NUTRITIONAL VALUE AND *IN VITRO* DRY MATTER DEGRADABILITY IN MEXICAN SUNFLOWER: *Tithonia diversifolia* Helms (Gray) †

[VALOR NUTRICIONAL Y DEGRADABILIDAD DE LA MATERIA SECA *IN VITRO*, EN GIRASOL MEXICANO: *Tithonia diversifolia* Helms (Gray)]

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SUMMARY

Background: *Tithonia diversifolia* Helms (Gray), is a robust shrubby forage plant with high integral biomass yield (stem and leaves); susceptible to heliophany and conditioning of bromatological characteristics, nutritional value, and modification of the rumen environment is due to genetic variability between genotypes. **Objective:** To evaluate the macromolar quality of the biomass of introductions *T. diversifolia* Helms (Gray) established in two locations, and the nutritional value, gas production and *in vitro* rumen environment promoted by the whole plant biomass established in Ecuador. **Methodology:** The chemical composition of the full plant (PITD) was considered based on Dry Matter (DM), Organic Matter (OM), Mineral composition (MC), Crude Protein (CP), neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF). I*n vitro* gas production was evaluated according to Ankom Technology, considering Tiffon 85 there (*Cynodon dactylon*) at control, quantified or ammoniacal nitrogen (N-NH_3), short-chain fatty acids (SCFA) and pH. The experiment was developed in a completely randomized design in factorial arrangement with three repetitions for the variable chemical composition, and the *in vitro* digestibility of the date processed through a random complete block design with six repetitions, in the ruminal ambient and production SCFA was analyzed through a completely randomized block design with three repetitions. **Results:** There were variable responses in the DM content for the effect of interaction P <0.01, and higher mineral content in El Carmen, Ecuador for introduction 13.5, with respect to the rest of the cultivars in both locations. NDF was significantly expressed (p<0.001) in variety 1.2 that grew in Candelaria, Ecuador. For the rest of the variables, no differences were recorded (P>0.05). The gas production of the introductions, except for 1.2, was similar to that produced by the control (Tifton 85), however, in the degradability of the OM, net gas production (Net GP) of DM and OM, stood out. introduction 1.2 compared to the control (P<0.01) and the rest of the introductions. The net GP of NDF exceeded 1.2 (p<0.02) than the control, also quantitatively to produce Acetic and Propionic Fatty Acid. **Implications:** The introductions of *T. diversifolia* Helms Gray based on the results obtained preserve macromolar characteristics, gas production and SCVFA production (short chain volatile fatty acids) in contrasting ecosystems such as Valle del Cauca, Colombia and Manabi in Ecuador. **Conclusions:** The introductions of *T. diversifolia* Helms Gray, maintain a good protein composition, produce low methane contents, have

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high performance in the fermentation of organic matter and the cell wall, which suggests its future use as a complement to the ration in conditions in conditions Edaphoclimatic of the province of Manabi.
Key words: Forage quality; ruminant nutrition; Ecuador.

RESUMEN

Antecedentes: *Tithonia diversifolia* Helms (Gray), es una planta forrajera arbustiva robusta con alto rendimiento de biomasa integral (tallo y hojas); susceptible a heliofanía y condicionamiento de características bromatológicas, valor nutricional y modificación del ambiente ruminal se debe a la variabilidad genética entre genotipos. **Objetivo:** Evaluar la calidad macromolar de la biomasa de introducciones de *T. diversifolia* Helms (Gray) establecidas en dos localidades y el valor nutricional, la producción de gas y el ambiente ruminal *in vitro* promovidos por la biomasa vegetal completa de las establecidas en el Ecuador. **Métodología:** Se consideró la composición química de la planta íntegra (PI) con base a Materia Seca (MS), Materia Orgánica (MO), Materia Inorgánica (MI), Proteína Cruda (PC), Fibra Detergente Neutro (FDN), Fibra Detergente Ácido. (FDA). La producción de gas *in vitro* se evaluó según Ankom Technology, considerando al Tiffon 85 (*Cynodon dactylon*) como control; cuantificándose el nitrógeno amoniacal ($N\text{-}NH_3$) (%), ácidos grasos de cadena corta (AGCC) (mmol) y pH. El experimento se desarrolló en un diseño completamente al azar en arreglo factorial con tres repeticiones para la variable composición química, y se analizó la digestibilidad *in vitro* mediante un diseño de bloques completos al azar con seis repeticiones, en el ambiente ruminal y en la producción de AGCC mediante un diseño de bloques completamente al azar con tres repeticiones. **Resultados:** Hubo respuestas variables en el contenido de MS por el efecto de la interacción $P<0.01$, y mayor contenido de minerales en El Carmen, Ecuador para la introducción 13.5, con respecto al resto de los cultivares en ambas localidades. La FDN se expresó significativamente ($p<0.001$) en la variedad 1.2 que creció en Candelaria, Ecuador. Para el resto de las variables no se registraron diferencias ($P>0.05$). La producción de gas de las introducciones a execepción de 1.2, fue similar a la producida por el control (Tifton 85), sin embargo, en la degradabilidad de la MO, producción neta de gas (GP Neto) de MS y MO, se destacó la introducción 1.2 respecto al control ($P<0.01$) y el resto de las introducciones. La GP neto de FDN superó 1.2 ($p<0.02$) al control, también cuantitativamente para la producción de Ácido Graso Acético y Propiónico. **Implicaciones.** Las introducciones de *T. diversifolia* Helms Gray a partir de los resultados obtenidos conservan característica macromolares, producción de gas y producciones de AGVCC (ácios grasos volátiles de cadena corta) en ecosistemas contrastantes como lo constituye el Valle del Cauca, Colombia y Manabí en el Ecuador. **Conclusiones:** Las introducciones de *T. diversifolia* Helms Gray, mantienen una buena composición proteica, producen bajos contenidos de metano, tienen alto desempeño en la fermentación de la materia orgánica y la pared celular, lo que sugiere su uso futuro como complemento a la ración en condiciones de condiciones edafoclimáticas de la provincia de Manabí.
Palabras claves: Calidad de forraje; nutrición de rumiantes; Ecuador.

INTRODUCTION

Tithonia diversifolia Helms (Gray), known as Golden Button (GB), is a robust shrubby forage plant with a high integral biomass yield (stem and leaves). It exhibits good palatability and digestibility for ruminants, both when grazed and included in pre-designed rations (Nieves *et al.*, 2011; Meza-Bone *et al.*, 2021). Depending on heliophany, GB demonstrates rapid recovery in grazing (every 50 days) and after cutting (every 60 days), yielding substantial biomass(Mahecha and Rosales, 2005; Cuartas *et al.*, 2015). Nutritional disparities exist between stems and leaves, primarily linked to the pre-flowering phenological stage (Medina *et al.*, 2009; Nieves and Aspuria, 2011; Lezcano *et al.*, 2012; Londoño, Mahecha and Angulo, 2019).

In this context, reported Crude Protein (CP) values include 29.8%, 24.0%, and 11.0% at 30, 40, and 60 days respectively (Mahecha and Rosales, 2005; La O. O *et al.*, 2012; Calsavara *et al.*, 2016; Galindo Blanco *et al.*, 2019; Kato-Noguchi, 2020). Meanwhile, Calsavara *et al.*, (2016) obtained values of 22.5%, 41.0%, and 26.1% for CP, NDF, and ADF respectively, under São João del-Rei edaphoclimatic conditions in Brazil (21°05' 11"; 44° 13' 33" W, 950 masl). Reports on soluble carbohydrate and starch content indicate variations between 9.65% to 12.92% and 4.55% to 6.73% respectively (Medina *et al.*, 2009), which likely accounts for cattle's ad libitum consumption without disturbing ruminal fermentation balance (Gallego-Castro, Machena-Ledesma and Angulo-Arizala, 2014; Galindo *et al.*, 2017; Galindo Blanco *et al.*, 2019).

