

Full Length Research Paper

Proximate composition, amino acid profile and some anti-nutrients of *Tithonia diversifolia* cut at two different times

T. A. Oluwasola* and F. A. S. Dairo

Department of Animal Production & Health Sciences, Faculty of Agricultural Sciences, Ekiti State University, P. M. B. 5363, Ado-Ekiti, Nigeria.

Received 17 February, 2016; Accepted 16 June, 2016

The experiment assessed the proximate and amino acid compositions, metabolizable energy and anti-nutrient constituents of *Tithonia diversifolia* leaf meal (TDLM) harvested separately in the months of May and September, respectively using standard laboratory methods. The crude protein ($20.5 \pm 0.1\%$), crude fibre ($18.8 \pm 0.01\%$) and ash ($14 \pm 0.1\%$) significantly ($p < 0.05$) increased in TDLM for September cutting than that of May ($18.05 \pm 0.1\%$, $11.17 \pm 0.3\%$ and $13.01 \pm 0.1\%$, respectively). All the anti-nutrient factors (ANFs), namely, oxalate, phenols, phytin, phytin P, saponins and tannins except alkaloids and flavonoids were significantly ($p < 0.05$) affected by the cutting time of the plant leaf. The ANFs contents in May cutting were oxalate (1.88 ± 0.1 mg/100 g), phenols (0.31 ± 0.01 mg/100 g), phytin (77.3 ± 0.1 mg/100 g), phytin P (21.8 ± 0.1 mg/100 g), saponins (21.8 ± 0.1 mg/100 g) and tannins (0.5 ± 0.01 mg/100 g). The metabolisable energy (ME) for May cutting of TDLM was 2908.27 kcal/kg while that of September was 2565.18 kcal/kg. Glutamic acid had the highest value of 10.94 ± 0.02 mg crude protein among the determined amino acids in TDLM whereas cystine was the lowest (0.94 ± 0.01 mg crude protein for both May and September cuttings). TDLM is limiting in methionine with a chemical score of 30 in both cuttings. Proximate composition of TDLM was higher for September cutting than May and could be used as vegetable protein source.

Key words: *Tithonia diversifolia* leaf, proximate composition, amino acid profile, anti-nutrients, May cutting, September cutting.

INTRODUCTION

Plants' leaves contain nutrients that are of importance in nutrition for growth, development and production in man and animals. They are sources of protein, carbohydrates,

pigments and minerals in human and animal food/feed. However, the presence of anti-nutritional factors such as oxalate, saponins and tannins limit the effective utilization

*Corresponding author. E-mail: ademolaoluwasola1@gmail.com.

of the nutrients in some of these leaves as sole ingredients or components of animal feeds by binding to digestive enzymes and some dietary proteins particularly in monogastrates (Aletor, 1999; Fasuyi et al., 2007). *Tithonia diversifolia*, Mexican sunflower plant, grows as weeds in many part of Southwest Nigeria acting as fallows on farmland, component of compost for organic manure and as aphrodisiac where the extracts are used as medicaments in human and animal (Ayeni et al., 1997; Adoyo et al., 1997; Inoti, 2000; Olabode et al., 2007; Akinola, 2014). *T. diversifolia* leaves have been documented to contain 18 to 21% crude protein (CP), 15 to 18% crude fibre (CF), 13 to 15% ash, 2.81 to 5.6% ether extract (EE) and 44.38 to 52% nitrogen free extract (NFE) by various workers (Olayeni, 2005; Fasuyi et al., 2007; Ekeocha, 2012). The proximate compositions of leaves have been reported to vary with cutting interval or age. Tannin content in cassava foliage was reported to increase at longer cutting intervals as high as 3.5% of the dry matter for 45 days cutting intervals when compared with the root harvest of 4.3% dry matter (Khang et al., 2005). Hiep et al. (2008) also documented the differences in the yield and chemical compositions of leaves of tropical kudzu grass (*Pueraria phaseoloides*). The study showed a decrease in the crude protein content and increasing dry matter and acid detergent fibre (ADF) in the leaves as the cutting interval progressed. However, there is dearth of information on the proximate composition at the different cutting times of the year, particular at young and maturity stages for *T. diversifolia*. This study therefore investigated the different compositions at this various times.

