



EFFECT OF A SILVOPASTORAL SYSTEM WITH *TITHONIA DIVERSIFOLIA* (HEMSL.) A. GRAY ON THE RUMINAL MICROBIAL COMMUNITY OF SIBONEY DE CUBA MALE CATTLE

EFFECTO DE UN SISTEMA SILVOPASTORIL CON *TITHONIA DIVERSIFOLIA* (HEMSL.) A. GRAY EN LA COMUNIDAD MICROBIANA RUMINAL DE MACHOS VACUNOS SIBONEY DE CUBA

✉ JUANA L. GALINDO BLANCO*, ✉ J. IRAOLA JEREZ, ✉ MAGALY HERRERA VILAFRANCA

Instituto de Ciencia Animal, Carretera Central, km 47½, San José de las Lajas, Mayabeque, Cuba

*Email: juanaluzgblanco@gmail.com

To evaluate the effect of silvopastoral system with *Tithonia diversifolia* on the ruminal microbial community of Siboney de Cuba genotype cattle in growth-fattening, a total of 24 non-castrated animals were used. Two treatments were established: 1) grazing on improved and natural grasses and 2) grazing in a silvopastoral system of *T. diversifolia* grasses, associated in 100 % of the area. The animals grazed for 24 hours on 10 hectares (ha) of improved grasses, divided into two systems of 5 ha each. The crude protein in the control treatment was 9.54 %, while in the SPS it was 10.5 % in the grass and 22.7 % in *Tithonia*. In the rumen of animals that grazed in the SPS with *Tithonia*, the total number of viable bacteria was higher ($p < 0.0001$), while the proteolytic bacteria were 47.17×10^5 CFU/mL and those of the control treatment, 19.42×10^5 CFU/mL, which represents 2.43 times fewer bacteria. There were no effects on the number of cellulolytic fungi. The silvopastoral system increased the number of cellulolytic bacteria and the protozoa were reduced 3.5 times. Methane production was 37.0472 g/kg of digested OM with grasses and in the SPS with *Tithonia* 34.788 g/kg of digested OM. It is concluded that the rumen microbial community of Holstein x Zebu non-castrated cattle that grazed in SPS with *Tithonia* showed a higher number of total viable bacteria, proteolytic bacteria, cellulolytic bacteria, a lower number of protozoa and less CH_4 compared to the control that grazed in grass areas.

Key words: cellulolytic bacteria, proteolytic bacteria, total bacteria, methane, protozoa

Para evaluar el efecto del silvopastoreo con *Tithonia diversifolia* en la comunidad microbiana ruminal de vacunos del genotipo Siboney de Cuba en crecimiento-ceba, se utilizaron 24 animales machos enteros. Se establecieron dos tratamientos: 1) pastoreo en gramíneas mejoradas y naturales y 2) pastoreo en sistema silvopastoral de gramíneas *T. diversifolia*, asociada en 100 % del área. Los animales pastaron 24 horas en 10 hectáreas (ha) de gramíneas mejoradas, divididas en dos sistemas de 5 ha cada uno. La proteína cruda en el tratamiento control fue 9.54 %, mientras que en el SSP fue 10.5 % en la gramínea y 22.7 % en *Tithonia*. En el rumen de los animales que pastaron en el SSP con *Tithonia*, el número de bacterias viables totales fue más numeroso ($p < 0.0001$), mientras que las proteolíticas fueron 47.17×10^5 UFC/mL y las del tratamiento control, 19.42×10^5 UFC/mL, que representa 2.43 veces menos bacterias. No se encontró efectos en el número de hongos celulolíticos. El sistema silvopastoral incrementó el número de bacterias celulolíticas y los protozoos se redujeron 3.5 veces. La producción de metano fue 37.0472 g/kg de MO digerida con las gramíneas y en el SSP con *Tithonia* de 34.788 g/kg de MO digerida. Se concluye que la comunidad microbiana del rumen de vacunos enteros Holstein x Cebú que pastan en SSP con *Tithonia* presentó mayor número de bacterias viables totales, proteolíticas, celulolíticas, menor número de protozoos y menos CH_4 con respecto al control que pastó en áreas de gramíneas.

