ESSENTIAL AND SULFUR AMINO ACID COMPOSITION OF FIVE COMMONLY FED TREE LEAVES TO RABBITS IN THE COASTAL SAVANNAH ZONE OF GHANA

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ABSTRACT
Despite the importance of essential amino acids (EAA) as building blocks of protein and the critical role played by sulfur-containing amino acids (SAA) in protein synthesis and functions, there is not much information available on the contents of these amino acids in leaves of trees grown in Ghana and fed to rabbits. The EAA and the major SAA (methionine and cysteine) contents in Moringa oleifera, Gliricidia sepium, Carica papaya and Musa paradisiaca leaves were determined. The EAA contents ranged 0.28 to 2.45% dry matter (DM) for methionine in Gliricidia sepium and leucine in Carica papaya leaves respectively. The total EAA contents ranged from 7.24% to 12.86% DM for Gliricidia sepium and Carica papaya leaves respectively. The EAA contents of crude protein (CP) in the leaves ranged from 1.59 to 8.58% for methionine in Gliricidia sepium and leucine in Musa paradisiaca leaves respectively. The methionine plus cysteine % DM was lowest for Gliricidia sepium leaves, and that of total nitrogen was lowest in Musa paradisiaca leaves. The mean methionine plus cysteine and total nitrogen percentage DM in the leaves were 0.68 ± 0.3 and 0.42 ± 0. 1% respectively, whilst the corresponding mean percentage CP were 3.0 ± 0.3 and 1.89 ± 0. 2% respectively. The results obtained in the study suggest that the leaves of the selected trees are good sources of CP, EAA and methionine plus cysteine, and are capable of meeting the requirements of rabbits when fed as sole diets or incorporated into concentrate diets at appropriate inclusion levels.

Keywords: *Amino acids, Moringa oleifera, Gliricidia sepium, Carica papaya, Musa paradisiaca leaves.*

INTRODUCTION

The major trees providing leaves for feeding rabbits in the Coastal Savannah zone of Ghana include *Moringa oleifera*, *Gliricidia sepium*, *Carica papaya* and *Musa paradisiaca*. *Moringa oleifera* leaves are rich in nutrients and some important medicinal values (Taher, 2017). *Gliricidia sepium* is a perennial, medium-sized legume tree, and mostly deciduous during the dry season but remains evergreen in humid areas (Heuzé and Tran, 2015a). Fresh *Carica papaya* leaves are of high nutritive quality and are suitable feed ingredient for inclusion in grower rabbit diets without any deleterious effects (Machoko *et al.,* 2019). *Musa paradisiaca* is a herbaceous plant with a robust treelike pseudostem and a crown of large elongated oval deep-green leaves with a prominent midrib (Imam and Akter,

2011). The fruit of the plant is widely used as food, with more than 90% of the cultivated area belonging to smallholder farmers (SRID, 2021; Dzomeku *et al.,* 2011).

Amino acids are the building blocks of protein with ten of them, comprising arginine, isoleucine, histidine, leucine, methionine, lysine, phenylalanine, tryptophan, threonine and valine, being essential because animals cannot produce these amino acids themselves and are thus required in the diet. (Halls, 2010). Although cysteine is a non-essential amino acid, it can replace methionine to a large extent in the provision of immune responses (Tsiagbe *et al*., 1987).

The sulfur amino acids (SAA), comprising methionine, cysteine, homocysteine, and taurine, play critical role in protein synthesis, structure and function. However, mainly methionine and cysteine are incorporated into proteins (Kim *et al*., 2014; Brosnan and Brosnan, 2006). The nutritive parameters and productivity of forages, including amino acids, could differ as they are influenced by environmental factors such as air humidity, temperature and soil pH (Melo *et al.,* 2022).

Despite the importance of essential amino acids (EAA) and SAA in animal nutrition, there is not much locally generated information available on amino acid contents in tree leaves used as rabbit feed in Ghana. This study was undertaken to determine the EAA and the major SAA, methionine and cysteine, contents in leaves of four trees commonly fed to rabbits in the Coastal Savannah zone of Ghana to serve as a guide for plausible inclusion in concentrate diets for rabbit feeding.

