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Technical note

Dry matter yield and nutritional values of four herbaceous legumes in a humid tropical environment in Hueytamalco, Puebla, Mexico

Sergio Alberto Lagunes Rivera [a]

Juan de Dios Guerrero-Rodríguez [a*]

Josafath Omar Hernández-Vélez [b]

José de Jesús Mario Ramírez-González [b]

Dulce Violeta García-Bonilla [a]

Antonio Alatorre-Hernández [a]

[a] Colegio de Postgraduados, Campus Puebla. Boulevard Forjadores de Puebla, No. 205 Santiago Momoxpan, 72760 San Pedro Cholula, Puebla, México.

[b] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. México.

* Corresponding author: rjuan@colpos.mx

Abstract:

Dry matter yield and nutritional data are needed before forage legumes can be proposed for inclusion in livestock systems. An evaluation was done of the dry matter (DM) yield, concentrations of neutral and acid detergent fiber (NDF and ADF) and crude protein (CP), and *in vitro* DM digestibility (IVDMD) of the forage legumes *Stylosanthes guianensis* (SG), *Centrosema macrocarpum* (CM), *Pueraria phaseoloides* (PP) and *Arachis pintoi* (AP). During an eleven-month period six cuts were made of these legumes at 56-d intervals. Experimental plots were 3 x 7 m, and analyses were done using a completely random block design with four replicates. Overall DM yield was higher ($P \leq 0.05$) in SG (19,410 kg DM ha^{-1}) and CM (17,462 kg DM ha^{-1}), than in PP (14,704 kg DM ha^{-1}) and AP (12,466 kg DM ha^{-1}). *Arachis pintoi* had the lowest NDF (60%) and ADF (35 %) contents ($P \leq 0.05$). Digestibility was highest in AP (74 %), followed by SG (62%), PP (57 %) and CM (55 %). Crude protein content was highest ($P \leq 0.05$) in AP and CM

(21 %) followed by SG (19 %) and PP (17.8 %). Although DM yield was highest in SG and CM, and digestibility and crude protein content were highest in AP, all four forage legume species exhibited sufficiently high nutritional values and DM production to make them alternative protein sources for ruminants with low-nutrient diets.

Key words: Nutritional quality, Yield, Herbaceous legumes.

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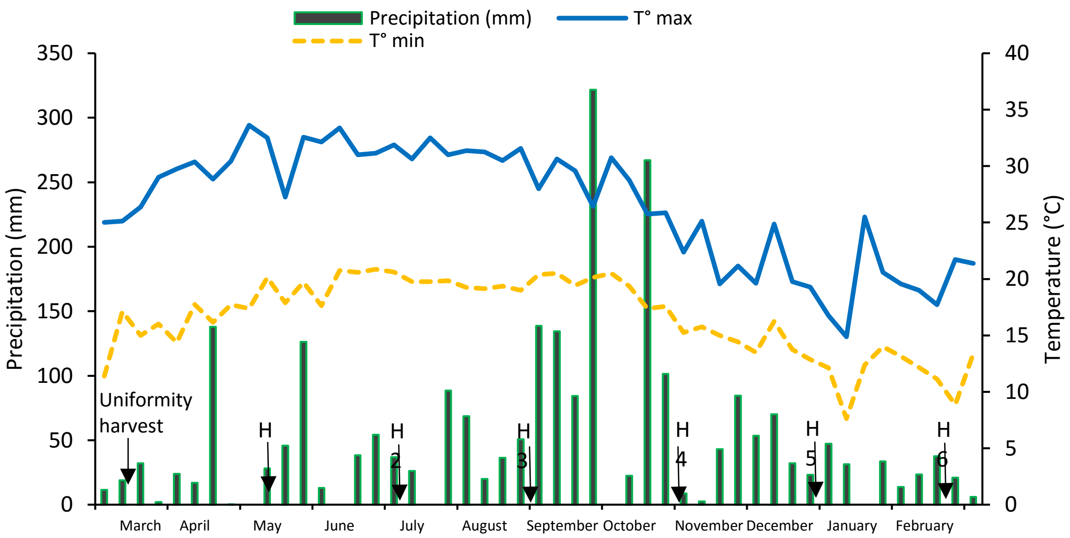
In tropical Mexico, ruminant livestock production depends largely on the grazing of native and introduced grasses. These usually require high levels of nitrogen fertilization and experience substantial declines in protein content and digestibility as they mature[1,2]. Indeed, depending on age, in some grass species protein content can be as little as 7 %, which can prove limiting to ruminant production[3].