Experience with *in vitro* fermentation kinetics in the rumen of cattle, utilizing the in sacco degradability technique (Orskov and Mcdonald, 1979; DHANOA, 1988; Ascencio-Rojas *et al.*, 2019), provides reliable data. However, while it doesn't measure pH stability and fermentation products such as ammoniacal nitrogen ($N\text{-}NH_3$), volatile fatty acids (VFA), and methane (CH4) (Dong *et al.*, 1999), the *in vitro* fermentation technique efficiently estimates biotic and abiotic ruminal indicators (Blümmel and Ørskov, 1993; France *et al.*, 1993; Lima *et al.*, 2018; Samir Attia Nagadi, 2019; González *et al.*, 2020).

Genetic variability among genotypes imparts diverse properties to the plant, including antiparasitic, antimicrobial, antitumoral, and insecticidal traits (Gama *et al.,* 2014; Pavela *et al.,* 2018), primarily due to tannins promoting ruminal fermentation and nutrient utilization (González-Castillo, Hahn von-Hessberg and Narváez-Solarte, 2014; Rivera *et al.,* 2018; López-Vigoa *et al.,* 2019).

Though edaphic factors minimally influence GB, variations in biomass quality and nutritional value are expected due to location-related environmental differences during growth (Gallego-Castro, Machena-Ledesma and Angulo-Arizala, 2014). These are explained by the Mendelian genetics model: F=G+A, where phenotype (F) results from genetic effects (G) and environmental effects (A) (Falconer, 1983; Ortiz Grisales *et al.,* 2014; Rivera *et al.,* 2018).

The phenotypic differences in BO and their implications were observed in a base collection of 44 BO introductions from six departments of central Colombia), among which six introductions stood out based on the yield of integral biomass (stems and leaves) and value nutritional, being noted as superior and promising (Holguín *et al.,* 2015; Holguín *et al.,* 2020). This collection is in the custody of the Agronomy and Vegetable Seed Production Program of the Faculty of Agricultural Sciences National University of Colombia Palmira Campus (Vegetable Program), and some of them were morphologically evaluated by the State University of Quevedo, Ecuador (Vivas-Arturo *et al.,* 2022) as part of the need to explore forage alternatives for animal feeding from shrub species, an aspect that is promoted as part of a strategy based on the use of pastures and forage to guarantee the base livestock nutrition in the province of Manabí, Ecuador (ESPAC, INEC, 2019; Meza-Bone *et al.,* 2022).

Based on what was stated above, the objective of the present study is to evaluate the agrostological potential, degradability, and *in vitro* gas production of different introductions of GB in different edaphoclimatic conditions.

MATERIAL AND METHODS

Vegetable Material

The plant materials came from the Germplasm Bank of the Vegetable Programme of the National University of Colombia (PHUNC), Palmira, represented by six Introductions of *Tithonia diversifolia* Helms (Gray), identified as 1.2, 1.3, 13.5, 17.9, 22.14A and 25.2 according to the reference of the PHUNC's Buttercup Collection.

Location

Six Introductions of *T. diversifolia* (Hemsl) A. Gray from the Vegetable Program, previously recognized by Holguín Castaño*et al.* (2015) for their superiority as forage species, were planted in two two locations:

a) Experimental Center of the Universidad Nacional de Colombia, Palmira Campus (CEUNP), located in Candelaria, Valle del Cauca (Colombia) (3º25'17" N and 76° 25'56" W), 972 m.a.s.l., with an average annual temperature of 26°C, 1100 mm of annual precipitation, and 76% relative humidity, characterized by a tropical dry forest climate (Holdridge, 1967).

b) El Carmen locality, Manabí (Ecuador) (0°80' S and 79°22'), at an elevation of 245 m.a.s.l., with an average annual temperature of 21°C, 2245 mm of annual precipitation, and characterized by a pre-montane humid forest climate (b.h. P.M.) (Holdridge, 1967).

The Introductions in the Candelaria locality, Valle del Cauca, Colombia were arranged in experimental plots of (10 x 10 m), where cultural practices for sowing in furrows were performed. Meanwhile, in the El Carmen locality, Ecuador, the growing conditions, and care were described by (Vivas-Arturo *et al.,* 2022).

Sample Preparation and Proximal and Fiber Analysis

In both locations (Candelaria, Valle del Cauca (Colombia) and El Carmen, Manabí (Ecuador)), whole plant samples of *T. diversifolia* (WPTd) in pre-flowering were taken for the estimation of chemical and mineral composition, simulating animal grazing (Paterson *et al.,* 1983). For the Candelaria location in Colombia, pre-flowering occurred at 60 days of plant age, and at 90 days in El Carmen, Ecuador. After sample collection (3 kg per introduction), the samples were placed in a forced air circulation oven at 60°C to remove excess moisture and they were transported to Seed Laboratory of the Universidad Nacional de Colombia, sede Palmira.

They were subsequently introduced into a Thomas Wiley blade mill (Thomas, model 3383, USA) using a 1 mm sieve and stored in plastic bags (10 x 5 cm); to subsequently determine dry matter (DM; Id 934.01), organic matter (OM), inorganic matter (IM), and crude protein (CP; Id2001.11) as described by the AOAC International, (2016). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin were determined according to the criteria of Van Soest, Robertson and Lewis, (1991) and Jaimes, Giraldo and Correa, (2018).

Gas Production *in vitro*

The samples collected in Manabí, Ecuador and Palmira, Colombia were transported to the Animal Nutrition Laboratory (LANA) at the Nuclear Energy Center for Agriculture (CENA) of the University of Sao Paulo (USP), Brazil. PITd was used as the substrate for the experiment. The donor animals were three adult male Santa Inés sheep with an average weight of 66± 2.33 kg/LW, equipped with a ruminal cannula. They were housed in individual pens with access to water, mineral salts, and ad libitum food (Vivas-Arturo *et al.,* 2022). The rumen content was extracted before morning feeding following the procedure described by Vivas-Arturo *et al.* (2022). To reduce variation due to the animal factor, three inocula were prepared using three different combinations of rumen content in a 25:25 proportion: Inoculum 1 (animal 1+animal 2); inoculum 2 (animal 3+animal 2), and inoculum 3 (animal 1+animal 3).

Incubation was carried out following the methodologies described by Theodorou *et al.* (1994) and Mauricio *et al.* (1999), with adaptations from Bueno and Lesmes, (2008). Ankom F57 bags (Ankom Technology Corp., Macedon, NY, USA) were identified and washed with acetone. They were then dried in an oven at 100 ºC for two hours to achieve their constant weight (La O *et al.,* 2012). The tare was obtained at that moment. Subsequently, 0.5 grams of the ground substrate (1 mm) was weighed on an analytical balance and placed within the filter bags in triplicate. These were then transferred, along with their respective contents, to 160 ml jars containing 50 ml of incubation medium and 25 ml of WPTd artificial saliva: rumen liquid ratio.

Additionally, a jar without substrate (Blank) and a jar with an internal laboratory standard (Tifton 85 Hay (*Cynodon dactylon*)) were included for each of the three inocula. Jars were sealed with rubber caps and placed in a forced ventilation incubator (MA 035 – Marconi, Piracicaba-SP, Brazil) at 39ºC. To estimate the accumulated profiles of *in vitro* gas production (GP), measurements of the internal pressure of each jar were captured at 0, 4, 8, 16, and 24 h post-incubation using a pressure transducer with a data logger (Pressure Press 800, LANA, CENA/USP, Piracicaba, Brazil), following the procedures described by Bueno and Lesmes (2007) and Lima *et al.* (2018).

