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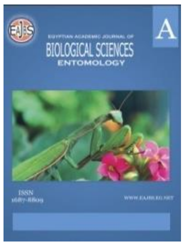

Comparative Amino Acid Content of *Pseudocanthotermes grandiceps, Macrotermis jeanelli* and *Allodontermes tenax* (Isoptera: Termitidea)

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ABSTRACT

The main objective of this study was to compare the amino acid profiles of *P.grandiceps, M. jeanelli and A. tenax.* The study was carried out in Trans-Nzoia at an altitude of 1,900 meters, with a latitude of 1°1'8.72"N, and longitude of 35°0'8.3"E. Termite alates was collected when swarming and placed in 500grams collection jars and taken to the laboratory for amino acid analysis. Some of the alates and a number of small or large soldiers and workers were preserved in 80% ethanol for identification at the National Museums of Kenya. The collected termite alates were analyzed for composition of amino acids using standard methods detailed in AOAC (2000). From the results, there was no significant difference in amino acid content, amino acid classes, amino acid groups and total essential amino acids and total non-essential amino acids of the three termite species. Glutamic acid was the most abundant of the three species. It ranged between 8.43g/100g - 15.5g/100g. The lowest concentrated amino acid was cysteine ranging between 0.60g/100g- 3.92g/100g. Leucine was found to be the most highly concentrated essential amino acid (eaa); 7.72g/100g crude protein in *P. grandiceps*, 7.48g/100g in *M. jeanelli* and 5.51g/100g in *A. tenax*. Methionine was the lowest eaa in *P. grandiceps* at 0.88g/100g crude protein while Histidine was the lowest eaa in both *M. jeanelli* and *A. tenax* at 2.15g/100g and 2.09g/100g respectively.

INTRODUCTION

Termite alates have been historically consumed in many parts of the world from time immemorial. Its delicacy is well known to many people in Kenya and has high protein content (Gahukar *et al.*, 2020) and its abundance is unquestionable (Makila *et.al.*, 2018). Kenya largely depends on agriculture to satisfy its food demands. In recent years there have been unforeseen and unpredictable weather changes prompting declining yields and resulting in poor harvests. There is therefore grave danger of chronic and sometimes acute food shortages that is likely to affect the nutritional and health status of the people. To reduce the chronic food shortages in Kenya, a variety of food sources are required with termites being one of them. They are a cheaper source of proteins as compared to fish, chicken and beef. Termites have a long history of consumption in western Kenya both during the rainy and dry seasons. They are a rich source of protein and thus serve as an important diet. While a rump steak yields 322calories per 100 gm. and codfish 74, termites provide 560 calories per 100gm. Termites rank among the highest in fat content (Itakura, 2006). Therefore, there is a need of making such food available to satisfy the demand of the general population and produced en-mass for packaging. The present study was carried out to evaluate amino acid content in the three most abundant termite species in western Kenya.

MATERIALS AND METHODS

Study Area:

The research was carried out in Trans-Nzoia and West Pokot counties at an altitude of 1,900 meters, a latitude of 1°1'8.72"N, and a longitude of 35°0'8.3"E and altitude of 1906 meters, latitude1.59244 and longitude 35.28536 respectively (Fig. 1). The main activity in the area is crop farming and livestock husbandry.

Fig. 1: Map of Kenya showing the location of the study area (Source: Adapted and modified from Microsoft Encarta 2008).

Collection of Termite Samples:

Termite alates were collected from Kitale and West Pokot Nasukuta area during long rain periods that occurred between April and September 2018. Colonies were marked and mapped, and 50% of the colonies were re-sampled at any time they were available. The selection of the sampling sites was guided by past experience and after consultation with various stakeholders in the region who are experts in the identification of areas where termite alates predominate. Collection of the termite alates was done using traps that were mounted on the termite mound to trap the alates a method that has been used traditionally for decades. Termite workers were collected by destructive sampling (Li, H. F. *et al.*, 2015). Collections were made by breaking open the termite mound (Li, H. F. *et al.*, 2015). The collected samples were put in collecting jars and taken to the laboratory for analysis. Some of the alates and a number of small or large soldiers and workers were preserved (in 80% ethanol) for identification at the National Museum of Kenya. Approximately 2000 live individuals from each colony were collected. Collections did not discriminate between small or large workers, or soldiers.