One way of improving the diet of grazing ruminants is to include herbaceous legume forage species, which, compared to grasses, maintain a high nutritional value throughout their biological cycle[1,4]. Depending on the species, tropical herbaceous legumes can attain crude protein contents ranging from 19 to 22 %[5], and dry matter digestibility values of 58 to 72 %[6]. When associated with grasses their ability to fix nitrogen in the soil can increase grass dry matter yield and nutritional quality[7,8]. They can also be used in monoculture or as protein banks. The legumes *Stylosanthes guianensis*, *Centrosema macrocarpum*, *Pueraria phaseoloides* and *Arachis pintoi* are proven to be important forage resources in the development of livestock production systems in wet and dry tropical regions[9,10,11]. *Stylosanthes guianensis* is a semi-erect evergreen herbaceous legume[9] similar to *C. macrocarpum*, although the latter is less woody than the former. *Pueraria phaseoloides* and *A. pintoi* are long-stemmed vines and with a creeping growth habit[12,13]. Both can adapt to soils ranging from clay to sandy, usually with an organic matter content above 3 %[12,14].

Introduction of these species to a given region requires prior knowledge of their productive behavior, and any possible environmental factors that could influence dry matter yield and nutritional value. Production data is available for each species but few comparisons have been made between them under the same edaphoclimatic conditions. This is vital when deciding which of them to plant. Evaluation of species dry matter yield and nutritional value is basic to developing supplementation strategies and optimizing use of available fodder[4]. The present study objective was to evaluate the dry matter yield and nutritional value of four herbaceous legume forage species in a warm humid tropical area of the state of Puebla, Mexico.

The experiment was run at the Las Margaritas Experimental Station of the National Institute of Agricultural and Livestock Forest Research (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias), in the Municipality of Hueytamalco, Puebla, under seasonal conditions. The station is located at 20°00’ N and 97°18’ W, and an altitude of 450 m asl. Regional climate is Af(c), with a 21 °C average annual temperature, 3,000 mm average annual rainfall and 90 % average relative humidity(15). During the study period, accumulated rainfall was 1200.7 mm, maximum temperature ranged from 14.9 to 33.6 °C, and minimum temperature from 7.6 to 21 °C (Figure 1). Soil in the study area was clayey with a pH of 4.4, EC of 0.26 mmhos cm^{-1} and organic matter content of 5.2 %. Nutrient content was poor in N (0.004 %), P (4.06 ppm), B (0.166 ppm) and Zn (4.66 ppm), rich in K (150 ppm), Ca (570 ppm) and Cu (520 ppm), and had average Mg levels (73.33 pm). The four legume species were evaluated for eleven months (March 2009 to February 2010). After an initial cut 45 d post-planting to create a homogenous height, successive cuts were done every 56 d, for a total of six cuts (Figure 1). Cut dates were 16 May, 12 July, 6 September, 2 November, and 28 December 2009, and 24 February 2010.

Figure 1: Temperature and rainfall during experimental period (March 2009 to February 2010). Arrows indicate cut dates of four forage legumes



Soils were prepared by plowing and raking. Fertilizer (00-80-60 N-P-K ha^{-1}) in the form of tricalcium superphosphate and potassium chloride was applied once at the time of planting. Seeds of three of the legumes [*Styloshanthes guianensis* (Aubl.) Sw. (cv Ubon); *Centrosema macrocarpum* Benth (CIAT 5713); and *Pueraria phaseoloides* (Roxb.) Benth (cv Jarocha)], were scarified with water at 75 °C for 3 min. They were then sown by aerial spreading in experimental units of 21 m^2 (3 x 7 m) at a density of 35 kg seed ha^{-1}; the seeds of these species have physical latency, and they were 3 yr-old prior to the trial. *Arachis pintoi* Krapov and W. C. Greg. (CIAT 17434) was planted using vegetative material (stems with roots of 2 to 4 nodules) with 10 cm between plants.