To convert the pressure data (PSI) into gas volume (mL), the following equation was used:

$$Y = -0.1375 + (5.385 * X) + (0.0777 * X^2)$$

Where: **X** is the pressure in PSI and **Y** is the gas volume in mL, and gas production was expressed per gram of incubated dry matter, degraded organic matter, and degraded NDF (mL g^-1 of DMi, DOM, and DFDN, respectively) (Longo *et al.,* 2006; Posada, Noguera Solano and Bolívar Vergara, 2006; Rodríguez, Fondevila and Castrillo, 2009; Hassen, Kelkay Tessema and Tolera, 2017; Jamarun *et al.*, 2017; 2019; 2020; Ningrat *et al.,* 2018; 2019; 2020; Ravhuhali, Msiza and Mudau, 2022; Hernández *et al.*, 2023).

After gas measurement, the internal pressure of each jar was released using hypodermic needles through the rubber cap, the contents of the jars were homogenized by shaking, and the jars were returned to the incubator.

Accumulated gas volume at the end of incubation was interpreted based on the logistic model described by Nelder (1961) and adapted for Vivas-Arturo *et al.* (2020).

$$\boldsymbol{y} = \frac{\boldsymbol{a}}{(\mathbf{1}+\boldsymbol{b}*\boldsymbol{exp}^{(-\boldsymbol{c}*\boldsymbol{t})})} \qquad (2)$$

Where:
Y = Accumulated gas volume at the end of the process at a given time.
a = Gas production at the end of dry matter degradation.
b = Slope of the gas accumulation curve (accelerating phase), up to the inflection point or change in dry matter degradation rate.
c = Speed of gas production, in a decelerating mode, after having reached the inflection point.
(a/2) = Inflection point of the curve.

Fermentation Products and *in vitro* Degradability

After pressure measurement for 24 hours, the jars were placed in ice water to halt fermentation. The lids were removed, and the bags were retrieved and placed in ice water. Two aliquots of the liquid content from each jar were stored in 20 mL vials and kept at -20ºC for later determination of ammoniacal nitrogen (N-NH_3), using the micro Kjeldahl method as described by Cadena-Villegas *et al.* (2020), and quantification of short-chain fatty acids (SCFAs) through chromatography, using chromatographic conditions described by Vivas-Arturo *et al.* (2022). Before freezing the material, the pH of the content in each jar was measured and recorded using a digital potentiometer (model TEC-2, Tecnal, Piracicaba, Brazil).

Upon retrieval of the bags and cessation of fermentation, the bags were treated with a neutral detergent solution for one hour at 90ºC using a fibre analyzer (TE-149, Tecnal Piracicaba – SP), followed by four 5-minute washes with water at 90ºC. The process was completed with a 5-minute wash using HPLC-grade acetone. The washed bags were placed in

a forced-air oven at 105ºC for 24 hours and subsequently weighed to calculate the true in vitro degradability of NDF (DIVFDN).

Experimental Design and Statistical Analysis

For chemical composition, the experiment was conducted using a completely randomized design in a factorial arrangement with three replicates. Factors considered were location (2) and Introductions of *T. diversifolia* (6), along with their 2*6 interaction, resulting in a total of 12 treatments, in addition to the positive control, Tifton 85. The mathematical model employed for the ANOVA was as follows:

$$Y_{ij} = \mu + Loc_i + Introd_j + (Loc \times Introd)_{ij} + e_{ij}$$

Where: μ = model average; Loc_i = *i*-th locality; $Introd_j$ = *j*-th introduction; Loc_i x $Introd_j$ = effect of the *i*-th locality on the *j*-th introduction; e_{ij} = error.

For the *in vitro* digestibility trial, the experimental design was a randomized complete block with 6 repetitions within each block, considering the inoculant as blocks and the substrate WPTd as treatments. For the modification of the ruminal environment and the production of SCFA, experiments were conducted in a completely randomized design with three repetitions.

Statistical analysis of the data was carried out using Statistics software v. 12.0. In all cases, the assumptions of normality and homoscedasticity were verified according to the criteria of Kolmogorov-Smirnov (Massey, 1951) and Bartlett (Bartlett, 1937), respectively. Mean comparisons were performed using Tukey's test at a 95% confidence level.

RESULTS

Macromolecular quality of whole-plant *Tithonia diversifolia* (Hemsl) A. Gray grown in two localities

The macromolecular content of PITd for the variables dry matter, mineral content, and NDF was affected by the second-degree interaction (Loc x Introd) (P≤0.05). The highest accumulation of dry matter in the whole plant of *T. diversifolia* was obtained in introduction 25.2, locality Candelaria, Colombia, with differences concerning 1.3 of Candelaria, Colombia and the 17.9 and 22.14A of El Carmen, Ecuador (P≤0.05). No significant differences (P≥0.05) were observed among the rest of the Introductions in both localities (Table 1).

Similarly, for NDF content, it was Introduction 1.2 grown in Candelaria, Colombia that showed the highest values (P≤0.05), surpassing the rest of the Introductions from this locality and those grown in El Carmen, Ecuador. A similar phenomenon occurred for mineral content, it was introduction 13.5 grown in El Carmen that yielded the highest percentages (P≤0.003), surpassing (P≤0.05) Introductions 1.3, 17.9, 22.14A, and 25.2 grown in this same locality, and those grown in Candelaria, Colombia.

For lignin (%), organic matter (%), and acid detergent fibre (%) values of the whole plant, no effects were observed from the interaction (Loc x Introd) (P≥0.05). Lignin values ranged between 10 and 20% in Introduction 25.2 and three of the Introductions located in Candelaria and Carmen respectively; organic matter content varied between 81 and 89% in Introductions 17.9 and 25.2 from Candelaria and Carmen respectively; ADF showed values of 38 and 54%, represented by Introductions 1.3 and 13.5; 17.9 and 22.14A in the localities Candelaria and Carmen respectively.

In general, mineral content, dry matter, *FDA*, and cell wall were significantly higher in El Carmen compared to Candelaria. This is indicative of, age, as well as a better performance of certain Introductions, which might be related to the specific responses of some Introductions to the prevailing edaphoclimatic conditions. Similarly, López *et al.* (1998); Ledea *et al.* (2016); Canul-Solis *et al.* (2020); Ku-Vera *et al.* (2020) reported very specific responses of some ecotypes of *Tithonia diversifolia*, suggesting that the variable results between Colombia and Ecuador could correspond to this behavior. However, Makkar *et al.* (1997) showed that 45% of the variation *in vitro* digestibility of some protein plants and tropical legumes was represented by the variation in the content of certain secondary compounds (Hoover and Stokes, 1991; Makkar, Aderibigbe and Becker, 1998; Martínez-Herrera *et al.*, 2006; Sauvant *et al.*, 2011; Leng, 2014; Holguín Castaño *et al.*, 2015). Nevertheless, these authors indicated that the beneficial or detrimental effect that these might have on some protein plants is not clear, recommending handling this aspect with utmost caution until concrete evidence is found.

