Chemical composition, *in situ* rumen degradability, and *in vitro* digestibility of *Tithonia diversifolia* ecotypes of interest for ruminant feeding

O. La O[1], H. González [2], A. Orozco[2], Y. Castillo[2]; O. Ruiz[3], A. Estrada[5], F. Ríos[5], E. Gutiérrez[4], H. Bernal[4], Dayki Valenciaga[1], Beatriz I. Castro[6] and Yasmila Hernánez[1]

[1]*Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba*
[2]*Universidad Autónoma de Ciudad Juárez, Avenida Plutarco Elías, Calle 1210 Fovissste Chamizal, Ciudad Juárez, Chihuahua, México*
[3]*Facultad de Zootecnia, Universidad Autónoma de Chihuahua, Perif. Fco. R. Almada, km 1, Chihuahua, México*
[4]*Facultad de Agronomía, Universidad Autónoma de Nuevo León, Campus Ciencias Agropecuarias, Escobedo, Nuevo León, México*
[5]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Sinaloa, Carretera Internacional, km. 3.5 sur, Apdo. Postal 1057, Culiacán, Sinaloa, México*
[6]*Instituto de Investigaciones en Ciencias Veterinarias de la UABC, Laguna Campestre s/n, Mexicali, BC. México*
Email: olao@ica.co.cu

An experimental sequence was performed with nine ecotypes of *Tithonia diversifolia* (3, 5, 6, 10, 13, 17, 23, 24, and 25) to determine chemical composition, *in situ* rumen effective degradability (ED) of dry matter (DM), and *in vitro* apparent and true digestibility of DM, OM, NDF, and ADF. Times of 6, 12, 36, 48, and 72 h were used for the degradability study. The kinetic performance was characterized by rise in the dynamics of DM disappearance, whereas the rumen effective DM degradability had values between 26.29 and 86.55 % for different rumen turnover constants. The fit of the data of *in situ* rumen degradability to the model proposed showed R^2 superior to 0.89. In the ecotypes under study, the estimates of DM and OM apparent digestibility (IVDMAD and IVOMAD) kept values inferior to the true digestibility of both constituents (IVDMTD and IVOMTD). The values of IVDMAD were within the range from 72.25 to 79.77 %, and those of IVOMAD were from 57.71 to 66.20 %, as compared with the values of IVDMTD and IVOMTD, which ranged from 81.08 to 85.66 %, and from 65.27 to 70.22 % respectively. There were differences between each of the plant materials per indicator ($P < 0.01$, $P < 0.001$). The chemical composition analysis results of the *in situ* DM rumen degradability and the *in vitro* apparent and true digestibility of the DM, OM, NDF, and ADF of the ecotypes of *T. diversifolia* suggest their nutritional value. However, physiological studies are required to link the cut frequency, the level of inclusion of these ecotypes, the degree of utilization of the nutrients by the animal and the effect of some secondary metabolites on the physiological and productive responses of the plant.

Key words: *in situ rumen degradability, digestibility, chemical composition, T. diversifolia*.

At present, worldwide, farmers should produce more efficiently to be competitive in the world's market. Cuba is not exempt from these demands. In order to fulfill this goal, alternatives are design to decrease the supplementation with imported concentrates. In this context, the supplementation with forage plants, whether legumes or not, is of special interest.

Many tree species have nutritional values that complement those of grasses and permit producing large amounts of edible biomass (Tun 2004, Mahecha *et al*. 2007, Ramírez *et al*. 2007, Medina *et al*. 2009 and Verdecia *et al*. 2011). There are evidences that *Tithonia diversifolia* is one of the non-leguminous plants that is promising for the feeding of different animal species (Pérez and Jiménez 2008 and Nieves *et al*. 2011), thereby being necessary to deepen into the knowledge of this feeding source. The object of this work was to determine the chemical composition, the *in situ* dry matter rumen degradability and the *in vitro* apparent and true digestibility of DM, OM, NDF, and ADF in different plant materials of *Tithonia diversifolia*, which are of interest for ruminant feeding.

