not determined in this study, have effects on rumen microbial efficiency and affect gas production *in vitro*, such as elemol and camphor, present in *Juniperus pinchotti* and *J. virginiana* (Stewart *et al*., 2015). The phenolic compound content of yellow mistletoe was lower than those reported by Hernández-Luna *et al*. (2017), and higher than that reported for mistletoe. *Viscum rotundifolium* with 7.3 g/kg DM of total tannins (Hawu *et al*., 2022). In traditional Mexican medicine of black mistletoe, as it is a medicinal plant used for the treatment of several ailments like an anti-inflammatory and nervous system relaxant (Biblioteca Digital de la Medicina Tradicional Mexicana, 2022), and has also been shown to have antimicrobial activity against *Clavibacter michiganenses* (García-García *et al.*, 2021). In addition, the inhabitants of the Loma Alta community in the APFFNT use it for the treatment of respiratory ailments (Sotero-García *et al*., 2016). Total gas production (B) had significant differences between mistletoe species and tree ages sampled (P<0.05), it is associated with total phenolics and condensed tannins content as it inhibits the activity of microorganisms due to their antibacterial and antiprotozoal properties, which reduce *in vitro* gas production (Fagundes *et al.*, 2020). The reduction in gas production is due to the fact that Zhang *et al*. (2021) indicate such the NDF fraction of tanniferous plants retains significant concentrations of condensed tannins which influence the digestion of cell contents and cell wall, which are included in the dry matter content of forages, which had significant differences between tree age categories and mistletoe species (P<0.05). In some studies, with other forage species, similar gas values have been reported for *Flemingia macrophylla* shrub with 81.99 mL (Fagundes *et al*., 2020). On the other hand, the *in vitro* gas production rate (c) did not show significant differences between mistletoe species or in the age categories of the trees sampled. The *in vitro* gas production rate is due to the nitrogen/crude protein and NDF content (Bureenok *et al*., 2019). Mistletoe shows better fermentation rates for both species compared to Ethiopian shrubs as *Ensete ventricosum*, *Erythrina brucei* and *Maesa lanceolata* with 0.03 mL/h (Mekuriaw *et al*., 2020). Lag time was significantly different between mistletoe species (P<0.05), the onset of NDF fermentation varies by about 4 hours between species, with a longer lag time observed in yellow mistletoe (11.24 h). This may be due to the differences found in the content of phenolic compounds of the mistletoe species in table 2, as it has been reported that they can modify the availability of structural carbohydrates due to the formation of lignocellulose complexes which delays their colonisation by ruminal bacteria (McSweeney *et al*., 2001), thus delaying the fermentation onset time. The digestibility of *in vitro* dry matter and *in vitro* organic matter was different between mistletoe species due to NDF and ADF content between species. The NDF content was lower in yellow mistletoe species, a better digestibility is observed because a higher quality of NDF in yellow mistletoe than in black mistletoe (P<0.05), as the amount of ADF in the yellow mistletoe was lower compared to the black mistletoe (P<0.05) due to its cellulose and lignin content. In comparison with other forage species such as *Juniperus pinchotti* at mature stage the digestibility of the dry matter of yellow mistletoe was 29.8% higher and when comparing the digestibility of black mistletoe it was 25% higher than *J. virginiana* at immature stage (Stewart *et al*., 2015). It was also 9.10% and 1.65% higher than the digestibility of *Typha latifolia* (Ávila-Gonzales *et al*., 2022). Because of this, mistletoe species are a better forage option than *Juniperus* and *Typha* species. The *in vitro* organic matter digestibility of both mistletoe species *Arceuthobium* spp, are equal to those observed in other shrub species utilized in livestock feed such as *Stereospermum kuthianum* and *Ekebergia capensis* (Mekuriaw *et al*., 2020) as well as *Acacia tortilis*, *Prosopis juliflora* and *Cordia africana* (Sisay *et al*., 2018), which like both species of mistletoe are considered plants of low nutritional quality and that complement the diet of small ruminants with little access to quality forage, so they represent a relevant source of food in extreme climates areas.