***In vitro* fermentation and gas production in the whole plant of *T. diversifolia* (Hemsl) A. Gray**

Ruminal degradability analysis of the dry matter of the whole plant *Tithonia diversifolia* from El Carmen, Ecuador, showed percentages for the soluble fraction (a) between 172% (intro. 17.9) and 189.67% (intro. 1.2) compared to 172.04% provided by the control (Tifton 85). Meanwhile, for the soluble but potentially degradable fraction (b), the introductions 1.2, 13.5, 17.9, and 22.14A were ≥8% with very little variability between the rest of the introductions and the Tifton 85 control; potential degradability, on the other hand,

Table 1. **Combined Effect of the Factors Location and Introductions of *T. diversifolia* from the Vegetable Program of the National University of Colombia, Palmira Campus, on certain Chemical Composition Variables in Whole Plant.**

Locations	Introductions (*T. diversifolia*)	Dry Matter DM (%)	Crude Protein (%)	Mineral composition (%)	[2]Neutre Fiber Detergent NFD (%)	[1]Lignine (%)	[2]Organic matter (%)	[2]Acid Fiber Detergent AFD (%)
Candelaria (Colombia)	1.2	20.65±1.89^{ab}	12.54±0.07	11.75±1.44^{bc}	83±3.72^{a}	17.83±6.60	86.24±5.03	44.07±7.04
	1.3	18.92±3.18^{b}	12.85±1.98	11.75±0.89^{bc}	57.30±14.19^{b}	14.20±0.85	87.97±2.26	38.53±1.88
	13.5	20.99±4.13^{ab}	14.31±1.9	12.14±0.79^{bc}	59.77±11.67^{b}	18.83±5.74	82.34±3.12	38.60±3.82
	17.9	20.80±2.31^{ab}	13.58±1.5	11.84±0.65^{bc}	51.82±4.19^{b}	13.19±1.67	81.74±2.52	43.34±5.31
	22.14A	23.76±3.43^{ab}	13.38±1.5	12.53±0.32^{bc}	53.95±2.36^{b}	12.42±4.45	86.16±2.62	42.33±3.16
	25.2	26.08±0.60^{a}	14.50±3.9	11.65±1.43^{bc}	54.65±9.0^{b}	10.62±3.75	84.57±2.97	42.22±5.40
El Carmen (Ecuador)	1.2	21.90±0.4^{ab}	13.71±0.04	13.09±0.002^{ab}	57.76±0.014^{b}	16.53±0.03	86.91±0.001	51.06±0.04
	1.3	22.50±1.11^{ab}	10.46±0.04	11.28±0.002^{bc}	64.42±0.024^{b}	16.72±0.04	88.73±0.001	53.02±0.01
	13.5	23.60±1.25^{ab}	14.30±0.01	14.72±0.003^{a}	62.91±0.01^{b}	20.91±0.05	85.28±0.001	53.86±0.04
	17.9	18.80±1.41^{b}	13.78±0.01	12.31±0.001^{bc}	64.67±0.03^{b}	18.39±0.04	87.69±0.001	54.81±0.58
	22.14A	19.40±0.87^{b}	13.02±0.02	12.39±0.001^{bc}	64.20±0.05^{b}	17.31±0.01	87.61±0.001	54.15±0.03
	25.2	20.50±0.56^{ab}	10.50±0.02	19.90±0.001^{c}	61±0.03^{b}	17.52±0.01	89.10±0.001	53.66±0.03
±SE		21.59	0.39	2.383	2.919	0.846	0.670	5.88
P		0.003	0.06	0.003	0.0001	0.28	0.27	0.36

[a, b, c] Similar letters within the same variable, do not differ statistically according to Tukey for ≤0.05.±SE: Standard error; P: Pvalue; [1]Resulted different (p<0.05) for location factor; [2]for the introduction factor. The analysis of the comparison of means for lignin was carried out utilizing the transformation $ArcCos(\sqrt{(\frac{x}{100})})$

ranged between values of 177.4% (intro. 13.5) and 198.3% (intro. 1.2), while the rate of degradation of the dry matter (c) of whole plant *Tithonia diversifolia* remained between 24-25%.h-1. (Table 2).

Table 2 shows the accumulated gas production from the fermentation of the DM of the studied samples during the *in vitro* incubation period. Kinetic behaviour was characterized by an increase in gas production with the exposure time of the samples to microbial attack, with values increasing with incubation time. The higher values in gas production in the last hour of incubation could be due to the concentration of carbohydrates and easily fermentable nutrients present initially, and because ruminal microorganisms and their enzymes first attack easily available carbohydrates. Then, with fibre colonization and fermentations, an increase in gas production is achieved.

Figure 1 describes the kinetic profiles of gas production per gram of incubated dry matter (mL.g^{-1} DM) in PITd compared to the laboratory control (CENA/USP, Piracicaba, Brazil), Tifton 85 hay, during the 24-hour incubation period. Uniform behaviour of the first four hours is highlighted, and from this point, a disparity in gas production from the introductions compared to the control begins to be observed, increasing as a function of time, and accentuating at the end of the 24 hours.

In vitro ruminal environment promoted by inoculation of whole plant of *T. diversifolia* (Hemsl) A. Gray

Figure 2 shows, in their various forms, the variables that promote a given rumen environment due to the inoculum of the whole plant *T. diversifolia*. This behaviour describes the general trends of *in vitro* indicators of nutritional value and fermentation.

Table 2. *In vitro* ruminal degradability of dry matter in the whole plant of T. diversifolia introductions originating from the Vegetable Program of the National University of Colombia, Palmira campus, cultivated in the locality of El Carmen, Ecuador.

***In vitro* degradability of dry matter (%)**	**Introduction (*T. diversifolia*)**						**Control (Tifton 85)**
	1.2	1.3	13.5	17.9	22.14A	25.2	
a	189.67	181.63	169.09	172.78	179	181.32	172.04
b	8.63	7.79	8.05	8.13	7.95	7.41	7.23
a+b	198.3	189.42	177.14	180.91	186.95	188.73	179.27
c (%, hours)	0.26	0.25	0.25	0.25	0.26	0.26	0.22
SEE	20.98	20.79	17.92	18.34	21.25	20.88	15.32
R^2	0.98	0.97	0.98	0.98	0.97	0.96	0.97

a: Soluble fraction; b: Insoluble but potentially degradable fraction; a+b: Potential degradation calculated according to model; c: Degradation rate of b; SEE: Standard error of the estimate; R²: Coefficient of determination of fit.

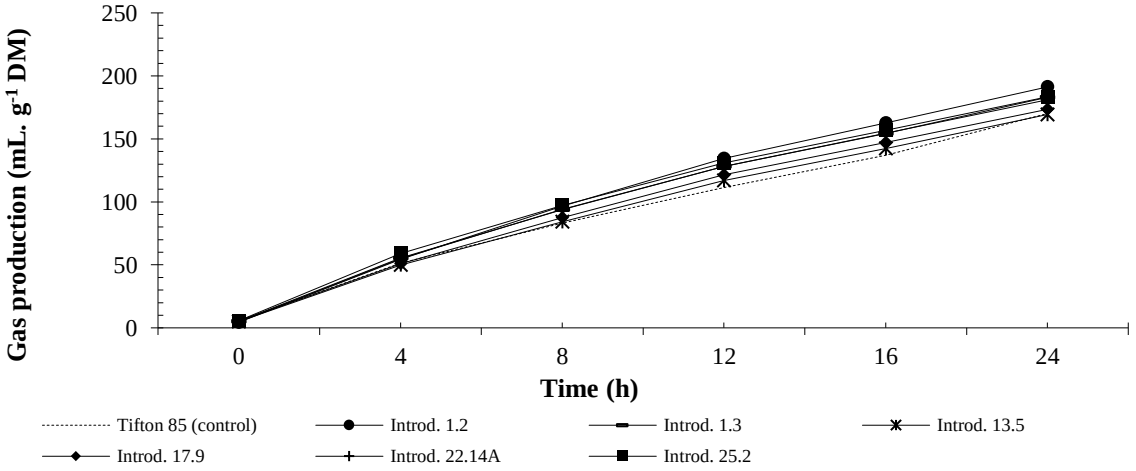

Figure 1. *In vitro* Fermentation Potential of Introductions of *T. diversifolia* Originating from the Vegetable Program of the Universidad Nacional de Colombia, Palmira Campus, Grown in the Locality of El Carmen, Ecuador.

