Biomass Production and Nutritional Profile of *Tithonia diversifolia* (Hemsl.) A. Gray. at varying stages of growth - 4, 8 and 12 weeks

[*1]Mako, A. A., [1]Kassim, H. G., [1]Oyegunwa, A. S., [1]Babalola, K. O., [1]Ettu, R. O., [1]Awobajo, O. K. and [2]Banjo, O. S.,

[1]Department of Animal Science, Tai Solarin University of Education, Ijagun. Ogun State Nigeria

[2] Department of Tourism and Hospitality, Tai Solarin University of Education, Ijagun. Ogun State Nigeria

Corresponding author:jokemako2006@gmail.com; 08023292736

Abstract

Tithonia diversifolia (Helms.) A. Gray., is a robust shrubby forage plant with high biomass production (stem and leaves) and nutritional value. This study was aimed at evaluating the biomass production and nutrient profile of Tithonia diversifolia (TD) at different growth stages in order to determine the optimal stage for maximum productivity and nutritional value of the plant as fodder for ruminants in the tropics. The study site is located within the tropical rainforest zone of southwestern Nigeria Tithonia diversifolia was established in a completely randomized block design with three replications and were harvested at 4, 8 and 12 weeks of growth. Results revealed that all parameters (fresh yield and dry matter yield (t/ha), leaf, stem and crude protein DM yield (t/ha), plant height (cm), no of leaves per stand, leaf:stem ratio) measured for production potential increased significantly ($p < 0.05$) with age of maturity, except leaf dry matter yield. Dry matter (DM) and crude protein (CP) yield ranged from 3.23 to 6.43and 0.73 to 1.82 t/ha for 4 - 12 weeks old plants, respectively. The DM increased significantly from 4 to 12 weeks old plants, ranging from 68.93 to 72.64; 81.03 to 82.88 and 73.35 to 78.12% for leaf, stem and leaf plus stem respectively. The CP content decreased significantly with age. Highest CP (28.00 %) was obtained in 4 weeks old leaf, while the lowest (0.73 %) was obtained in 12 weeks old stem. The neutral detergent fibre increased significantly with age, recording the highest (54.24 %) for 12 weeks old stem and lowest (39.73 %) for 4 weeks old leaf. Significant ($p<0.05$) differences were observed for macro and micro elements investigated. The macro and micro mineral concentrations of leaf, stem and leaf plus stem decreased with maturity, with the leaf recording highest concentrations of macro and micro elements than the stem and leaf plus stem, except for potassium that is highest in the stem. Phytochemical contents were similar for different growth stages, although the values obtained in this study were within limit that is safe for animal consumption. During the study period (January – June, 2025), average monthly temperatures ranged between 27.04 ^{0}C and 28.15 ^{0}C, with relative humidity increasing from 78.75% during dry months to 81.65% at the onset of rainy season. Rainfall typically begins in April, contributing to favourable growth conditions. Agro-meterological variables were not directly measured in this study, however, Tithonia diversifolia exhibited consistent biomass yield and nutrient composition across the 4-, 8-, and 12- week growth stages. This suggests that the plant can maintain a good productivity and nutritional quality under the prevailing climatic conditions typical of southwestern Nigeria during the study period. It can be concluded that Tithonia diversifolia plant maintains a good production yield and nutrient composition under the prevailing climatic conditions of the study region.

Keywords: Biomass production, nutrient profile, phytochemicals, stages of Growth and *Tithonia diversifolia*

Running title: Developmental variation in *Tithonia diversifolia* yield and nutrients

Résumé

Tithonia diversifolia (Helms.) A. Gray., est une plante fourragère arbustive robuste avec une production de biomasse (tige et feuilles) élevée et une valeur nutritionnelle élevée. Cette étude visait à évaluer la production de biomasse et le profil nutritionnel de Tithonia diversifolia (TD) à différents stades de croissance afin de déterminer le stade optimal pour une productivité et une valeur nutritionnelle maximales de la plante comme fourrage pour les ruminants sous les tropiques. Le site d'étude est situé dans la zone de forêt tropicale humide du sud-ouest du Nigeria. Tithonia diversifolia a été établi selon un plan en blocs entièrement randomisé avec trois répétitions et a été récolté à 4, 8 et 12 semaines de croissance. Les résultats ont révélé que tous les paramètres (rendement en frais et en matière sèche (t/ha), rendement en feuilles, en tiges et en protéines brutes MS (t/ha), hauteur de la plante (cm), nombre de feuilles par peuplement, rapport feuille:tige) mesurés pour le potentiel de production augmentaient significativement ($p < 0,05$) avec l'âge de maturité, à l'exception du rendement en matière sèche des feuilles. Les rendements en matière sèche (MS) et en protéines brutes (CP) variaient respectivement de 3,23 à 6,43 et de 0,73 à 1,82 t/ha pour des plants âgés de 4 à 12 semaines. La MS a augmenté de manière significative des plantes âgées de 4 à 12 semaines, allant de 68,93 à 72,64 ; 81,03 à 82,88 et 73,35 à 78,12 % pour la feuille, la tige et la feuille plus tige respectivement. Le contenu en CP diminuait significativement avec l'âge. Le CP le plus élevé (28,00 %) a été obtenu dans une feuille âgée de 4 semaines, tandis que le plus faible (0,73 %) a été obtenu dans une tige âgée de 12 semaines. La fibre au détergent neutre a augmenté de manière significative avec l'âge, enregistrant la plus élevée (54,24 %) pour une tige âgée de 12 semaines et la plus faible (39,73 %) pour une feuille âgée de 4 semaines. Des différences significatives ($p <0,05$) ont été observées pour les macro et micro éléments étudiés. Les concentrations de macro et micro minéraux de la feuille, de la tige et de la feuille et de la tige diminuaient avec la maturité, la feuille enregistrant les concentrations de macro et de micro éléments les plus élevées que la tige et la feuille et la tige, à l'exception du potassium qui est le plus élevé dans la tige. Les contenus phytochimiques étaient similaires pour les différents stades de croissance, bien que les valeurs obtenues dans cette étude se situent dans les limites de sécurité pour la consommation animale. Au cours de la période d'étude (janvier - juin 2025), les températures mensuelles moyennes variaient entre 27,04 0C et 28,15 0C, avec une humidité relative augmentant de 78,75 % pendant les mois secs à 81,65 % au début de la saison des pluies. Les pluies commencent généralement en avril, contribuant à des conditions de croissance favorables. Les variables agro-météorologiques n'ont pas été directement mesurées dans cette étude, cependant, Tithonia diversifolia a présenté un rendement en biomasse et une composition en éléments nutritifs constants tout au long des stades de croissance de 4, 8 et 12 semaines. Cela suggère que la plante peut maintenir une bonne productivité et une bonne qualité nutritionnelle dans les conditions climatiques typiques du sud-ouest du Nigeria pendant la période d'étude. On peut conclure que la plante Tithonia diversifolia maintient un bon rendement de production et une bonne composition nutritionnelle dans les conditions climatiques dominantes de la région d'étude.