Finally, NDF digestibility was low in both mistletoe species in different age categories, which is associated with the amount of condensed tannins as the formation of condensed tannin-extracellular microbial enzyme complexes reduces their digestive activity (Lagrange *et al*., 2021) and/or inhibits cellulolytic bacteria (*Fibrobacter succinogenes, Ruminococcus flavefaciens,* and *Ruminococcus albus*) and fungi (Gunun *et al*., 2019) leading to decreased NDF digestibility. This effect can be counteracted by including polyvinylpolypyrrolidone (PVPP) and/or polyethylene glycol (PEG) on *in vitro* tests, which inhibits the action of tannins and improves the digestibility of tanniferous plant nutrients by rumen microflora (Makkar, 2003b).

In conclusion, mistletoe *Arceuthobium globosum* subsp. *grandicaule* (yellow mistletoe) and *A. vaginatum* subsp. *vaginatum* (black mistletoe) have nutritional characteristics that differ between species and age of the tree they parasitise. The nutritional content of both species represents a forage alternative as they have a good content of fibre, crude protein and total phenols that would improve the nutrient utilisation of traditional diets and complement the feeding of small ruminants in the NTFFPA area.

The importance of using dwarf mistletoe species in animal feed is their effect on the control and management of this forest pest in the NTFFPA where much of it is affected. Livestock farmers in the area could collect the plants from the infected trees to include them in the diet of ruminants, at no extra cost to the production unit, only the transport from the collection area to the production unit.

Further research is needed on both species to find optimal inclusion levels in small ruminant feeds that can provide environmental, economic and productive benefits.

Acknowledgements
Thanks are due to the Instituto de Ciencias Agropecuarias y Rurales of the Universidad Autónoma del Estado de México where the laboratory analyses were carried out. We are grateful to the National Commission of National Protected Areas (CONANP) for allowing the collection of mistletoe specimens for this study (Of. No. DAPFFNT/515/2021). Student Maria Mitsi Nalleli Becerril Gil thanks CONACyT for the doctoral scholarship awarded (654795).

Funding. Part of this study was financed by the UAEM project 6460/2022CIB "Alternativas sostenibles para la alimentación de ovinos en el Estado de México" and also to the project 4938/2019C.

Conflicts of interests. Authors declare there are no conflicts of interest, and are fully responsible for the contents and writing of this manuscript.

Compliance with ethical standards. The experimental procedures followed the guidelines accepted by the Instituto de Ciencias Agropecuarias y Rurales of the Universidad Autónoma del Estado de México and were institutionally approved (DICARM-1221).

Data availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contribution statement (CRediT). M.M.N. Becerril Gil - Investigation, formal analysis, writing – original draft. **A. Olmedo Juárez** - Methodology, writing - review and editing. **A.R. Endara Agramont** - Methodology, writing - review and editing. **J.G. Estrada-Flores** - Conceptualization, resources, writing – review and editing, supervision, funding acquisition. All authors read and approved the final manuscript.

REFERENCES

AFRC (Agricultural Food Research Council)., 1993. *Energy and protein requirements of ruminants*, Wallingford: CAB international.

Ankom, 2023. Procedures for NDF and ADF. *Ankom Technology Method*. Operator´s manual. https://www.ankom.com. Accessed 18 April 2023.

AOAC (Association of Official Agricultural Chemists)., 2023. *Official Methods of Analysis* of AOAC INTERNATIONAL. 'Animal Feed—General', Wendt Thiex, Nancy J (ed.), New York. https://doi.org/10.1093/9780197610145.003.037

Atalay, A.İ., 2020. Determination of nutritive value and anti-methanogenic potential of mistletoe leaves (Viscum album) grown on different host, *International Journal of Agriculture Forestry and Life Sciences* 4, pp. 120-123.