Values for the partition factor (PF) are presented, a variable that expresses the ratio between truly degraded substrate and total gas volume produced (Figure 2a); for which no significant differences were obtained (P=0.07) promoted by the WPTd introductions compared to the control (Tifton 85). Average values for this variable ranged between 0.03 and 0.08. A similar condition (P≥0.05) was observed for net methane production linked to DM (Net CH_4 DM) (P=0.16) (Figure 2b), whose values were found between 4.83 - 10 mL g^{-1} DM.

Likewise, concerning neutral detergent fibre degradation (DFND) (P=0.06) (Figure 2c), net methane production linked to organic matter degradability (Net CH_4 DOM) (P=0.11) (Figure 2g) and net methane production linked to DNF (Net CH_4 NFD) (P=0. 28) (Figure 2i), no significant differences were obtained (P≥0.05), however, DNFD showed a tendency to increase its values (P≤0.10), this variable presented averages between 236.52 and 331.17 g kg^{-1}, while Net CH_4 OM showed ranges between 1.82-4.18 mL g-1 DOM, and Net CH_4 NFD 1.42-2.48 mL g-1 DNFD.

In the digestibility of organic matter (DOM) (Figure 2d) and net gas production per gram of degradable Organic Matter (Net GP DOM) (Figure 2f), significantly higher values were obtained in introductions 1.2 and 1.3, with averages of 555.48 and 493.61 g Kg^{-1} DOM for DOM; and 81.67- and 68.57 mL g^{-1} DOM for Net GP DOM, respectively. Between both introductions, no differences existed (P≥0.05), but there were differences compared to the rest of the introductions studied. Their values for DOM ranged between 471.73-485.79 g Kg^{-1} DOM, and 60.59-66.38 mL g^-1 DOM for Net GP DOM; however, all surpassed the Tifton 85 control (P≤0.05), which showed values of 336.17 g Kg^{-1} DOM and 42.32 mL g^{-1} DOM for Net GP DOM.

Net gas production associated with dry matter degradation (net GP DM) was significantly represented by introduction 1.2, with contributions of 146.29 mL g^{-1} DM, differing (P≤0.01) from introduction 13.5 (129.14 mL g^{-1} DM) and the control Tifton 85 (125.82 mL g^{-1} DM), which were similar (P≥0.05). Between introduction 1.2 and the rest of the introductions, there were no differences (P≥0.05) (Figure 2e). For gas production associated with the degradation of NFD (Net GP NFD), introduction 1.2 (48.75 mL g^{-1} NFD) also significantly surpassed the Tifton 85 control (33.09 mL g^{-1} NFD) (P≤0.05), as well as the rest of the introductions, except introduction 25.2 (32.90 mL g^{-1} NFD), which resembled the control (P≥0.05) (Figure 2h).

Generation of organic acids During the *in vitro* incubation of whole plant *T. diversifolia* (Hemsl) A. Gray

Figure 3 contains information related to the production of organic fatty acids and gases during *in vitro* digestion of whole plant *T. diversifolia*. In acetic acid production, the values obtained for all introductions were higher than the control Tifton 85 (P≤0.001). Among them, differences were also established (P≤0.05). In this way, significantly higher levels were promoted by the whole plant inoculum from introduction 1.2 compared to introduction 13.5 (P≤0.001). At the same time, both 13.5 and 1.2 shared superscripts with introductions 1.3, 22.14A, and 25 (P≥0.05) (Figure 3A).

However, in the production of propionic acid, in addition to surpassing (P≤0.01) all the introductions over the Tifton 85 control, except for 17.9, no significant differences were established among them (P≥0.05). As to produce butyric and valeric acids, no differences were recorded (P=0.91 and 0.98, respectively). The values for these ranged between 0.55-0.79 mmol Ac. Butyric, and 0.16-0.21 mmol Ac. Valeric. The ratio or relationship between levels of acetic ac: propionic did not show significant differences (P≥0.24), nor did ammonia nitrogen content (P≥0.34), nor hydrogen ion potential (pH) (P≥0.07) (Figure 3B).

DISCUSSION

Macromolecular quality of whole-plant *T. diversifolia* (Hemsl) A. Gray grown in two localities

Seasons, specifically the dry season characterized by high sunlight intensity and temperature, lead to changes in the fibrous components of plants, with a significant amount of structural carbohydrates (cellulose and hemicellulose) observed (Hassen *et al.*, 2017). However, the use and spread of protein plants are a favourable option for low-input systems (La O *et al.*, 2012; Carvalho-Costa *et al.*, 2020). These variable results obtained in Ecuador and Colombia with similar introductions contribute to the search for economically feasible and sustainable alternatives for Latin American regions. This continues to open opportunities where plants play a critical role, especially concerning specific nutritional effects.