MATERIALS AND METHODS

Collection and preparation of *T. diversifolia* foliage

T. diversifolia leaves were harvested within the Ekiti State University, Ado-Ekiti, Nigeria. It is located on longitude 7° 40' North and latitude 5° 15' East of the Greenwich Meridian in the rainforest zone with average rainfall of 1500 mm, ambient temperature between 22 and 38°C and relative humidity of 70%. The leaves of the plant were harvested in May (at the onset of growth of the leaves after the dry spell) and September (before the flowering stage) which are the two significant periods in the year that could influence the nutrient composition of the leaves of the plant. The leaves were chopped and sun dried to a crispy touch, milled in a commercial feed milling machine (Artec Model 20) to *T. diversifolia* leaf meal (TDLM). The dried samples were stored in transparent polythene bags and kept in the feed store house.

Determination of proximate composition

About 2 g of the dried *T. diversifolia* leaf meal (TDLM) of the two different cuttings (that is, May and September) were analyzed for proximate composition. The dry matter (DM) was determined by drying in a Gallenkamp draught oven at 105°C for 24 h to a constant weight. The crude protein (CP) of each of the samples was determined by Kjeldahl method, ether extract (EE) by using petroleum ether with a boiling point range of 40 to 60°C for

extraction in a Soxhlet apparatus for 6 h, all as described by AOAC (2005). The crude fibre (CF) was analyzed using the method of van Soest and Rorbertson (1985). The ash was determined by burning the defatted sample in a muffle furnace at 600°C and percent ash of the TDLM calculated. Nitrogen free extract (NFE) was obtained by difference while the metabolisable energy (ME) was calculated using prediction equation of Ponzenga (1985), that is, $ME = 35 \times \%CP + 81.8 \times \%EE + 35.5 \times \%NFE$.

Amino acid analysis

Dried 2 g samples of TDLM was hydrolyzed at 150°C for about 90 min and the solution used for the determination by the modification of waters 'pictotag system' (Bidlingmeyer et al., 1984; Gardner et al., 1991). The amino acid score was calculated as shown according to Crampton and Harris (1969) and WHO (1973).

Amino acid score = mg of amino acid per gram of test diet / mg of amino acid per gram of reference protein

Determination of anti-nutrients

Tannin

The tannin in the two samples were quantified by macerating 200 g of each of the TDLMs in 10 cm³ Folin Ciocalteu reagent (sigma) and 2.5 cm³ NaCO₃ (Sodium carbonate). The absorbent of the solution was measured at 725 nm after 40 min using the method of Makkar and Goodchild (1976). Tannin equivalent was calculated from the standard curve.

Phytin, phytin P and oxalate

Phytin contents were determined using 8 g of each of the samples of TDLM soaked in 200 cm³ of 2% HCl and allowed to stand for 3 h. The extract of each of the samples was filtered through a double layer filter paper, 50 cm³ of the duplicate samples of each of the filtrates were pipette into 400 cm³ beakers. 10 cm³ of 0.3% NH₄SCN (Ammonium thiocyanate) was used as an indicator and 107 cm³ of distilled water to obtain acidity of pH 4.5. Ferrous chloride (FeCl₂) solution containing 0.00195 g/cm³ of Fe²⁺ was then titrated against each of the solutions of the test samples until brownish yellow colouration persisted for 5 min. Phytin content was calculated by multiplying the titre value with a factor of 3.55 as described by Young and Greaves (1940). The oxalate contents were also analyzed as described by Huang and Tanudjaja (1992).

Statistical analysis

All the data collected except the amino acid composition were analyzed for mean values, standard deviation, coefficient of variation and t-test using the General linear model computer package as described by SAS (1987).