Motsclés: production de biomasse, profil nutritionnel, composés phytochimiques, stades de croissance et Tithonia diversifolia

Introduction

Livestock production plays a vital role in the livelihoods of millions of people in tropical regions, contributing significantly to food security, rural income and socio-economic development (FAO, 2019). However, livestock production in the tropics is constrained by inadequate availability of high-quality feed,

especially during the dry season when pasture biomass and nutritive value decline significantly (Mako *et al.*, 2018). In this context, the use of multipurpose fodder shrubs such as *Tithonia diversifolia* plant (commonly known as Mexican sunflower) has gained attention due to their potential to enhance ruminant nutrition (Kruger *et al*., 2024) *Tithonia diversifolia* plant is a fast-growing perennial shrub native to Central America, but widely naturalized in tropical regions of Africa, Asia and South America (Lukuyu *et al*., 2023). It is recognized for its high biomass yield, rapid regrowth and rich nutrient content, particularly crude protein, minerals and digestible fibre (Baloyi *et al.*, 2022).

One of the critical factors influencing the suitability of *Tithonia diversifolia* plant as livestock feed is its stage of growth. As with many forage species, both biomass yield and nutritive value of *Tithonia diversifolia* plant vary significantly with maturity. Typically, younger foliage exhibits higher crude protein and digestibility, while older plants yield more biomass but with declining nutritional quality due to increased fibre and lignifications (Ojo *et al*., 2022). Understanding the optimal growth stage for harvesting *Tithonia diversifolia* plant is therefore crucial to maximize both yield and feed quality for ruminants.

Recent research efforts have focused on evaluating the adaptability, productivity and the feeding value of *Tithonia diversifolia* plant under different agro-ecological conditions in the tropics (Paumier *et al*., 2022). However, there remain a knowledge gap concerning the precise changes in biomass yield and nutritional profile of different plant parts (leaf, stem and leaf plus stem) across specific phonological stages, especially under tropical field conditions.

This study was aimed at assessing the biomass production and nutritive composition of morphological parts of *Tithonia diversifolia* harvested at different growth stages, with the goal of identifying the optimal harvest time for use as a high-quality ruminant feed in the tropical environments.

Materials and methods

Cultivation of Tithonia diversifolia

Tithonia diversifolia Hemsl.A. Gray was planted in the Teaching and Research Farm of College of Agriculture and Hospitality Management, Tai Solarin University of Education, Ijagun, Ijebu-Ode. The location is 7º21' N and 3º 45' S at an altitude of between 200m and 300m above sea level. The mean temperature is 25-29 ^{0}C with an average rainfall of about 1250mm. The plant was established in plots (10 x 12 m) with 30cm long cuttings with nodes from green matured stems at a spacing of 0.75mwithin row and2m across row. The stem cuttings were inserted vertically at 10cm deep into the soil. The soil was loamy fine sand and its pH was 6. Weeding was carried out weekly in experimental plots. *Tithonia diversifolia* was sampled approximately 5 cm above ground level using 1x1 m quadrant (three replicates for each sampling) on 4, 8 and 12 week old plant. Plants within the quadrant were immediately weighed to determine fresh weight. Six plants were randomly selected from each quadrant and measured for plant heights, number of leaves, then the leaves were separated from the stems to determine the leaf to stem ratio.

Determination of the yield of dry matter

The yield of dry matter per unit area of leaf, stem and leaf plus stem fractions and CP yield per unit area of leaf, stem and leaf plus stem were estimated from fresh weight, DM and CP contents of each fraction

Collection and Processing of Weather Data

Daily rainfall, air temperature, relative humidity, and sunshine hours were collected from February to May 2025 at the Ijebu-Ode Office of the Nigeria Meteorological Agency. An Excel Pivot Table was used to process the data into weekly means.

Chemical Analysis

Chemical Composition

Leaf, stem and leaf plus stem samples from each quadrant were chopped and dried at 60 ^{0}C to constant weight to determine dry matter (DM).Dried samples at each stage of growth were ground through 1-mm mesh and analyzed for the contents of Crude Protein (CP), Ether Extract (EE) ash (AOAC, 2023). Neutral detergent fibre (NDF), Acid detergent fibre (ADF) were determined according to Van Soest, 1995 procedure.

