Enfoque UTE, V.13-N.4, Oct. 2022, pp. 1-10
http://ingenieria.ute.edu.ec/enfoqueute/
e-ISSN: 1390-6542


Received: 2022/04/25
Accepted: 2022/07/19
Online: 2022/07/19
Published: 2022/10/01
https://doi.org/10.29019/enfoqueute.856

# Chemical composition of the foliage meal of *Tithonia diversifolia*

## *Composición química de la harina de follaje de Tithonia diversifolia*

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### *Abstract*

*With the objective of determining the nutritional constituents, amino acids and the content of secondary metabolites of the foliage meal of Tithonia diversifolia. For this, plant material was collected 70 days after regrowth. The percentages of DM, CP, P, Ca, Si, CF, NDF, ADF, ADL, Cel, Hcel, CC were determined; amino acid profile (methionine, cystine, methionine + cystine, lysine, threonine, aspartic acid, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, serine, phenylalanine, arginine, histidine), and content of TT, TP, TCT, TBCT, FCT, Flv, Sap, Alk, Trit and TS. For statistical processing, descriptive techniques (mean and standard deviation) were used. The plant material of Tithonia diversifolia analyzed showed percentages of 22.23, 2.62, 0.013, 45.7, and 29.8% and 6.09 MJ/kg (CP, Ca, P, NDF, ADF and ME). For the secondary metabolites, they presented concentrations of 5.35, 12.38, 13.7, 9.58, 4.15, 24.49, 0.86, 1.36, 7.71 and 10.75 g/kg MS for total tannins, total phenols, total condensed tannins, total bound condensed tannins, free condensed tannins, flavonoids, alkaloids, saponins, triterpenes and total steroids). The highest concentrations of amino acids were for lysine, aspartic acid, glutamic acid, proline, glycine, alanine, valine, leucine, serine and phenylalanine with values of 13-30 g/kg of protein. It is concluded that the foliage meal of Tithonia diversifolia presents an adequate relation in its quality.*

### *Keywords*

*Foliage meal; basic bromatology; amino acids; secondary metabolites.*

### Resumen

El presente estudio se enfoca en determinar los constituyentes nutritivos, aminoacidico y el contenido de metabolitos secundarios de la harina de follaje de *Tithonia diversifolia.* Para esto se recolectó el material vegetal a los 70 días de rebrote. Se determinaron los porcentajes de MS, PB, P, Ca, Si, FB, FND, FAD, LAD, Cel, Hcel, CC; perfil de aminoácidos (metionina, cistina, metionina + cistina, lisina, treonina, ácido aspártico, ácido glutámico, prolina, glicina, alanina, valina, isoleucina, leucina, serina, fenilalanina, arginina, histidina), y contenido de TT, FT, TCT, TCLT, TCL, Flv, Sap, Alc, Trit y ET.
Para el procesamiento estadísticos se utilizaron técnicas descriptivas (media y desviación estándar). El material vegetal de *Tithonia diversifolia* analizado mostró porcentajes de 22 .23, 2.62, 0.013, 45.7, y 29.8 % y 6.09 MJ/kg (PB, Ca, P, FND, FAD y EM). Para los metabolitos secundarios presentaron concentraciones de 5.35, 12.38, 13.7, 9.58, 4.15, 24.49, 0.86, 1.36, 7.71 y 10.75 g/kg MS para taninos totales, fenoles totales, taninos condesados totales, taninos condensados ligados totales, taninos condensados libres, flavonoides, alcaloides, saponinas, triterpenos y esteroides). Las mayores concentraciones de aminoácidos fueron para lisina, ácido aspártico, glutámico, prolina, glicina, alanina, valina, leucina, serina y fenilalanina con valores de 13-30 g/kg de proteína.
Se concluye que la harina de follaje de *Tithonia diversifolia* presenta una adecuada relación en su calidad.

### Palabras clave

Alimentación, aminoácidos, metabolitos secundarios, pared celular, valor nutritivo.

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## 1. Introduction

The tropical zone contains the greatest genetic diversity in the world, which is expressed in a large number of vascular plants per unit area. However, despite this richness, animal feeding models have been based mainly on the use of few plant species. This is more valid in the case of fodder trees and shrubs that have not been properly used as animal feed (Valdivié-Navarro et al., 2020). In the study of feed systems used in hot climates, it is suggested that the sustainability of the system depends, largely, on the use of different local biological resources as an alternative for feeding non-ruminant species (Verdecia et al., 2018).