MATERIALS AND METHODS

Study Location

Moringa oleifera, Gliricidia sepium, Carica papaya and *Musa paradisiaca* leaf were collected from the environs of the Council for Scientific and Industrial Research (CSIR) - Animal Research Institute (ARI) Frafraha Station, situated on latitude 5^0 43′ 48″ North and longitude 0^0 9′ 0″ East, about 20 km from Accra, the capital of Ghana in the Coastal Savannah zone. The average annual rainfall in the zone is about 730 mm with two rainy seasons (i.e. May to mid-July and mid-August to October). There is very little variation in temperature throughout the year. The mean monthly temperatures range from 24.7°C (in August) to 28°C (in March) with an annual average of 26.8 °C. The relative humidity is generally high with values ranging from 65% (mid-afternoon) to 95% (night time) (AMA, 2006).

Sampling, drying of leaves and amino acid analysis of leave samples

Triplicates of 300 g of each tree leaf (matured), picked from the environs of the study site on the same day between 8.00am to 10.00am, were taken to the CSIR-ARI laboratory immediately after harvest (a maximum delay of 60 min) and dried in a Precision laboratory oven at 60^oC for 48 h. A subsample, l0-l2 g, from each of the three dried samples of a forage species was ground to pass through a 40 mm mesh screen. The dry matter (DM), crude protein (CP) and amino acid analysis of the subsamples were undertaken at the AminoLab, Evonik Nutrition & Care GmbH, Germany using high performance liquid *chromatography (HPLC) method (*AOAC, 2005).

Statistical analyses

The data generated were subjected to Analysis of Variance (ANOVA) as outlined by the Generalized Linear Model of the GenStat Discovery Edition (VSN International, 2010). Least Significant Difference (LSD) Test was used to separate means at 0.05 level of significance and the results were expressed as means ± Standard Error (SE).

RESULTS AND DISCUSSION

Dry matter and crude protein contents of tree leaves

The DM and CP contents of the tree leaves are presented in Table 1. There were no significant differences ($p>0.05$) between the DM contents of the tree leaves. The DM contents in the tree

leaves, ranging from 91.91 to 92.72%, were comparable to the values of 91.31 and 92.56% in *Molus alba* and *Leuceana leucocephala* leaves respectively (Simbaya *et al.*, 2020), but higher than the values of 16.92 to 56.60% in the leaves of various fodder trees and shrubs used for feeding livestock in India (Gaikwad *et al.,* 2017), 40.2 to 70.2% in leaves of four browse plants (*Adogla-Bessa et al*., 2022), and 34.54 to 44.83 % DM in the leaves of eight browse plants (Ansah and Issaka, 2018) in the Coastal and Guinea Savannah zone of Ghana respectively.

Carica papaya had significantly (P<0.05) highest CP contents among the tree leave. The CP contents of DM in the tree leaves were higher than the values of 15.9 to 31.4% recorded for the browse species studied by Adogla-Bessa *et al.* (2022), and 9.22 to 22.92% in the study by Ansah and Issaka, (2018). The CP contents in the leaves in the present study, with the exception of the value for *Carica papaya*, were lower than the mean value of 26.87% obtained for some fodder tree leaves in Pakistan (Sultan *et al.,* 2008). The CP contents of *Moringa oleifera, Carica papaya* and *Gliricidia sepium* were higher whilst that of *Musa paradisiaca* was lower than the values of 19.4% and 19.5% obtained for *Albizia lebbeck* leaves from South-east México (Solorio-Sánchez *et al*., 2000) and *Musa paradisiaca* leaves in Nigeria (Okareh *et al*., 2015).

A study by Sarkwa *et al.* (2021) of the chemical composition of leaves of seven fodder trees in the Coastal Savannah zone showed that though dry matter content was higher crude protein con-

Table 1: Dry matter and crude protein contents of tree leaves

Parameter	Type of tree leaves				
	Moringa oleifera	***Gliricidia. Sepium***	***Carica papaya***	***Musa paradisiaca***	**P values**
DM %	92.79 ±1.3	91.91 ±1.3	92.39 ±1.3	92.56 ±1.3	0.922
CP %	22.19^{b} ±0.8	17.68^{d} ±0.8	30.71^{a} ±0.8	19.52^{c} ±0.8	<.001

Figures standardized to a DM content of 88%. CP is based on Dumas combustion (Leco CNS 2000) method (CP = 6.25). Means within the same row with different superscripts are significantly different (p<0.05)