Analysis of minerals

A total of ten minerals were analysed. Plant parts were digested with HNO_3 / $HClO_3$ mixtures (nitric acid and perchloric acid) (20:5 v/v). The digest was made up to 100 mL in standard volumetric flask with deionzed water. Ca, Na, K, Fe, Cu, Mn, Zn, Mg and Pb in the digest were determined with the atomic absorption spectrophotometer model 420. (Gallenkemp and Co. Ltd). Phosphorus in the digest was estimated with vanadomolybdate solution. The colour so developed was read with spectrophotometer at 420 m/u.

Quantitative determination of tannin, saponin, phenol and oxalate

Tannin contents were determined as described by Bohm and Kocipai-Abyazan (1994). The method of Peng and Kobayasli (1995) was used for saponin analysis. While oxalate and phytate contents were determined as described by Oke (1969) and Maga (1983), respectively.

Statistical analysis

Data generated were analyzed in a Completely Randomized Design using the general linear model procedure (SAS, 2023) according to the following statistical model: $Y_{ij} = \mu + A_{i +} e_{ij}$, where A is effect of age of *Tithonia diversifolia* plant. The differences among means were separated using Duncan's New Multiple Range Test (Steel and Torries, 1980)

Results and Discussion

Table 1 presents the forage biomass of *Tithonia diversifolia* (TD) at 4, 8 and 12 week old. The fresh and dry matter yield of TD increased significantly ($p<0.05$) with forage maturity. This is in agreement with the findings of Paumire *et al.* (2022) who reported increase in the total edible and dry matter biomass with forage maturity of *Tithonia divesifolia* cutoff at 60, 90, 120, 150 and 180days. The value obtained here ranged from 14.88 – 58.21 and 2.32 - 6.43 t/ha for fresh and dry matter yield respectively. This result compared well with the values (14.33 - 43.00 and 2.08 – 6.25 t/ha for fresh and dry matter yield respectively) reported for *Tithonia diversifolia* plant by Nguyen *et al*. (2010). However these values are higher and at variance to values of 0.77- 4.98 and 0.47 – 2.52 t/ha for fresh and dry matter respectively reported by Mako *et al*. (2018) for *Alternanthera brasiliana* plant. Similar trend of progressive increase with maturity was observed for leaf and stem dry matter, they ranged from 1.25 – 3.18 and 0.68 – 1.12 t/ha respectively. This result is higher than the value (2.68t/ha/yr) reported for leaf dry matter yield of TD (Farinu *et al*., 1999). Although the CP content of each plant part decreased with increasing maturity (Paumire *et al.*, 2022), the CP yield of TD increased ($P<0.05$) significantly. This response is attributable to the progressive increased yield of the plant. However the CP yield (0.60 – 1.82 t/ha for 4 and 12 week old plant) obtained in this study were similar to values of 0.50 – 1.62 t/ha for 4 and 12 week old plant as reported for TD (Nguyen *et al*., 2010), but higher than values (0.03 – 0.16 t/ha for 4 and 12 week old plant) reported for *Alternanthera brasiliana. (*Mako *et al*., 2018). The leaf- stem ratio decreased significantly ($p<0.05$) with increasing maturity of TD. Normally, as forage advance in maturity, the lower leaves senesced and this resulted in decreasing proportion of leaves with increasing proportion of stem (Webber, 1993). Plant height and number of leaves also follow same trend. This result is consistent with the report of Nguyen *et al*. (2010). Plant height and number of leaves ranged from

25.4 – 78.35 cm and 35 - 90, respectively. This result is higher than values reported elsewhere (Nguyen *et al*., 2010), but lower than the value range (31.97 – 122.27 cm and 15 – 53, respectively) reported by Mako *et al*., (2018) for *Alternanthera brasiliana* at same age of cutting. The 12 week old TD is leafier than 4 and 8 week old plant. Forage production, which is the process of growing and harvesting plant matter for livestock feed, is influenced by multiple factors. These include the specific forage species and cultivars, soil quality, fertilization practices, temperature, rainfall, and the season of growth as reported by Perotti *et al*., 2021. Additionally, factors like grazing intensity, plant density, and the stage of maturity at harvest also significantly impact forage yield and quality (Melo *et al.*, 2022).

Table 1: Production characteristics of 4, 8 and 12 week old *Tithonia diversifolia*

	Stage of *T. diversifolia* growth (Weeks)			
Production characteristics	4	8	12	SEM
Fresh yield (t/ha)	14.88^{c}	28.34^{b}	58.21^{a}	0.25
DM yield (t/ha)	3.23^{c}	4.20^{b}	6.43^{a}	0.10
Leaf DM yield (t/ha)	1.25	2.56	3.81	0.10
Stem DM yield (t/ha)	0.68^{c}	0.72^{b}	1.12^{a}	0.01
Crude protein DM yield(t/ha)	0.73^{c}	1.35^{b}	1.82^{a}	0.02
Plant height (cm)	25.00^{c}	56.25^{b}	78.35^{a}	0.15
Number of leaves/plant stand	45.00^{c}	59.00^{b}	78.00^{a}	2.00
Leaf-stem ratio	3.71^{a}	2.81^{b}	2.10^{c}	0.02

^{a,b,c} =means on the same row with different superscript differed significantly ($p<0.05$)