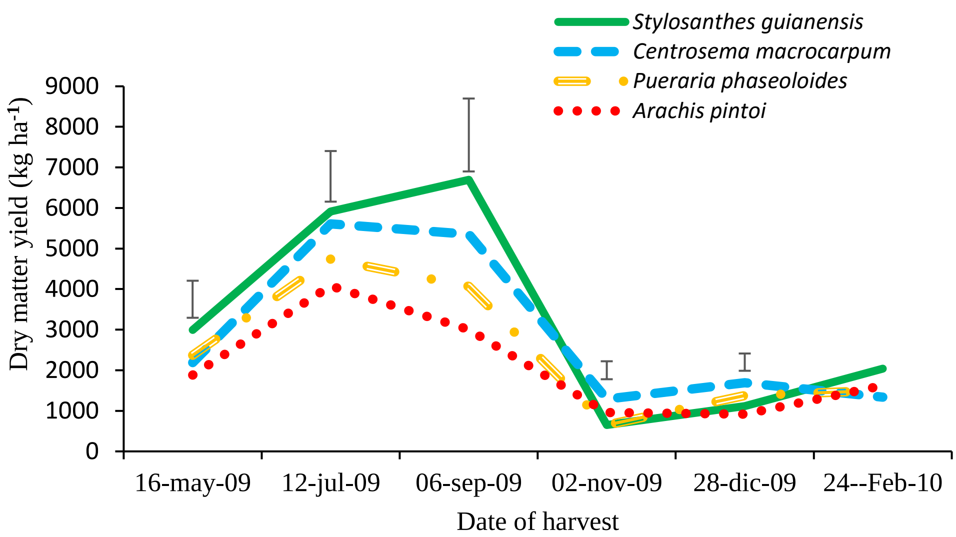
Vegetal material samples were collected every 56 d by randomly placing a 0.25 x 0.25 m metal frame inside each experimental unit. Within this quadrant all aerial biomass was removed from each plant(11); for *A. pintoi* this was done at 10 cm above soil surface, and for *S. guianensis*, *C. macrocarpum* and *P. phaseoloides* at 15 cm above soil surface. The collected matter was weighed fresh and stored in marked paper bags. The leaf-to-stem ratio of each species was measured by separately weighing the leaf and stem fractions of each plant and dividing leaf weight by stem weight. All samples were dried in a forced-air stove at 55 °C to constant weight.

Dried samples were ground in a cyclone mill until passing through 1 mm mesh. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured sequentially and in duplicate with a fiber analyzer (ANKOM 200/220) following manufacturer protocols(16). Crude protein (CP) was measured in duplicate with the Kjeldahl method, multiplying nitrogen percentage by 6.25(17). *In vitro* dry matter digestibility (IVDMD) was measured in duplicate following the pepsin-cellulase enzymatic method(18,19).

Because the experimental parcel was sloped, a completely random block design with four repetitions was used; each legume species constituted a treatment. Data were analyzed with a divided plots arrangement in which the largest plots were each legume species and the smallest plots were each cut. An analysis of variance was run and the means compared with the Tukey test. All statistical analyses were done with the SAS ver. 9.0 statistical package(20).

Dry matter (DM) production differed ($P \leq 0.05$) between the four studied legume species and between cuts. In most of the cuts AP had the lowest yields, although it did not differ from PP. Overall, the four legumes tended to increase DM production from cut 2 to cut 3 (Figure 2), but from cut 4 to cut 6 production decreased substantially. Total DM production during the study period was highest in SG (19,410 kg DM ha^{-1}) and CM (17,462 kg DM ha^{-1}), and lowest in PP (14,704 kg DM ha^{-1}) and AP (12,466 kg DM ha^{-1}).

Figure 2: Dry matter (DM) yield (kg ha^{-1}) of the herbaceous legumes *Stylosanthes guianensis*, *Centrosema macrocarpum*, *Arachis pintoi* and *Pueraria phaseoloides*



Average leaf production was higher in SG, CM and PP than in AP ($P \leq 0.05$), while stem production was highest in SG ($P \leq 0.05$). The leaf-to-stem ratio was highest in PP, followed by CM ($P \leq 0.05$), and was consistently the lowest in SG (Table 1).

