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]

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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; neutral 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 36.45-467.43 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 rate c on average was 0.04 g/kg DM. The TP, TT and CT content was different between M (P<0.05), the highest was found in YM with no effect observed in AC. The gas production rate c on average was 0.04 g/kg DM. The TP, TT and CT content was different between M (P<0.05), the highest was found in BM. The NDF and ADF content was different between M and the AC, containing secondary metabolites such as total phenols and condensed tannins and have an impact on in vitro fermentation. 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 vitro.


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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; Permentky 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 (≥ 30 cm < 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 Thedodorou 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):

\[ G = A \times (1 \Leftrightarrow 0.5^{(-e - c(t - L))}) \]

where:
- \( G \) = total cumulative gas production over time (mL);
- \( A \) = 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
- \( L \) = 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:

\[ Y_{ijk} = \mu + M_i + AC_j + (M \times AC)_{ij} + e_{ijk} \]

Where:
- \( \mu \) = overall mean of the treatments
- \( M_i \) = effect of the i-th mistletoe species (i=2).
- \( AC_j \) = effect of the j-th tree age category (j=4).
- \( (M \times AC)_{ij} \) = 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 age 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 established 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.
was observed in the yellow mistletoe, and in general categories of trees sampled. Significant differences (P<0.05) were observed in B between mistletoe species and age categories. Signiﬁcant differences (P<0.05) were found between mistletoe species in total tannins content; total tannins content had the highest values in yellow mistletoe. Significant differences (P<0.05) were found between mistletoe species for condensed tannins content, with the highest values found in yellow mistletoe.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mistletoe species</th>
<th>Small sapling</th>
<th>Large sapling</th>
<th>Juvenile</th>
<th>Adult</th>
<th>SEM</th>
<th>M</th>
<th>AC</th>
<th>M*AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>Yellow</td>
<td>352.39</td>
<td>351.30</td>
<td>351.83</td>
<td>385.90</td>
<td>14.80</td>
<td>&lt;0.0001</td>
<td>0.0048</td>
<td>0.2897</td>
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<tr>
<td></td>
<td>Black</td>
<td>412.62</td>
<td>401.62</td>
<td>406.46</td>
<td>417.01</td>
<td>&lt;0.0001</td>
<td>0.9569</td>
<td>0.5885</td>
<td></td>
</tr>
<tr>
<td>OM</td>
<td>Yellow</td>
<td>925.58</td>
<td>928.05</td>
<td>932.12</td>
<td>922.24</td>
<td>10.85</td>
<td>&lt;0.0001</td>
<td>0.0146</td>
<td>0.0097</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>949.59</td>
<td>948.20</td>
<td>948.83</td>
<td>953.31</td>
<td>&lt;0.0001</td>
<td>0.1391</td>
<td>0.0345</td>
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</tr>
<tr>
<td>NDF</td>
<td>Yellow</td>
<td>364.45</td>
<td>391.37</td>
<td>399.73</td>
<td>409.23</td>
<td>12.62</td>
<td>&lt;0.0001</td>
<td>0.1920</td>
<td>0.8851</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>467.43</td>
<td>464.53</td>
<td>459.72</td>
<td>459.46</td>
<td>9.20</td>
<td>&lt;0.0001</td>
<td>0.1002</td>
<td>0.1920</td>
</tr>
<tr>
<td>ADF</td>
<td>Yellow</td>
<td>270.18</td>
<td>292.93</td>
<td>291.52</td>
<td>300.66</td>
<td>9.75</td>
<td>&lt;0.0001</td>
<td>0.1920</td>
<td>0.8851</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>337.05</td>
<td>334.91</td>
<td>331.17</td>
<td>333.22</td>
<td>7.75</td>
<td>&lt;0.0001</td>
<td>0.1920</td>
<td>0.8851</td>
</tr>
<tr>
<td>CP</td>
<td>Yellow</td>
<td>65.33</td>
<td>66.12</td>
<td>62.08</td>
<td>62.94</td>
<td>5.78</td>
<td>0.1002</td>
<td>0.9206</td>
<td>0.8851</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>56.88</td>
<td>63.63</td>
<td>60.25</td>
<td>58.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mistletoe species</th>
<th>Small sapling</th>
<th>Large sapling</th>
<th>Juvenile</th>
<th>Adult</th>
<th>SEM</th>
<th>M</th>
<th>AC</th>
<th>M*AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>Yellow</td>
<td>6.93</td>
<td>6.74</td>
<td>6.68</td>
<td>6.75</td>
<td>0.43</td>
<td>&lt;0.0001</td>
<td>0.9556</td>
<td>0.888</td>
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<tr>
<td></td>
<td>Black</td>
<td>5.59</td>
<td>5.86</td>
<td>5.66</td>
<td>5.70</td>
<td>&lt;0.0001</td>
<td>0.5076</td>
<td>0.5314</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>Yellow</td>
<td>6.16</td>
<td>6.07</td>
<td>5.97</td>
<td>6.06</td>
<td>0.46</td>
<td>&lt;0.0001</td>
<td>0.5076</td>
<td>0.5314</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>4.65</td>
<td>5.34</td>
<td>4.82</td>
<td>5.25</td>
<td>&lt;0.0001</td>
<td>0.5076</td>
<td>0.5314</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Yellow</td>
<td>1.44</td>
<td>1.35</td>
<td>1.32</td>
<td>1.31</td>
<td>1.03</td>
<td>&lt;0.0001</td>
<td>0.6898</td>
<td>0.8766</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>1.14</td>
<td>1.10</td>
<td>1.15</td>
<td>1.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
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* (Gebeeyew 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

**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* (Gebeeyew 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

**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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mistletoe specie</th>
<th>Age Category</th>
<th>SEM</th>
<th>M</th>
<th>AC</th>
<th>M*AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Yellow</td>
<td>Small sapling</td>
<td>84.06&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>4.68</td>
<td>0.0001</td>
<td>0.7352</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Large sapling</td>
<td>85.50&lt;sup&gt;A&lt;/sup&gt;</td>
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<td>0.01</td>
<td>0.5869</td>
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<td>0.01</td>
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<td>0.0071</td>
<td>0.2256</td>
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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).
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 antiprotzol 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 lignocellulosic 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 (Avila-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.

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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.

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