Palabras clave: bacterias celulolíticas, bacterias proteolíticas, bacterias totales, metano, protozoos

Received: May 05, 2025

Accepted: September 03, 2025

Conflict of interests: The authors declare that there is no conflict of interest.

CRedit Authorship Contribution Statement: Conceptualization, Investigation, Data curation, Writing - original draft: Juana L. Galindo Blanco. Conceptualization, Investigation, Data curation: J. Iraola Jerez. Conceptualization, Formal analysis: Magaly Herera Villafranca.



This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial (CC BY-NC 4.0). <https://creativecommons.org/licenses/by-nc/4.0/>



Introduction

Ruminants have a consortium of microorganisms in their rumen-reticulum able of fermenting food, mainly fibrous food (da Silva-Macedo et al. 2022 and Pazla et al. 2024). Currently, efforts are being made to diversify the forage supply and silvopastoral systems are being promoted for this purpose. *T. diversifolia* (Hemsl.). Gray, is an herbaceous plant of the Compositae (Asteraceae) family, native to Central America and naturalized in Cuba. This species has promising characteristics for animal production, improves the environment and adapts to diverse climatic conditions (Rai et al. 2023 and Hernández-Arboleda et al. 2024)

Tithonia diversifolia is a multifunctional species, with tolerance to various soil conditions, including low fertility, acidity and low phosphorus levels (dos Santos Silva et al. 2021). Its properties identify it with pharmacological principles, which give it potential as an anti-inflammatory, antibacterial, antitumor and antimalarial agent (Zhao et al. 2020), due to a varied composition of secondary metabolites. The mentioned plant offers abundant biomass, with values from 14.4 to 30.6 t DM/ha, defined by the variety (Guatusmal-Gelpud et al. 2020), growth conditions and cultural care (dos Santos Silva et al. 2021).

Among its nutritional characteristics, the content of protein, soluble carbohydrates and the level of tannins highlighted, conditions of great importance, because these components can help to improve the food balance in terms of the contribution of energy and protein in the diet of dairy cattle (Argüello et al. 2020 and Rivera et al. 2021). In addition to contributing to improving rumen balance and increasing the efficiency of ammonia transformation into microbial protein. This would imply lower energy costs due to losses of ammonia, CH₄ and ruminal CO₂, an aspect that reduces the possible environmental pollution.

The objective of this research was to evaluate the effect of silvopastoral system with *T. diversifolia* on the microbial community of the rumen from Siboney de Cuba non-castrated, intended for beef production.

Materials and Methods

Location: The experiment was carried out at Ayala feedlot from Instituto de Ciencia Animal (ICA). This farm is located at 22° 53' north latitude, at 82° 02' west longitude and 92 m o.s.l., belongs to San José de las Lajas municipality, Mayabeque province, Republic of Cuba. The study complied with the ethical standards from ICA experimental farm commission for working with animals. It was approved with the project code PN131LH001 49. The animals had no health intervention during the experiment.

Edaphoclimatic characteristics: The climatic conditions of the region were characterized by an average annual temperature of 24.86 °C, with the highest values in June

(27.17 °C) and August (27.48 °C), with maximums between 32.65 °C and 33.48 °C. December, January and February showed the lowest average temperature values (22.18, 21.47 and 22.50 °C, respectively), with minimums below 17 °C and, on some days, with records of 5 to 10 °C.

The annual rainfall was 1361 mm and from May to October 77.13 % of the annual rainfall was recorded, with a monthly average of 174.95 mm, while from November to April 22.92 % occurred with a monthly average of 51.99 mm. The average annual relative humidity was 80.88 % and its extreme values were in March (77.15 %) and June (82.86 %) (data taken from the Meteorological Station of Instituto de Ciencia Animal, San José de las Lajas, Mayabeque).

Animals: A total of 24 non-castrated male from a Siboney de Cuba genetic group (5/8 Holstein x 3/8 Zebu) were used in the growth-fattening stage, 12-month-old yearlings. They started the experiment with 198 kg of LW and finished with 414 kg. They grazed for 24 h on 10 ha of improved grasses (*Cynodon nlemfuensis*) and natural grasses (*Paspalum notatum*, *Sporobolus indicus* and *Dichantium sp*), divided into two 5 ha systems, one with grasses and the other made up of a silvopastoral system (SPS) of grasses - *T. diversifolia* associated in 100 % of the area.