Ávila-González R., Arriaga-Jordán C., Estrada-Flores, J. and López González, F., 2022. Evaluación nutricional de tule (*Typha latifolia*) en la alimentación de ovinos en el altiplano central de México. *Tropical and Subtropical Agroecosystems*. 25, p. #073. http://doi.org/10.56369/tsaes.4075

Biblioteca Digital de la Medicina Tradicional Mexicana, 2022. *Atlas de las Plantas de la Medicina Tradicional Mexicana. Universidad Nacional Autónoma de México* (UNAM) http://www.medicinatradicionalmexicana.unam.mx/apmtm/index.html Accessed 28 August 2022.

Bureenok, S., Langsoumechai, S., Pitiwittayakul, N., Yuangklang, C., Vasupen, K., Saenmahayak, B., and Schonewille, J. T., 2019. Effects of fibrolytic enzymes and lactic acid bacteria on fermentation quality and in vitro digestibility of Napier grass silage, *Italian Journal of Animal Science*, 18:1, pp. 1438-1444, http://doi.org/10.1080/1828051X.2019.1681910

Costa-Santos, A. C., Rejane-de Almeida, W., Maldonado-López, Y., Cuevas-Reyes, P., and Santos, J. C., 2021. Variation in the co-occurrence of pathogen and herbivores between ontogenetic stages of *Miconia albicans*. *Trees*, 35, pp. 1001-1011. https://doi.org/10.1007/s00468-021-02097-9

Díaz-Medina, L. K., Colín-Navarro, V., Arriaga-Jordán, C. M., Brunett-Pérez, L., Vázquez-de-Aldana, B. R., and Estrada-Flores, J. G., 2021. In vitro nutritional quality and antioxidant activity of three weed species as feed additives for sheep in the Central Highlands of Mexico. *Tropical Animal Health and Production*, 53 (3), pp. 394. https://doi.org/10.1007/s11250-021-02819-8

Endara-Agramont A., Heredia-Bobadilla R., Garcia-Almaraz L., Luna-Gil A., Franco-Maass S. and Cibrián-Llanderal V., 2022. Factores asociados con la distribución espacial de muérdagos enanos en dos poblaciones de *Pinus hartwegii* del centro de México. *Revista Mexicana de Biodiversidad*. https://doi.org/10.22201/ib.20078706e.2022.93.5008

Fagundes, G. M., G. Benetel, K. C. Santos, K. C. Welter, F. A. Melo, J. P. Muir, and I. C. S. Bueno., 2020. Tannin-Rich Plants as Natural Manipulators of Rumen Fermentation in the Livestock Industry, *Molecules.* 25, pp. 2943. https://doi.org/10.3390/molecules25122943

France, J.K., Dijkstra, J., Dhanoa, M.S., López, S., and Bannink, A., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. *The British Journal of Nutrition*, 83 (2), pp. 143-150. https://doi.org/10.1017/s0007114500000180

García-García, J. D., Anguiano-Cabello, J. C., Arredondo-Valdés, R., Candido del Toro, C. A., Martínez-Hernández, J. L., Segura-Ceniceros, E. P., and Govea-Salas, M., 2021. Phytochemical Characterization of *Phoradendron bollanum* and *Viscum album* subs. *austriacum* as Mexican Mistletoe Plants with Antimicrobial Activity. *Plants*, 10 (7), pp. 1299. http://dx.doi.org/10.3390/plants10071299

Gebeyew, K., Abera, B., Bajigo, A., Gebresilassie, G., Martínez, Y., and Adebowale, T. 2020. Indigenous medicinal uses, toxicity, and chemical composition of browsing plant used by camel in Ethiopia Somali Regional State: a survey. *Tropical Animal Health and Production*, 52 (3), pp. 1459–1466. https://doi.org/10.1007/s11250-019-02152-1

Giridhar K.S., Prabhu T.M., Singh, K.C., Nagabhushan, V., Thirumalesh, T., Rajeshwari, Y.B. and Umashankar, B.C., 2018. Nutritional potentialities of some tree leaves based on polyphenols and rumen in vitro gas production. *Veterinary World*. 11 (10), pp. 1479-1485. https://doi.org/10.14202/vetworld.2018.1479-1485