Table 1: Average yield for dry matter(DM) per cut, leaves and stems, and leaf-to-stem ratio values for *Stylosanthes guianensis* (SG), *Centrosema macrocarpum* (CM), *Pueraria phaseoloides* (PP) and *Arachis pintoi* (AP)

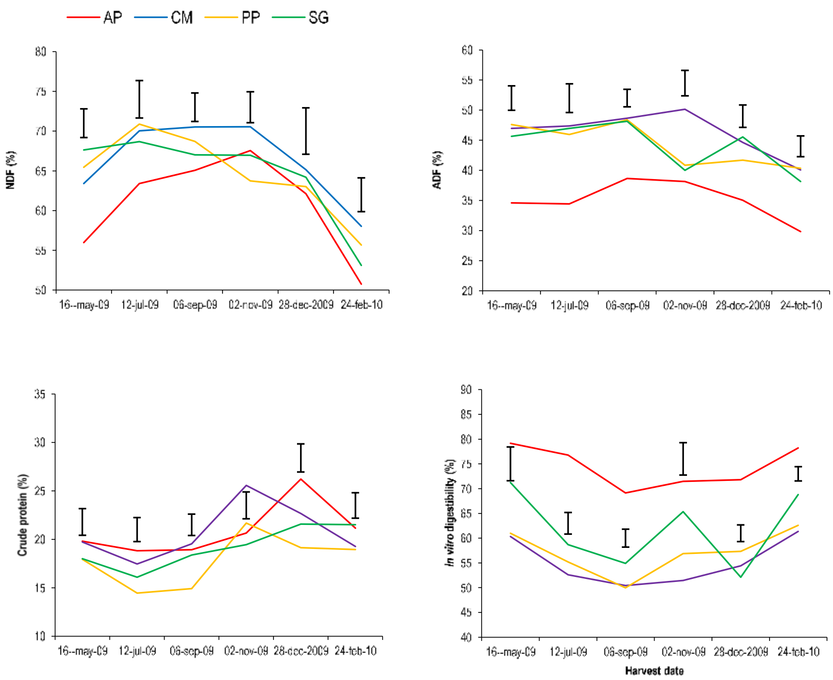
Variable	SG	CM	PP	AP	SEM
DM, kg ha^{-1}	3235.3^{a}	2910.3^{a}	2450.6^{b}	2077.8^{c}	196.0
Leaf, kg ha^{-1}	1601.5^{a}	1641.2^{a}	1467.5^{a}	1171.5^{b}	99.4
Stem, kg ha^{-1}	1633.6^{a}	1269.1^{b}	983.2^{c}	906.1^{c}	95.5
Leaf:stem	1.07^{c}	1.38^{ab}	1.57^{a}	1.45^{b}	0.048

SEM= standard error of the mean.

^{a,b,c} Different letter superscripts in the same row indicate significant difference ($P \leq 0.05$).

Average neutral detergent fiber (NDF) content ranged from 61 to 66 % and differed ($P \leq 0.05$) between all four species. Acid detergent fiber (ADF) content ranged from 35 to 46 %, and again differed ($P \leq 0.05$) between all four species. The lowest fiber content in all but one of the cuts was in AP (Figure 3).

Figure 3. Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein and *in vitro* dry matter digestibility (IVDMD) in *Stylosanthes guianensis* (SG), *Centrosema macrocarpum* (CM), *Pueraria phaseoloides* (PP) and *Arachis pintoi* (AP)



Crude protein (CP) content varied from 18 to 21 %, and was highest in AP and CM ($P \leq 0.05$) in most cuts (Figure 3; Table 2). Average IVDMD ranged from 55 to 74 %, with AP consistently having the highest levels ($P \leq 0.05$).

Table 2. Average NDF, ADF, CP and IVDMD percentages (n=24) for *Stylosanthes guianensis* (SG), *Centrosema macrocarpum* (CM), *Pueraria phaseoloides* (PP) and *Arachis pintoi* (AP)

Variable	SG	CM	PP	AP	SEM
NDF	65[a]	66[a]	65[a]	61[b]	0.591
ADF	44[b]	46[a]	44[b]	35[c]	0.575
CP	19[b]	21[a]	18[c]	21[a]	0.300
IVDMD	62[b]	55[d]	57[c]	74[a]	0.945

SEM= standard error of the mean.

[a,b,c,d] Different letter superscripts in the same row indicate significant difference ($P \leq 0.05$).