Materials and Methods

Experimental procedure. The study was performed in the laboratory of feed analysis and rumen physiology, of the department of Veterinary Sciences of the Institute of Biomedical Sciences from the Autonomous University of Juárez City, Chihuahua, Mexico. The samples were collected during the rainy season. They were collected from 15 individual adult plants at random (7 kg/ plant), at the vegetative stage, at 77 d of age. They came from a typical red ferrallitic soil (Hernández *et al*. 1999), which was at the Experimental Station of Pastures and Forages of the Institute of Animal Science. In the sampling, the animal browsing was simulated (Paterson *et al*. 1983). Part of the harvested material, previously homogenized and dried for 48 h in air-forced oven at 55 ºC, was ground at 1 mm to determine the bromatological composition and the *in vitro* digestibility, and at 2 mm for the *in situ* rumen degradability.

Chemical composition. In the analysis of the samples from the ecotypes, the content of dry matter (DM), ashes, organic matter (OM), and crude protein (CP) (Labconco) was determined according to the techniques of AOAC (2000). The content of neutral detergent fiber (NDF)

and acid detergent fiber (ADF) was calculated by the method of Goering and van Soest (1970) through a fiber extractor device (Ankom 2007).

In situ degradability. The degradability of the samples was determined by the dacron bag technique (Ørskov *et al*. 1980). Sealed with heat and free of nitrogen, the dacron bags were of white monofilament, with pore size of 53 (±10) microns. They measured 5 x 10 cm and contained 2 g of 1 mm sample. The samples were incubated in the rumen of two rams, with initial average weight of 40 kg and at 36 months of age. The animals were fitted a permanent rumen cannula of 7.5 cm of diameter. They were given a diet of 70 % of alfalfa hay and 30 % commercial balanced feed (12 % CP). They were allocated in individual metabolic cages with cement floor, bedding of wood shavings and surface area of 1.8 m^2. The diets were given at 8:00 a.m. and 5:00 p.m. The animals had free access to a mineral block. The water was provided *ad libitum*. The bags were removed at 0, 6, 12, 24, 48, and 72 h to be washed with water and dry them in oven at 60 °C for 24 h.

In vitro digestibility. The technique of ANKOM Technology (1998) was used. Five Pelibuey rams were used as rumen liquor donors. They were on a fast, and had 40 kg of initial average weight and were 36 months old. They were fitted with a permanent rumen cannula of 7.5 cm of diameter. They were given a diet described under the heading of *in situ* degradability.

For the *in vitro* test, bags of FN° 57 were used, with pore of 25 µm and dimensions of 5 x 4 cm. They were of polyester/ polyethylene, with filaments extracted from a matrix of three dimensions. The bags were previously identified and washed with acetone. They were introduced into an oven at 100 °C ± 5 °C during two hours to be dried and attain constant weight. In each bag, 0.25 g of sample were deposited to obtain an effective area of 36 cm^2. It corresponded to the ratio between simple size and the bag surface of 14.4 mg/cm^2. Later, they were sealed with heat.

Four digestion jars were used, and two forage samples were incubated in each at random (25 bags/jar). One bag was included as blank (empty and sealed bag without sample) to generate the correction factor by input of particles or loss of weight in the bags.

The rumen inoculum needed for the procedure (4:1 ratio of culture medium solution: rumen inoculum) was collected through vacuum pump. The rumen liquid processing included the mixing in a blender for 30 seconds. It was filtered through double gauze layers and later added (400 mL) to the buffer solution (1.600 mL/jar). CO_2 was added to maintain the anaerobic conditions in the rumen.

The samples were incubated for 48 h in the Daisy, at 39.2 ± 0.5 °C of temperature, with constant circular agitation. After the incubation, the bags were washed with water to stop the fermentation. They were processed in the fiber analyzer. The residues from the incubation wee in a neutral detergent solution at 100 °C for one hour. They included three successive washes with water at 90 °C and they were dried in air-forced oven at 105 °C for at least two hours. Later, the bags were weighed to obtain results in terms of *in vitro* DM true digestibility of (IVDMTD), which are considered as estimates of the real digestibility of the feeds.