The chemical composition of different parts (leaf, stem and leaf plus stem) of *Tithonia diversifolia* plant at 4, 8 and 12 week old is shown in Table 2. Significant ($p<0.05$) variations was observed for all parameters measured. The dry matter ranged between 68.93 and 82.88 % from 4 week old leaf and 12 week old stem respectively. The crude protein (CP) content of the different plant parts decreased with increasing age of maturity, the highest (23.03 – 28.00 %) CP content was obtained in the leaf for12 and 4 week old plant respectively, while the lowest (0.76 – 1.23 %) was obtained in the stem for12 and 4 week old plant respectively. This result is in agreement with values reported in previous studies on *Alternanthera brasiliana* by Mako *et al.,* (2018), who reported a value range of 21.53 – 25.53% CP in 12 and 4 week old leaf respectively. This result is also similar to the CP value (20.15%) reported for *Tithonia diversifolia* leaf (Firsoni *et al.,* 2021), however, this result is higher than the CP content of 13.61 – 15 91% and 13.33 – 15.95%reported for *Tephrosia bracteolata and Gmelina arborea* at 8 and 12 weeks old respectively (Ojo *et al.*,2022). The reduced CP content of the stem as the plant matures was mainly due to rapid accumulation of fibrous content (Mendez-Martinez *et al.*, 2023). Maturity has large influence on the CP content of forage and its reduction with advancing maturity may be attributed to (i) fodder mature increases proportionally in the stem (ii) CP content fall in all fractions with advancing maturity (Mendez-Martinez *et al.*, 2023).

The ash content of all plant parts decreased as the plant matures; this is in agreement with reports of Ojo *et al*. (2022). The highest range (12.63- 14.01 %) was observed in the leaf for 12 and 4 weeks old plant respectively, while the lowest range (5.14 – 8.00%) was recorded for 12 and 4 weeks old stem, respectively. These ranges are in agreement with the values reported elsewhere for tropical legumes (Olayinka *et al*., 2015).

The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents of leaf, stem and leaf plus stem obtained here increased

significantly ($p<0.05$) with maturity. Highest NDF and ADF (53.24 and 20.01%) was obtained in 12 old stem, while the lowest (39.73 and lower18.37%) was recorded for 4 week old leaf respectively. This result is lower than the findings of Mako *et al.,* (2018) who reported highest value of 66.81 and 49.94 % for NDF and ADF in 12 weeks old stem and lowest value of 38.24 and 20.36 % in 4 weeks old leaf respectively for their studies in *Alternanthera brasiliana* plant.

Table 2: Chemical composition (%) of leaf plus stem, leaf and stem of 4, 8 and 12 week old *Tithonia diversifolia*

	Stage of *T. diversifolia* growth (Weeks)			
Chemical composition (%)	4	8	12	SEM
Leaf				
Dry matter	68.93^{c}	70.03^{b}	72.64^{a}	0.02
Crude protein	28.00^{a}	25.15^{b}	23.03^{c}	0.03
Ether extract	4.00^{a}	2.86^{b}	2.00^{c}	0.01
Ash	14.01^{a}	13.83^{b}	12.63^{c}	0.01
Neutral detergent fibre	39.73^{c}	41.23^{b}	43.20^{a}	0.02
Acid detergent fibre	18.37^{c}	20.15^{b}	22.00^{a}	0.01
Stem				
Dry matter	81.03^{c}	81.97^{b}	82.88^{a}	0.02
Crude protein	1.23^{a}	1.00^{b}	0.76^{c}	0.06
Ether extract	0.30^{a}	0.22^{b}	0.18^{a}	0.01
Ash	8.00^{a}	6.82^{b}	5.14^{c}	0.01
Neutral detergent fibre	46.23^{c}	48.14^{b}	53.24^{a}	0.01
Acid detergent fibre	23.58^{c}	26.13^{b}	28.01^{a}	0.01
Leaf plus Stem				
Dry matter	73.35^{c}	75.83^{b}	78.12^{a}	0.02
Crude protein	25.22^{a}	22.28^{b}	20.61^{c}	0.05
Ether extract	1.15^{a}	1.10^{b}	1.00^{c}	0.01
Ash	11.23^{a}	10.34^{b}	9.25^{c}	0.01
Neutral detergent fibre	43.20^{c}	44.13^{b}	46.27^{a}	0.01
Acid detergent fibre	20.86^{c}	22.98^{b}	24.01^{a}	0.01

^{a,b,c} =means on the same row with different super script differed significantly ($p<0.05$)

Presented in Table 3 is the macro and micro mineral contents of different parts of *Tithonia diversifolia* plant at 4, 8 and 12 week old. This result revealed that significant ($p<0.05$) differences occurred among the leaf, stem and leaf plus stem for macro and micro mineral content of leaf, stem and leaf plus stem *Tithonia diversifolia* plant. It was observed that, the contents of the minerals decreased with increasing maturity, with the highest value occurring in the early stage of cutting (Mako *et al.,* 2018). However, calcium on the contrary increased with maturity. This could be attributed to the fact that calcium is needed for proper growth and functioning of root tips and meristems, thus increasing mechanical strength of the plant and the amount of cellular material which is composed principally of this element (Markovic *et al.,* 2009). The highest (2.79%) value of calcium was obtained in the 12 week old leaf, while it was lowest (1.93%) was recorded in the 4 weeks old stem. The performance and health

status of grazing animals is dependent on the adequacy and availability of essential minerals from forages. It was observed that the leaf contained higher concentrations of macro-elements than the stem and leaf plus stem, except for potassium, this report is in agreement with the findings of Mako *et al.*, (2018), who reported that concentrations of most minerals are greater in the leaves than in stems, but who also found potassium concentrations to be greater in stems than in leaves.

