Article

Chemical composition and digestibility of four Mexican tropical legumes

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Abstract

In Mexico, the nutritional properties of tropical legumes are not widely known. Therefore, the objective of the present study was to evaluate the nutritional value of *Arachis pintoi*, *Clitoria ternatea*, *Macroptilium atropurpureum* and *Stylosanthes guianensis*. Crude protein (CP), crude fiber (CF), ether extract (EE), ash (ASH), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude lignin (CL), *in vitro* digestibility of dry matter (IVDDM) and *in situ* digestibility DM (DISDM). The samples were collected in the experimental plots of the National Institute of Forest, Agricultural and Livestock Research, Veracruz state, Mexico. The evaluations were carried out in the facilities of the Zootechnics Department, Autonomous University Chapingo, State of Mexico, Mexico. Each species was evaluated, with three repetitions and each repetition was considered the experimental unit, resulting in twelve observations per variable. The means were separated using the Tukey test ($p< 0.05$). *Arachis pintoi* showed the highest percentages of CP (25.2%), ASH (9.0%), IVDDM (83.2%) and ISDDM (88.0%), and the lowest values of NDF (32.8%), ADF (26.9%) and CL (5.5%). *Macroptilium atropurpureum* showed the lowest percentages of CP, IVDDM, IVDDM and ASH (6.4%), and the highest values of NDF (50.9%), ADF (37.6%) and CL (11.8%). The other legumes showed intermediate or low values of all variables. Therefore, based on chemical composition and, *in vitro* and *in situ* dry matter digestibility, *Arachis pintoi* was the species with the best nutritional value.

Keywords: *Arachis pintoi*, *Clitoria ternatea*, *Macroptilium atropurpureum* and *Stylosanthes guianensis*.

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Introduction

The nutritional quality of forages in the Mexican tropics is decisive for feeding ruminants (Estrada *et al*., 2019). In tropical areas, livestock production improves when high nutritional value forage is available, which meets the animal's requirements (Rincón *et al*., 1992). Due to their wide productivity and quality, native legumes can be used in animal production systems in Mexico (Alatorre-Hernández *et al*., 2018).

In most of Mexico's tropical ecosystems, there is an abundance of legumes (Piñeiro-Vázquez, 2017), compared to grasses, these plants have more protein and less fiber (Solati *et al*., 2017). Legumes increase voluntary consumption and improve rumen functioning (Sahay *et al*., 2016). Legumes, in addition to improving animal production, fix atmospheric nitrogen (Clua *et al*., 2018), both for their own growth and for that of grasses and other crops (Sahay *et al.*, 2016; Burger and Zipper, 2018). Legumes with a temperate climate, such as lucerne (*Medicago sativa*), white clover (*Trifolium repens*) and red clover (*Trifolium pratense*), have been widely studied and are still being studied in Mexico (Camacho-García and García-Muñiz, 2003; Castrejon *et al.*, 2017).

However, in tropical conditions there is a diversity of legumes such as the genera: *Arachis*, *Clitoria*, *Centrosema*, *Cratylia*, *Desmodium*, *Lablab*, *Leucaena*, *Macroptilium*, *Pueraria*, *Stylosanthes* and *Vigna* (Castrejon *et al.*, 2017; Singh *et al.*, 2018). In the state of Veracruz, animal production is based on native pastures composed of the grasses *Paspalum* sp., *Axonopus* sp., *Setaria* sp. and *Leucaena* sp., *Desmodium* sp. and *Centrosema* sp. legumes (Hernandez *et al*., 1990; Castrejon *et al*., 2017).

Although the numerical ranges of the evaluated variables have already been determined, no information was found on the relationship between these variables. The CP varied from 12.6 g $(100 g)^{-1}$ to 21.8 g $(100 g)^{-1}$, with the lowest value for *S. guianensis* and the highest for *A. pintoi*. In the literature consulted, the ASH variable was 6.7 g $(100 g)^{-1}$, 10.9 g $(100 g)^{-1}$ and 12.0 g $(100 g)^{-1}$ for *C. ternatea*, *A. pintoi* and *S. guianensis*, respectively. The CC variable was 47.7 g $(100 g)^{-1}$ and 45.2 g $(100 g)^{-1}$ for *S. guianensis* and *A. pintoi*, respectively (Kavana *et al.*, 2005; García-Ferrer *et al.*, 2015).