Data processing and statistical analysis. Fort he rumen degradability, the results were fitted to the exponential model of Orskov and McDonald (1979):

$P1 = a + b\ (1 - e^{-ct})$ where:

P1 = Real degradation in time (t)

a = Intersect of the degradation curve at time zero. It represents the component that degrades rapidly.

b = Potential degradability of the component.

e = Bases of the natural logarithms (2.71828).

c = Constant degradation rate.

a + b = Total degradability of the component.

For the determination of the rumen effective degradability, the model of Mc Donald (1981) was applied:

$ED = A + ((B^* c) / (c+k))$ where,

k = Fractional rate of rumen passage. Different values of k were assumed.

For the chemical composition, only the standard deviation of the observations was determined in respect to the mean. For the estimation of the rumen degradation, the interactive process of the algorithm of MARQUARDT was conducted, with the help of the procedure for non-linear models PROC NLIN of the SAS software, version 6,12 (SAS 1993). For the *in vitro* apparent and true digestibility of DM, OM, NDF and ADF, a one-way analysis of variance was applied for each indicator in the ecotypes under study. Duncan's test (1955) was used for the comparison of the means.

Results and Discussion

Table 1 presents the results of the chemical composition of the ecotypes of *T. diversifolia*. The values of protein and NDF, with figures from 18.26 to 26.40 %, and from 14.79 to 25.74 %, respectively, were those having the greatest standard deviation compared with the rest of the indicators. These outcomes are related, partially, to the report of Stewart and Dunsdon (1998), La O *et al*. (2003 a), and Verdecia *et al*. (2011) about the influence of the environment on the quality performance. However, in the ecotypes, there was high value of the nutritive constituents of interest for animal feeding in the conditions under study.

In general, the ecotypes of *T. diversifolia* had adequate nutritional value of foliage for ruminants (table 1). The contents of proteins were within the range reported by Devendra (1995) for leaves from twelve species of

Table 1. Chemical composition (%) of the ecotypes of *T. diversifolia*

Ecotypes	DM	Ashes	OM	NDF	CP
TITONIA 3	88.76	21.97	78.02	38.38	18.26
TITONIA 5	89.12	20.11	79.88	34.09	19.21
TITONIA 6	88.41	17.72	82.27	37.57	23.61
TITONIA 10	88.87	20.15	79.84	36.24	19.72
TITONIA 13	88.85	16.88	83.11	32.62	25.91
TITONIA 17	88.12	16.04	83.95	37.41	26.40
TITONIA 23	89.21	19.04	80.95	38.31	24.62
TITONIA 24	88.65	19.15	80.84	41.83	20.81
TITONIA 25	88.77	17.51	82.48	38.54	20.79
Standard deviation	0.33	1.86	1.86	2.68	3.03

tropical trees (14-36.6 %) and by La O *et al*. (2003b) for 12 varieties or ecotypes of *Leucaena leucocephala* (14-30.6 %). Also, they agreed with the range noted by Verdecia *et al*. (2011) for *T. diversifolia*, with different cut ages in the Granma conditions, Cuba. Nevertheless, the values of structural carbohydrates were similar to those of Rosales (1996), who found contents of 35.5 % for the NDF in this shrub species.

The evolution in the dynamics of *in situ* DM rumen degradation (figure 1) showed progressive increase in time of the asymptotic type up to the last incubation time for all the ecotypes. This performance could be related to, up to a large extent, with Barry and Manley (1986). These authors reported that the type and amount of phenolic compounds in some tropical plants can limit or favor the rumen degradability of the nutrients. Although this