Amino Acid Analysis:

The collected termite alates were analyzed for the proximate composition of amino acids using standard methods according to AOAC (2000). Analysis of the amino acids was performed with a Waters C18 column (3.9 x 150 mm). The solvents utilized were the sodium acetate buffer and acetonitrile (300 ml ACN, 200 ml water, 0.2 ml CaEDTA). Twenty microliter aliquots were injected onto the column. Elution of the amino acids was achieved by increasing the acetonitrile concentration in the eluent, causing individual amino acids to be eluted at predetermined times. Quantitation was achieved by monitoring the absorption of the column at 254 nm and comparing the absorbance of individual peaks with that of the corresponding amino acid standard.

Statistical Analysis:

Amino acid content for the three different termite species was done using Chi-square with significance set at P=0.05.

RESULTS

The Table1 shows the amino acid content of the samples obtained from the three termite species in (g/100g).

The protein content of the three termite species is shown in Table 1. The protein content obtained from the whole termite showed *Pseudocanthotermes grandiceps* had the highest at 94.11g/100g while *Allodontermes tenax* was the lowest at 79.92/100g. From the results, the protein was the major nutrient component of the three termite species that were analyzed. Glutamic acid was the most abundant in the three samples. It ranged between 8.43g/100g - 15.5g/100g of the total amino acids that were analyzed. The lowest concentrated amino acid was cysteine with values ranging between 0.60g/100g-3.92g/100g. Leucine was found to be the most highly concentrated essential amino acid. It was 7.72g/100g of crude protein in *P. grandiceps*, 7.48g/100g in *M. jeanelli* and 5.51g/100g in *A. tenax*. Methionine was the lowest essential amino acid in *P. grandiceps* at 0.88g/100g crude protein. Methionine is important in the synthesis of choline among other substances. Histidine was the lowest essential amino acid in both *M. jeanelli* and *A. tenax* at 2.15g/100g and 2.09g/100g respectively. Lysine was also relatively high 4.16g/100g – 7.80g/100g. Tryptophan was found to be available in considerable amounts1.70g/100g- 6.43g/100g, an amino acid absents in most other protein foods (DeFoliart, 1991). A summary of essential amino acids is shown in Figure 2. The most abundant essential amino acid in *P. grandiceps* was Leucine at 7.72g/100g, in *M. jeanelli* was lysine at 7.80g/100g and *A. tenax* was methionine at 6.86g/100g. According to FAO (1985), preschool children aged below 5 years require Leu (6.6 g/100g), Ile (2.8 g/100g), Lys (5.8 g/100g), Met + Cys (2.5 g/100g) and His (1.9g/100g). From the results obtained, the three termite species have the potential to provide the required amino acid and even exceed the requirements. There was no significant difference in the amino acid content of the three termite species as shown in Table 1.

Figure 2 shows a summary of essential amino acids for the three termite species.Leucine was the most highly concentrated essential amino acid 7.72g/100g crude protein in *P. grandiceps*, 7.48g/100g in *M. jeanelli* and 5.51g/100g in *A. tenax*. Methionine was the lowest essential amino acid in *P. grandiceps* at 0.88g/100g crude protein. Histidine was the lowest essential amino acid in both *M. jeanelli* and *A. tenax* at 2.15g/100g and 2.09g/100g respectively.