Lagunes-Rivera *et al*. (2019) found that *A. pintoi* showed the highest values of CC (39 g $(100 g)^{-1}$ against 35 g $(100 g)^{-1}$ of *S. guianensis*), the lowest of NDF (61 g $(100 g)^{-1}$ against 65 g $(100 g)^{-1}$ of *S. guianensis*), the lowest in the ADF (35 g $(100 g)^{-1}$ against 44 g $(100 g)^{-1}$ of *S. guianensis*), the highest of CP (21 g $(100 g)^{-1}$ against 19 g $(100 g)^{-1}$ of *S. guianensis*) and the highest of IVDDM (74 g $(100 g)^{-1}$ against 62 g $(100 g)^{-1}$ of *S. guianensis*). In general, in the literature consulted, high values of CP correspond to high values of CC, low NDF, high IVDDM and high DISDM.

In addition, tropical forage legumes have been little used in animal feed, probably due to the lack of commercial seed and lack of knowledge of its management, among other causes. Although there is abundant information on agronomic evaluations in forage legumes, the attributes of these species for the animal are poorly understood (Alatorre-Hernández *et al*., 2018). So, knowing the characteristics of these legumes is essential to improve animal production (Valles-de la Mora *et* *al*., 2017). Therefore, the objective of the study was to evaluate the chemical composition and *in vitro* and *in situ* digestibility of *Arachis pintoi*, *Clitoria ternatea*, *Macroptilium atropurpureum* and *Stylosanthes guianensis*.

Materials and methods

Location

Samples were obtained from the 'La Posta' Experimental Field belonging to the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), located in Paso del Toro, Medellin, Veracruz, between parallels 18° 50' and 19° 09' north latitude and the meridians 96° 02' and 96° 16' west longitude, 50 to 55 masl, average annual temperature 24 to 28 °C, precipitation 1 100 to 1 600 mm, average relative humidity 31% (INEGI, 2009).

Obtaining the samples

Plants (30 days old) were manually cut, bagged and labeled and dried at 55 °C in a forced air oven to constant weight. Subsequently, they were ground and identified, placed in plastic bags for analysis in the Animal Nutrition laboratory of the Department of Zootechnics of the Autonomous University Chapingo (UACH), state of Mexico.

Determined variables

The following variables were determined (AOAC, 1990; Van Soest *et al.*, 1994; Giraldo *et al.*, 2007; Navarro-Ortiz and Roa-Vega, 2018), all in g $(100\ g)^{-1}$ of sample: dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), ash (ASH), organic matter (OM), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude lignin (CL), hemicellulose (HEM), cellulose (CEL), cellular content (CC), *in vitro* digestibility of dry matter (IVDDM) and *in situ* digestibility of DM (DISDM).

In vitro and in situ digestibility of dry matter

In a 100 cm^3 plastic tube, 0.5034 ±0.002 g of sample was weighed, 20 cm^3 of Mc Dougall's saliva were added as buffer and 5 cm^3 of rumen liquid. The mixture was incubated at 39 °C for 48 hr, with shaking every 12 hr. The residual NDF was determined and the following formula was applied to calculate the digestibility of the sample in g $(100\ g)^{-1}$: IVDDM= 100-(residual NDF/g of sample)×100. Anaerobic conditions were achieved using a CO_2 stream. The ruminal fluid was obtained from a fistulated Holstein bovine (Giraldo *et al.*, 2007; Navarro-Ortiz and Roa-Vega, 2018), who was consuming an alfalfa-based diet. DISDM was determined similarly but 5 × 5 cm Ankom® bags (Ankom Co., Fairport, NY, USA) were used instead of a plastic tube. 0.5402 ± 0.0472 g of sample were placed in the bags, the same fistulated Holstein bovine was used and the bags remained 48 h in the rumen. After this time, the bags were dried at 100 °C, the residual NDF was determined, and a formula similar to the above was used for the calculation of DISDM.

Experimental design and statistical analysis

A completely randomized experimental design was used with four treatments (four legumes), three repetitions for the variables DM, CP, CF, EE, ASH, MO, NDF, CL, CEL, HEM and CC and four repetitions for the IVDDM variables and DISDM. After performing the analysis of variance, the Tukey test was used for the separation of means ($p< 0.05$), using the Statistical Package for the Social Sciences (SPSS, 2011) version 8.0.