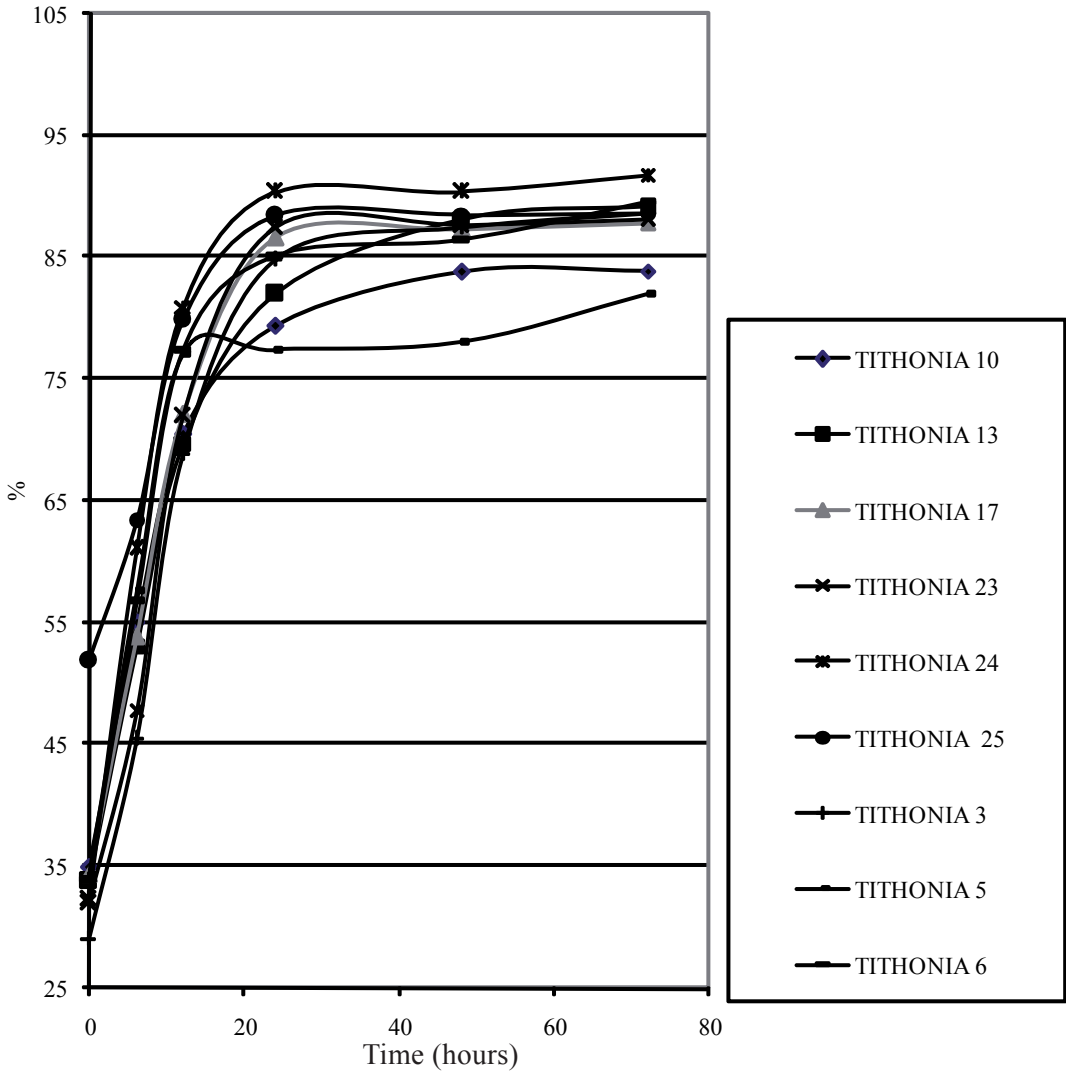

Figure 1. Dynamics *in situ* DM rumen degradation of different plant materials of *Tithonia diversifolia*

has not been proved in *Tithonia diversifolia*, it should be highlighted that the contents of tannins are inferior to the averages reported for the edible fraction of this species (Verdecia *et al*. 2011) and of some typical legumes of the production systems in the tropics (Devendra 1995 and Medina *et al*. 2009). This proves the quality of this species for its use in animal feeding.

The parameters a, b, a + b and c (table 2) were very variable in each of the ecotypes, with values from 1.95 to 24.99; 58.20 to 95.53, 83.62 to 99.5 and 0.09 to 0.39 for a, b, a + b and c, respectively. This performance can be related to the report of Pedraza (2000) and La O (2001), who by studying *Gliricidia sepium* and *Leucaena leucocephala* noted the great inter-specific variability in ecotypes of the same species, as well as the structures and relations established between the different macromolecules of the feed in their interaction with rumen bacteria.

