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Technical note

Yield, agronomic parameters and nutritional quality of *Tithonia diversifolia* in response to different fertilization levels

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Abstract:
Tithonia diversifolia is a bushy forage plant with high biomass production, rapid post-harvest recovery, and good feed composition values (especially protein levels). It is a promising alternative raw material for incrementing protein content in livestock feeds, but its response to different fertilization regimes has received limited attention. An evaluation was done of this species' yield, composition and nutritional quality values at six different fertilization levels. Experimental design was a completely random block design with six treatments to measure nutrient extraction and its relationship to plant agronomic parameters (biomass production, leaf:stem ratio, plant height at cut, stem count per plant), nutritional value and *in vitro* digestibility. Fertilization produced an overall improvement in *T. diversifolia* agronomic and composition values: compared to the control, biomass increased five-fold in response to fertilization, protein production four-fold and energy production was significantly higher. The best overall response was observed with fertilization using 28.1 g urea, 15.8 g DAP and 10.1 g KCl after each harvest cut.

Key words: Feed analysis, Forages, Ruminant nutrition**.**

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Cattle ranching requires huge areas with consequently profound environmental impacts such as deforestation, soil degradation from over-grazing and subsequent erosion and nutrient loss[1]. Current solutions focus on using closed systems based on production of high biomass forage with nutritional content. These can prove to be economically, ecologically and socially sustainable, but require extensive study of bush forage species[2]. Feed costs represent the largest proportion of expenses in livestock production, the principal contributing factor being the high cost of protein-containing raw materials. High protein content forages such as the tree marigold *Tithonia diversifolia* hold promise as alternatives to costly feed concentrates, and could reduce dependency on them. Inclusion of *T. diversifolia* in ruminant diets can also contribute to reducing methane production during ruminal fermentation, helping to mitigating the impact of this greenhouse gas[3].

A robust bushy plant belonging to the Compositae (Asteraceae) family, *T. diversifolia* is widely distributed throughout the tropics from sea level to 2500 m[4,5]. It is also highly adaptable to tropical conditions, particularly the coffee-growing regions of Columbia. It is very resistant to permanent harvest conditions, improves nutrient recycling and prevents erosion. It is therefore a promising resource even in hillside conditions. Areas of *T. diversifolia* reduce the effects of animal trampling on soils, it has high biomass production and is reported to be an ideal forage in cutting and hauling systems on dairy farms[6]. Extracts from this species have insecticidal properties, which means it protects plants in proximity, including food and timber crops[7]; indeed, it has been used in silvopastoral systems in Antioquia, Colombia[8]. Many recent studies of *T. diversifolia* have focused on its medicinal properties[9-12].

Data on the nutrient requirements of forages is fundamental to developing crops that produce high levels of biomass and are sustainable over time[13]. The present study objective was to determine the nutrient extraction potential of *Tithonia diversifolia* receiving different levels of fertilization, and the effects of fertilization levels on agronomic parameters (biomass production, leaf:stem ratio, plant height at harvest, and number of stems per plant), nutritional value and *in vitro* digestibility.

The project was carried out in Andisol soil in the Colombian Coffee Axis, on La Esmeralda farm in the municipality of Circasia, Quindío Department, Columbia (04°38’24” N; 75°38’26” W). Study area elevation is 1,680 m asl, with annual rainfall ranging from 2,000 to 3,000 mm and an average annual temperature of 19 ºC (Barbas Bremen Meteorological Station).

Before formulating the treatments, which were based on fertilization levels, a sample of twelve *T. diversifolia* plants was collected from a previously established crop of 100 plants which had received no fertilization. This sample was analyzed to quantify biomass production (dry basis) and leaf nutrient content. Soils were also analyzed. Based on these analyses six treatments were formulated: initial nutrient extraction (INE); INE + 25%; INE + 50%; INE + 75%; INE + 100%; and INE + 200%. These were formulated using urea, diammonium phosphate (DAP) and potassium chloride (KCl), and applied after each harvest cut in the following proportions:

T1) no fertilization;
T2) 28.3 g fertilizer per plant / cut (16.0 g urea, 8.4 g DAP, 3.9 g KCl)
T3) 36.7 g fertilizer per plant / cut (20.0 g urea, 10.8 g DAP, 5.9 g KCl)
T4) 45.4 g fertilizer per plant / cut (24.1 g urea, 13.3 g DAP, 8.0 g KCl)
T5) 54.0 g fertilizer per plant / cut (28.1 g urea, 15.8 g DAP, 10.1 g KCl)
T6) 88.5 g fertilizer per plant / cut (44.3 g urea, 25.7 g DAP, 18.5 g KCl).