Results and discussion

Proximal analysis

The variables evaluated from this analysis (Table 1) were: crude protein (CP), ash (ASH), ethereal extract (EE), crude fiber (CF), organic matter (OM) and nitrogen-free extract (NFE). Only CP, ASH and OM were statistically different between legumes. *A. pintoi* 25.2 g (100 g)$^{-1}$, showed the highest value of CP, followed by *C. ternatea* 22.7 g (100 g)$^{-1}$; while *S. guianensis* 16.2 g (100 g)$^{-1}$ and *M. atropurpureum* 16.5 g (100 g)$^{-1}$, showed the lowest values. The ASH variable was ($p< 0.05$) the highest in *A. pintoi* 9 g (100 g)$^{-1}$, intermediate in *S. guianensis* 8.1 g (100 g)$^{-1}$ and the lowest in *C. ternatea* 6.8 g (100 g)$^{-1}$ and *M. atropurpureum* 6.4 g (100 g)$^{-1}$. These CP and ASH values were close to those of the consulted literature, with plant ages between 30 and 60 days.

Table 1. Proximal analysis on a dry basis, g (100)$^{-1}$, of four legumes from the state of Veracruz, Mexico.

Species	CP	ASH	CF	EE	OM	NFE
Clitoria ternatea	22.7 b	6.8 c	40.3	3.1	93.2 a	26.9
Macroptilium atropurpureum	16.5 c	6.4 c	35.9	3.2	93.5 a	37.9
Stylosanthes guianensis	16.2 c	8.1 b	37.5	2.4	91.8 b	35.5
Arachis pintoi	25.2 a	9.0 a	27.7	2.7	90.9 c	35.4

Means in the same column are statistically different literal different (Tukey, $p< 0.05$). CP= crude protein; ASH= ashes; CF= crude fiber; EE= ethereal extract; OM= 100-ASH= organic material; NFE = nitrogen free extract.

García-Ferrer *et al*. (2015) reported 21.8 g (100 g)$^{-1}$ of CP for *A. pintoi*, 18.3 g (100 g)$^{-1}$ for *C. ternatea* and 14.5 g (100 g)$^{-1}$ for *S. guianensis*. Kavana *et al*. (2005) reported 15.3 g (100 g)$^{-1}$ for *C. ternatea*, 13.4 g (100 g)$^{-1}$ for *M. atropurpureum* and 12.6 g (100 g)$^{-1}$ for *S. guianensis*. That is, the order of CP found in the present study is conserved between species, with *A. pintoi* showing the highest values of CP. Sotelo *et al*. (2018) and Oyekunle *et al*. (2018) reported 12.0 g (100 g)$^{-1}$ and 10.9 g (100 g)$^{-1}$ of ASH for *S. guianensis* and *A. pintoi*, respectively (high values). La O *et al*. (2006) reported 8.3 g (100 g)$^{-1}$ of ASH for *M. atropurpureum* and Bugarin *et al*. (2009) reported 6.7 g (100 g)$^{-1}$ of ASH for *C. ternatea* (low values). In this study also, *A. pintoi* and *S. guianensis* showed the highest ASH values and the other two legumes the lowest values.

All the legumes studied showed CP values greater than 16 g (100 g)$^{-1}$. But *A. pintoi* and *C. ternatea* showed CP values greater than 22 g (100 g)$^{-1}$, which makes them good sources of protein for animals in the tropics. Probably due to the nutritional quality of *A. pintoi* and *C. ternatea*, Jusoh and Nur-Hafifah (2018) found a higher preference in rabbits for *A. pintoi*, followed by *C. ternatea* and less for *S. guianensis*.

Van Soest's analysis

The variables evaluated from this analysis (Table 2) were: neutral detergent fiber (NDF), acid detergent fiber (ADF), crude lignin (CL), hemicellulose (HEM), cellulose (CEL) and cellular content (CC).

Table 2. Van Soest analysis on dry basis g (100)$^{-1}$, of four legumes from the state of Veracruz, Mexico.