Thus, this concept calls for expanding the use of the diversity of species as providers of large volumes of feed for the animal. Given the diversity of forage species, there is a vital need to study and recommend promising species for specific agro-ecological environments and biomass production systems, mainly considering their nutritional value (Herrera et al., 2017). In this sense, one of the species with high potential and widespread in the tropics is *T. diversifolia*, commonly known as buttercup, false sunflower, or Mexican sunflower, it has a wide edaphoclimatic adaptation as it has been reported in more than 50 countries. This fodder plant as a high biomass production and more than chemical composition compared to most tree species in tropical conditions. This perennial shrub contains low values of acid detergent fiber (ADF) and neutral detergent fiber (NDF), high content of nitrogen and calcium, as well as acceptable percentages of degradation and content of non-structural carbohydrates.

Although, some secondary metabolites responsible for biological activity such as saponins, tannins, essential oils, flavonoids that act as antioxidants, anticancer, antiparasitic and reproductive stimulants are known, however, studies are insufficient to elucidate the impact of the concentration of these secondary metabolites on the biological response of poultry (Rivera et al., 2018). Thus, the shrubby plant *Tithonia diversifolia* could contribute to reducing the use of imported conventional foods and lowering production costs in poultry and other non-ruminant species (Martínez et al., 2011). Therefore, it is necessary to increase knowledge about the nutritional value of foliage to be used in animal diets. The aim Therefore, the objective of this research was to evaluate the effect of regrowth age on the nutritional quality of *Tithonia diversifolia* foliage meal.

## 2. Metodology

### *Experimental location, sample collection and preparation*

The research was carried out in the period from May to June 2017, in the area of The Dátil, Bayamo-Granma, Cuba. For the determination of the chemical composition, amino acids, and quantification of the secondary metabolites of the flour of the green material of Tithonia diversifolia, the forage was taken at 70 days of age, in an area of 20 hectares of the crop. The soil of the experimental area was Fluvisol (Soil Survey Staff, 2014), with a 6.4 (pH). The content of 2.2 ($P_2O_5$), 33.05 ($K_2O$), 3.7 (total Nitrogen) of mg/100g of soil and organic matter of 3.1%.

During this so-called rainy period in the region, rainfall was 893.67 mm; the average, minimum, and maximum temperature registered values of 26.73, 22.31, and 33.92 ºC, respectively. The material was manually separated from the leaves and petioles less than 2 mm from the stems, considered as edible biomass (Verdecia et al., 2018). After drying the sample in a dark, and ventilated place for a period of 12 days; the milling was carried out in a hammer mill to a particle size of 1mm and they were stored in amber bottles until analysis in the laboratory.

### *Determination of chemical composition, amino acids and secondary metabolites*

Dry matter (DM), crude protein (CP), crude fiber (CF), P, Ca, and Si according to the AOAC (2005), while neutral detergent *fiber* (NDF), *acid detergent fiber* (ADF), acid detergent lignin (ADL), cellulose (Cel), hemicellulose (Hcel), cell content (CC) according to Goering & Van Soest (1970). Metabolizable energy (ME) according to the Nutrition of the European Federation of Branches and Subcommittee Energy of the E.F.B.S.E.W.G (1989) was calculated. In addition, the amino acid profile in the foliage of *Tithonia diversifolia* was quantified by high performance liquid chromatography (HPLC) (Reverter et al., 1997).

Secondary metabolites were quantified according to the following analyses: The Folin-Ciocalteu method for total phenols (TP) and total tannins (TT), before and after treatment of the extracts with polyvinylpolypyrrolidone (PVPP) described by Makkar (2003). Total condensed tannins (TCT), free condensed tannins (TCL) and total condensed tannins attach (TCTA) by the n-butanol/HCl/Fe3+ method (Porter et al. 1985). Flavonoids (Flav) according to Boham and Kocipai-Abyazan (1994). Saponins (Sap) by the method described by Obdoni and Ochuko (2002). Triterpenes (Trit) according to Jie-Ping & Chao-Hong (2006). Total steroids (TS) by Galindo et al. (1989) and the alkaloids (Alk) by the method described by Muzquiz et al. (1994).