Each system was in turn divided into eight paddocks of 0.63 ha, with free access to the watering place and saltshaker. The grazing was established with 42 days of rest, six days of occupation in each paddock and a stocking rate of 2.40 animals/ha. The treatments were: control 1) free grazing of grasses + mineral salts and an experimental group 2) free grazing of the SPS *T. diversifolia* + mineral salts.

Treatments: The treatments were compared: 1) grazing on improved grasses (*C. nlemfuensis*) and natural grasses (*P. notatum*, *S. indicus* and *Dichantium sp*) and 2) grazing on an SPS of grasses - *T. diversifolia* associated in 100 % of the area.

In the SPS, the vegetative material (VM) No. 10 of *T. diversifolia*, proposed by Ruiz et al. (2010) for grazing, was used. Galindo et al. (2018) reported that VM 10, join to VM 23 of *T. diversifolia*, were among twelve materials that produced the fewest methanogens and protozoa in the rumen. The initial weight of the animals was homogeneous in the yearling category (animals of one year).

Sampling: Rumen fluid samples were taken from each animal via esophageal tube. All sampling coincided with the animals' weighing days, once a month. For this purpose, two animals were selected from each treatment each time. The rumen fluid samples were transferred to the rumen biochemistry and microbiology laboratory, located in the Central Laboratory Unit from ICA, with the help of hermetically sealed thermos to maintain the temperature and anaerobic conditions of the rumen fluid. The contents of the thermos were filtered through muslin. Preservations were made for the selected determinations and samples were taken for the culturing of rumen microorganisms.

Measurements: The following microbiological indicators were determined: number of total viable bacteria, cellulolytic bacteria, proteolytic bacteria, cellulolytic fungi, and protozoa. For the culturing of rumen microorganisms, the [Hungate \(1950\)](#) culture technique was used in rolled tubes and under strict anaerobic conditions. Total viable, cellulolytic and proteolytic bacteria were inoculated in the culture media of [Caldwell and Bryant \(1966\)](#), modified by [Elias \(1971\)](#). In the case of proteolytic bacteria, 10 % sterile skimmed milk was added, according to [Galindo \(1988\)](#). The [Joblin \(1981\)](#) culture medium was used to determine the number of fungi. Three dilutions were used for the inoculation of microorganisms, and each dilution was replicated three times. Total viable bacterial, cellulolytic, proteolytic and fungal colony counts were performed by placing the rolled tubes under a magnifying glass and counting the colonies that showed a digestion halo ([Galindo 1988](#)). The results were expressed in colony forming units (CFU) for bacteria and in thallus forming units (TFU) for fungi.

Rumen protozoan count: The protozoa were directly counted under a Neubauer chamber optical microscope, after staining them with a 0.01 % gentian violet solution in glacial acetic acid. To perform protozoan counts, rumen fluid samples were preserved in a 10 % formaldehyde solution at a 1:1 (v/v) dilution.

Determination of the chemical composition of foods: The analysis of the chemical composition of the food intake by the animals was carried out according to the techniques described by the [AOAC \(2016\)](#). The fibrous fractions were analyzed using the procedure of [Goering and van Soest \(1970\)](#). [Table 1](#) shows the chemical composition of each of the diets.

In this experiment, it was not possible to determine the methane concentration, which led to its estimation using the equation of [Sauvant et al. \(2011\)](#), considering that methane production in the rumen is related to the CP content of the food intake by the animals. That is: $CH_4, \text{ g/kg of digested OM} = 40.1 - (0.32 \times \text{CP}) \%$ of DM.

The experimental design was completely randomized.

Statistical treatment of microorganism counts: Viable microorganism counts were transformed according to Log N to ensure normal conditions in the microbial growth curve.

The formula $(K+N).10X$ was applied for the analysis where:

K: constant that represents the logarithm of the dilution where the microorganism was inoculated,

N: logarithm of the colony count, expressed as CFU/mL, CFU/mL, or cells/mL

10 is the base of logarithms

X: dilution at which the inoculation was carried out ([Galindo 1988](#))

Statistical analysis: The theoretical assumptions of the analysis of variance were verified for the original variables total bacteria, cellulolytic bacteria, fungi, proteolytic bacteria, and protozoa. For the homogeneity of variance of the treatments, [Levene \(1960\)](#) test was applied. The normality of the errors was analyzed using the [Shapiro-Wilk \(1965\)](#) test, both assumptions were unfulfilled, so the generalized linear mixed model was used with the help of GLIMMIX procedure of [SAS \(2007\)](#).