Gunun, P., Gunun, N., Khejornsart, P., Ouppamong, T., Cherdthong, A., Wanapat, M., Sirilaophaisan, S., Yuangklang, C., Polyorach, S., Kenchaiwong, W., and Kang, S., 2019. Effects of *Antidesma thwaitesianum* Muell. Arg. pomace as a source of plant secondary compounds on digestibility, rumen environment, hematology, and milk production in dairy cows. *Animal Science Journal*, 90 (3), pp. 372–381. https://doi.org/10.1111/asj.13147

Hawksworth, F.G. and Wiens, D., 1996. *Dwarf mistletoes: biology, pathology, and systematics*. US Department of Agriculture, Forest Service, USA.

Hawu, O., Ravhuhali, K. E., Musekwa, M. G., Sipango, N., Mudau, H. S., Mokoboki, K. H., & Moyo, B., 2022. Utilization of the Viscum Species for Diet and Medicinal Purposes in Ruminants: A Review. *Animals*, 12 (19), pp. 2569. https://doi.org/10.3390/ani12192569

Hernández-Luna, G.B., Endara-Agramont, A.R., González-Ronquillo, M., Martínez-Hernández, J., Vilmar-Kozloski, G. and Estrada-Flores, J.G., 2017. La utilización de muérdago enano (*Arceuthobium globosum*) como forraje en la alimentación de rumiantes. In: Sustentabilidad Agropecuaria. Brunett-Pérez, L., Gómez Demetrio, W., Gutiérrez Castillo, A.C., Jaimes Arriaga, E. (eds). *Experiencias de investigación para el desarrollo agropecuario, forestal y rural*. Universidad Autónoma del Estado de México. pp. 49-60.

Huang H., Szumacher-Strabel M., Kumar Patra A., Ślusarczyk S., Lechniak D., Vazirigohar M., Varadyova Z., Kozłowska M. and Cieślak A., 2021. Chemical and phytochemical composition, in vitro ruminal fermentation, methane production, and nutrient degradability of fresh and ensiled Paulownia hybrid leaves. *Animal Feed Science and Technology*, 279, pp. 115038. http://doi.org/10.1016/j.anifeedsci.2021.115038

Jibril, J.A., Gazali, Y.M., Dantani, M., Alamin, H. and Zannah, B.B., 2020. Performance of Balami Rams Fed Graded Levels of Mistletoe Leaves (*Viscum album*) and Sorghum Stover in Semi-Arid Zone of Borno State, Nigeria. Niger. *Nigerian Journal of Animal Science and Technology*, 3, pp. 25- 285.

Lagrange S.P., MacAdam J.W., Villalba J.J., 2021. The use of temperate tannin containing forage legumes to improve sustainability in Forage-Livestock Production. *Agronomy*, 11, pp. 2264. https://doi.org/10.3390/agronomy11112264

Lázaro-González A., Hódar, J.A., and Zamora, R., 2018. Mistletoe Versus Host Pine: Does Increased Parasite Load Alter the Host Chemical Profile? *Journal of Chemical Ecology*. 45. pp. 95-105. https://doi.org/10.1007/s10886-018-1039-9

Makkar, H.P., 2003a. *Quantification of Tannins in Tree and Shrub Foliage*. A Laboratory Manual. Kluwer Academic Pulishers. Dordrecht, The Netherlands.

Makkar, H.P.S., 2003b. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research*, 49 (3), pp. 241-256. https://doi.org/10.1016/S0921-4488(03)00142-1

McSweeney, C.S., Palmer, B., McNeill, D.M., and Krause, D.O. 2001. Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology*, 91, pp. 83-93. http://doi.org/10.1016/S0377-8401(01)00232-2

Mekuriaw S., Tsunekawa A., Ichinohe T., Tegegne F., Haregeweyn N., Nobuyuki K., Tassew A., Mekuriaw Y., Walie M., Tsubo M. and Okuro T., 2020. Mitigating the anti-nutritional effect of polyphenols on in vitro digestibility and fermentation characteristics of browse species in north western Ethiopia. *Tropical Animal Health and Production*. 52, pp. 1287–1298. https://doi.org/10.1007/s11250-019-02126-3

Menci, R., Coppa, M., Torrent, A., Natalello, A., Valenti, B., Luciano, G., Priolo, A., and Niderkorn, V., 2021. Effects of two tannin extracts at different doses in interaction with a green or dry forage substrate on in vitro rumen fermentation and biohydrogenation. *Animal Feed Science and Technology*, 278, pp. 114977. https://doi.org/10.1016/j.anifeedsci.2021.114977.