At the beginning of the experiment, 80 cm-long cuttings were planted at 20 cm depth, in an area of 1.0 m x 1.0 m blocks with a total of 50 plants per block. The first uniform harvest cut was made at 140 days. After this first cut, fertilizer was applied according to the respective treatments. Four consecutive cuts were made every 50 d at 30 cm above soil surface.

Experimental design was a completely random block design using 50 m^2 experimental units with 50 plants per unit, and four blocks with six treatments each for a total of 24 experimental units. Thirteen variables were analyzed: nutrient extraction; biomass production; plant height at harvest cut; leaf:stem ratio; stem count per plant; dry matter; crude protein; crude energy; neutral detergent fiber (NDF); acid detergent fiber (ADF); lignin; ash; and *in vitro* dry matter digestibility (IVDMD). An analysis of variance (ANOVA) was applied, and any differences (P<0.05) analyzed with a Duncan multiple range test to compare means. All statistical analyses were run with the SAS package[14].

Yield and agronomic parameters were analyzed by randomly collecting twelve plants per experimental unit. Each plant was individually measured and weighed, and biomass production measured initially on a fresh basis and then on a dry basis. Measurements were made of the leaf:stem ratio, plant height at cut and stem count per plant.

Feed composition analysis was done using twelve plants randomly collected from each experimental unit per cut; a total of approximately 2 kg was collected per sample. Samples were weighed and left to dry in the sun. Feed analyses were done following the Weende and Van Soest methodology, and IVDMD quantified with the prececal method and hydrolysis kinetics via enzymatic simulation[15].

Biomass production increased (P<0.05) as fertilization levels increased; for example, at 50 days average biomass production was 79.9 g dry matter (DM) in treatment T1 (no fertilizer) but 304.5 g in T6 (highest fertilization level). Estimated production per hectare/year was 31,075 kg fresh matter (FM) in T1 but 147,408 kg in T6 (Table 1), clearly demonstrating the importance of fertilization in management of *T. diversifolia*. Correlation values indicated that biomass production (fresh base) was related to levels of N (90.3 %), P_2O_5 (90.5 %) and K_2O (90.9 %).

Table 1: Biomass production per plant at 50 d and estimated annual biomass yield by fertilization treatments

Treatment	(g plant/cut)		(kg ha^{-1} yr)	
	Biomass FM	Biomass DM	Biomass FM	Biomass DM
T1	425.7 ^{f}	79.9 ^{f}	31,075 ^{f}	5,829 ^{f}
T2	774.5 ^{e}	135.7 ^{e}	56,538 ^{e}	9,908 ^{e}
T3	1004.5 ^{d}	169.1 ^{d}	73,326 ^{d}	12,347 ^{d}
T4	1307.1 ^{c}	205.9 ^{c}	95,416 ^{c}	15,027 ^{c}
T5	1732.6 ^{b}	268.6 ^{b}	126,481 ^{b}	19,609 ^{b}
T6	2019.3 ^{a}	304.5 ^{a}	147,408 ^{a}	22,229 ^{a}
CV	6.4	7.2	6.4	7.2

FM= fresh matter; DM= dry matter; CV= coefficient of variation.

^{abcdef} Different letter superscripts in the same column indicate significant difference (P<0.05).

Plant height clearly increased (P<0.05) with greater fertilization, ranging from 90.65 cm in T1 to 154.88 cm in T6, with differences (P<0.05) between all treatments except T5 and T6 (Table 2). Plant height at cut was highly correlated to biomass production (94.6 %).

Table 2: Agronomic characteristics of *T. diversifolia* by fertilization treatment

Treatment	Plant height at cut (cm)	Leaf length (cm)	Leaf width (cm)	Stem count per plant	Leaf:stem ratio	Leaf weight (g)
T1	90.65 [e]	20.88 [e]	15.05 [f]	14.03 [f]	1.46 [a]	1.59 [e]
T2	111.88 [d]	23.38 [d]	16.84 [e]	17.08 [e]	1.10 [b]	1.99 [d]
T3	129.63 [c]	25.83 [c]	18.33 [d]	19.55 [d]	0.97 [bc]	2.37 [c]
T4	141.40 [b]	28.98 [b]	20.17 [c]	23.55 [c]	0.93 [bc]	2.97 [b]
T5	151.93 [a]	37.19 [a]	22.12 [b]	27.73 [b]	0.82 [cd]	4.12 [a]
T6	154.88 [a]	37.19 [a]	23.01 [a]	29.18 [a]	0.79 [d]	4.32 [a]
CV	2.8	2.7	2.9	3.0	10.8	6.6

CV= coefficient of variation.