Table1: Amino Acid Content

Amino Acid	*P. grandiceps* (g/100g)	*M.jeanelli* (g/100g)	*A. tenax* (g/100g)	Mean	SD	CV%	χ2
Lysine	6.44	7.8	4.16	6.13	1.84	30	0.607532
Threonine	4.36	3.91	3.2	3.82	0.58	15.3	0.044325
Valine	4.6	3.98	4.09	4.22	0.33	7.8	0.077451
Methionine	0.88	2.48	6.86	3.41	3.09	90.8	7.145698
Isoleucine	4.22	4.96	3.3	4.16	0.83	20	0.133807
Leucine	7.72	7.48	5.51	6.9	1.21	17.6	4.053052
Phenylalanine	4.58	3.36	2.54	3.49	1.02	29.4	0.355341
Tryptophan	2.47	1.7	6.43	3.53	2.54	71.9	4.888299
Histidine	3.09	2.15	2.09	2.44	0.56	23	0.174922
Arginine	6.9	6.6	4.15	5.88	1.51	25.7	0.343642
Aspartic acid	6.71	7.87	4.96	6.51	1.46	22.5	0.287363
Serine	5.39	4.06	4.14	4.53	0.75	16.5	0.183868
Glutamic acid	13.7	15.5	8.43	12.5	3.67	29.4	1.123141
Proline	5.7	4.01	4.05	4.58	0.96	21.1	0.29152
Glycine	6.09	5.76	3.25	5.03	1.55	30.9	0.50788
Alanine	5.86	6.97	5.79	6.21	0.66	10.7	0.111876
Cysteine	0.6	1.89	3.92	2.14	1.67	78.2	3.37496
Tyrosine	4.8	3.33	3.05	3.72	0.94	25.3	0.30558
Total						***P* = 0.05**	24.01026 χ2=**43.773**

SD = standard variation, CV% = coefficient of variation percent, χ2 = Chi-square, α = 0.05.

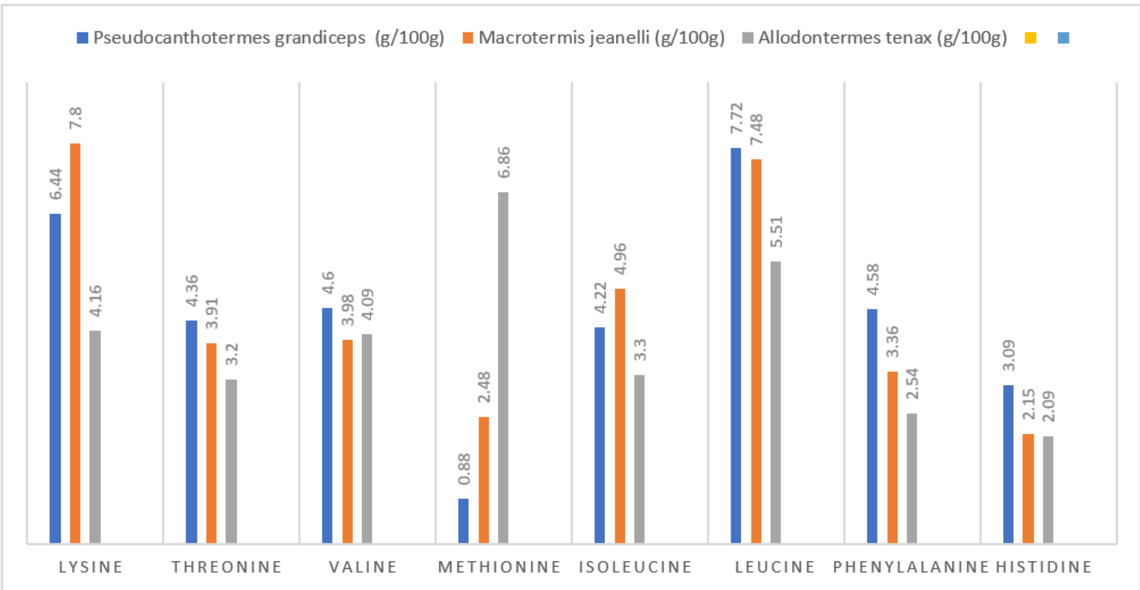

Fig 2. Essential Amino Acids

The Table 2 shows the classification of amino acids into Total essential amino acid (TEAA); Total conditionally essential amino acid (TCEA); Total nonessential amino acid (TNEAA); Total amino acid (TAA); Total acidic amino acids (TAAA); Total basic amino acids (TBAA); Total neutral amino acids (TNAA); Total essential aliphatic amino acids (TEAlAA) and Total Sulphur
amino acids (TSAA).