In the model, the treatments were considered as fixed effects, the rumen fluid repetitions as random effects, and the animal was the subject. To know the performance of the data, the Normal, Poisson, log normal, Igauss and Gamma distributions were tested. In the case of total bacteria, cellulolytic bacteria, fungi and protozoa, the best fit was Igauss and for proteolytic bacteria Gamma, both distributions with log link function. The variance-covariance structures tested were Toeplitz (Toep), variance component (VC), composite symmetry (CS), autoregressive of order 1 (AR [1]) and unstructured (UN), with Toep performing best. For the selection of the best fit structure to the data, the information criteria [Akaike (AIC), Corrected Akaike (AICC) and Bayesian (BIC)] were used, for which the smallest value was considered. For the comparison of means, the fixed-rank test ([Kramer 1956](#)) was applied. The data were analyzed using the [SAS \(2013\)](#), statistical package version 9.3.

Results and Discussion

[Table 2](#) shows the results for total viable bacteria, proteolytic bacteria, and cellulolytic fungi in the rumen. It was observed in the rumen of the animals that were in the SPS with *T. diversifolia*, that the total number of viable bacteria was more numerous ($p < 0.0001$) than in the rumen of those that were grazing on the improved grasses mixture. The population values were 17.20 and 27.98 x 10¹¹ CFU/mL for the grasses system and SPS, respectively.

Table 1. Chemical composition of the experimental diets

Indicators, %	Treatment 1 (control)		Treatment 2 (SPS with <i>T. diversifolia</i>)	
	grasses		grasses	<i>T. diversifolia</i>
Dry matter, DM	35.2		33.1	19.6
Ashes	13.4		11.5	16.8
Crude protein, CP	9.54		10.5	22.7
Neutral detergent fiber, NDF	71.8		70.3	56.9
Acid detergent fiber, ADF	38.3		39.6	33.7

Table 2. Effect of SPS with *T. diversifolia* on the number of total viable bacteria, proteolytic bacteria and cellulolytic fungi in the rumen

Treatments Microbial groups	Improved grasses +naturals	Improved grasses +Tithonia	SE (\pm), Signif.
Number of total viable bacteria, 10^{11} CFU/mL	2.84 (17.20)	3.33 (27.98)	0.12 $p=0.0001$
Number of fungi, 10^4 TFU/mL	2.21 (9.12)	2.20 (9.03)	0.14 $p=0.9442$
Number of proteolytic bacteria, 10^5 CFU/mL	2.97 (19.42)	3.85 (47.17)	0.11 $p<0.0001$

CFU/mL: colony forming units per milliliter of ruminal fluid, TFU/mL: thallus forming units per milliliter of ruminal fluid. Values in parentheses are the original means of the number of microbial populations

Galindo-Blanco *et al.* (2018) found increases in the total number of viable bacteria, which were attributed to the defaunating effect produced by this plant, as a consequence of ecological relations of predation exerted by the protozoa on the total viable bacteria. However, when evaluating different plant materials or ecotypes, there were differences among them (Galindo *et al.* 2022). By reducing the protozoan population, the total number of bacteria is favored. In other experiments, a reduction has been observed, which can be attributed to the secondary metabolites of *T. diversifolia* and to other more complex ecological relations that exist in the organ.

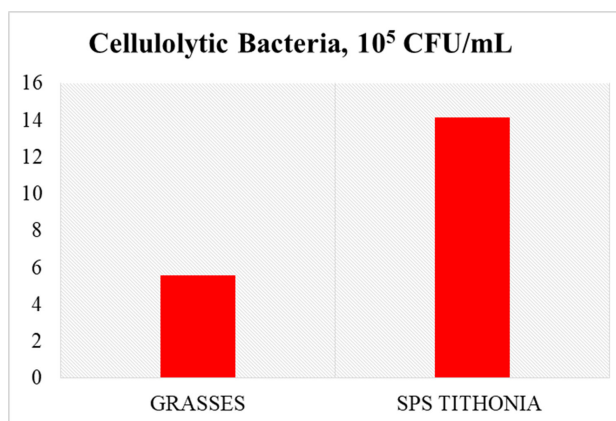
In the rumen of the animals that were in the SPS, the number of proteolytic bacteria was 47.17×10^5 CFU/mL, while in those that grazed in the control treatment, the population was 19.42×10^5 CFU/mL, which represents 2.43 times fewer bacteria that degrade proteins.