Monteiro, G. F., Boanares, D., Novais, S., França, M. G., Antonini, Y., Barbosa, M., Oki Y., and Fernandes G. W., 2022. Imbalance of water potential and photosynthetic efficiency in the parasitic relationship between *Struthanthus flexicaulis* and *Baccharis dracunculifolia*. *Folia Geobotanica*, 57 (1), pp 71-82. https://doi.org/10.1007/s12224-022-09410-5

Mosley, J.C., Frost, R.A., Roeder, B.L., Kottet R.W., 2017. Targeted Sheep Grazing to Suppress Sulfur Cinquefoil (*Potentilla recta*) on Northwestern Montana Rangeland, *Rangeland Ecology & Management*, 70 (5), pp. 560-568. http://dx.doi.org/10.1016/j.rama.2017.03.002.7

Pell, A.N. and Schofield, P., 1993. Computerized Monitoring of Gas Production to Measure Forage Digestion in Vitro. *Journal of Dairy Science*, 76, pp. 1063-1073, http://dx.doi.org/10.3168/jds.S0022-0302(93)77435-4

Pernitsky, K.Y., Mason, Q.D., Cinel, B. and Friedman, C.M.R., 2011. Discovery and partial purification of an antibiotic from lodgepole pine dwarf mistletoe (*Arceuthobium americanum*) active against Gram-positive organisms including Methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of Medicinal Plants Research*, 5 (9), pp. 1722-1727.

PKB (PlantwisePlus Knowledge Bank)., 2021a. *Arceuthobium americanum* (lodgepole pine dwarf mistletoe). Technical Factsheet. https://doi.org/10.1079/pwkb.species.6824

PKB (PlantwisePlus Knowledge Bank)., 2021b. *Arceuthobium oxycedri* (juniper dwarf mistletoe). Technical Factsheet. https://doi.org/10.1079/pwkb.species.6851

Porter, L.J., Hrstich, L.N. and Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyaniding and delphinidin. *Phytochemistry* 25, pp. 223-230.

Queijeiro-Bolaños, M.E., Cano-Santana, Z. and García-Guzmán, G., 2014. Incidence, severity, and aggregation patterns of two sympatric dwarf mistletoe species (Arceuthobium spp.) in Central Mexico. *European Journal of Forest Research*, 133, pp. 297–306. https://doi.org/10.1007/s10342-013-0762-6

Queijeiro-Bolaños M.E and Cano-Santana Z., 2018. Dwarf mistletoes as a relevant component in temperate forest: an integral view. *Forestry*

Research and Engineering: International Journal. 2 (1), pp. 31–33. https://doi.org/10.15406/freij.2017.02.00023

Ramantsi, R., Mnisi, C.M. and Ravhuhali, K.E., 2019. Chemical composition and in vitro dry matter degradability of mistletoe (*Viscum verrucosum* (Harv.)) on *Vachellia nilotica* (L.) in North West Province of South Africa. *Tropical Agriculture*, 96, pp. 53-60. https://doi.org/10.37234/TA96012019/0000960106

Sáenz-Romero, C., Mendoza-Maya, E., Gómez-Pineda, E., Blanco-García A., Endara-Agramont, A.R., Lindig-Cisneros, R., López-Upton, J., Trejo-Ramírez, O., Wehenkel, C., Cibrián-Tovar, D., Flores-López, C., Plascencia-González, A. and Vargas-Hernández, J.J., 2020. Recent evidence of Mexican temperate forest decline and the need for ex situ conservation, assisted migration, and translocation of species ensembles as adaptive management to face projected climatic change impacts in a megadiverse country. *Canadian Journal of Forest Research*, 50 (9), pp. 843-854. https://doi.org/10.1139/cjfr-2019-0329.