The values of effective DM degradability (table 2) for different constants of rumen turnover rates (k) did not have similar performance in all the plant materials, and ranged from 26.29 up to 86.55 %. These results were within the range of values for tropical plants, as reported by Mupangwa *et al*. (2003), La O *et al*. 2003 (a and b) and La O *et al*. (2008) in studies on different seasonal legumes at different cut ages; among them, there were creeping plants and *T. diversifolia*.

The low degradation values in the ecotype 5 could be associated with the possible effect of some secondary compounds such as the tannins, in respect to certain macronutrients and to the concentration of nitrogen and other macromolecules linked to the lignocellulosic compound in the plant material. La O *et al*. (2003a) studied this aspect in leucaena. Likewise, Savón and Scull (2002), Scull (2004) and Ramírez *et al*. (2007) dealt with it in trees and legumes. Verdecia *et al*. (2011) and Nieves *et al*. (2011) studied it in *T. diversifolia*. Therefore, it is a challenge to identify the factors that could limit the utilization of this element in the rumen.

Stewart and Dunsdon (1998) noted that 45 % of the variation in the *in vitro* digestibility of tropical legumes was represented by the variation in the content of tannins. Nevertheless, these authors reported that it is not clear the beneficial or harmful effect that tannins could have on several genera of tropical legumes, thus, they recommended to deal with this aspect with utmost care until there are evidences on the specific nutritional effects.

The estimates of DM and OM apparent digestibility (IVDMAD and IVOMAD) kept values inferior to the true digestibility of both constituents (IVDMTD and IVOMTD) (table 3). Figures of IVDMAD were found within the range from 72.25 to 79.77 %, and of IVOMAD from 57.71 to 66.20 % in respect to the values of IVDMTD, which ranged from 81.08 to 85.66 %, and to those of IVOMTD, which were between 65.27 and 70.22 %. These variations were expected, considering the nature and the chemical characteristics (table 1) of the ecotypes under study, as well as the diverse sources of variation that may affect the digestibility of the constituents. Among these characteristics, there are those intrinsic to each ecotype and the relations established between the macromolecules in their interaction with the rumen

Table 2. Kinetic characteristics of the *in situ* rumen degradability of different ecotypes of *Tithonia diversifolia* according to the model Y= a+b*(1-exp-(c*t)).

Parameter	Ecotypes								
	3	5	6	10	13	17	23	24	25
a	2.27	10.2	1.57	25.42	24.99	9.17	6.33	1.95	14.85
b	95.53	89.3	89.14	58.20	63.86	78.33	88.73	89.28	73.97
a+b	97.80	99.5	90.71	83.62	88.85	87.50	95.06	91.23	88.82
c	0.13	0.39	0.17	0.11	0.09	0.13	0.15	0.18	0.17
R^2	0.97*	0.89*	0.90*	0.99*	0.99*	0.92*	0.95*	0.89*	0.99*
RSD	0.64	2.49	1.84	0.94	0.72	1.29	1.22	0.73	0.53
k									
0.01	81.62	73.57	82.82	79.19	82.92	82.71	82.15	86.55	84.89
0.02	75.84	68.22	78.55	75.34	78.00	78.07	76.60	82.33	81.36
0.03	70.76	63.14	74.70	92.10	73.85	73.98	71.64	78.51	78.17
0.04	66.27	58.28	71.19	69.08	70.31	70.36	67.19	75.04	75.27
0.05	62.27	53.65	68.00	66.50	67.24	67.12	63.16	71.89	72.63
0.06	58.68	49.22	65.07	64.21	64.56	64.21	59.51	68.99	70.21
0.07	55.44	44.98	62.38	62.16	62.20	61.58	56.17	66.30	67.98
0.08	52.51	40.93	59.90	60.32	60.11	59.19	53.12	63.83	65.92
0.09	49.84	37.04	57.60	58.65	58.24	57.01	50.31	61.54	64.02
0.10	47.39	33.31	55.47	57.14	56.56	55.02	47.73	59.42	62.25
0.11	45.15	29.73	53.49	55.16	55.04	53.19	45.33	57.41	60.61
0.12	43.09	26.29	51.64	54.49	53.66	51.50	43.11	55.59	59.08