Generally, there is rapid uptake of mineral during early growth and gradual dilution as the plant matures. The present investigation has revealed that concentration of microelements changed in all plant parts, as well as in whole plant with plant maturation. The considerable decline in the iron concentration of *Tithonia diversifolia* stem with increasing forage maturity and the consistently high iron concentration of leaf are in agreement with earlier study (Mako *et al.*, 2018). The value of iron was lowest in the stem, it ranged between 120.25 and 197.11 ppm in 12 and 4 weeks old *Tithonia diversifolia* stem respectively, while the highest (284.10 and 291.25 ppm) value was obtained in the leaf for 12 and 4 weeks old *Tithonia diversifolia* leaf, respectively. It was observed that values obtained for all macro and micro minerals are within the recommended range for proper functioning of organs of ruminants in the tropics (NRC, 2002).

Table 3: Macro and micro mineral content of leaf plus stem, leaf andstem of 4, 8 and 12 week old *Tithonia diversifolia*

Minerals	Stage of *T. diversifolia* growth (Weeks)			SEM
	4	8	12	
Leaf				
Calcium (%)	2.430^{b}	2.500^{b}	2.790^{a}	0.002
Phosphorus (%)	0.015^{a}	0.014^{b}	0.013^{c}	0.001
Potassium (%)	0.730^{a}	0.700^{b}	0.660^{c}	0.003
Sodium (%)	0.451^{a}	0.450^{a}	0.410^{b}	0.0002
Magnesium (%)	0.614^{a}	0.590^{a}	0.510^{b}	0.003
Iron (ppm)	291.25^{a}	286.13^{b}	284.10^{c}	0.15
Manganese (ppm)	181.10^{a}	180.20^{a}	176.10^{b}	0.10
Zinc (ppm)	51.20^{a}	50.01^{b}	49.98^{b}	0.05
Copper (ppm)	14.30^{a}	13.67^{b}	13.21^{b}	0.15
Stem				
Calcium (%)	1.930^{c}	1.986^{b}	2.00^{a}	0.003
Phosphorus (%)	0.003^{a}	0.002^{b}	0.001^{c}	0.0001
Potassium (%)	0.810^{a}	0.820^{b}	0.783^{c}	0.002
Sodium (%)	0.135^{a}	0.130^{b}	0.128^{c}	0.001
Magnesium (%)	0.325^{a}	0.320^{b}	0.289^{c}	0.002
Iron (ppm)	197.11^{a}	194.00^{b}	120.25^{c}	1.125
Manganese (ppm)	135.2^{a}	123.30^{b}	98.85^{c}	1.000
Zinc (ppm)	23.30^{a}	20.10^{b}	18.82^{c}	0.150
Copper (ppm)	13.18^{a}	10.32^{b}	9.23^{c}	0.050
Leaf plus tem				
Calcium (%)	2.105^{b}	2.109^{b}	2.265^{a}	0.0001
Phosphorus (%)	0.015^{a}	0.012^{b}	0.011^{b}	0.001

Potassium (%)	0.853[a]	0.831[b]	0.800[c]	0.003
Sodium (%)	0.234[a]	0.229[b]	0.221[c]	0.002
Magnesium (%)	0.483[a]	0.478[b]	0.463[c]	0.002
Iron (ppm)	263.11[a]	255.82[b]	241.33[c]	0.51
Manganese (ppm)	158.11[a]	155.01[b]	151.83[c]	0.35
Zinc (ppm)	40.20[a]	38.45[b]	36.63[c]	0.15
Copper (ppm)	11.81[a]	11.01[b]	10.23[b]	0.05

[a,b,c] =means on the same row with different super script differed significantly ($p<0.05$)

Shown in Table 4 is the result of phytochemical contents in the different parts (leaf, stem and leaf plus stem) of *Tithonia diversifolia* at different growth stages. It was observed that the values for all phytochemicals investigated were similar. It was observed that the leaf recorded the highest values for all phytochemicals tested for 4, 8 and 12 weeks old plant. This assertion is in agreement with the findings of Olayinka *et al.,* (2015), however, this is contrary to the findings of Mako *et al.,* (2018), who reported highest values of phytochemicals in the leaf plus stem of *Alternanthera brasiliana* plant. Tannin in this study varied from 0.11 % in 12 week old stem to 0.42 % in 4 week old leaf. These values are higher than the findings of Oluwanisola and Dairo (2016), who reported 0.38 mg/100g for tannin in *Tithonia diversifolia* cut at two different times. The level of tannin obtained in this study is lower than the range of 6 – 10 % considered to depress feed intake and growth (Besharati *et al.,* 2022). Diets containing 2 to 3% tannin for ruminants have been shown to have beneficial effects because they reduce the protein degradation in the rumen by forming a protein-tannin complex (Brutti *et al*., 2023) thereby increasing the amount of by-pass protein that will be available in the small intestine for the animals (Sanket *et al*., 2023). The presence of tannin in this plant is an added advantage. Saponin content ranged from 0.65% in 12 week old stem to 2.38% in 4 week old leaf. These values are in agreement with value range of 2.25 to 3.31% reported for *Moringa oleifera ,Gmelina arborea* and *Tithonia diversifolia* (Aye, 2016). Same trend was observed for oxalate and phytate. It was observed that phytate was high in *Tithonia diversifolia*, this might cause bitter taste, that will make animals reject it (Olayinka *et al*., 2015), but processing like ensiling, sun drying; boiling or wilting might reduce the level of these phytochemicals especially phytate (Ikusika *et al*., 2023).