Species	NDF	ADF	CL	HEM	CEL	CC
Clitoria ternatea	46.5 ab	38.7 a	8.8 b	7.8 bc	38.9 a	53.4 bc
Macroptilium atropurpureum	50.9 a	37.6 a	11.8 a	13.3 a	37.7 a	49.1 c
Stylosanthes guianensis	44.3 b	32.4 b	5.1 c	11.8 ab	34 b	55.7 b
Arachis pintoi	32.8 c	26.9 c	5.5 c	5.8 c	28 c	67.2 a

Means in the same column are statistically different literal different (Tukey, $p< 0.05$). NDF = neutral detergent fiber; ADF = acid detergent fiber; CL= crude lignin; HEM= hemicellulose; CEL= cellulose; CC= 100- NDF= cellular content.

Cell content

Legume *A. pintoi* produced the highest values 67.1 g (100 g)$^{-1}$ of CC (non-fibrous compounds) and *M. atropurpureum*, showed the lowest values 49 g (100 g)$^{-1}$. *S. guianensis* was intermediate and *C. ternatea* was low-intermediate in this variable that represents the non-fibrous or soluble compounds (Table 2). The CC values shown by *A. pintoi* were higher than those reported by Castaño and Cardona (2015) who found 47.7 g (100 g)$^{-1}$. At different ages (20 to 80 days old) and times of the year, García Ferrer *et al*. (2015) found CC values from 32.8 g (100 g)$^{-1}$ to 55.2 g (100 g)$^{-1}$ in *A. pintoi*; likewise, in *S. guianensis* they found values from 25.9 g (100 g)$^{-1}$ to 42.9 g (100 g)$^{-1}$. That is, according to these authors, *A. pintoi* tends to be better than *S. guianensis* in this variable.

Fiber variables

Contrary to cellular content, *A. pintoi* produced the lowest values of all the fiber variables: NDF, ADF, CL, HEM, and CEL and *M. atropurpureum* showed the highest values. *A. pintoi* together with *S. guianensis* showed the lowest CL values (Table 2). García-Ferrer *et al*. (2015) found NDF values of 54.8 g (100 g)$^{-1}$ for *A. pintoi*, 57.1 g (100 g)$^{-1}$, for *S. guianensis* and 57 g (100 g)$^{-1}$ for *C. ternatea*. Lagunes-Rivera *et al*. (2019) found ADF values of 35 g (100 g)$^{-1}$ for *A. pintoi* and 44 g (100 g)$^{-1}$ for *S. guianensis*.

These values are greater than those of the present study, probably because the intervals between cuts were greater than 30 days in the works of these authors. But, although these values of the fiber variables are high compared to those of the present study, they maintain the relationship of low values of *A*. *pintoi* and high values of the other legumes. In a study by Lagunes-Rivera *et al*. (2019), the species *A*. *pintoi* had lower fiber content, contrasting with the other herbaceous legumes that they studied.

In vitro and *in situ* digestibility of dry matter

Legume *A*. *pintoi* showed the highest values of IVDDM and DISDM, and *M*. *atropurpureum* showed the lowest values of these variables. *S*. *guianensis* and *C*. *ternatea* showed intermediate values (Table 3). The IVDDM values for *A*, *pintoi*, *S*. *guianensis*, *C*. *ternatea* and *M*. *atropurpureum* were: 84.5 g (100) $^{-1}$, 74 g (100) $^{-1}$, 68.9 g (100) $^{-1}$ and 63 g (100) $^{-1}$, respectively. The DISDM values, in the same order of these legumes, were: 88 g (100) $^{-1}$, 73.7 g (100) $^{-1}$, 69.9 g (100) $^{-1}$ and 56.6 g (100) $^{-1}$, respectively. That is, *A*, *pintoi* showed the highest digestibility and *M*. *atropurpureum* showed the lowest value of this variable.

Table 3. *In vitro* and *in situ* digestibility on a dry basis, g (100 g)$^{-1}$, of four legumes from the state of Veracruz, Mexico.

Species	IVDDM	DISDM
Clitoria ternatea	68.9 c	69.9 b
Macroptilium atropurpureum	63 d	56.6 c
Stylosanthes guianensis	74 b	73.7 b
Arachis pintoi	84.5 a	88 a

Means in the same column are statistically different literal different (Tukey, $p< 0.05$). IVDDM= *in vitro* digestibility of dry matter, DISDM = *in situ* digestibility of dry matter. Both were determined using Holstein bovine ruminal fluid, with a diet based on alfalfa, the main forage legume in the study area.