Table 2: Essential amino acid contents in the dry matter of tree leaves

Parameter % DM	Type of tree leaves				
	Moringa oleifera	***Gliricidia. sepium***	***Carica papaya***	***Musa paradisiac***	**P values**
Phenylalanine	1.06^{b} ±0.09	0.89^{b} ±0.09	1.62^{a} ±0.09	1.05^{b} ±0.09	<.001
Methionine	0.36^{bc} ±0.06	0.28^{c} ±0.06	0.56^{a} ±0.06	0.41^{b} ±0.06	0.007
Histidine	0.41^{b} ±0.06	0.37^{b} ±0.06	0.60^{a} ±0.06	0.38^{b} ±0.06	0.003
Lysine	0.88^{c} ±0.05	0.94^{bc} ±0.05	1.51^{a} ±0.05	0.98^{b} ±0.05	0.001
Threonine	0.88^{b} ±0.05	0.74^{c} ±0.05	1.34^{a} ±0.05	0.38^{d} ±0.05	<.001
Arginine	1.12^{b} ±0.01	0.91^{c} ±0.01	1.72^{a} ±0.01	1.13^{b} ±0.01	<.001
Isoleucine	0.89^{bc} ±0.05	0.79^{c} ±0.05	1.38^{a} ±0.05	0.91^{b} ±0.05	<.001
Leucine	1.64^{b} ±0.04	1.38^{c} ±0.04	2.45^{a} ±0.04	1.67^{b} ±0.04	<.001
Valine	1.09^{b} ±0.06	0.94^{c} ±0.06	1.68^{a} ±0.06	1.14^{b} ±0.06	<.001
Total	8.33	7.24	12.86	8.05	9.12 ± 2.5

Figures standardized to a DM content of 88%. Means within the same row with different superscripts are significantly different (p<0.05).

tent was lower in the dry season than the wet season, the seasonal changes in chemical composition of the fodder tree leaves were found to be less drastic, which implies that, the fodder tree leaves can be used as supplementary feed throughout the year regardless of the season. The observed differences between current results and data from other studies could be due to species, age at harvesting, soil nutrient status and season of harvest differences.

Essential amino acid contents of dry matter and crude protein in tree leaves

EAA contents in DM of the tree leaves are presented by Table 2. *Carica papaya* had significantly ($p<0.05$) highest EAA contents of DM in the tree leaves. Phenylalanine, histidine, lysine, threonine and arginine percentages of DM in the tree leaves were lower, whilst isoleucine, leucine and valine contents were higher than the corresponding values in dried *Moringa oleifera* leaves in the study by Moyo *et al.* (2011). The mean EAA percentages of DM in the leaves were found to be lowest (0.40 ± 0.1%) in methionine and highest (1.79 ± 0.5%) in leucine. The total EAA percentages of DM in the leaves varied from 7.24 to 12.86% in *Gliricidia sepium* and *Carica papaya* leaves respectively.

The EAA percentages of CP in the tree leaves, ranging from 1.59 to 8.58% for methionine and leucine in *Gliricidia sepium* and *Musa paradisiaca* leaves respectively (Table 3), were generally lower than the values for forages (Tedeschi *et al*., 2001) and Monteiro-Motta *et al.*, (2013). *Musa paradisiaca* had the highest EAA percentages of CP in all the leaves with the exception of histidine and lysine which were highest in *Gliricidia sepium* leaves, and arginine and isoleucine which were highest in *Carica papaya* leaves. The mean EAA percentages of CP in the leaves ranged from 1.78 ± 0.2 to 7.94 ± 0.5% for methionine and leucine leaves respectively, and the total EAA contents of CP was lowest (37.56%) and highest (43.89%) for *Moringa oleifera* and *Carica papaya* leaves respectively.

Methionine plus cysteine and Total Nitrogen contents in dry matter and crude protein of tree leaves

The Methionine plus cysteine and percentages of DM in the leaves are shown in Table 4. *Carica papaya had* significantly ($p<0.05$) highest methionine plus cysteine and total nitrogen percentages of DM in the leaves. *Gliricidia sepium* and *Musa paradisiaca* had significantly ($p>0.05$)