*P< 0.05, RSD- Residual Standard Deviation

Table 3. *In vitro* DM and OM apparent and true digestibility of different plant materials of *Tithonia diversifolia*

Ecotypes	IVDMAD	IVDMTD	IVOMAD	IVOMTD
TITONIA 3	73.96^{a}	83.65^{b}	57.71^{a}	65.27^{a}
TITONIA 5	76.99^{b}	83.94^{bc}	61.50^{b}	67.05^{ac}
TITONIA 6	75.52^{ab}	81.47^{a}	62.14^{b}	67.03^{ac}
TITONIA 10	73.25^{a}	83.39^{b}	58.48^{a}	66.58^{ab}
TITONIA 13	79.77^{c}	84.48^{c}	66.30^{c}	70.22^{d}
TITONIA 17	76.33^{b}	83.28^{b}	64.08^{bc}	69.92^{d}
TITONIA 23	78.35^{bc}	85.66^{d}	63.43^{b}	69.34^{d}
TITONIA 24	72.25^{a}	83.38^{b}	58.41^{a}	67.41^{bc}
TITONIA 25	74.58^{ab}	81.08^{b}	61.52^{b}	66.87^{ac}
Standard error	0.63**	0.22**	0.68**	0.54**

^{abcd} Means on the same column differ at $P < 0.05$ (Duncan 1955). $P < 0.01$**

environment.

The values were higher than those of Verdecia *et al*. (2011) for digestibility studies in *T. diversifolia* coming from poor-drained soils in the Granma province. In this respect, D´Mello (1992) and La O *et al*. (2003a) noted the importance of knowing the variability in the chemical composition and the degree of utilization of some tropical forage sources. These authors reported variabilities up to 45 % in some phytogenetic sources, according to the large amount of ecosystems where this plant can grow. This aspect could be seen in *Tithonia diversifolia*.

La O *et al*. (2008) reported the importance of making *in vitro* evaluations with fast methodologies and with great reproducibility, such as being non-invasive to the animals and permitting to represent the nutritive differences of any feed.

When studying the apparent and true digestibility of the cell wall and the ADF (table 4), the same performance of the DM and the OM was evidenced, with analogous values as to the tendency of the true and apparent digestibility, and significant ($P < 0.01$, $P < 0.1$) differences in the plant materials. Later works will be addressed to determine the correspondence and validation of these results from the productive point of view.

The values of IVDMAD, and those of OM, NDF, and ADF, as well as of the IVDMTD, and those of OM, FND and FAD evidenced differences between the ecotypes under study, with trustworthy digestibility results and able to be compared. The variations between the true and the apparent digestibility were in the range of 5 % for NDF and up to 2 % for ADF.

The results from the analysis of the chemical composition and the *in situ* DM rumen degradability in the ecotypes of *T. diversifolia* suggested the nutritional value of this species. However, physiological studies are needed to link the cut frequency and the level of inclusion of the ecotype with the degree of utilization of these nutrients by the animal. Also, they should make reference in tests in animals to the effect of some secondary metabolites on the physiological and productive responses of this plant.

Acknowledgments

Thanks are due to the project International

Table 4. *In vitro* apparent and true digestibility of NDF and ADF of different plant materials of *Tithonia diversifolia*

	IVADNDF	IVTDNDF	IVADADF	IVTDADF
TITONIA 3	28.39^{bc}	32.11^{c}	11.55^{a}	13.06^{a}
TITONIA 5	26.25^{a}	28.62^{ab}	12.20^{a}	13.31^{a}
TITONIA 6	28.38^{bc}	30.61^{bc}	14.97^{b}	16.15^{c}
TITONIA 10	26.54^{a}	30.22^{b}	11.34^{a}	12.91^{a}
TITONIA 13	26.03^{a}	27.56^{a}	14.22^{b}	15.06^{bc}
TITONIA 17	28.56^{bc}	31.16^{b}	13.68^{b}	14.93^{bc}
TITONIA 23	30.02^{c}	32.82^{c}	11.60^{a}	12.68^{a}
TITONIA 24	30.22^{c}	34.88^{d}	11.91^{a}	13.75^{a}
TITONIA 25	28.74^{bc}	31.25^{bc}	14.84^{b}	16.13^{c}
Standard error	0.67**	0.69**	0.40 ***	0.40 ***