In this experiment, there were no effects due to the feeding system on the number of ruminal cellulolytic fungi (table 2). In this regard, it should be reported that, although these microbial groups are numerically smaller than cellulolytic bacteria, their extracellular enzymes are capable of degrading, to a greater extent, the cellulosic materials contained in plants. It is estimated that approximately 58 % of ruminal cellulolysis is due to the presence of the mentioned microorganisms, which are capable of adhering to, colonizing and degrading cellulosic materials and even modifying the structure of lignin.

Previous studies by Galindo-Blanco *et al.* (2018) and Galindo *et al.* (2022) have found that the effect of Tithonia on the cellulolytic fungal population of the rumen varies depending on the cultivable variety and other factors, such as the age of the plant, aspects that are in study phase.

The number of cellulolytic bacteria was modified as a consequence of the effect of the SPS with Tithonia. Figure 1 shows that the populations of these cellulolytic organisms were 5.55 and 14.10×10^5 CFU/mL, for the control and the SPS, respectively ($p<0.0001$).

The evaluation of the population dynamics of cellulolytic bacteria in the rumen has been the subject of study since 2012, when the first studies with this plant began. Increases have been observed, which vary depending on the plant material



Transformed data: Grasses 1.71 and SPS with Tithonia 2.55 CFU/mL; Original means 5.55 CFU/mL and 14.10 CFU/mL for grasses and Tithonia, respectively; SE (\pm) 0.16, $p<0.0001$

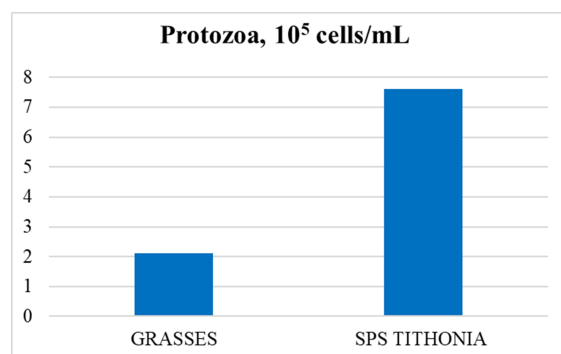
Figure 1. Effect of grazing in SPS with *T. diversifolia* on the number of cellulolytic bacteria in the rumen (10^5 CFU/mL)

and the cutting time. It is important to highlight that the higher nutritional value of this vegetable significantly contributes to these results. Similar results were obtained in studies shown by Ruiz *et al.* (2017) with *T. diversifolia* materials collected in the western region of Cuba in 2006 and subsequently in 2015 in the eastern region.

By performing a comprehensive analysis of the effect of SPS on the number of cellulolytic bacteria, it can be reported that these plant materials promote higher fiber degradability, an aspect of vital importance in the evaluation of fibrous sources, because although *T. diversifolia* has a high CP content, the presence of fiber places it as a fibrous source, reasons that require it to be degraded at the ruminal level by the microorganisms that have that capacity.

Figure 2 shows that the number of rumen protozoa was reduced 3.5 times when the animals grazed in the SPS. This effect is not an isolated fact, since it has been shown that in the plant materials of *T. diversifolia* collected in the western and eastern region of Cuba, as well as in more than 20 shrubs and leguminous or non-leguminous trees, there are smaller protozoan populations.

Among the advantages of protozoan reduction or defaunation are the increase in the population of cellulolytic microorganisms, the stabilization of the rumen pH, the decrease in free ammonia, the reduction of methanogenesis and the increase in the efficiency of digestive use of different diets, mainly fibrous ones (Dai and Faciola 2019). These results coincide with those showed by Króliczewska *et al.* (2023), in which it is asserted that the most outstanding contribution of the reduction of protozoa in the rumen is that it improves energy metabolism and reduces losses due to methane production, which is an environmental pollutant. Palangi and Lackner (2022) reported that defaunation reduces enteric CH₄ emission, due to the flow of microbial cells from the rumen and the reduction in the acetate/propionate ratio, events that are considered electron sinks.



