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Preliminary analyses and amino acid profile of wild sunflower (*Tithonia diversifolia*) leaves

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ABSTRACT

Tithonia diversifolia (wild sunflower) leaves were harvested, sundried and milled to obtain *Tithonia diversifolia* leaf meal (TDLM). Samples of the TDLM were analysed for proximate composition, amino acid profile and certain antinutrients. Analysis revealed a composition of 20.6% CP, 18.9% CF, 4.0% EE, 42.5% CHO and 14.0% ash. Phytin was high at 79.10 mg/100 g. Saponin, oxalate and alkaloids were also present at 2.36, 1.76 and 1.23 mg/100 g, respectively. Tannin and flavonoid were in traces of 0.39 and 0.87mg/100g, respectively. The contents of lysine, arginine, aspartate, glutamate, methionine+cystine, isoleucine, tyrosine and phenylalanine were remarkably high at 5.3, 6.19, 13.32, 12.19, 2.60, 4.28, 7.55, 3.53 and 5.47g/100g protein. The ample presence of ANFs (phytins, oxalates, flavonoids and saponnins) portends a negative nutritional evaluation for TDLM as a potential non-ruminant feed ingredient. ANFs have been suggested as a probable militating factor hindering the digestibility of crude protein (CP) and amino acids (AAs) in most plant products. The ample presence of some essential amino acids particularly isoleucine, leucine and lysine and other aromatic amino acids like phenylalanine and valine were remarkable suggestion of the potentiality of TDLM as a veritable alternative protein resource in non-ruminant feeding.

Keywords: Antinutrients, potential protein resource, nutritional evaluation.

INTRODUCTION

The recognition of protein from leaf sources is fast gaining prominence because of its availability and perhaps, because it is the cheapest and the most abundant potential source of protein for animal nutrition (Fasuyi, 2007). The world wide shortage of animal protein sources, particularly in developing countries in Africa, has necessitated investigations of several novel protein sources for possible incorporation into animal feeds (particularly poultry) as replacements for the expensive conventional sources such as fish meal, groundnut cake and soybeans. The acute shortage of protein has been attributed to the phenomenal rise in the prices of animal feeds which account for about 75-85% of the recurrent production inputs in intensive monogastric animal production (Fetuga, 1977). Therefore, it is necessary to explore other less competitive plant resources and by-products for use in monogastric nutrition, especially poultry. Wild sunflower (*Tithonia diversifolia*) is a green plant that originated from Mexico, and it is now widely distributed throughout the humid and sub-humid tropics in Central and South America, Asia and Africa (Sonka, 1997). *Tithonia* was probably introduced into Africa as an ornamental plant and can be found on roadsides and as invader of field crops in the forest savanna transition zones in Nigeria (Ayeni et al., 1997). *Tithonia* has been a subject of research interest because of the relatively high nutrient concentrations found in its biomass and because of its ability to extract relatively high amounts of nutrients from the soil. *Tithonia diversifolia* (wild sunflower) has been suggested as a potential green forage plant that can be utilized as feed stuff for animals due to its protein content (Farinu, 1986; Dutta et al., 1986).

This study therefore investigated the chemical, amino acids and antinutritional constituents in *Tithonia diversifolia* as a prelude to incorporation into non-ruminants feeds.

MATERIALS AND METHODS

Preparation of *Tithonia diversifolia* leaf meal (TDLM)

The test ingredients *Tithonia diversifolia* leaf meal (TDLM) was prepared by harvesting daily, the fresh and matured leaves of *Tithonia diversifolia* plants of different ages before flowering. The whole leaves were chopped manually using sharp knives, evenly sun dried for 4 days by regular turning using a rake. The chopped leaves were intermittently weighed to achieve 12 -13% moisture content. Properly sun-dried TDLMs were stored in an air-tight container from which samples were periodically taken for laboratory analyses.

Phytochemical screening of TDLM

The extraction and precipitation of phytin in the fresh and dried leaves was done by the method of Wheeler and Ferrel (1971), while iron in the precipitate was determined as described by Makower (1970). Phytin was determined by using a 4:6 Fe/P ratio to calculate phytin phosphorous and multiplying the phytin phosphorous by 3.55 as suggested by Young and Greaves (1940). Oxalate content was determined by the titrimetric method of Moir (1953) as modified by Ranjhan and Krishna (1980). Where extracts were intensely coloured, they were decolourised with activated charcoal (Balogun and Fetuga, 1980).