^{abcd} Means in the same column differ at $P < 0.05$ (Duncan 1955). ** $P < 0.01$ *** $P < 0.001$

Network on Ruminant Nutrition and Feeding, which belongs to the Program of Improvement of the Professor Staff (PROMEP), of the Secretary of Public Education (SEP) in Mexico, for the support to the mobility for the development of this research.

References

ANKOM Technology. 1998. *In vitro* true digestibility using ANKOM's DAISYII. Fairport, NY. p. 6-8

ANKOM Technology. 2007. Procedures for fiber and *in vitro* analysis. Available: <http://www.ankom.com/homepage.html> [Consulted: 26/11/2007]

AOAC. 2000. Official Methods of Analysis. Ass. Off. Anal. Chem. 17 th. Ed. Arlington, VA

Barry, T. N. & Manley, T.R.1986. Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in *Lotus sp*. and their possible consequences in ruminant nutrition. J. Sci. Food Agric. 37:248

D´Mello, J. F. P. 1991. Chemical constraints to the use of tropical legumes in animal nutrition. Anim. Feed Sci. Tech. 38: 237

Devendra, C. 1995. Composition and nutritive value of legumes tropical animal nutrition. Eds. J. D´Mello y C. Devendra. CAB INTERNATIONAL, UK. p. 49-66

Duncan, D.B. 1955. Multiple range and multiple F. test. Biometrics 11: 1

Goering, H. K. & van Soest, P. J. 1970. Forage fibre analysis. Agricultural Handbook No. 379. Agric. Res. Service, US. Dept. Agriculture, Washington, D.C.

Hernández, A., Pérez, J.M. & Boch, D. 1999. Nueva versión de la clasificación genética de los suelos de Cuba. AGROINFOR-Ministerio de la Agricultura. Ciudad de La Habana. Cuba. p. 26

La O, O., Chongo B., Delgado, D., Valenciaga D., Rodríguez, Y., Scull, I., Ruiz, T.E. & Oramas, A. 2003b. Influence of polyethyleneglycol-3500 on the ruminal degradability of *Leucaena leucocephala* cv CIAT-7929. Cuban J. Agric. Sci. 37:271

La O, O., Valenciaga, D., Ruiz, T.E., Ruiz, O. , Castillo, Y. , González, H., Rodríguez, C., Alfonso, D., Chongo, B., Arzola, C. & Cairo, J. 2008. Efecto de la edad de corte en la capacidad fermentativa *in vitro* y la dinámica de degradación ruminal *in situ* de materia seca de *Tithonia diversifolia*. Zoot. Trop. 26:243

La O, O. 2001. Contribución al estudio del valor nutritivo de diferentes ecotipos del género Leucaena para la alimentación de rumiantes. PhD Thesis. Instituto de Ciencia Animal. La Habana, Cuba

La O, O., Chongo B., Delgado, D., Ruiz, T.E., Valenciaga D. & Oramas, A. 2003a. Composición química y degradabilidad ruminal de leguminosas de importancia para la alimentación animal. II Foro Latinoamericano de Pastos y Forrajes. San José de las Lajas. La Habana, Cuba

Mahecha, L. 2003. Valor nutricional y utilización del botón de oro *Tithonia diversifolia* en la alimentación animal. In: Tres especies vegetales promisorias nacedero, botón de oro, Bore. Available: <http://www.utafoundation.org/botondeoro.htm> [Consulted: 21/5/12]

Mahecha, L., Escobar, J. P., Suárez, J.F. & Restrepo, L. F. 2007. *Tithonia diversifolia* (Hemsl.) Gray (botón de oro) como suplemento forrajero de vacas F1 (Holstein por Cebú). Liv. Res. Rural Dev. 19:2007