Table 3: Essential amino acid content in crude protein of tree leaves

Parameter % CP	Type of tree leaves				
	Moringa oleifera	***Gliricidia. sepium***	***Carica papaya***	***Musa paradisiaca***	**P values**
Phenylalanine	4.79^{c} ±0.09	5.03^{b} ±0.09	5.28^{a} ±0.09	5.39^{a} ±0.09	<.001
Methionine	1.63^{bc} ±0.06	1.59^{c} ±0.06	1.81^{b} ±0.06	2.10^{a} ±0.06	<.001
Histidine	1.83^{c} ±0.05	2.10^{a} ±0.05	1.97^{b} ±0.05	1.97^{b} ±0.05	0.003
Lysine	3.98^{c} ±0.08	5.33^{a} ±0.08	4.93^{b} ±0.08	5.03^{b} ±0.08	<.001
Threonine	3.95^{d} ±0.06	4.18^{c} ±0.06	4.36^{b} ±0.06	4.52^{a} ±0.06	<.001
Arginine	5.05^{c} ±0.07	5.14^{c} ±0.07	5.61^{b} ±0.07	5.77^{a} ±0.07	<.001
Isoleucine	4.00^{b} ±0.13	4.46^{a} ±0.13	4.48^{a} ±0.13	4.67^{a} ±0.13	0.004
Leucine	7.40^{c} ±0.18	7.79^{c} ±0.18	7.98^{b} ±0.18	8.58^{a} ±0.18	0.001
Valine	4.93^{c} ±0.1	5.34^{b} ±0.1	5.48^{b} ±0.1	5.86^{a} ±0.1	<.001
Total	37.56	40.96	41.90	43.89	41.08 ± 2.6

Figures standardized to a DM content of 88%. Means within the same row with different superscripts are significantly different ($p<0.05$)

Table 4: Methionine plus cysteine and total nitrogen contents in dry matter of tree leaves

Parameter % DM	Tree leaves content (% DM)				
	Moringa oleifera	***Gliricidia. Sepium***	***Carica papaya***	***Musa paradisiaca***	**P values**
Meth. + Cystine	0.65^{b} ±0.05	0.47^{c} ±0.05	0.98^{a} ±0.05	0.63^{b} ±0.05	<.001
Total Nitrogen	0.41^{b} ±0.02	0.38^{b} ±0.02	0.59^{a} ±0.02	0.32^{c} ±0.02	<.001

Figures standardized to a DM content of 88%. Means within the same row with different superscripts are significantly different (p<0.05)

Table 5: Methionine plus cysteine and total nitrogen contents in crude protein of tree leaves

Parameter	Type of tree leaves (% CP)				
	Moringa oleifera	***Gliricidia. sepium***	***Carica papaya***	***Musa paradisiaca***	**P values**
Meth. + Cystine	2.93^{bc} ± 0.12	2.68^{c} ±0.12	3.18^{ab} ±0.12	3.23^{a} ±0.12	0.007
Total Nitrogen	1.83^{bc} ±0.1	2.13^{a} ±0.1	1.93^{ab} ±0.1	1.65^{c} ±0.1	0.02

Figures standardized to a DM content of 88%. Means within the same row with different superscripts are significantly different (p<0.05)

lowest methionine plus cysteine and total nitrogen contents in the leaves respectively. The mean methionine plus cysteine and total nitrogen contents in the tree leaves were 0.68 ± 0.2% and 0.42 ± 0.1% DM respectively.

The methionine plus cysteine percentages of DM for *Moringa oleifera* (0.65%) and *Musa paradisiaca* (0.63%) were comparable to the recommended value of 0.60% of DM for a balanced feed for all types of rabbits (Lebas, 2013) and also fell within the recommended range of 0.52 to 0.73% of DM for rabbit diets (Spreadbury, 1978; Monteiro-Motta, 2013), whilst the values for *Gliricidia sepium* and *Carica papaya* were lower and higher respectively than recommended values.

Table 5 shows that *Musa paradisica* tended to have higher methionine plus cysteine whilst *Gliricidia sepium* tended to have the lower methionine plus cysteine %CP content in the leaves of the tree leaves. *Gliricidia sepium* teneded to have the higher total nitrogen whilst *Musa paradisiaca* tended to have lower total nitrogen %CP content in the leaves of the tree leaves. The mean methionine plus cysteine content of CP in the tree leaves, 3.01%, was higher than the value of 2.2% for *Manihot esculanta* foliage (Heuzé and Tran, 2016) and lower than the value of 3.6% CP for *Leucena leucocephala* leaves (Heuzé and Tran, 2015b).

CONCLUSION

The generally high contents of DM, CP, EAA, major SAA and total nitrogen in DM of *Moringa oleifera*, *Gliricidia. sepium*, *Carica papaya* and *Musa paradisiaca* leaves suggest that the leaves when fed as supplements to concentrate diets will meet the EAA requirements of rabbits, and could also be incorporated in concentrate diets at appropriate inclusion levels to achieve satisfactory rabbit performance.

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