[abcdef] Different letter superscripts in the same column indicate significant difference (P<0.05).

Stem count per plant increased (P<0.05) with fertilization from 14.03 stems per plant in T1 to 29.18 in T6 (Table 2). The leaf:stem ratio steadily decreased (P<0.05) as fertilization levels increased (1.46 in T1 to 0.79 in T6). Both these variables respond to the fact that as *T. diversifolia* develops it generates a greater number of stems (Table 2). This is confirmed by the correlation matrix which produced negative percentages (-85.1 % for plant height at cut, and -85.2 % for stem production). The correlation was also negative (-82 %) for biomass production since this variable increases as the leaf:stem ratio decreases. Leaf diameter and weight also increased (P<0.05) with greater fertilization. Length increased from 20.88 cm in T1 to 37.19 cm in T6, while width grew from 15.05 cm in T1 to 23.01 cm in T6. Leaf weight also increased (P<0.05), from 1.59 g per leaf in T1 to 4.32 g in T6. Treatments T5 and T6 did not differ for the variables leaf length and leaf weight (Table 2).

The parameters leaf length, width and weight are important indicators in most production parameters. Compared to the other analyzed variables, these three had high correlations to biomass production (92 %), agronomic parameters (75 %), and protein and energy content (84 %), and negative correlations to NDF, ADF and lignin (-72 %).

Dry matter (DM) content decreased as fertilization increased (P<0.05), dropping from 18.54 % in T1 to 15.07 % in T6, but did not differ from T4 onward (Table 3). In contrast, protein content rose with fertilization levels (P<0.05), from 27.31% in T1 to 30.53 % in T5 and 30.25 % in T6 treatments. This was due to the progressively higher nitrogen content in the fertilization treatments (8.85 g N per plant in T1 to 24.99 g N in T6) since nitrogen is biochemically transformed into protein. Fertilization also affected protein levels (Table 3) in protein content per plant per hectare, which grew substantially (P<0.05) from 22 g per plant in T1 to 92 g in T6. Likewise, estimated protein yield per hectare increased from 1,610 kg protein per year in T1 to 6,726 kg in T6; all the treatments differed (P<0.05). Fertilization level impacted protein production in *T. diversifolia*. This is perhaps its greatest contribution to livestock production since protein is the most expensive raw material in feed.

Gross energy also increased (P<0.05) with fertilization from 4,163.0 kcal/kg gross energy in T1 to 4,570.8 kcal/kg in T6, although treatments T4, T5 and T6 differed little if at all (Table 3). This was unexpected because higher fertilization levels produced higher fiber content and lower leaf:stem ratio values. However, the higher nutrient concentrations in response to increased fertilization levels may have been responsible for this.

Table 3: Feed composition analysis of *Tithonia diversifolia* plants at 50 days by fertilization treatment

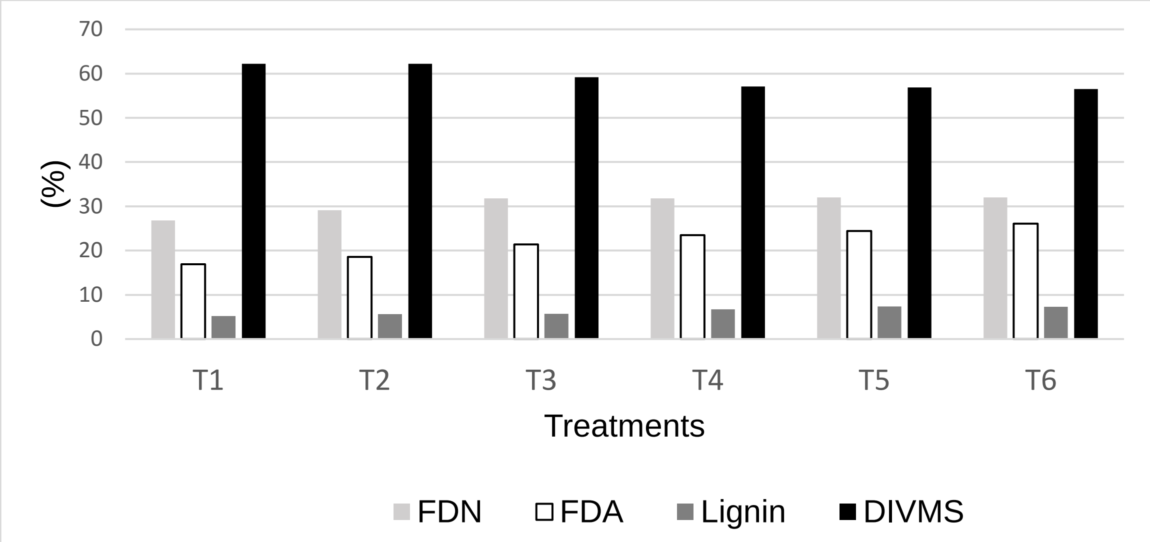
Treatment	(%)				(kcal/kg)
	Dry matter	Crude protein	Ether extract	Ash	Gross energy
T1	18.54 [a]	27.31 [c]	3.59	15.57	4163.0 [e]
T2	17.59 [b]	27.62 [c]	3.1	15.84	4346.5 [d]
T3	16.87 [b]	28.80 [b]	3.17	14.33	4404.5 [c]
T4	15.79 [c]	29.32 [b]	3.47	14.05	4500.0 [b]
T5	15.45 [c]	30.53 [a]	3.26	15.3	4550.5 [ab]
T6	15.07 [c]	30.25 [a]	2.97	15.39	4570.8 [a]
CV	3.8	1.5	5.06	3.53	6.85