Total essential amino acids (TEAA) with His ranged between 31.75g/100g to

36.12g/100g; without His, it ranged between 29.66 g/100g to 33.97 g/100g.WHO requirements for essential amino acids (EAA) with His are as follows: infant, 46.0 g/100g; pre-school 2-5 years, 33.9 g/100g; school child 10-12 years, 24.1 g/100g and adult 12.7 g/100g. Without His: infant 43.4 g/100g; pre-school 2-5years, 32.0 g/100g; school child 10-12years, 22.2 g/100g and adult 11.1 g/100g. The three termite species contain the EAA in the required proportions to supply the human diet satisfactorily to all age groups except infants. Percentage TEAA was considerably higher with Histidine 38.1-39.7g/100g; without Histidine 34.9 - 37.1g/100g as compared to percentage TNEAA 29.2 - 36.7g/100g in the three termite species; similarly, the TAAA was higher 13.39 - 23.37g/100g as compared to TBAA 10.4-16.55g/100g. TNAA range was 57.4 – 70.2g/100g showing that they formed the bulk of all the amino acids. TCEAA ranged between 18.42 to 24.0g/100g while the percentage Cys/TSAA was between 36.4 to 43.2g/100g. The ratios of Leu/Ile were between 1.51 to 1.83g/100g and this was lower than the required amount of 2.36. This study also showed that Lys/Trp ranged between 0.65 to 4.59g/100g while Met/Trp range was 0.36 to 1.46g/100g. The termites' amino acid classes were not significantly different in all the three termite species.

Table 2. Classification of Amino Acids (g/100g Crude Protein) of the Termite Samples and their Percentages.

Amino Acid	*P. grandiceps* (g/100g)	*M. jeanelli* (g/100g)	*A. tenax* (g/100g)	Mean	SD	CV%	$\chi 2$
TAA	94.11	93.81	79.92	89.28	8.12	9.1	0.240886
TEAA With His	35.89	36.12	31.75	34.59	2.46	7.1	0.017995
No, His %TEAA	32.8	33.97	29.66	32.14	2.23	6.9	0.011351
With His	38.1	38.5	39.7	38.7	0.83	2.6	0.482691
No His	34.9	36.2	37.1	36.1	1.11	3.1	0.49989
TNEAA	31.66	34.4	23.32	29.8	5.77	19.4	1.141375
%TNEAA	33.6	36.7	29.2	33.2	3.77	11.4	0.237791
TAAA	20.41	23.37	13.39	19.1	5.13	26.8	1.758641
%TAAA	21.7	24.9	16.8	21.1	4.08	19.3	0.81495
TBAA	16.43	16.55	10.4	14.46	3.56	24.3	1.044602
%TBAA	17.5	17.6	13	16	2.63	16.4	0.384109
TNAA	57.27	53.89	56.13	55.8	1.72	3.1	0.619503
%TNAA	60.9	57.4	70.2	62.97	6.84	10.9	3.419493
TCEAA	24.09	21.59	18.42	23.4	2.84	12.1	0.370757
%TCEAA	25.6	25.6	23	24.7	1.5	6.1	0.00561
TEAlAA	28.1	27.4	22.74	26.1	2.91	11.2	0.187885
%TEAlAA	29.9	29.2	28.5	29.2	0.7	2.4	0.104163
TSAA	1.48	4.37	10.78	5.54	4.76	85.9	9.489002
%Cys/TSAA	40.5	43.2	36.4	40	3.42	8.6	0.0838
Leu/Ile ratio	1.83	1.51	1.67	1.67	0.16	9.6	0.04701
Lys/Trp	2.6	4.59	0.65	2.61	1.97	75.5	2.594804
Met/Trp	0.36	1.46	1.07	0.96	0.56	58.1	0.635441
TOTAL					24.19175 ***P* = 0.05, $\chi 2$= 55.758**		

Group I ranged between 21.94- 29.15g/100g; group II, 7.34- 9.75g/100g; group III, 1.48- 10.78g/100g; group IV, 13.39 – 23.37g/100g; group V, 10.4- 16.55g/100g; group VI, 10.54 – 14.94g/100g; group VII, 4.01-5.70. There was no significant difference in amino acid groups in the three termite species.