RESULTS

The proximate compositions of *T. diversifolia* harvested in May and September are shown in Table 1. Crude protein (CP), crude fibre (CF) and ash contents (20.50±0.1 g/100 g, 18.80±0.1 g/100 g and 14.10±0.1 g/100 g, respectively) were significantly higher (P<0.05) for

Table 1. May and September cuttings' proximate composition of *T. diversifolia* leaf meal.

Parameter (%)	May cutting	September cutting	t-test	Coefficient of variation (CV)
Dry matter	91.03	88.72	0.7 ^{NS}	4.15
Crude protein	18.05± 0.1 ^b	20.5± 0.1 ^a	0.01*	6.98
Crude fibre	11.17± 0.3 ^b	18.8± 0.01 ^a	0.01*	27.93
Ash	13.01± 0.01 ^b	14.1± 0.1 ^a	0.01*	4.42
Ether extract	5.5± 0.1 ^a	4.1± 0.1 ^b	.0001**	16.08
Nitrogen free extract (NFE)	51.2± 0.1 ^a	42.6± 0.1 ^b	0.01*	10.15
†ME (Kcal/kg) (Calculated)	2908.27 ^a	2565.18 ^b	0.01*	15.02

^{a,b}Means with different alphabets on the same row differ significantly (P<0.05). *Significant at (P<0.05); NS= Not significant (P>0.05). †ME: Metabolisable energy.

Table 2. Anti-nutrient content of *T. diversifolia* (mg/100 g).

Parameter	May cutting	September cutting	t-Test	Coefficient of variation (%)
Alkaloids	1.24± 0.01	1.24± 0.01	100 ^{NS}	0.72
Flavonoids	0.87± 0.01	0.87± 0.01	100 ^{NS}	1.02
Oxalate	1.88± 0.01 ^a	1.75± 0.01 ^b	0.01*	3.95
Phenols	0.31± 0.01 ^b	0.51± 0.01 ^a	0.01*	26.8
Phytin	77.3± 0.01 ^b	79.2± 0.01 ^a	0.01*	1.33
Phytin P	21.8± 0.1 ^b	23.3± 0.01 ^a	0.01*	3.65
Saponins	2.82± 0.01 ^a	2.37± 0.01 ^b	0.01*	9.50
Tannins	0.51± 0.01 ^a	0.38± 0.01 ^b	0.01*	16.12

^{a,b}Means with different alphabets differ significantly (p<0.05). *Significant at (p<0.05); NS= Not significant (p>0.05).

September compared to May cuttings (18.05±0.13 g/100 g CP, 11.17±0.3 g/100 g CF, 13.01±0.01 g/100 g ash).

The NFE value was significantly lower (P>0.05) for September cutting (42.60±0.1 g/100 g) than that of May (51.20±0.1 g/100 g). The dry matter was not affected (P>0.05). The determined ANFs in *T. diversifolia* for May and September cuttings are shown in Table 2. The alkaloids and flavonoids were not significantly affected (P>0.05) as they recorded same values for May and September cuttings (1.24±0.01 mg/100 g and 0.87±0.01 mg/100 g, respectively). Oxalate, saponins and tannin had significantly higher values (p<0.05) in the May cutting (1.88±0.01 mg/100 g, 2.82±0.01 mg/100 g and 0.51±0.01 mg/100 g, respectively) than that of September (1.75± 0.01 mg/100 g, 2.37± 0.01 mg/100 g and 0.38± 0.01 mg/100 g, respectively). However, phenols, phytin and phytin P exhibited higher values (P<0.05) in September cutting (0.51±0.01 mg/100 g, 79.2±0.01 mg/100 g and 23±0.01 mg/100 g, respectively) compared to those of May (0.51± 0.01 mg/100 g, 79.2± 0.01 mg/100 g, 23.3± 0.01 mg/100 g, respectively). The coefficient of variation varied between 1.33 for phytin and 26.8 for phenol.

The amino acids in May and September are shown in Table 3. There were no significant differences (P>0.05%) in the values of amino acids and chemical scores. Generally, the order of ranking of the amino acids in TDLM for May and September cuttings is glutamic

acid>leucine>aspartic acid> alanine> arginine> lysine>isoleucine> phenylalanine> proline> glycine> serine> tyrosine>threonine>histidine>methionine. Methionine had the lowest score of 30 in both cuttings.