Throughout the six cuts the four legume species exhibited differential DM yield patterns in response to environmental temperature and humidity. Yields were highest when minimum temperatures exceeded 18 °C and humidity was high, but as these two environmental variables declined so did DM accumulation. This is similar to the results reported in a study of *S. guianenesis*, *A. pintoi*, *P. phaseoloides* and *Clitoria ternatea* which found that during a regrowth period of 21 to 84 days DM increase was higher (1.17 to 6.52 t ha^{-1}) during the rainy season than the dry season (0.749 to 4.37 t ha^{-1})[(9)]. These results are interpreted to indicate that these legumes' high forage potential depended on favorable humidity and temperature conditions. In the present case, the lower DM yield from November to February may have been due to the studied species inability to adapt to the cold season. The lowest average temperature during these months (November-February) was 12.9 °C. These months also coincide with the last three cuts, in which the four species exhibited the lowest DM yield. However, average DM yields per cut for the studied legumes were similar to those reported in other studies for *Stylosanthes macrocephala* (2,701 kg MS ha^{-1}), *P. phaseoloides* (2,404 kg), *A. pintoi* (1,470 kg) and *Centroscema pubescens* (2,172 kg)[(9,11,21)].

Leaf yield did not differ between SG, CM and PP, although SG and CM had higher stem production. The latter two species have a semi-erect growth habit which apparently provided them with higher cumulative DM production than the creeping vines AP and PP. The highest leaf-to-stem ratio was observed in PP. This agrees with a study of *P. phaseoloides* in which it was found to have a higher leaf-stem ratio than *S. macrocephala* and *Macrotyloma axillare*(11). Leaves apparently provide a greater portion of DM production in PP, which directly influences its advantageous composition for livestock feed. The lowest leaf-to-stem ratio values among the studied species were observed in SG. Similar observations have been made in a study of *S. guianensis* which found it to have a lower leaf-to-stem ratio than *A. pintoi*, *P. phaseoloides* and *C. ternatea* largely due to its semi-erect, semi-woody growth habit and short branches(9).

The lower NDF and ADF contents in AP compared to the other species were probably due to differences in growth habits. Semi-erect legumes such as CM and SG generally accumulate more structural carbohydrates in stems to support foliage than do creepers such as AP(22). Of note is that PP has the same growth habit as AP but its NDF and ADF contents did not differ from SG and CM. This indicates that, despite having a creeping growth habit and a higher leaf-to-stem ratio, PP contains more fibrous components than AP. The NDF and ADF values observed here for SG and CM are comparable to those reported for *Stylosanthes scabra* (NDF= 50%; ADF= 40%) and *C. pubescens* (NDF= 53 %; ADF= 48%)(10).

Given its lower NDF and ADF contents the high CP content of AP is to be expected. In another report with the same observation this higher CP content was attributed to the stable leaf-to-stem ratio of AP throughout its growth period and its consequently higher cellular content(9). The leaves of AP accumulate a higher proportion of N derived from reserves in the roots and mature stems(23). This may explain the similar CP content in AP and CM since the latter has a larger proportion of leaves than AP but they still have very similar leaf-to-stem ratio values. In contrast, PP had more leaves and a higher leaf-to-stem ratio than AP but had the lowest CP content; even SG, which had the highest stem proportion, had a higher CP content than PP. There is evidence that PP has a low CP content even though it produces extensive foliage during growth(9). Despite the differences in CP content between the studied legumes all their levels surpassed the estimated 7 % CP minimum maintenance requirements for ruminants(3).

Throughout all six cuts IVDMD remained highest in AP, which is directly related to its lower NDF and ADF contents during growth. In contrast, PP and CM had the lowest IVDMD values due to their high fiber contents. The low digestibility of these species (PP, CM) may also be due to high lignin concentration (not evaluated here) since this can considerably reduce forage IVDMD[3]. Further supporting this possibility is the low digestibility reported for *C. pubescens* and *S. scabra* due partially to high NDF and ADF contents, but primarily caused by these species' high lignin concentration (17 and 18 %, respectively)[10]. However, a forage plant's nutritional value can vary in response to factors such as species, climatic conditions, sampling site and vegetative stage[24].

Dry matter production was variable in the four studied legumes and responded mainly to rainfall and temperatures at the experimental site. Inter-species variation in chemical composition was affected mainly by differences between species. *Stylosanthes guianensis* and *Centrosema macrocarpum* had the highest dry matter production, but *Arachis pintoi* exhibited the highest nutritional quality due mainly to its high crude protein content and digestibility. Although they differed in terms of the evaluated variables, the four studied legumes had high nutritional value, are promising alternative forage sources and apt for use as complements to low nutritional quality diets for ruminants in the study region.

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