Transformed data: Grasses 2.03 and SPS with Tithonia 0.76, Original means 7.58 grasses and 2.16 SPS with Tithonia, SE (\pm) 0.10, $p < 0.0001$

Figure 2. Effect of grazing in SPS with Tithonia on the number of rumen protozoa

Considering the results of the CP content shown in table 1, the methane (CH₄) production for the grasses treatment was 37.0472 g/kg of digested OM and for the SPS with *T. diversifolia*, 34.788 g/kg of digested OM. Similar results were reported by Delgado *et al.* (2011), Galindo *et al.* (2018) and Pérez-Can *et al.* (2021) when foliage from different tropical plants was evaluated. These authors found that *Leucaena leucocephala* and *T. diversifolia* were the plants that produced the least methane (mL/gDM) compared to ten others, and showed that the response is associated with the higher content of condensed tannins and saponins, which act on methanogens and protozoa, and also have the ability to increase the molar ratio of propionic acid. However, it seems that the mechanism of action is different in each case.

Conclusions

It is concluded that the microbial community of the rumen of Holstein x Zebu non-castrated cattle, grazing in a silvopastoral system with *T. diversifolia*, showed a higher population of total viable bacteria, proteolytic bacteria,

cellulolytic bacteria, a lower population of protozoa and less CH₄ compared to the control that grazed in grass areas.

Acknowledgments

Thanks for the valuable collaboration of technicians Onidia Moreira, Aned Capó, Ybeth Orta, Jorge Luis Hernández and Lucía Sarduy in carrying out the research.

References

- AOAC. 2016. Official methods of analysis of AOAC International. 20 ed., Rockville MD: AOAC International., Latimer, George W. Jr., ISBN: 9780935584875. <http://www.worldcat.org/title/official-methods-of-analysis-of-aoac-international/oclc/981578728?referer=null&ht=edition>.
- Argüello, R., Mahecha- Ledesma, L. & Angulo- Arizala, J. 2020. Perl nutricional y productivo de especies arbustivas en trópico bajo, Antioquia (Colombia). *Ciencia y Tecnología Agropecuaria*, 21(3): e1700, ISSN: 2500-5308. https://doi.org/10.21930/rcta.vol21_num3_art:1700.
- Caldwell, D.R. & Bryant, M.P. 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Applied Microbiology*, 14(5): 794-801, ISSN: 2717-5936. <https://doi.org/10.1128/am.14.5.794-801>.
- Dai, X. & Faciola, A.P. 2019. Evaluating strategies to reduce ruminal protozoa and their impacts on nutrient utilization and animal performance in ruminants - A meta analysis. *Frontiers in Microbiology*, 10: 2648, ISSN: 1664-302X. <https://doi.org/10.1007/sl.1250-011-0045-5>.
- Delgado, D., Galindo, J., González, R. Savón, D., Scull, I., González, N. & Marrero, Y. 2011. Feeding of tropical trees and shrub foliage as a strategy to reduce ruminal methanogenesis: studies conducted in Cuba. *Tropical Animal Health and Production*, 44(5): 1097-1104, ISSN: 1573-7438. <https://doi.org/10.1007/sl.1250-011-0045-5>.
- dos Santos Silva, A.M., Santos, M.V., da Silva, L.D., dos Santos, J.B., Ferreira, E.A. & Santos, L.D.T. 2021. Effects of irrigation and nitrogen fertilization rates on yield, agronomic efficiency and morphophysiology in *Tithonia diversifolia*. *Agricultural Water Management*, 248: 106782, ISSN: 1873-2283. <https://doi.org/10.1016/j.agwat.2021.106782>.
- Elías, A. 1971. The rumen bacteria of animals fed on a high-molasses urea diet. Thesis PhD. Aberdeen.
- Galindo, J. 1988. Efecto de la Zeolita en la población microbiana ruminal de vacas que consumen ensilaje. Tesis DrC. Instituto de Ciencia Animal. La Habana.
- Galindo, J., González, N., Ruiz, T., Herrera, M., Moreira, O., Capó, A. & Díaz, H. 2022. Effect of three collections of *Tithonia diversifolia* on the ruminal microbial population of cattle. *Cuban Journal of Agricultural Science*, 56(1): 1-12, ISSN: 2079-3480. <https://cjas.science.com/index.php/CJAS/article/view/1039>.