The polyphenols (tannic acid) was determined by extracting the dried and finely blended TDLM (250 mg in 10 ml of 70% aqueous acetone) for 2 hr~~s~~ at 30 ^oC using Gallenkamp orbital shaker (Survey, UK). Pigments and fats were first removed from the leaves by extracting with di-ethyl ether containing 1% acetic acid. Thereafter, the total polyphenols (as tannic equivalent) were determined in 0.05, 0.2 or 0.5 ml aliquot using Folin Ciocalteu (Sigma) and standard tannic acid (0.5 mg/ml) as described by Makkar and Goodchild (1996). Alkaloid determination was done using the method of Harbone (1973) while flavonoid determination was by the method described by Boham and Kocipai-Abyazan (1974). Saponin content was assayed by the techniques of Rodriguez et al. (1986).

Determination of proximate composition and amino acid profile of TDLM

Proximate constituents of the TDLM (previously sun dried and milled to pass through 0.5 mm sieve) were determined by the method of Association of Official Analytical Chemist (AOAC, 1995). The amino acid profile was subsequently determined using methods described by Speckman et al. (1958). The TDLM sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino acid Analyzer (TSM).

RESULTS

Proximate composition and phytochemical screening of *T. diversifolia* leaf meal (TDLM)

Analysed TDLM contained 20.6% CP, 18.9% CF, 4.0% EE, 42.5% CHO and 14.0% ash (Table 1). Phytin was noticeably higher than all other antinutrients (ANFs) at 79.10 mg/100 g. Saponin, oxalate and alkaloids were also present at 2.36, 1.76 and 1.23 mg/100 g, respectively. Other ANFs values were in traces of 0.39 and 0.87 mg/100 g for tannin and flavonoid, respectively (Table 2).

Amino acid profile of TDLM

Amino acid profile is shown on Table 3. The contents of lysine, arginine, aspartate, glutamate, methionine+cystine, isoleucine, tyrosine and phenylalanine were appreciably high at 5.3, 6.19, 13.32, 12.19, 2.60, 4.28, 7.55, 3.53 and 5.47 g/100 g protein.

Table 1: Proximate Composition of Fresh/Dried *T. diversifolia* Leaves (% dry matter).

Nutrient	Percentage Composition
Moisture	11.0 ± 0.01
Crude Protein	20.6 ± 0.03
Crude fibre	18.9 ± 0.10
Ether extracts	4.0 ± 0.01
Carbohydrate	42.5 ± 0.10
Ash	14.0 ± 0.02

Values are means of 3 determinations ± SEM.

Table 2: Composition of Antinutrients (mg/100 g) in *T. diversifolia* leaves.

Antinutrients	Concentration (mg/100 g)
Phytin	79.10 ± 0.09
Tannin	0.39 ± 0.01
Oxalate	1.76 ± 0.02
Saponin	2.36 ± 0.01
Alkaloid	1.23 ± 0.03
Flavonoid	0.87 ± 0.02

Values are means of 3 determinations ± SEM.

Table 3: Amino Acid Profile of TDLM Compared with Groundnut Cake, Whole Egg and FAO/WHO Recommended Pattern (g/100 g protein).

Amino Acids	*TDLM	[a]GNC	[b]Hen's whole egg	FAO/WHO
Lysine	5.35	3.80	3.94	3.44
Histidine	2.25	2.40	1.50	-
Arginine	6.19	11.00	3.81	-
Aspartic acid	13.32	-	-	-
Threonine	4.26	2.80	3.19	2.50
Serine	5.13	-	-	-
Glutamic acid	12.19	-	-	-
Proline	3.91	-	-	-
Glycine	5.09	6.00	6.00	-
Alanine	6.08	-	-	-
Valine	5.29	5.20	4.75	3.13
Methionine	1.56	1.10	2.00	-
Cystine	1.04	1.60	1.13	-
Meth. + Cys.	2.60	2.70	3.13	2.19
Isoleucine	4.28	-	3.50	2.50
Leucine	7.55	6.50	5.19	-
Tyrosine	3.53	3.90	2.50	3.75
Phenylalanine	5.47	5.20	3.19	-