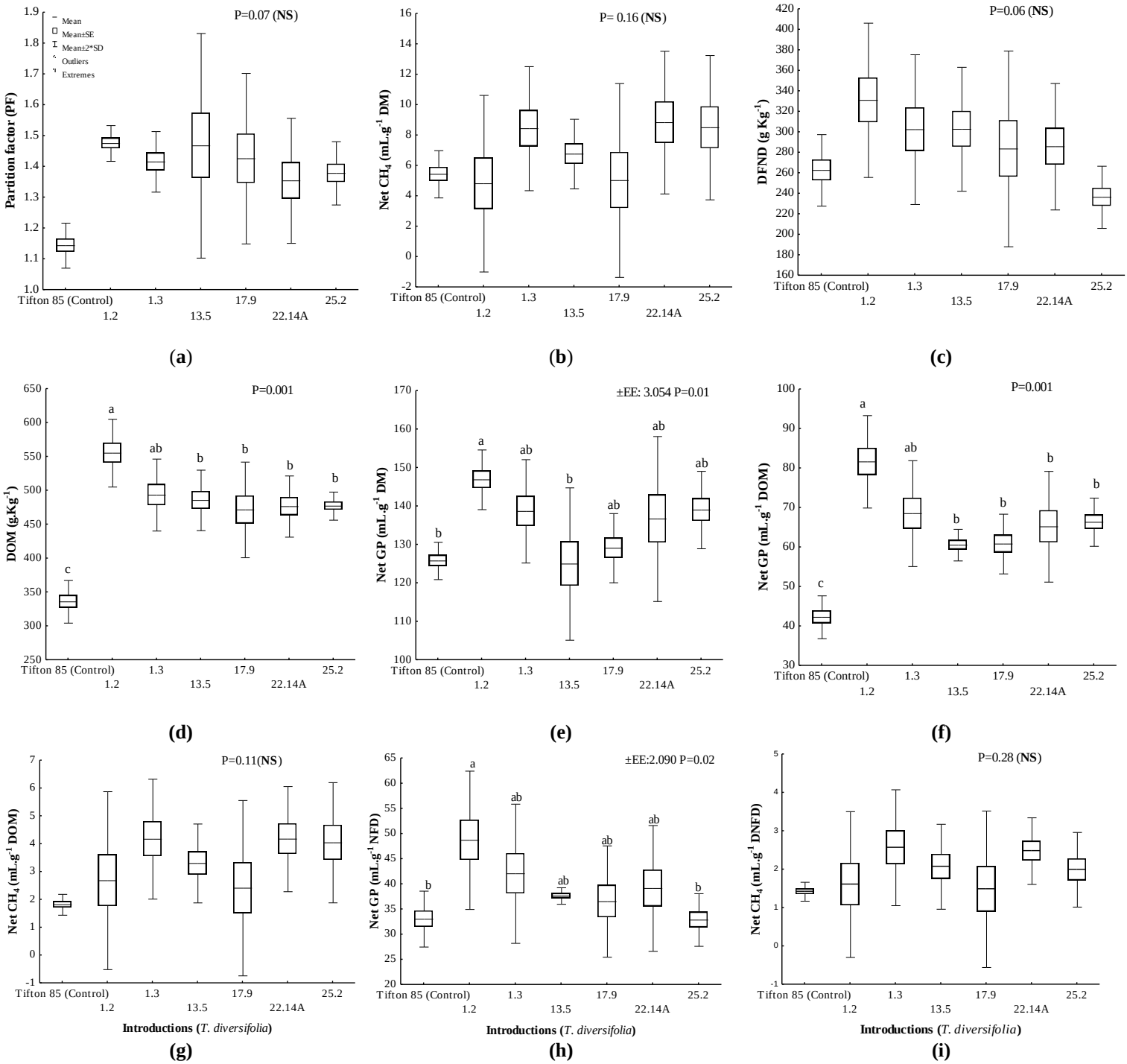

Figure 2. Rumen environment promoted by *T. diversifolia* whole plant inoculums from the Vegetable Programme of the Universidad Nacional de Colombia, Palmira, grown in El Carmen, Ecuador. [a, b] Similar letters within the same figure do not differ statistically according to Tukey for P≤0.05. **(a)** Partition factor; **(b)** Net CH_4 (mL.g^{-1} DM)= Net methane production, in millilitres per gram of digestible dry matter; **(c)** DFDN (g.Kg^{-1})= Degradation of Detergent Neutral Fibre in grams per kilogram; **(d)** Digestibility of organic matter (g kg^{-1}); **(e)** Net GP DM (mL.g^{-1} DM)= Net gas production in millitres per gram of degradable dry matter; **(f)** Net GP DOM (mL.g^{-1} DOM)= Net gas production in millitres per gram of degradable organic matter; **(g)** Net CH_4 DOM (mL.g^{-1} DOM)= Net methane production per unit of degradable organic matter; **(h)** Net GP (mL.g^{-1} NDF)= Net gas production in millilitres per gram of neutral detergent fibre; (i) Net CH_4 (mL.g-1 DFDN)= Methane production in millilitres per gram degraded of Neutral Detergent Fiber. **NS** Not significant.

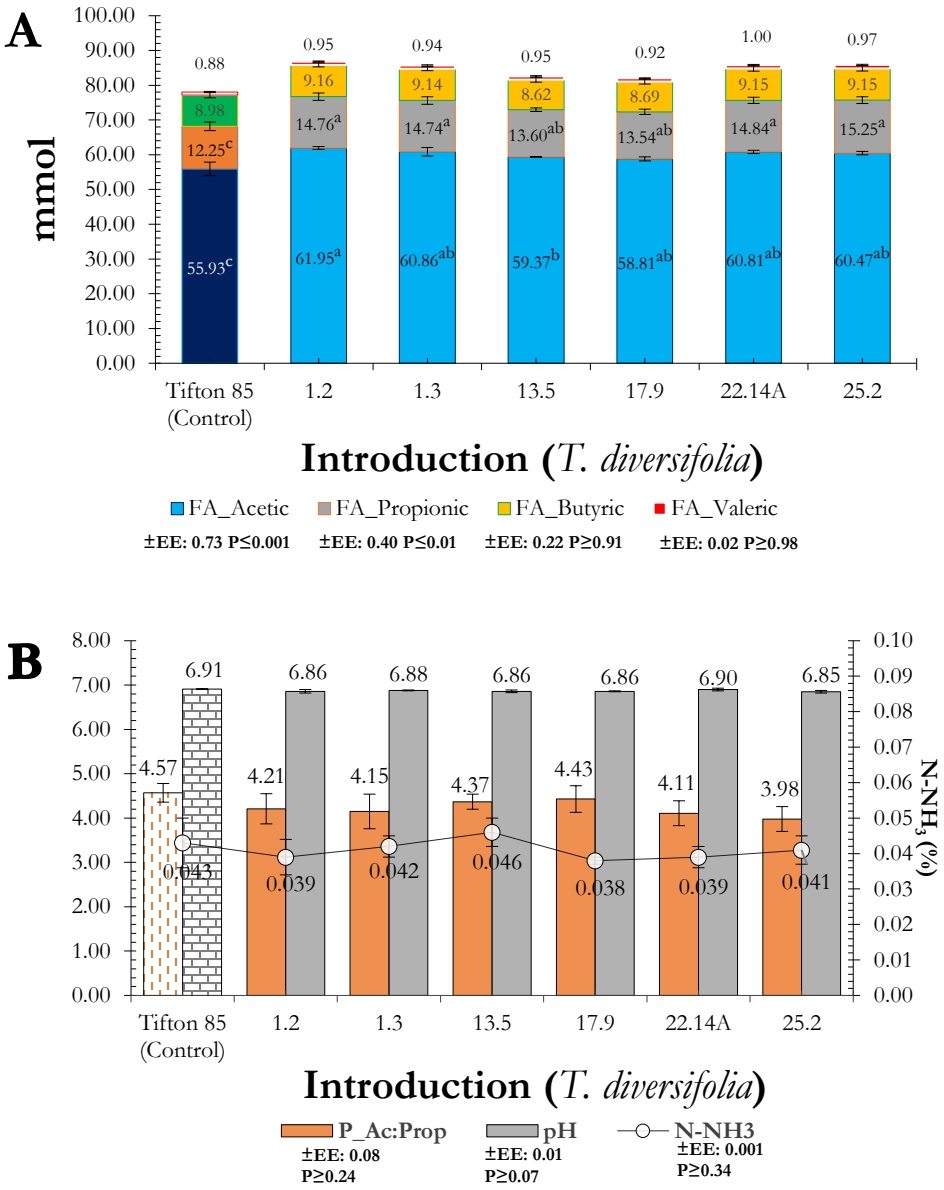

Figure 3. Proportion (**A**) and quantification (**B**) of organic acid production *in vitro* condition in whole-plant introductions of *T. diversifolia* from the Vegetable Program of the National University of Colombia, Palmira Campus, grown in El Carmen, Ecuador. A- FA_Acetic= Fatty acid Acetic; FA_Propionic= Fatty acid Propionic; FA_Butyric= Fatty Acid Butyric; FA_Valeric= Fatty Acid Valeric. Vertical lines within each bar indicate the standard deviation (SD) of the mean. B- pH: Hydrogen ion potential in the in vitro solution; P_Ac: Pr. The ratio between Acetic and Propionic production. PF= Partition factor or the ratio between truly degraded substrate and total gas volume produced; N-NH_3= Ammoniacal Nitrogen ±SE: Standard Error; P= P-value; vertical lines within each bar indicate the standard deviation (SD).

In summary, the differences between regions in the performance of these introductions are not yet fully supported by experimental lab and animal tests. Despite variable and/or matching results in chemical evaluation, the multidimensional factors affecting these outcomes present an ongoing challenge for future research in both regions.

Studies developed by Herrera *et al.* (2017), in ecotypes of *T. diversifolia* in different regions of Cuba. In the rainy period, only multiple linear equations could be established for NDF, ADF, lignin, cellulose and cellular content, where climatic elements of greatest preponderance were the maximum temperature, total rainfall and days with rain. It is also necessary to point out its high coefficients of determination and statistical

probability, as well as its low estimation error, which demonstrates the effect of climatic seasons on the nutritional behaviour of *T. diversifolia*, in the content of the fibre fractions. Subsequent studies will be necessary to identify the behaviour of lignin in this plant since, even though it is made up of high molecular weight alcohols and polyphenolic compounds, it maintained a stable trend between both regions and introductions.

Tithonia presents secondary metabolites that can negatively influence its digestibility (Verdecia 2014). However, Lezcano *et al.* (2012) also studied the secondary metabolites of this plant but did so through qualitative analysis and this depends on the sensitivity of the reagents used, the age of the plant, the content of secondary metabolites and the leaf: stem ratio. Therefore, it offers information of certain value, but with less precision than when the quantitative determination of said substances is carried out.