CV= coefficient of variation.

[abcd] Different letter superscripts in the same column indicate significant difference (P<0.05).

Neutral detergent fiber (NDF) was lowest (P<0.05) in T1 (26.80 %), increased in T2 (29.9 %) and then remained essentially unchanged from T3 (31.8 %) to T6 (32.01 %) (Figure 1). This may be due to the lower leaf:stem ratio, as suggested by the negative correlation with this variable (-84 %). Values for ADF steadily increased from 16.91 % in T1 to 26.05 % in T6. Again, this is most probably due to the lower leaf:stem ratio, which had a negative correlation (-83 %).

Figure 1: Fiber percentages and *in vitro* DM digestibility in *T. diversifolia* by fertilization treatment



Very much like ADF levels, lignin levels increased (P<0.05) steadily with higher fertilization levels. The lower leaf:stem ratio affected this variable as shown by its negative correlation (-75 %). Overall, ADF levels rose from 5.23 % in T1 to 7.39 % in T5, and 7.30 % in T6; treatments T1, T2 and T3 did not differ (P>0.05) and neither did treatments T5 and T6 (Figure 1).

As fertilization levels increased digestibility generally decreased from 62.18 % in T1 to 56.45 % in T6 (P<0.05), although T1 and T2 did not differ and neither did T4, T5 and T6. This variable correlated negatively with NDF (-83 %), ADF (-93 %) and lignin (-88 %) concentrations, but positively (76 %) with the leaf:stem ratio (Figure 1).

Tithonia diversifolia has high biomass production and rapid post-cut recovery, both of which depend on planting density, soils and vegetative condition(4). The present results coincide with biomass production reported for *T. diversifolia* fertilized with 100 g fertilizer (12-24-12, N-P-K) in which production was 0.82 at the 30-day cut, 1.73 at the 60-d and 2.58 at the 85-d(16). In another study addressing the effects of distance between plants (0.5 and 1.0 m), cut frequency (40, 60 and 80 d) and cut height (5, 10 and 15 cm), yields ranged from 0.85 to 5.5 t ha^{-1} DM, which is at the lower limit of the present results(17). An evaluation of planting area on establishment and production in *T. diversifolia* found yields of 10.31 t ha^{-1} DM in a 0.5 m x 1.0 m area, 10.28 t ha^{-1} DM in a 0.75 m x 1.0 m area, and 13.52 t ha^{-1} DM in a 1.0 m x 1.0 m area(18); these are consistent with the present results. A study of annual yield and nutritional assessment in five bushy forage species found a 114.2 t ha^{-1} FM in *Thichanthera gigantea*, 68.2 in *Gliricidia sepium*, 16.6 in *Erythrina peruviana*, 17.4 in *Leucaena leucocephala* and 9.3 in *Moringa oleifera*. The yields for *E. peruviana*, *L. leucocephala* and *M. oleifera* exceeded those of *T. diversifolia* in the present study, but those for *T. gigantea* and *G. sepium* were similar to them[19].