DISCUSSION

CP, CF and ash of *T. diversifolia* as shown in Table 1, showed significant increase in the September cutting. This indicated that these nutrients increased correspondingly as the plant aged. The result is comparable with reports of previous workers on other leaf meals such as those of *gliricidia*, cassava and amaranthus that were adjudged to be good source of protein in broiler or monogastric diets (Agbede and Aletor, 2003; Fasuyi et al., 2008). It also agrees with the findings of Kitaba (2003) and Bayble et al. (2007) that noted higher crude protein yield as Napier grass increases in age. The observed increase in the proximate values of the September cuttings may not be unconnected with the fertility of the soil which could be influenced by the pH, organic matter content and the soil type (Muya et al., 2011).

The presence of ANFs in TDLM agreed with the result of other workers (Fasuyi et al., 2010; Ekeocha, 2012). However, the ANFs content appeared to be within the

Table 3. Amino acid composition of *T. diversifolia* Leaf meal (TDLM) cut in May and September.

Amino acids	Mean value \pm SD (mg/100 g) May	Mean value \pm SD (mg/100 g) September	Coefficient of variation (%)	Chemical score May	Chemical score September	t-test
Alanine	6.90 \pm 0.01	6.80 \pm 0.2	0.22	-	-	12.3 ^{NS}
Arginine	5.70 \pm 0.01	5.75 \pm 0.03	0.27	89	89	10.7 ^{NS}
Aspartic acid	7.05 \pm 0.03	6.95 \pm 0.1	0.75	-	-	8.75 ^{NS}
Glutamic acid	10.94 \pm 0.02	11.04 \pm 0.01	0.36	-	-	14.1 ^{NS}
Histidine	2.55 \pm 0.03	2.45 \pm 0.01	1.96	121	121	7.89 ^{NS}
Iso-leucine	4.21 \pm 0.02	3.81 \pm 0.05	0.76	53	53	9.45 ^{NS}
Lysine	5.39 \pm 0.07	4.91 \pm 0.01	2.14	75	75	7.84 ^{NS}
Phenylalanine	4.08 \pm 0.01	4.11 \pm 0.04	0.49	65	65	6.67 ^{NS}
Proline	3.96 \pm 0.01	5.96 \pm 0.03	0.38	-	-	8.61 ^{NS}
Serine	3.55 \pm 0.02	4.15 \pm 0.02	1.41	-	-	7.02 ^{NS}
Threonine	3.06 \pm 0.01	3.06 \pm 0.01	0.49	63	63	4.68 ^{NS}
Tyrosine	3.43 \pm 0.06	4.03 \pm 0.01	2.91	76	76	5.99 ^{NS}
Glycine	3.93 \pm 0.02	3.53 \pm 0.03	0.96	-	-	5.10 ^{NS}
Leucine	8.02 \pm 0.04	7.92 \pm 0.01	0.87	87	87	10.44 ^{NS}
Methionine	1.25 \pm 0.02	1.17 \pm 0.02	2.01	30	30	2.93 ^{NS}
Cystine	0.93 \pm 0.01	0.73 \pm 0.01	1.61	39	39	3.01 ^{NS}
Valine	4.05 \pm 0.01	3.95 \pm 0.01	0.62	55	55	4.78 ^{NS}

NS: Not significant ($p > 0.05$).