### *Statistical analysis*

Data were analyzed using descriptive statistics and the mean and standard deviation (SD) were determined using the Statistic Version 6.0 for Windows program.

## 3. Results

The foliage meal of *Tithonia diversifolia* showed in its chemical composition percentages of 22.23% in CP, 2.62% Ca, 0.013% P, 45.7% NDF, 29.8% ADF and 6.09 MJ/kg of ME (Table 1).

Regarding the results of the concentration of secondary compounds (table 2), low amounts of 5.35 were found; 12.38; 13.7; 9.58; 4.15; 24.49; 0.86; 1.36; 7.71 and 10.75 g/kg MS for TT, TP, TCT, TCTA, FCT, Flv, Sap, Alk, Trit and TS, respectively; which can be considered low compared to other species.

***Table 1***. Chemical composition of the foliage meal of *Tithonia diversifolia*

| Components | Content | SD± |
|---|---|---|
| DM, % | 88,91 | 0,34 |
| CP, % | 22,23 | 0,04 |
| CF, % | 18,51 | 0,06 |
| Ca, % | 2,62 | 0.003 |
| P, % | 0,013 | 0.005 |
| Si, % | 3,44 | 0,012 |
| NDF, % | 45,7 | 0,45 |
| ADF, % | 29,8 | 0,36 |
| ADL, % | 7,18 | 0,23 |
| Hcel, % | 16,48 | 0,05 |

| | | |
|---|---|---|
| Cel, % | 16,74 | 0,06 |
| CC, % | 59,65 | 0,76 |
| ME, MJ/kg | 6.09 | 0,049 |

Dry matter (DM), Crude protein (CP), Crude fiber (CF), Calcium (Ca), Phosphorus (P), Silica (Si), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), Hemicellulose (Hcel), Cellulose (Cel), Cell content (CC) and Metabolizable energy (ME)

***Table 2***. Secondary metabolites in the foliage meal of *Tithonia diversifolia*

| Secondary metabolites | (g/kg MS) | SD± |
|---|---|---|
| TT | 5,35 | 0,003 |
| TP | 12,38 | 0.678 |
| TCT | 13,7 | 0.598 |
| TCTA | 9,58 | 0,023 |
| FCT | 4,15 | 0,045 |
| Flv | 24,49 | 2,988 |
| Sap | 1,36 | 0.002 |
| Alk | 0,86 | 0.023 |
| Trit | 7,71 | 0,122 |
| TS | 10,75 | 0,892 |

Total tannins (TT), Total phenols (TP), Total condensed tannins (TCT), Total condensed tannins attach (TCTA), Free condensed tannins (FCT), Flavonoids (Flv), Saponins (Sap), Alkaloids (Alk), Triterpenes (Trit) and Total steroids (TS)

The highest concentrations of amino acids (table 3) in the foliage meal of *T. diversifolia* were for phenylalanine, serine, lysine, proline, leucine, aspartic acid, valine, glutamic acid, alanine and glycine with values of 13-30 g/kg of protein.

***Table 3***. Amino acid content in the foliage meal of *Tithonia diversifolia*

| Amino acid | (g/kg of crude protein) | SD± |
|---|---|---|
| Methionine | 6,60 | 0,012 |
| Cystine | 5,12 | 0.234 |
| Methionine + Cystine | 11,72 | 0.894 |
| Lysine | 21,30 | 1,023 |
| Threonine | 12,96 | 0,466 |
| Aspartic acid | 29,98 | 1,883 |
| Glutamic acid | 21,06 | 0.987 |
| Proline | 21,21 | 1.011 |
| Glycine | 26,44 | 1,665 |
| Alanine | 19,12 | 0,887 |
| Valine | 24,11 | 1,112 |

| | | |
|---|---|---|
| Isoleucine | 8,26 | 0,332 |
| Leucine | 20,67 | 0.765 |
| Serine | 19,55 | 0,643 |
| Phenylalanine | 13,32 | 0,534 |
| Arginine | 9,15 | 0,226 |
| Histidine | 3,33 | 0,045 |