The effects of planting density in *T. diversifolia* on plant height at 90- and 100-d cuts have been found to produce heights ranging from 158 to 176 cm[18], which are consistent with the present results considering the longer cutting intervals. A study of *T. diversifolia* production using 60-d cuts and a 1.0 m x 2.0 m planting area reported plant heights from 214 to 262 cm (higher than in the present results), leaf weight between 0.42 and 0.66 g (lower than the present results), leaf:stem ratio values from 1.13 to 3.41 (higher than in the present data), and stem counts of 7.6 to 15.4 stems per plant (lower than in the present study)[20]; these discrepancies are probably due to the longer cutting intervals and lower plant density used in the present methodology. This influence can also be seen in a study evaluating 44 *T. diversifolia* introductions aimed at identifying promising plants in which a 1 m planting density was used between sites and 2 m between rows, and cuts were done at 40 cm height every 60 d[21]. Average plant height was 246 cm (higher than in the present study), stem count per plant was 12.08 (lower than the present study), average leaf weight was 0.52 g (lower than in the present results), and the leaf:stem ratio was 1.70 (higher than in the present study); the differences between the studies are probably due to lower planting density and higher luminosity.

Cut height and frequency have a significant effect on crude protein content in *T. diversifolia*; for instance, cutting at 20 cm height and at shorter intervals is reported to produce the highest protein concentrations[16]. The present protein content results are consistent with the 28.5 to 29.8 % reported at 30 days' regrowth[5,22], but higher than those reported in other studies using various cut intervals, plant heights at cutting and fertilization regimes[23,24,25]. Application of biofertilizers and irrigation significantly increases nutrient accumulation[26].

The gross energy content in the present results is higher than reported for the forage bushes *M. oleifera* (3,764 kcal/kg), *Gmelina arborea* (3,755 kcal/kg) and *T. diversifolia* (3,912 kcal/kg)[27]. Neutral detergent fiber (NDF) contents reported for *T. diversifolia* are higher than those found in the present study, possibly due to the longer cut intervals employed. The values nearest the present results are 33.3 to 34.5 % at a 56-d interval[28], but many others are higher, such as 43.9 to 54.5 %[21], 35.2 %[23], and 55.5 %[29]. The same is true of ADF values, with previous reports being higher than the present results: 45.8 to 56.7 %[21,29], with 26.3 and 27.7 % at a 56-d interval[28]. In a study of lignin concentrations in fodder from *Morus alba* (4.8 %), *Erythrina poeppigiana* (4.8 %), *T. diversifolia* (4.4 %) and *Hibiscus rosa-sinensis* (5.2 %), the lowest values were in *T. diversifolia*, which were even lower than in the present study[23].

In vitro dry matter digestibility (IVDMD) values for *T. diversifolia* in the present study were higher than reported for *M. oleifera* (52.44 %), *T. gigantea* (37.18 %) and *L. leucocephala* (51.99 %), but lower than those for *Morus alba* (79.76 %)[30]. In an evaluation of ten potential forage species for ruminants (*Albizia niopoides*, *G. sepium*, *L. leucocephala*, *Samanea saman*, *Acacia farnesiana*, *Mimosa pigra*, *M. oleifera*, *Brosimun alicastrum*, *Cordia dentata* and *Guazuma ulmifolia*) both *C. dentata* and *G. sepium* had IVDMD values largely similar to those of the present results for *T. diversifolia*, although they ranged from 39.1 to 74.8 %[31].

Tithonia diversifolia demonstrated a high nutrient absorption capacity as shown in its greater biomass production as fertilization levels increased. Indeed, this variable increased fivefold between treatment T1 (no fertilizer) and T6 (the highest fertilizer level – 88.5 g per plant). Fertilization also had a significant impact on this crop's agronomic characteristics, such as accentuated plant growth, higher stem counts per plant, and greater leaf length, width and weight. Leaf characteristics were found to be highly correlated to other productive characteristics, making them an easy-to-measure benchmarks for determining productive characteristics and nutritional properties in *T. diversifolia*.

Application of fertilizers in *Tithonia diversifolia* significantly increased leaf content of protein, energy and ash, combined with increased biomass production. The present results suggest that optimizing crop performance requires interpretation of soil analyses and plant nutrient extraction capacity, which allow calculation of optimal fertilization levels. Optimal levels result in the highest biomass production, nutrient contents and digestibility. Based on the present results the optimal fertilization level was that in treatment T5 (28.1 g urea, 15.8 g DAP, 10.1 g KCl) which had the best biomass and nutrient yields. This treatment is preferable to T6 since they did not differ in most variables. The data provided here on optimum fertilization levels for *Tithonia diversifolia* is important to promoting cultivation of this high protein content forage, which can help to reduce the need for protein concentrates in livestock feed systems.

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