tolerable dietary level for monogastric animals as the data obtained in this study were within the range 0.36 to 0.38 mg/100 g for tannin, 1.5 to 2.93 mg/100 g for oxalate (Dairo, 2008; Fasuyi and Ibitayo, 2011). In addition, TDLM has been investigated as components of diets in poultry, swine and ruminant to give reasonable results (Odunsi et al., 1996; Farinu et al., 1999; Togun et al., 2006; Fasuyi and Ibitayo, 2011; Ekeocha, 2012). TDLM as shown in Table 3, has high content of amino acids notably glutamic acid (10.94 \pm 0.1 g/100 g) which is similar to the findings of Fasuyi and Ibitayo (2011). The order of occurrence of amino acid in TDLM has been noted under the aforementioned result. The limiting amino acid is methionine for both cuttings as indicated by the chemical score of 30. This shows that TDLM must be supplemented with good quality animal protein and/or vegetable sources if it must be used as vegetable protein source farm animal feeds. TDLM is analyzed to contain reasonable content of lysine which has been reportedly required in breast muscle development for a good carcass cut and general body growth in broiler chicken (Tang et al., 2007). The low values of some of the essential amino acids; however, could be improved upon through processing to make TDLM a veritable vegetable protein source in farm animal feeding.

Conclusion

T. diversifolia leaf meal has been reportedly investigated

as component of animal feeds. The nutrient compositions at the start of foliation at about May were lower than for the month of September even though the crude fibre was higher. *T. diversifolia* cut in September could be a good vegetable protein source and if adequately supplemented with methionine, it could be effectively used in animal feeding.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

- Adoyo F, Mukalama JB, Enyola M (1997). Using *Tithonia* concoction for termites control in Busia District, Kenya. ILELA Newsletter 13:24-25.
- Agbede JO, Aletor VA (2003). Evaluation of fishmeal replaced with leaf protein concentrate from glyricidia in diets for broiler-chicks: Effect on performance, muscle growth, haematology and serum. Int. J. Poult. Sci. 2:242-250.
- Akinola BA (2014). Prevalent growth habits and management options of Mexican sunflower (*Tithonia diversifolia*) Hemsl A Gray. Ph.D Thesis in the Department of Crop, Soil and Environmental Sciences, Ekiti State University, Ado-Ekiti, Nigeria P 233.
- Aletor VA (1999). Anti-nutritional factors as nature's paradox in food and nutrition Securities. 15th Inaugural Lecture delivered on August 12, 1999 at the Federal University of Technology, Akure.
- AOAC (2005). Official Methods of Analysis (18th edn). AOAC, International Maryland: USA.
- Ayeni AO, Lombanjou, DT, Majek BA (1997). *Tithonia diversifolia* (Mexican flower) in south western Nigeria: Occurrence and growth habit. Weed Res. (Oxford) 37:443-449.