Saponin-rich forages are effective defaunating agents in the rumen, reducing protozoal populations and disrupting methanogenic archaea. They achieve this by interacting with cell membranes, disrupting their integrity, and potentially killing microorganisms. This reduction in protozoa can improve rumen fermentation and reduce methane production (Yanza 2024). This anti-protozoa activity can decrease enteric methane emissions by up to 50%, recovering energy otherwise lost by animals, while also lowering greenhouse gas contributions that impacts the ozone-depleting processes in the atmosphere (Morales-Mendez and Silva-Rodríguez, 2018) The level of tannin and saponin obtained in this study fall within safe limit that support optimal physiological functioning in animals (NRC, 2021).

This study found that oxalate and phytate levels were within acceptable limits; however, these compounds have been reported to reduce the bioavailability of minerals like calcium, phosphorus, magnesium, zinc, iron, and molybdenum (Akter *et al*., 2020) Specifically, phytate can form insoluble complexes with these minerals, hindering their absorption, while oxalate can bind to calcium, reducing its uptake (Boda *et al*., 2025).

Tale 4: Phytochemical contents (%) of leaf, stem and leaf plus stem of 4, 8 and 12 week old *Tithonia diversifolia*

	Stage of *T. diversifolia* growth (Weeks)			
Anti-nutrients	4	8	12	SEM
Leaf				
Tannin	0.42	0.39	0.37	1.10
Saponin	2.38	2.33	2.30	1.55
Oxalate	1.85	1.83	1.80	1.50
Phytate	7.11	6.51	5.10	2.55
Stem				
Tannin	0.18	0.15	0.11	1.00
Saponin	0.89	0.73	0.65	1.10
Oxalate	0.80	0.65	0.43	1.20
Phytate	4.03	3.67	2.31	2.00
Leaf plus stem				
Tannin	0.25	0.22	0.20	1.05
Saponin	1.53	1.31	1.26	1.21
Oxalate	1.00	0.87	0.73	1.20
Phytate	5.13	4.82	3.80	2.11

Table 5 shows the average monthly climatic conditions during the growth period of *Tithonia diversifolia*. Rainfall exhibited a significant increase from week 4 to week 8, peaking at 144.7 mm, before declining by 58.9 mm in the subsequent period from weeks 8 to week 12. This pattern suggests an initial phase of abundant water availability, which is crucial for the establishment and early vegetative growth of *Tithonia diversifolia*. The fresh and dry matter yields of *Tithonia diversifolia* demonstrated a marked increase as the forage matured, with fresh yield ranging from 14.88 to 58.21 t/ha and dry matter yield from 3.32 to 6.43 t/ha (Table 1). This substantial increase, particularly between Weeks 4 and 8, could be attributed to the higher rainfall during the period. This report is in agreement with the findings of Zheng *et al*. (2024), who reported that increasing rainfall can promote biomass and nutrient accumulation of *Fraxinus malacophylla* Hemsl seedlings.

During the 12-weeks study, the average air temperature gradually declined, decreasing by 0.83 °C between Weeks 4 and 8 and by an additional 0.28 °C from Weeks 8 to 12. The CP content of *Tithonia diversifolia* decreased with increasing maturity. In contrast, the NDF and ADF contents increased (Table 2), indicating that air temperature is a key weather factor that influences plant metabolic processes, including photosynthesis, respiration, and enzymatic activity, and could be responsible for these changes in the plant's nutritional profile (Van Der Zanden, 2008). Additionally, lower air temperature was found to affect the rate of protein synthesis and the accumulation of structural carbohydrates, leading to a reduction in CP and an increase in fiber content as the plant matures.

Relative humidity was found to consistently increase throughout the growth period, rising by 1.61% from Week 4 to Week 8, and by an additional 1.30% from Week 8 to Week 12. Higher relative humidity reduces the vapour

pressure deficit between the plant and the atmosphere, thereby lowering the transpiration rate. This can be particularly beneficial during periods of reduced rainfall, as it helps *Tithonia diversifolia* conserve water and maintain turgor pressure, which is crucial for cell expansion and plant growth. The plant's ability suggests its resilience and efficient water-use mechanisms, thus making it a suitable forage species for humid tropical environments. In addition, the water-conserving mechanism supports the sustained growth of plants and can indirectly aid in biomass accumulation.

During Weeks 4 and 8, there was a slight decrease in sunshine hours, followed by an increase from Weeks 8 to 12. The periods of increased sunshine enhance photosynthetic activity, thereby resulting in greater dry matter production, which corresponds with the observed increase in biomass yield in the study. Ogren (1993) reported that variations in sunshine hours have a direct impact on the photosynthetic rate and, consequently, on biomass accumulation. Evapotranspiration rates generally decreased from Week 4 to Week 8 and then experienced a slight increase from week 8 to week 12. Lower evapotranspiration rates, particularly when accompanied by sufficient rainfall, indicate efficient water use by the plant and favourable conditions for soil water retention.