However, Herrera *et al.* (2017) clarify studies carried out with this plant, evaluating several hypotheses about its behaviour, suggesting that the rainy period is the stage of highest temperatures, solar radiation, duration of light and rain, so the conditions are conducive to the growth and development of plants; From a metabolic point of view, there are no limiting factors for photosynthesis. However, the dry period is characterized by lower temperatures (including the minimum), solar radiation, duration of light and rainfall. This imposes limiting factors for growth, and the plant must adapt to the limiting factors limitations for photosynthesis. However, the dry period is characterized by lower temperatures (including the minimum), solar radiation, duration of light and rainfall. This imposes limiting factors for growth, and the plant must adapt to the stress factors that predominate in the period.

Additionally, when considering the NDF value in introduction 1.2, which exceeds by 39% the introduction with the closest value, the hypothesis could be established that there are variations in the intensity and elements that intervene in the synthesis of the compounds. of the cell wall in this plant (Herrera *et al.*, 2017).

In Vitro Fermentation and Gas Production in Whole Plant *T. diversifolia* (Hemsl) A. Gray

Gas production characteristics show data fitting an exponential model (R^2>97), with similar behaviour across all introductions and little influence from factors inherent to the plant and the fermentation process itself. It is crucial to consider that gas accumulation during fermentation, particularly hydrogen, could inhibit ruminal cellulolysis and its relationship with high concentrations of secondary plant compounds. This aspect has not been confirmed in studies with different introductions of *T. diversifolia*. Additionally, Rodríguez *et al.* (2009) stated that under physiological stress conditions, plants can create defence mechanisms ranging from producing various secondary metabolites to changing certain storage and utilization patterns, an aspect not explored in this study but represents a challenge for future research, considering adaptability to soil and climatic conditions.

In this regard Cadenas-Viellegas *et al.* (2020), state that the protein tannin bond is very stable and as it approaches the abomasum it becomes unstable, so it can potentially be used as a strategic option for the protection of protein from rumen degradation and increase the by-pass; these sources of tannins, where it is also present in *Tithonia diversifolia*, can increase the efficiency of protein use of these feeds as an alternative for farm animals (Ningrant *et al.*, 2018; 2019; 2020).

Tannins can also bind to carbohydrates and at high levels in plants like Calliandra can affect the rate of degradation or digestibility of this nutrient and protein. This infers that the theory that ruminants can fully counteract the antinutritional effect of tannins is not entirely accurate (Rodríguez *et al.*, 2009). Similar opinions are held by Jamarum et al. (2018; 2019; 2020a,b) who studied some ingredients in alborea seeds and found negative effects when exceeding optimal levels tolerable by animals of productive interest.

Rates of gas production speed (c) found are in the range reported for some tropical legumes like *Leucaena leucocephala* (La O *et al.*, 2012) and similar for transit speeds in cattle fed with tropical pastures. Meza-Bone *et al.* (2022) reported lower gas production in Botón de Oro, whereas in legumes it was higher, with the cutting age affecting gas production, showing lower production in the dry season at 30 and 45 days.

In vitro gas productions found in the studied introductions indicate a potential use of these for animal feed. Ravhuhali *et al.* (2022) have indicated that the dynamics of the season or time of the year markedly affect nutritional value, chemical composition estimation, and the in vitro digestibility of dry matter and its intrinsic nutrients.

The evolution of gas production dynamics (Figure 1) showed a progressive asymptotic increase. This behaviour is characteristic of most protein plants, as they show exponential growth over time until the last incubation time (Galiendo *et al.*, 2011; La O *et al.*, 2012). This trend could be related to the type and amount of nutritional compounds available in the organic matter of these introductions, which in larger

measures are present in some tropical plants that can either limit or favour the ruminal degradability of nutrients (Rodríguez *et al.,* 2009; La O *et al.,* 2012 and Galindo *et al.,* 2019).

In vitro ruminal environment promoted by whole-plant inoculum of *T. diversifolia* (Hemsl) A. Gray

The nutritional value of any feed is related to the nutrient contribution determined by chemical and biological methods that allow having a reference for the possible use that ruminants can make in terms of nutrients (Cadena-Villegas *et al.,* 2020). Among these methods, the gas production technique is one of the most used in determining the use of organic compounds through the fermentation process of nutrients; ruminal degradability is another element to consider when determining possible effects of foods on the first portion of the ruminant's digestive system.

Figure 2a describes the net methane production, in millilitres per gram of digestible dry matter (Net CH_4 mL g^{-1} DM) and degradation of Neutral Detergent Fiber in grams per kilogram (DFDN (g Kg^{-1})) of the different introductions studied in the research; without existing differences between these. The degradability obtained in the different introductions is like the range of values reported by La O *et al.* (2012) *in situ* tests, with plant materials of *T. diversifolia*, using cannulated bulls; and are within favourable limits for ruminal microbial fermentation when using trees, shrubs, and tropical legumes. Something similar happened with the net methane productions, which coincides with what was found by Galindo *et al.* (2011; 2019), when studying different ecotypes of Golden Button in the western region of Cuba, who report values of up to 23.5; 10^{10} CFU/mL, which infers measured contents of this compound under "*in vitro*" conditions; however, it has been shown that methane-producing bacteria have biochemical and genetic characteristics distinct from the rest of the microorganisms that coexist in the rumen. On the other hand, Holguín *et al.* (2015) reported lower methane values as the inclusion of Golden Button increased in the mixture.

Variable plant responses exist to nutritional value indicators associated with 1- the time of year; 2- the physiological age of the plants associated with changes in cell wall components and protein composition; as well as 3- the presence of bioactive components (condensed tannins, polyphenols, and others) and their influence on the rate of degradation and digestibility of foods (Canul-Solis *et al.,* 2020; Ravhuhali *et al.,* 2022).

The population density of bacteria in the rumen varies with diet and mainly with the fibre content of foods (13). On the other hand, it is known that the use of foliage from tree plants or their extracts is a current practice used to reduce methanogens and methane production in the rumen (Galindo *et al.,* (2011). It has also been reported (Galindo *et al.,* 2019), that both *in vitro* and *in vivo* conditions can have direct effects due to the presence of tannins, as well as indirect effects because on fibre digestion.

For NDF digestibility (g kg^{-1}), the values obtained in the introductions ranged from 230 to 340 g kg^{-1} of digestible neutral detergent fibre, significantly lower than those obtained by Gutiérrez *et al.* (2017), Galindo *et al.* (2011; 2017), La O *et al.* (2012) y Cadena-Villegas *et al.* (2020). This is largely related to differences in standardization and the type of technique used in the study, as well as the ratio of buffer: ruminal fluid, sample size, and container. However, methane production (2 to 10 mlg^{-1} DM) not corresponds with the results obtained by Galindo *et al.* (2017), who assert that *T. diversifolia* has a significant impact on reducing methanogenic bacteria present in the rumen. Nevertheless, it is necessary to validate these results under our conditions, which will be a challenge for subsequent studies to prove this assertion.

The *in vitro* degradability of organic matter reached maximum values ($p<0.001$) for all introductions up to 49.3% OM and cell walls up to 35.0% NDF, yet these were significantly lower than those obtained by La O *et al.* (2012) and Gutiérrez *et al.* (2017). This is perhaps related to the effects of factors such as season, different edaphoclimatic conditions, and other uncontrolled components.

Indeed, it is proven that organic matter is where all the nutritive compounds present in any food are found. The differences in the digestibility of organic matter among the studied introductions are related to several factors that affect its composition and the percentage of utilization. In this regard, authors such as Canul-Solis *et al.* (2020); and Ravhuhali *et al.* (2022); refer to the effects of edaphoclimatic conditions, age, stress responses, secondary compounds, or antinutritional factors. In plants, these secondary metabolites are defence mechanisms against the presence of pathogenic microorganisms and predation by insects or herbivores, and their role as a defaunation agent in the rumen is linked to their composition in tannins, saponins, and essential oils, as is the case with methanogens.