- Galindo, J.L., La O, O., Ruiz, T., González, A. & Narváez, W. 2018. Efecto de diferentes materiales vegetales de *Tithonia diversifolia* (Hemsl.) Gray en la población de metanógenos y protozoos del rumen. *Revista UNESUM-Ciencias. Revista Científica Multidisciplinaria*, 2(3): 98, ISSN: 2602-8166. <https://doi.org/10.47230/unesum-ciencias.v2.n3.2018.98>.
- Galindo-Blanco, J.L., Rodríguez-García, I., González-Ibarra, N., García- López, R. & Herrera-Villafranca, M. 2018. Sistema silvopastoril con *Tithonia diversifolia* (Hemsl.) A. Gray: efecto en la población microbiana ruminal de vacas. *Pastos y Forrajes*, 41(4): 273-280, ISSN: 2078-8452. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-03942018000400006&Ing=es&tIng=es.
- Goering, H.K. & Van Soest, P.J. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). US. Agricultural Research Service. 20p.
- Guatusmal-Gelpud, C., Escobar-Pachajoa, L.D., Meneses-Buitrago, D.H., Cardona-Iglesias, J.L. & Castro-Rincón, E. 2020. Production and quality of *Tithonia diversifolia* and *Sambucus nigra* high andean colombian tropic. *Agronomía Mesoamericana*, 31(1): 193-208, ISSN: 2215-3608. <https://doi.org/10.15517/ma.v31i1.36677>.
- Hernández-Arboleda, X., Ortiz-Grisales, S., Vivas-Arturo, W.F., Fernández-Romay, Y., La O-León, O., Luiz-Abdalla, A., Pérez-Márquez, S. & Ledea Rodríguez, J.L. 2024. Nutritional value and invitro dry matter degradability in Mexican sunflower: *Tithonia diversifolia* Hemsl (Gray). *Tropical and Subtropical Agroecosystems*, 27(3): 094, ISSN: 1870-0462. <https://doi.org/10.56369/tsaes.5211>.
- Hungate, R.G. 1950. The anaerobic, mesophilic cellulolytic bacteria. *Bacteriological Reviews*, 14(1): 1-49, ISSN: 2691-9443. <https://doi.org/10.1128/br.14.1.1-49.1950>.
- Joblin, K.N. 1981. Isolation, enumeration and maintenance of rumen anaerobic fungi in roll tubes. *Applied and Environmental Microbiology*, 42(6): 1119-1122, ISSN: 1098-5336. <https://doi.org/10.1128/aem.42.6.1119-1122.1981>.
- Kramer, C.Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics*, 12(3): 307-310, ISSN: 0006-341X. <https://doi.org/10.2307/3001469>.
- Króliczewska, B., Pecka-Kielb, E. & Bujok, J. 2023. Strategies used to reduce methane emissions from ruminants: Controversies and issues. Review. *Agriculture*, 13(3): 602, ISSN: 2077-0472. <https://doi.org/10.3390/agriculture13030602>.
- Levene, H. 1960. Robust tests for the equality of variance. Contributions to Probability and Statistics. Stanford University Press.
- da Silva-Macedo, A.J., Costa-Campos, A., Nascimento-Coutinho, D., Soares-Freitas, C.A., dos Anjos, A.J. & Rocha-Bezerra, L. 2022. Efecto de la dieta sobre los parámetros ruminales y la microbiota ruminal: revisión. *Revista Colombiana de Ciencia Animal (Recia)*, 14(1): e886, ISSN: 2027-4297. <http://doi.org/10.24188/recia.v14.n1.2022.886>.
- Palangi, V. & Lackner, M. 2022. Management of enteric methane emissions in ruminants using feed additives: A Review. *Animals*, 12(24): 3452, ISSN: 2076-2615. <https://doi.org/10.3390/ani12243452>.