- Bayble T, Solomon M, Prasad NK (2007). Effects of cutting dates on nutritive value of Napier (*Pennisetum purpureum*) grass planted sole and in association with Desmodium (*Desmodium intortum*) or Lablab (*Lablab purpureus*). Livest. Res. Rural Dev. 19:1.
- Bidlingmeyer BA, Cohen SA, Tervin TLJ (1984). Rapid analysis of amino acids using precolumnderivatization. Chromatography 336:93-100.
- Crampton EW, Harris LE (1969). Applied animal nutrition. The use of feedstuffs in the formulation of livestock rations (2nd Ed).
- Ekeocha AH (2012). Utilization of mexican sunflower leaf meal-based diets by pre weaned West African Dwarf lambs. Poljoprivreda 18(1):41-46.
- Farinu GO, Odunsi AA, Akinola JO, Togun VA (1999). Yield and chemical composition of wild sunflower (*Tithonia diversifolia*) Hemsl. A. Gray and feeding value of wild sunflower forage meal in broiler chicken diets. Trop. J. Anim. Sci. 2:31-37.
- Fasuyi AO, Ibitayo FJ (2011). Nitrogen balance and morphometric traits of weaning pigs fed graded levels of wild sunflower (*Tithonia diversifolia*) leaf meal. Afr. J. Food Agric. Nutr. Dev. 11(5):5125-5141.
- Fasuyi AO, Dairo FAS, Adeniji AO (2008). Protein supplementary quality of tropical vegetable (*Amarantus cruentus*) leaf meal in broiler starter diets: Bionutritional evaluation. J. Cent. Eur. Agric. 9(1):23-34.
- Fasuyi AO, Dairo FAS, Ibitayo FJ (2010). Ensiling wild sunflower (*Tithonia diversifolia*) leaves with sugar cane molasses. Livest. Res. Rural Dev. 22:3.
- Fasuyi AO, Dairo FAS, Olujimi OT (2007). Protein supplementary quality of vegetable leaf meal (*Amaranthus cruentus*) in the diets of laying hens: Egg laying performance and haematological implications. J. Food. Agric. Envir. 5 (3/4):294-300.
- Gardner B, Anstee DJ, Mawby MJ, Tanner JA, Borne-Vomdem AE (1991). The abundance and organization of polypeptides associated with antigens of the Rh blood group system. Transfusion Med. 1:77-85.
- Hiep NV, Wiktorsson H, Man NV (2008). The effect of molasses on the quality of Kudzu silage and evaluation of feed intake and digestibility of diets supplemented with Kudzu silage or Kudzu hay by heifers. Livest. Res. Rural Dev. P. 20.
- Huang AS, Tanudjaja IS (1992). Application of anion exchange high performance Liquid Chromatography in determining oxalate in Taro (*Colocasia esculenta*) corms. J. Agric. Food Chem. 40:2123-2126.
- Inoti SK (2000). The response of maize to split application of urea and *Tithonia diversifolia* green leaf manure in western Kenya. Eldoret, Kenya: Moi University. 108 p.
- Khang DN, Wiktorsson H, Preston TR (2005). Yield and chemical composition of cassava foliage and tuber yield as influenced by harvesting height and cutting interval. Asian-Aust. J. Anim. Sci. 18:1029-1035.
- Kitaba A (2003). Effect of stage of growth and fertilizer application on dry matter yield and quality of natural grassland in the highlands of North Shoa, Oromia Region. M. Sc. Thesis, Alemaya University, Ethiopia P 104.
- Makkar AOS, Goodchild AV (1976). Quantification of tannins. A laboratory manual. International Centre for Agriculture Research in the Dry Area (ICARDA) Aleppo Syria IV + 25 p.
- Muya EM, Obanyi S, Ngutu M, Sijali IV, Okoti M, Maingi PM, Bulle H (2011). The physical and chemical characteristics of soils of Northern Kenya Aridlands: Opportunity for sustainable agricultural production. J. Soil Sci. Environ. Manag. 2(1):1-8.
- Odunsi AA, Farinu GO, Akinola JO (1996). Influence of dietary wild sunflower leaf meal on layer's performance and egg quality. Nig. J. Anim. Prod. 23:28-32.
- Olabode OS, Ogunyemi S, Akanbi WB, Adesina GO, Babajide PA (2007). Evaluation of *Tithonia diversifolia* (Hemsi A Gray) for soil improvement. World J. Agric. Sci. 3(4):503-507.
- Olayeni TB (2005). Effects of wild sunflower (*Tithonia diversifolia*) leaf meal on growth performance, blood and carcass characteristics of weaner pigs. M.Tech. Thesis, Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomosho, Nigeria.
- Pauzenga U (1985). Feeding parent stock. Zootech. Int. pp. 22 -24.
- SAS (1987). SAS/STAT. Guide for personal Computers. Version 6 Ed. pp. 697-978.
- Togun VA, Farinu GO, Olanbiji RO (2006). Feeding graded levels of wild sunflower (*Tithonia diversifolia* Hemsl. A. Gray) meal in replacement of maize at pre-pubertal age, negatively impacts on growth and morphometric characteristics of the genitalia of Anak 2000 broiler cocks at their pubertal age in Nigeria. World Appl. Sci. J. 1(2):115-118.
- Van Soest PJ, Robertson JB (1985) Analysis of Forages and Fibrous Foods. A Laboratory Manual for Anim. Sci. 613. Cornell University, Ithaca, NY.
- World Health Organisation (1973). Energy and protein requirement. WHO Technical Report Series No 522. Geneva, World Health Organisation.
- Young SM, Greaves JS (1940). Influence of variety and treatment on phytic acid content of wheat. Food Res. 5:103-105.