The values obtained for gas production showed differences among the introductions ($p<0.001$) (ml. g^{-1} of degradable organic matter) and demonstrated high fermentation capacity, which can reach up to 85 ml of gases g^{-1} of fermentable organic matter of *T. diversifolia* introduction 1,2. This indicates a high content of nutrients available for ruminal

microorganisms, allowing an increase in microbial synthesis efficiency.

However, for CH_4 (ml. g^{-1} of degradable organic matter), the values obtained are relatively low and can range between 2 to 5 ml of CH_4 g^{-1} of fermentable organic matter. These results do not correspond with those found by Galindo *et al.* (2017), who observed a reduction of approximately twice the population of methane-producing microorganisms in the rumen with a depressive effect, with significant differences, in ruminal methanogens in cows integrated into a silvopastoril system with *T. diversifolia*. The response to the use of Silvopastoral Systems (SPS) with *T. diversifolia* concerning the depressive effect on the population of methanogens can be explained by the fact that these live endosymbiotically on the rumen protozoa (Galindo *et al.*, 2011), and any effect that helps eliminate rumen protozoa will decisively contribute to reducing methane-producing populations.

Gas production values, 85 and CH_4, 2 (ml. g^{-1} MOD), indicate the possibility of efficiently utilizing the cell wall of the Golden Button in productive systems. The fermentative potential of the fibrous fraction is one of the most significant aspects of manipulating ruminal fermentation. Therefore, the gas production technique has been used as a measure of ruminal food degradation and as an indicator of digestible DM intake. The fractional degradation rate has been a means of predicting the voluntary intake of ruminants (La O *et al.*, 2012).

Methane production (ml g-1 DNFD) showed no significant differences among the studied introductions, which is consistent with the findings of this research and what has been proposed by some researchers using certain tropical forage species in ruminant feeding, which have been shown to decrease CH4 synthesis due to the presence of compounds such as tannins and saponins. These have some anti-methanogenic effects, thus showing a significant decrease in CH4 production per unit of digested forage (Carvalho-Costa *et al.*, 2020). In this regard, Tithonia diversifolia is a plant with good nutritional value (protein-soluble carbohydrate balance) (Makkar *et al.*, 1995; Galindo *et al.*, 2019) and control of methanogenic microorganisms with the consequent reduction of CH4 observed in in vitro tests (Mauricio *et al.*, 1999; Galindo *et al.*, 2011). However, these results coincide with those reported by Molina *et al.* (2015), who found that the inclusion of *T. diversifolia* in the diet of bovines was not associated with reductions in net enteric methane emissions, but with increases in consumption. of dry matter and nutrients in animals with higher nutritional requirements and conclude that it is necessary to identify the impact of the presence of *T. diversifolia* at the level of the total production system, to quantify the true impact of the adoption of silvopastoral systems based on this species by contrasting them against the productive benefits that are normally observed in these productive agroecosystems.

On the other hand, Golden Button stands out for its ability to accumulate nitrogen (Navas, 2008) and for its nutritional characteristics, such as protein content, soluble carbohydrates (Makkar *et al.*, 1995; Galindo *et al.*, 2019), and tannin content (La O *et al.*, 2012). These can contribute to improving the nutritional balance in animals, concerning energy and protein supply in the dairy cattle diet. In addition, studies conducted by Sauvant *et al.* (2011), based on a compilation of 59 experiments, showed that CH_4 production per kilogram of digested OM decreased linearly when the crude protein content of the foods increased (CH_4, g kg OM digestible $^{-1}$ = 40.1 0.32 × CP, percentage of DM).

In Figure 2a, part of the main fermentation profile results of the Golden Button introductions are reported with a partition factor (PF) ranging from 1.50 to 1.60 mg. ml^{-1}, without showing differences between the introductions. In this regard, generally, substrates that record higher degradation DM and OM, per unit of gas generated, are associated with higher PFs and microbial protein formation but with a progressive decrease in methane production. There is an inverse relationship (Longo *et al.*, 2006), which corresponds to what is suggested by some authors (Makkar *et al.*, 1995, 1997, 1998; Martínez-Herrera *et al.*, 2006).

They stated that when plants have high tannin contents, partition factors range from 2.75 to 4.41 mg. ml^{-1}; however, for balanced feeds, they range from 2.65 to 4.41 mg. ml^{-1} due to the high concentration of easily fermentable carbohydrates, much higher than those found in our work. This could be possibly related to the relatively low tannin concentration in the studied introductions, which will be necessary to study in future research. In this regard, studies by La O *et al.* (2012) with various ecotypes and/or cultivars of *T. diversifolia* reported that this plant generally maintains relatively low tannin concentrations and suggest that tannin: macromolecule bonds may not have high efficiency concerning the tannin complex: proteins and tannins: fibre.

Generation of organic acids during the *in vitro* incubation of whole *T. diversifolia* (Hemsl) A. Gray plant

Values obtained for pH, ammoniacal nitrogen, acetic acid, propionic acid, butyric acid, valeric acid, total fatty acids, and acetic: propionic ratio were lower for pH; short-chain total fatty acids, acetic acid, propionic acid, and valeric acid compared to those reported by

Galindo *et al.* (2017) in cows in a silvopastoral system with Golden Button. In this regard, it is proposed that microbial relationships in the rumen are complex, and even more so are the fermentative pathways that use or produce H_2, as microorganisms can change their fermentation patterns in response to small differences in energy conservation, ceasing to use thermodynamically less favourable routes.

Volatile fatty acid values were lower than those reported by Cardona *et al.* (2017), probably related to Rodríguez *et al.* (2009), who reported that the VFA profile is mainly modified by the type of diet, the forage: concentrate ratio, the level of consumption, and the use of additives, among other factors; according to the authors, an increase in fibrous sources generally leads to acetic fermentation.

The aforementioned could explain the responses found in the concentrations of volatile fatty acids in the rumen when *T. diversifolia* is consumed, as it has been shown that the increase in the partial pressure of hydrogen in the rumen also reduces the deamination of reduced amino acids, including branched-chain ones (Galindo *et al.*, 2019).

Results of the relationships between the VFAs in the rumen seem to indicate that the mechanism by which the foliage of this plant acts is very complex and involves other factors, such as the degradability of the protein in the rumen (La O *et al.*, 2012). In this regard, Leng (2014) proposed a possible mechanism by which soluble proteins cause higher volumes of methane in the rumen, while those less soluble may pass to the lower parts of the GIT, a factor that could be related to the efficiency of VFA utilization.

Galindo *et al.* (2011) demonstrated that when shrub foliage, legumes capable of providing protein sources, is made available to rumen microorganisms, specifically cellulolytic ones, compounds such as ammonia, amino acids, peptides, and branched short-chain fatty acids are generated. These substances favour fibre degradation (Hoover *et al.*, 1991) and justify the high fermentability of dry matter and, therefore, a rapid availability of nutrients, and fermentation products, aspects reported by Mahecha and Rosales (2005).

CONCLUSIONS

From the results obtained, it can be concluded that the Golden Button introductions maintain an acceptable protein composition, high degradability, and low methane production, which would reduce the population of protozoa and ruminal methanogens when evaluated under in vitro conditions; with the consequent benefit for the energy utilization of the feed; there is high performance in the fermentation of organic matter and the cell wall, which positively denotes and rules out its future use as a ration supplement under edaphoclimatic conditions for Colombia and Ecuador.

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Compliance with ethical standards. Compliance with ethical standards through the authorization by an ethical or bioethical committee.

Data availability. The databases and information related to the project are under the responsibility of walter.vivas@utm.edu.ec upon reasonable request.

Conflicts of interest. The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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original draft preparation. All authors have read and agreed to the published version of the manuscript.

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