- Pazla, R., Jamarum, N., Agustin, F., Arief, A., Elihasridas, E., Ramaiyulis, R., Yanti, G., Ardani, L.R., Sucitra, L.S. & Ikhlas, Z. 2024. Nutrition profile and rumen fermentation of *Tithonia diversifolia* fermented with *Lactobacillus bulgaricus* at different dosis. *Journal of Advanced Veterinary and Animal Research*, 11(1): 146-152, ISSN: 2311-7710. <https://doi.org/10.5455/javar.2024.k759>.
- Pérez-Can, G.E., Tzec-Gamboa, M., Albores-Moreno, S., Sanginés-García, J., Aguilar-Urquizo, E., Chay-Canul, A., Canul-Solis, J., Muñoz-Gonzalez, J., Diaz-Echeverria, V. & Piñero-Vázquez, A.T. 2021. Degradabilidad y producción de metano *in vitro* del follaje de árboles y arbustos con potencial en la nutrición de rumiantes. *Acta Universitaria*, 30: e2480, ISSN: 2007-9621. http://scielo.org.mx/scielo.php?script=sci_arttext&pid=S0188-62662020000100129&Ing=es&tIng=es.
- Rai, P.K., Soo Lee, S., Bhardwaj, N. & Kim, K-H. 2023. The environmental, socio-economic, and health effects of invasive alien plants: Review on *Tithonia diversifolia* (Hemsl.) A. Gray in Asteraceae. *South African Journal of Botany*, 62: 461-480, ISSN: 1727-9321. <http://doi.org/10.1016/j.sajb.2023.09.038>.
- Rivera, J.E., Ruiz, T.E., Chara, J., Gómez- Leiva, J.F. & Barahona, R. 2021. Biomass production and nutritional properties of promising genotypes of *Tithonia diversifolia* (Hemsl.) A. Gray under different environments. *Tropical Grasslands-Forrajes Tropicales*, 9(3): 280-291, ISSN: 2346-3775. [http://doi.org/10.17138/tgft\(9\)280-291](http://doi.org/10.17138/tgft(9)280-291).
- Ruiz, T.E., Alonso, J., Febles, G.J., Galindo, J.L., Savón, L.L., Chongo, B.B., Martínez, Y., La O, O., Cino, D.M., Crespo, G.J., Mora, L., Valenciana, N., Padilla, C., Rodríguez, B., Muir, L., Rivero A. & Hernández, N. 2017. Evaluación de materiales recolectados de *Tithonia diversifolia* (Hemsl) Gray en Cuba. Editores: Chará J, Peri P y Rivera J. IX Congreso Sistemas Silvopastoriles. Aporte a los objetivos de desarrollo sostenible, CIPAV. Cali, Colombia. Fundación CIPAV, pág. 486.
- Ruiz, T.E., Febles, G., Torres, V., González, J., Achang, G., Sarduy, L. & Díaz, H. 2010. Assessment of collected materials of *Tithonia diversifolia* (Hemsl.) Gray in the center-western región of Cuba. *Cuban Journal of Agricultural Science*, 44(3): 291-296, ISSN: 2079-3480. <https://cjas.com/index.php/CJAS/article/view/223>.
- SAS. 2007. SAS/STAT Software. Version 9 Edition, SAS Institute Inc., Cary, NC. Available at: <http://www.sas.com/>.
- SAS 2013. SAS/IML 9.3 User's Guide.SAS Institute Inc., Cary, NC. Available at: <http://www.sas.com/>.

- Sauvant, D., Giger-Reverdin, S., Serment, A. & Broudiscou, I. 2011. Influences des régimes et de leur fermentation dans le rumen sur la production de méthane par les ruminants. *Productions animales*, 24(5): 433-446, ISSN: 2824-3633. <http://doi.org/10.20870/productions-animales.2011.24.5.3276>.
- Shapiro, S. & Wilk, B. 1965. An analysis of variance test for normality (complete simples). *Biometrika*, 52(3-4): 591-611, ISSN: 1464-3510. <https://doi.org/10.2307/2333709>.
- Zhao, L., Hu, Z., Li, S., Zhang, L., Yu, P., Zhang, J., Zheng, X., Rahman, S. & Zhang, Z. 2020. Tagitinin A from *Tithonia diversifolia* provides resistance to tomato spotted wilt orthotospovirus by inducing systemic resistance. *Pesticide Biochemistry and Physiology*, 169: 104654, ISSN: 1095-9939. <https://doi.org/10.1016/j.pestbp.2020.104654>.