Table 5: Average monthly (in weeks) weather parameters during the growing period of *Tithonia diversifolia*

Weather parameters	Age of *T. diversifolia* (Weeks)		
	4	8	12
Rainfall (mm)	24.6	169.3	110.4
Relative humidity (%)	78.75	80.36	81.65
Sunshine (hours)	8.99	8.54	9.14
Air Temperature (°C)	28.15	27.32	27.04
Evapotranspiration (mm)	4.13	3.85	3.91

Conclusion

The study evaluated the biomass production and nutritional profile of *Tithonia diversifolia* at 4, 8 and 12 weeks of growth, focusing on the leaf, stem and combined leaf-stem components. The results revealed that biomass yield and nutritional quality increased progressively from 4 to 12 weeks. However, younger plants generally showed higher concentration of certain nutrients including crude protein, some macro and micro minerals (e.g. calcium, potassium, zinc) and beneficial phytochemicals, suggesting better nutritional quality at earlier stages. Among the plant parts, the leaf consistently exhibited superior nutritional and phytochemicals profiles compared to the stem and the combined leaf-stem. It can be concluded that, *Tithonia diversifolia* harvested at 8 weeks in this study appears to offer an optimal balance between biomass yield and nutrient density, making it suitable for use as a resource or green fodder in sustainable agricultural systems.

References

Akter, S., Netzel, M., Tinggi, U., Fletcher , M[1], Simone Osborne, S., Sultanbawa, Y. 2020. Interactions Between Phytochemicals and Minerals in *Terminalia ferdinandiana* and Implications for Mineral Bioavailability Frontiers Nutrition. 7: 598219. doi: 10.3389/fnut.2020.598219

AOAC 2023. Official methods of Analysis, 22nd ed. (Association of Officials Analytical Chemists, Arlington, VA).

Aye, P. A. 2016. Comparative nutritive value of Moringa oleifera, Gmelina arborea and Tithonia diversifolia leaf meal. *American Journal of Food and Nutrition*, 6 (1): 23 - 32

Baloyi, J. J., Nherera-Chokuda, F. V., and Manyawu, G. J. 2022. The role of fodder shrubs in improving livestock productivity in tropical and subtropical regions. *Tropical Animal Health and Production.* 54 (5): 120. https://doi.org/10.1007/s11250-022-03148-2

Besharati, M., Maggiolino, A., Palangi, V., Kaya, A., Jabbar, M., Eseceli, H., De Palo, P., Lorenzo, J. M. 2022. *Molecules*. 27 (23): 8273. doi: 10.3390/molecules27238273.PMID: 36500366

Boda, A. H., Dowell, B. F., Malgwi, D. W. and Emmanuel, W. 2025. Phytochemicals and Effect of Blanching Time on Oxalate and Phytate Content in Leaves of Some Non-conventional Vegetables in Gombi Local Government Area, Adamawa State, Nigeria. *Journal of Applied Science and Environmetal. Management.* 29 (4) 1039-1047

Bohm, BA and Kocipai-Abyazan 1994. Flavonoid and Condensed tannins from leaves of *Vaccinum raticulation* and *Vaccinum calcyimium*. *Pacific Scientific journal*. 48: 458-463

Brutti, D.D., Canozzi, M. E. A., Sartori, E. D. Colombatto, D and Barcellos, J.O.J. 2023.
Effects of the use of tannins on the ruminal fermentation of cattle: A meta-analysis and meta-regression, *Animal Feed Science and Technology*, Vol 306: 115806, https://doi.org/10.1016/j.anifeedsci.2023.115806.

Farinu, G. O., Odunsi, A. A., Akinola, J. O. and Togun, V. A. 1999. Yeild and chemical composition of wild sunflower forage meal in Broiler Chicken diets. *Tropical Journal of Animal Science*, 2 (2): 31 – 37

FAO, 2019: Food and Agricutural Organizations of United Nations. The role of Livestock in food security, poverty reduction and wealth creation in West Africa.

Firsoni, F., Sasongko, W. T., & Wahyono, T. 2021. Nutritive value and fermentation characteristics of Tithonia diversifolia and Moringa oleifera evaluated by gas production technique in vitro. *Advances in Biological Sciences Research*, 1(1), 5. doi: 10.2991/absr.k.220309.052.

Ikusika, O.O., Mako, A. A. & Mpendulo. T. 2023. Effect of different processing methods on nutrient content and acceptability of Hog Plum (*Spondias mombin.* Linn) leaf by West African dwarf Sheep. *Journal of Animal and Feed Research* 13 (1): 46 – 54 DOI: https//dx.doi.org/10.51227/ojafr.2023.8

Kruger, A. M., Lima, P. D. M. T., Ovani, V., Perez-Marquez, S., Louvandini, H. and Abdalla, A. L. 2024. Ruminant grazing lands in the tropics: Silvopastoral ***systems*** and *Tithonia diversifolia* as tools with potential to promote sustainability. *Agronomy* . 14 (7), p. 1386https://doi.org/10.3390/agronomy14071386

Lukuyu, B., Kaitho, R., and Mwendia, S. 2023. Evaluating fodder shrubs for sustainable livestock feeding in East Africa. *Animal Feed Science and Technology*, 296: 115547

Maga 1983. Phytate: It's Chemistry: Occurrence, Food Interactions, Nutritional

Mako, A. A., Akinwande, V.O.,Ettu, R.O., Kassim, H.G and Adegbite, A.S. 2018. Evaluation of Yield and nutritive value of *Alternanthera brasiliana* at various stages of growth as a potential fodder for

ruminants in the tropics. .*Ethiopian Journal of Applied Sciences and Technology*. 9 (1): 11-17

Markovic, J, Strbanovic, R, Cvetkovic, M, Andelkovic, B and Zivkovic, B. 2009. Effects of growth stage on the mineral concentrations in alfafa (*Medicago sativa* L.) leaf, stem and whole plant. *Biotechnology in Animal Husbandry*, 25 (5 - 6): 1225 - 1231