SAS., 2006. SAS Institute. *SAS User´s guide: Statistics*. Ver 9.0 Cary NC, USA.

SEMARNAT., 2022. Secretaría del Medio Ambiente y Recursos Naturales. https://www.gob.mx/semarnat. (Accessed 07 November 2022).

Shah, S. S., Rehman, Y. U., Iqbal, A., Rahman, Z. U., Zhou, B., Peng, M., and Li, Z., 2017. Phytochemical screening and antimicrobial activities of stem, leaves and fruit extracts of Viscum album L. *Journal of Pure Applied Microbiology*, 11 (3), pp. 1337-1349. https://doi.org/10.22207/JPAM.11.3.14

Sisay, A., Negesse, T. and Nurfeta, A., 2018. Short chain fatty acid production, organic matter digestibility and metabolisable energy content of indigenous browses from Ethiopian rift valley. *IOSR Journal of Agriculture and Veterinary Science*, 11 (1), pp. 61-68.

SMN., 2022. Servicio Meteorológico Nacional. En: https://smn.conagua.gob.mx/es/climatologia/temperaturas-y-lluvias/resumenes-mensuales-de-temperaturas-y-lluvias. (Accessed 07 November 2022).

Sotero-Garcia AI., Gheno-Heredia YA., Martínez-Campos AR. And Arteaga-Reyes T., 2016. Plantas medicinales usadas para las afecciones respiratorias en Loma Alta, Nevado de Toluca, México. *Acta Botanica Mexicana* 114: pp. 51-68. https://doi.org/10.21829/abm114.2016.1102

Sotero-García, A.I., Arteaga-Reyes, T.T., Martínez-Campos, A.R., Galicia, L., 2018. Efecto de las podas sobre Arceuthobium spp. en bosques densos y semidensos de Pinus hartwegii (Lindl.) *Madera y Bosques*, 24 (2), pp. e2421582. https://doi.org/10.21829/myb.2018.2421582

Stewart W. C., Whitney T. R., Scholljegerdes E. J., Naumann H. D., Cherry N. M, Muir J. P., Lambert B. D., Walker J. W., Adams R. P., Welch K. D., Gardner D. R., Estell R. E., 2015. Effects of Juniperus species and stage of maturity on nutritional, in vitro digestibility, and plant secondary compound characteristics, *Journal of Animal Science*, Volume 93, Issue 8, August, Pages 4034–4047. https://doi.org/10.2527/jas2015-9274

Theodorou, M.K., Williams, B.A., Dhanoa, M.S. and McAllan, A.B. and France J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant's feeds, *Animal Feed Science and Technology*, 48, pp. 185-197, https://doi.org/10.1016/0377-8401(94)90171-6

Van Soest, P.J., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, pp. 3583-3597, https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vázquez-Carrillo M. F., Montelongo-Pérez H. D., González-Ronquillo M., Castillo-Gallegos E., and Castelán-Ortega O. A., 2020. Effects of Three Herbs on Methane Emissions from Beef Cattle. *Animals*, 10 (9), pp. 1671. https://doi.org/10.3390/ani10091671

Zaidi, M., Ahsan, H. and Crow, S., 2006. Pharmacological Screening of Arceuthobium oxycedri (Dwarf Mistletoe) of Juniper Forest of Pakistan. *OnLine Journal of Biological Sciences*. https://doi.org/10.3844/ojbsci.2006.67.70

Zhang, Y., MacAdam, J. W., Villalba, J. J., and Dai, X., 2021. In vitro digestibility of mountain-grown irrigated perennial legume, grass and forb forages is influenced by elevated non-fibrous carbohydrates and plant secondary compounds. *Journal of the science of food and agriculture*, 101 (1), pp. 334–340. https://doi.org/10.1002/jsfa.10648