## 4. Discussion

The reported of chemical composition (table 1) values are in the range described scientific reports for CP (16-23%); DM (5-10 MJ/kg DM); NDF (33-47%); ADF (15-30%); ADL (5-8%), Si (2-4%), Hcel (11-25), Cel (14-19%) and CC (40-60%) in *Piptocoma discolor, Hibiscus rosa-sinensis, Tithonia diversifolia, Clitoria fairchildiana, Trichanthera gigantea, Solanum rugosum, Gliricidia sepium, Leucaena leucocephala* and *Erythrina variegata* (Moriones-Ruíz & Montes-Rojas, 2017; Rivera et al., 2018; Riascos-Vallejo et al., 2020). These variabilities are attributed to the age of forage, and to the intrinsic characteristics of each species, although it is worth noting that it is within the range of many species used in livestock, so Tithonia constitutes an alternative for animal feed in production systems in the tropics.

The chemical characterization of feeds is one of the most important aspects for the successful development of livestock production and complements the *in vitro* and *in vivo* results. Thus, these chemical studies are essential to formulate diets considering the animal species, productive category, physiological state, and productive purpose (Betancourt et al., 2017), which will allow taking advantage of the available raw materials and lowering the production costs associated with feeding (Martínez et al, 2021).

In tropical countries there is a great diversity of plants with these characteristics, among them *Tithonia diversifolia* (Hemsl.), which can be used in more environmentally friendly environments, with better responses in animal welfare and better feed conversion, which could contribute to more competitive livestock systems (Parra-Ortiz et al., 2019; Cabrera-Núñez et al., 2019). In this sense, there is evidence that species of non-legume plants such as *Tithonia diversifolia* accumulate similar nitrogen in their leaves as legumes, have high levels of phosphorus, high number of roots, good ability to take up the few nutrients from the soil, wide range of adaptation, tolerate conditions of acidity and low fertility in the soil, it can withstand pruning at ground level, it has fast growth and low demand for inputs and management for its cultivation. In addition, information is available on its use in the feeding of non-ruminant species such as birds in the form of flour (Mejía-Díaz et al., 2017).

The protein values (22.23%) coincide with those reported by Verdecia et al. (2021) who reported that *Tithonia diversifolia* has high protein values of 22.6%, which show that this plant can be used as daily feed in ruminants and non-ruminants. Thus, due to the protein content (22.23%), its low cell wall content (45.7; 29.8; 7.18, 16.48; 16.74%; FDN, ADF, ADL, Cel and Hcel, respectively) and the adequate cell content (59.65%), this forage (*Tithonia diversifolia*) is a good candidate as raw material for feed due to its excellent ratio of nutrients in its composition. Likewise, Cardona-Iglesias et al. (2017); Gallego-Castro et al. (2017), point out that this species is recognized for its high capacity to accumulate nitrogen in its leaves, phosphorus, potassium, and soluble carbohydrates.

In studies by Cabanilla-Campos et al. (2021), when evaluating the effect of the cutting system (30 to 75 days) on the nutritional value of *Tithonia diversifolia*, the CP ranged between 12 and 24 %, NDF between 41 and 47% and ADF between 29 and 35%, which denoted the possibility of considering this species as promising of interest for animal production. While Lodoño et al. (2019); Navas and Montaña (2019), reported similar values in CP, NDF, ADF and ADF (18-25; 40-64; 17-48 and 6-22%) and higher in energy with 8.75 MJ/kg of DM. This shows that the cutting age, selected plant material, management and soil and climatic conditions can directly influence the chemical composition. Authors as Pérez et al. (2009) and Verdecia et al. (2011) stated that *T. diversifolia* is a plant that is characterized by excellent quality, by its adequate ratio of nutrients in its foliage and reported values of NDF (43.79 ± 2.96) and ADF (27.81 ± 3.60) respectively. The NDF levels in the investigated plant (45.7%) are higher than those reported by Rosales (1996), who found values of 35.3%, while for FDA (29.8-30.4%) the results were similar, these differences may be due to the material collected, analytical technique and quality of the reagents used.