Melo, C. D., Cristiana S. A. M., Maduro, D., Sophie, W., Alfredo, E. S. B., João, M., Paulo, A. V.B., Maria, T. F. and Rui, B. E. 2022. Influence of Climate Variability and Soil Fertility on the Forage Quality and Productivity in Azorean Pastures, *Journal of Agriculture*, *12* (3):358 https://doi.org/10.3390/agriculture12030358

Mendez-Martinez, Y., Rosa I. Narváez-Narváez, R.I., Angulo, C., Cortés-Jacinto, E., Botello-Leon, A, Verdecia-Acosta, D. M., Torres-Navarrete, Y. G. 2023. Chemical composition of Tithonia diversifolia (Hemsl.) and its effect on growth performance, feed efficiency and metabolic biochemistry of juvenile hybrid tilapia Oreochromis mossambicus × Oreochromis niloticus. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 51,(3,): 13337 DOI:10.15835/nbha51313337 Research ...

Morales-Mendez, J. D. and Silva-Rodríguez, R. 2018. Environmental assessment of ozone layer depletion due to the manufacture of plastic bags Heliyon 4 (2018) e01020.

Nguyen Van Sao., Nguyen Thi Mui and Dinh Van Bin. 2010. Biomass Production of *Tithonia diversifolia* (wild Sunflower), soil improvement on sloping land and use as high protein foliage for feeding goats. *Livestock Research for Rural Development* 22 (8)

NRC, 2002. Nutrient requirements for dairy cattle seventh revised edition. National Research Council. **National Academy of Science press. Washington DC, USA**

NRC, 2021. (National Research Council). Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. The National Academies Press. Washington

Ogren, E. 1993. Convexity of the photosynthetic light-response curve in relation to intensity and direction of light during growth. Plant physiology, 101 (3): 1013 – 1019. https://doi.org/10.1104/pp101.3.1013

Ojo, V. O. A., Lamidi, A. A. and Aina, A. B. J. 2022. Influence of age at harvest on the organic nutrients degradability of shrub and tree forage plants by West African dwarf sheep. *Nigerian Journal of Animal Production.* 49(2): 307-316. https://doi.org/10.51791/njap.v49i2.3492

Oke, C. L. 1969. Oxalic acid in plants and in nutrition. World Review of nutritional and Dietetics, New York

Olayinka, B. U., Raiyemo, D. A. and Etejere, E. O. 2015. Phytochemical and Proximate composition of *Tithonia diversifolia* (Helms.) A. Gray. Annals:*Food Science and Technology*. 16 (1): 195 – 200. www.afst.valatia.ro

Oluwanisola, T. A and Dairo, F. A. S. 2016. Proximate composition, amino acid profile and anti nutrients of Tithonia diversifolia cut at two different times. African Journal of Agricultural Research. 11 (38): 3659 – 3663

Paumier, M., Herrera-Herrera, R. C., Toapanta-Mendoza, E.O., Gonzalez-Aguilera, D. M., Verdecia, D. M.,

Ramirez, J. L. and Herrera, R.S. 2022. Productivity of Tithonia diversifoliaplant under Edaphoclimatic conditions of Eastern Cuba. *New Countryside* 1 (01): 21-27. https://doi.org/10.55121/nc.v1i1.24

Peng and Kobayasli 1995. Novel furastonol glycosides from allium anacros tenon plant. *Media.*, 6: 58-61

Perotti, E., Huguenin-Elie, O., Marco Meisser, M., Sébastien Dubois, S., Massimiliano Probo, M., Mariotte, P. 2021. Climatic, soil, and vegetation drivers of forage yield and quality differ across the first three growth cycles of intensively managed permanent grasslands. *European Journal of Agronomy*, vol. 122: 126194 Elsevier

Sanket M. K., Priyal , D., Lokendraand Shubham D. N. 2023. Revolutionizing Livestock Feeding with Bypass Protein Technology. *Acta Scientific Veterinary Sciences* (ISSN: 2582-3183). Volume 5 issue 5

SAS, 2023. Statistical Analytical Systems. SAS Version 9.4 user's guide. Carry, NC: SAS Institute Inc

Steel, R. G. D. and Torrie, J. H. 1980. Principles and procedures of statistics. A biometrical approach, 2nd Edition, McGraw-Hill Book Company, New York.

Van Soest, P. J. 1995. Nutritional Ecology of Ruminants, 2nd ed. Cornell University Press

Van Der Zanden, A. M. 2008. Environmental factors affecting plant growth. https://extension.oregunsttae.edu/gardening/techniques/environmetal-factors-affecting-plant-growth

Webber, C. L, 1993. Crude protein and yield components of six kenaf cultivars as affected by crop maturity. *Ind. Crops Prod.*, 2: 27 – 31

Yanza, Y. R., Irawan, A., Jayanegara, A., Ramadhani, F., Respati, A. N., Fitri, A., Hidayat, C., Niderkorn, V., Cieslak, A., Szumacher-Strabel, M., Hidayat, R. and Tanuwiria, U. H. 2024 Saponin Extracts Utilization as Dietary Additive in Ruminant Nutrition: A Meta-Analysis of In Vivo Studies.*Animals*, 14(8): 123. https://doi.org/10.3390/ani14081231

Zheng S, Cha X, Dong Q, Guo H, Sun L, Zhao Q and Gong Y 2024. Effects of rainfall patterns in dry and rainy seasons on the biomass, ecostoichiometric characteristics, and NSC content of *Fraxinus malacophylla* seedlings. *Front. Plant Sci*. 15:1344717. doi: 10.3389/fpls.2024.1344717

Date received: 16th September, 2025
Date accepted: 31st October, 2025