*TDLM, *Tithonia diversifolia* leaf meal, amino acid profile was determined using methods described by Speckman et al (1958).[a] *Amino acid profile for G/nut meal was adopted from Ravindran and Blair (1992).* [b]*Amino acid profile for hen's whole egg was cited by Robinson (1987). FAO/WHO and whole egg amino acid profiles were finally converted to g/100g from initial unit of g 16 g^{-1} N by Robinson (1987) before converted to g $kg^{-1}$and cited by Fasuyi (2006). GNC is groundnut cake.*

DISCUSSION

Proximate composition and phytochemical screening of TDLM

The 20.6% CP obtained for analysed TDLM was appreciable and at face value qualifies TDLM as a potential plant protein source in livestock nutrition. This quantity was higher than 18.9% CP reported by Olayeni et al. (2006). It was also higher compared to other leaf meals like *Colliandra sp* (18.9% CP), *Alfalfa* leaf meal (20.0% CP) and sweet potato leaf meal (14.0% CP) (Katto and Salazar, 1995). The above variation in the concentration of nutrients in *T. diversifolia* can conceivably be influenced by plant part, age of plant, position of the leaf within the plant canopy, soil fertility and provenance (Jama et al., 2000). The low fat content of 4.0% recorded for TDLM in this study may be a nutritional advantage in farm animal nutrition as higher fat contents in feed ingredients have been reported to be the probable causes of retardation of normal digestion and metabolism (Atteh, 2002). The crude fibre content was appreciably high at 18.9% and calls for nutritional concern, especially when considering TDLM as a potential ingredient in monogastric feeds. This level may be a hindrance to the bioavailability of other nutrients, intestinal irritation, feed bulkiness and overall decreased nutrient utilization (Fasuyi and Nonyerem, 2007).

The major antinutrients in TDLMs (phytin, tannin, oxalate, alkaloid and flavonoid) in this study had higher levels compared to previous report by Fasuyi et al. (2010). Fasuyi et al. (2010) suggested that the antinutrients in TDLM can be further reduced when TDLM is subjected to ensiling with molasses addition. The presence of some anitnutritional factors (ANFs) in TDLMs is of negative nutritional relevance. The ample presence of ANFs (phytins, oxalates, flavonoids and saponnins) portends a negative nutritional value on the bioavailability of TDLM in animal nutrition. These ANFs have been suggested as probable factor that militated against the digestibility of crude protein (CP) and amino acids (AAs) in most plant products. Phytic acid levels in the TDLMs were high and similar to those earlier reported (Proll et al., 1998). When ingested by non-ruminants, phytic acid can bind with proteins to form phytate protein complexes (Saio et al., 1967). This complex can adversely affect the digestibility of proteins (Reddy et al., 1982) by inhibiting a number of digestive enzymes in the gastrointestinal tract. ANFs have been reported as having inhibiting effects on the digestive enzyme activity in chickens and rats (Longstaff and Mcnab, 1991; Welsch et al., 1989). Meanwhile, the high saponin content of the seed may potentiate it as an aphrodisiac.

Amino acid profile of TDLM

Amino acid profile indicates that TDLM is rich in some essential amino acids, particularly isoleucine, leucine and lysine; also rich in aromatic amino acids like phenylalanine and valine when compared with amino acid profiles of commonly used conventional protein sources like groundnut cake. The comparison with whole egg and FAO/WHO recommended pattern also indicated a fairly balanced amino acid profile.

The abundant protein in some green leaves (Fasuyi and Aletor, 2005), could be attributed to the ability of green leaves to synthesize amino acids from a wide range of virtually unlimited and readily available primary material such as water, carbon-dioxide and atmospheric nitrogen. Utilization may however be hindered by the presence of anti-nutritional factors (Fasuyi and Aletor, 2005). However, there seemed to be a deficiency in the quantities of histidine, arginine, glycine and tyrosine when compared to that of groundnut.

Conclusion

With the overwhelming possibility of reducing the antinutritional factors in TDLM by bio-fermentation and other processing techniques, TDLM can be considered a potential plant protein source in livestock feeding particularly in monogastric animals which naturally compete with man for conventional food/feed materials. With its rich amino acid profile, TDLM can be further processed and investigated in feeding trials for various categories of farm livestock.

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