For secondary metabolites (table 2) In this sense, Verdecia et al. (2014); Verdecia et al. (2021) when stud-ying *L. leucocephala, G. sepium, E. variegata, T. diversifolia, N. wightii* and *T. labialis* found that T.diversifolia and G*. sepium* comprehensively presented the best results in terms of the chemical composition and the lowest in the content of secondary metabolites. Likewise, Millán-Domín-guez et al. (2017); Quintanilla-Medina et al. (2018); Verdecia et al. (2020) reported high concen-trations of secondary metabolites (total tannins, condensed, flavonoids, saponins) in *Moringa oleifera, Samanea saman*, *Leucaena leucocephala* and *Erythrina variegata*, which exceed con-centrations of 40, 60, 60 and 50 g/kg DM, respectively, and could affect the metabolism and productive performance of non-ruminant species.

On the other hand, Riascos-Vallejos et al. (2020), when evaluating several tree species, such as *Piptocoma discolor, Hibiscus rosa-sinensis, Tithonia diversifolia, Clitoria fairchildiana, Trichantera gigantea, Solanum rugosum*, found that *Tithonia diversifolia* reported the lowest results in terms of phenol concentration, condensed tannins and total alkaloids (31.6; 2.8 and 6.3 g/kg DM), considering the comparison with the average of the rest of the species with 33.5; 3.62 and 5.58 g/kg DM. This confirms that the values of these compounds in *T. diversifolia* are below those reported in the edible fraction of some legumes of production systems in the tropics, which demonstrates the quality of this species to be used in the animal feeding.

Lezcano-Más et al. (2016); Rivera et al. (2018); Verdecia et al. (2018) who found in the foliage of *T. diversifolia* the presence of Alk, Sap, cardiotonic carbohydrates, tannins, Flv and TS, from low to medium concentrations, quantities that do not affect consumption and digestibility. These compounds are the product of the action of the defense mechanism of plant species; hence, their fluctuations will depend on the behavior of climatic variables, types of soil and nutrient intake; as well as the attack of pests, diseases, and herbivores (Valdivié-Navarro et al., 2020).

Although tannins (table 2) are considered anti-nutritional factors, however, when used in small concentrations in diets, they are efficient bactericides, fungicides, antioxidants, mineral binders, and astringents (Nawab et al. 2020), thus the inclusion of tannins is suggested. These beneficial polyphenols in small amounts in poultry and other non-ruminant diets (Martínez et al., 2021). The results found in the current investigation ratify the low levels of this compound in the foliage of Tithonia, aspects that ratify it as a good local raw material for the formulation of alternative breaths for non-ruminants

The results for amino acid (table 3) coincide with those reported by Fuentes-Martínez et al. (2019) which reported low concentrations of essential amino acids, when compared to other plant sources such as *Phaseolus lunatus*, *Vigna unguiculata*, *Cajanus cajan* and *Vigna radiata* with amounts of 42-122 g/kg of protein. These differences are due to the intrinsic characteristics of each species, management, age, part of the plant and geographic area (Miquilena & Higeura-Moros, 2012; Marrugo-Ligardo et al., 2016).

In this sense, Mejías-Díaz et al. (2017) obtained similar results, when carrying out a comparative study of *T. diversifolia* with several forage species (*M. sativa, M. alba, C. clandestinus*) and soybean meal where, they found no differences in amino acid content between forages, although it was lower than soybean meal, where on average the forages presented 19 % of protein notably below the 47 % of soybean meal. Hence, the nutritional potential depends to a great extent on its protein quality, on the type and quantity of amino acids it contains, which is a determining factor in its nutritional assessment, since protein quality establishes the contributions of nitrogen and essential amino acids for the animal organism. From a nutritional point of view, protein is a macronutrient present in feed. The importance of the protein present in the diet is due to its ability to provide amino acids to attend to the maintenance of body protein and the increase of this during growth, thus, a better amino acid profile will be able to predict the impact of a new food on productivity and edible production (Basyuni & Wati, 2017; Zhou et al., 2017; Feng et al., 2020).

## 5. Conclusions and recommendations

The foliage meal of *Tithonia diversifolia* has an adequate relationship in its chemical composition, with a low content of secondary metabolites and a good balance of essential and non-essential amino acids, these chemical characteristics make this forage an alternative protein feed in the diet of non-ruminant animals.

We recommend carrying out research where the animal response is evaluated, and the inclusion percentages from which the biological response is affected by the presence of secondary metabolites.

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