Mc Donal, I.M. 1981. A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. 92:499

Medina, M., García, D., Gónzalez, M., Cova, L. & Moratinos, P. 2009. Variables morfoestructurales y de calidad de la biomasa de *Tithonia diversifolia* en la etapa inicial de crecimiento. Zoot. Trop. 27:121

Mupangwa, J.F., Nogongoni, N.T. & Harmidikuwanda, H. 2003. The effect of stage of growth and meted of drying fresh herbage on *in sacco* dry matter degradability of three tropical forage legumes. Liv. Res. Rural Dev. 15:2003. Available: <http://www.cipav.org.co/lrrd/lrrd15/2/mupa152.htm> [Consulted: 13/8/2011]

Nieves, D., Terán, O., Cruz, L., Mena, M., Gutiérrez, F. & Ly, J. 2011. Nutrientes digestibilty in *Tithonia diversifolia* foliage in fattening rabbits. Tropical and Subtropical Agroecosystems. 14: 309

Ørskov, E. R., Deb, F. Hovell, D. & Mould, F. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5:195

Ørskov, E. R. & McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499

Paterson, R.T., Quiroga, L., Sauma, G. & Samur, C. 1983. Crecimiento de novillas Cebú Criollo en la época de seca con acceso limitado a la Leucaena. Prod. Anim. Trop. 8:150

Pedraza, R.M. 2000. Valoración nutritiva del follaje de *Gliricidia sepium* (Jacq.) kunth ex walp. y su efecto en el ambiente ruminal. PhD Thesis. Vet. Instituto de Ciencia Animal. La Habana, Cuba

Pérez, J. & Jiménez, N. 2008. Uso de follaje fresco de arnica (*Tithonia diversifolia*) en conejos de engorde. Tesis Ingeniería en Producción Animal. Universidad Nacional Experimental de Los Llanos Occidentales Ezequiel Zamora (UNELLEZ). Venezuela. 29 pp.

Ramírez, R.U., Escobedo, J.G., Lara, P.E., Cen, C.F. & Sanginés, J.R. 2007. Digestibilidad y balance de nitrógeno de dietas para ovinos con niveles crecientes de *Tithonia diversifolia*. Available: <http://www.dict.isch.edu.cu/.../agroforesteria%202007/data/posters/3lossistem> [Consulted: 21/8/ 2009]

Rosales, M. 1996. *In vitro* assessment of the nutritive value of mixtures of leaves from tropical fodder trees. PhD Thesis. Departament of Plant Sciences. Oxford University. Oxford, UK. 214 pp.

SAS 1993. Statistical Analysis System. Statistical Analysis System Institute. Version 5.0. Cary, North Carolina. U.S.A.

Savón, L. & Scull, I. 2002. Valor potencial de elementos fibrosos tropicales para especies monogástricos. II Congreso de Cunicultura de las Américas. La Habana. Cuba. pp. 69

Scull, I. 2004. Metodología para la determinación de taninos en forrajes de plantas tropicales con potencialidades de uso en la alimentación animal. Master Thesis. Universidad de La Habana. Cuba

Stewart, J.L. & Dunsdon, A.J. 1998. Evaluación preliminar de la calidad potencial como forraje de un rango de especies de Leucaena. Pasturas Tropicales. 20:3

Tun, M. 2004. Efecto de la edad de corte en la distancia de siembra en el rendimiento y calidad de *Tithonia diversifolia*. Master's Thesis. Inst. Tecnológico Agropecuario Nº 2. Conkal, Yucatán. México

Verdecia, D., Ramírez, J.L., Leonard, I., Álvarez,Y., Bazán,Y., Bodas, R., Andrés, S., Álvarez, J., Giráldez, F. & López, S. 2011.Calidad de la *Tithonia diversifolia* en una zona del valle del Cauto. Rev. Electrónica Vet. 12:5. Available: <www.veterinaria.org/revista/redvet/n050511/051113.pdf> [Consulted: 13/8/ 2011]

Received: July 4, 2011