García-Ferrer *et al*. (2015) found, at 21 days of regrowth, DISDM values of 80.2 g (100), 74 g (100)$^{-1}$ and 76 g (100) $^{-1}$ for *A*. *pintoi*, *S*. *guianensis* and *C*. *ternatea*, respectively. Values similar to those of the present study (Table 3). Lagunes-Rivera *et al*. (2019) found that *A*. *pintoi* showed the highest IVDDM values compared to three other forage legumes from the tropical region of the state of Puebla, Mexico.

The IVDDM and DISDM values of *A*. *pintoi* were very good, which implies that, if the cellular content is digested by 99 g (100) $^{-1}$ (NRC, 2001), then the *in vitro* and *in situ* digestibilities of the NDF it will be 51.1 g (100) $^{-1}$ and 65.8 g (100) $^{-1}$, respectively.

Correlations between the variables studied

IVDDM and DISDM showed a positive correlation with ASH ($p< 0.05$). On the other hand, ASH showed a positive correlation ($p< 0.05$) with CC. Consequently, CC had ($p< 0.05$) positive correlation with IVDDM and DISDM (Table 4). This correlation was also deduced from the positive correlation ($p< 0.05$) between CC and CP. In other words, the greater the cellular content, the greater the digestibility.

Table 4. Significant correlations between the determined variables of four legumes from the state of Veracruz, Mexico.

Correlations	r	Significance
ASH vs. IVDDM	0.945	**
ASH vs. DISDM	0.904	**
CC vs. ASH	0.89	*
CC vs. IVDDM	0.938	**
CP *vs* CC	0.703	*
CP *vs* DISDM	0.707	*
IVDDM *vs* CP	0.707	*
DISDM *vs* IVDDM	0.974	**
ADF *vs* CEL	0.992	**
CP *vs* NDF	-0.703	*
NDF *vs* IVDDM	-0.938	**
NDF *vs* DISDM	-0.936	**
HEM *vs* DISDM	-0.673	*

CP= crude protein; ASH = ashes; CC= cellular content; NDF= neutral detergent fiber; ADF= acid detergent fiber; IVDDM= *in vitro* digestibility of dry matter; DISDM= *in situ* digestibility of dry matter; CEL= cellulose; HEM= hemicellulose; r= Pearson's correlation coefficient. *= $p< 0.05$; **= $p< 0.01$.

CP was positively correlated with CC and this variable had a positive correlation with IVDDM and DISDM (Table 4), therefore, CP had a positive correlation with digestibilities ($p< 0.05$). In other words, the greater the protein, the greater the digestibility.

As expected, the ADF was positively correlated ($p< 0.05$) with CEL (Table 4). This is because cellulose is a component of the cell wall (NDF). The NDF was negatively correlated ($p< 0.05$) with CP and the digestibilities ($p< 0.05$). In agreement, García-Ferrer *et al*. (2015) found a negative correlation ($p< 0.01$) between NDF and DISDM. Likewise, in this study, HEM (component of the NDF) was ($p< 0.05$) negatively correlated with DISDM (Table 4). That is, the higher the content of the fiber variables, the lower the protein and the lower the digestibility.

These negative correlations indicate that as the fibrous fraction of the plant is high, either due to its older age, due to environmental differences (Castaño and Cardona, 2015) or for genetic reasons, its fibrous components increase and its cellular contents decrease. The first two factors are excluded, since the four legumes were the same cutting age and were affected by the same environmental factors. As the fiber is partially soluble and the cellular content is highly soluble (NRC, 2001), it follows that the digestibility decreases because the fibrous fraction of the legume increases.

Conclusions

The four legumes presented an acceptable percentage of crude protein; however, *Arachis pintoi* and *Clitoria ternatea* presented the highest levels. Also *A. pintoi* presented the lowest levels of neutral detergent fiber, acid detergent fiber, hemicellulose and lignin, as well as the highest levels of cellular content and digestibility, being in this sense the species with the best nutritional value. If the variables neutral detergent fiber, cellulose and hemicellulose, increase, the *in vitro* and *in situ* digestibilities decrease. On the contrary, if the crude protein and the cellular content increase, the digestibilities increase.

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