Table 3. Shows amino acid groups of the termite samples.

Groups	*P. grandiceps* (g/100g)	*M.jeanelli* (g/100g)	*A. tenax* (g/100g)	Mean	SD	CV%	χ^2
I	28.49	29.15	21.94	26.53	3.99	15	0.208774
II	9.75	7.97	7.34	8.35	1.25	15	0.160994
III	1.48	4.37	10.78	5.54	4.76	85.9	10.48238
IV	20.41	23.37	13.39	19.1	5.13	26.8	1.37478
V	16.43	16.55	10.4	14.46	3.52	24.3	0.708189
VI	14.94	10.54	14.11	13.2	5.64	42.8	1.290547
VII	5.7	4.01	4.05	4.59	0.96	21	0.275194
TOTAL				14.50086 **P = 0.05, χ2= 21.026**			

[Group I = amino acids with aliphatic side chains (Gly, Ala, Val, Leu and Ile); group II = amino acids with side chains containing OH group (Ser and Thr); group III = amino acids with side chains containing Sulphur atom (Cys and Met); group IV = amino acids with side chains containing acidic groups or their amides (Asp and Glu); group V = amino acids with side chains containing basic groups (Lys, His and Arg); group VI = amino acids with side chains containing aromatic rings (His, Phe, Tyr and Trp) and group VII = amino acids (Pro)].

Table 4 gives a summary of amino acid content. Total essential amino acids and total non-essential amino acids were not significantly different in the three termite species.

Table 4. Summary of the amino acid content

Groups	*Pseudocanthotermes grandiceps* (g/100g)	*Macrotermis jeanelli* (g/100g)	*Allodontermes tenax* (g/100g)	χ^2
Total essential amino acids	32.8	33.97	29.66	0.247638
Total non-essential amino acids	31.66	34.4	23.32	0.267171
			P=0.05, χ^2=5.991. 0.51481	

DISCUSSION

The three termite species contained essential amino acids in considerable amounts. Leucine was the highest among all the essential amino acids, followed closely by lysine. Leucine is important for general muscle health (Oliveira *et al.*, 2018). It can stimulate protein synthesis and reduce muscle protein breakdown, especially after injury (Brestensky et al., 2019). Additionally, Leucine also raises insulin levels in the blood (Oliveira, A.G, *et.al,* 2018; Brestensky *et. al.,* 2019) Leucine was the most abundant amino acid in the three termite species with *P. grandiceps* leading at 7.72g/100g and the lowest was observed in *A. tenax* 5.5g/100g. The three termite species, therefore, have the potential to provide the required 5.5g/100g daily intake (Borack, M.S., and Volpi, E., 2016).

Lysine being an essential amino acid can only be obtained from food (Goyal *et al.,* 2017), it is important in normal growth and muscle turnover. It's also important in the formation of carnitine a substance found in most cells of the body; it also transports fats across cells for energy production. Animal protein foods rich in lysine include beef steak 3.3g/100g, lean chicken breast 3.08g/100g and lean pork 2.76g/100g (Goyal, a., *et al*, 2017; Yang, Q.Q., 2017). Comparably *M. jeanelli* has the highest content of lysine at 7.8g/100g closely followed by *P. grandiceps* at 6.44g/100g and *A. tenax* at 4.16g/100g. The three termite species showed to have more than what the conventional proteins could provide therefore the insects' protein could be used as an alternative, especially for malnourished households who cannot afford most of the other animal protein foods.

Arginine is a very important amino acid to the heart (Albaugh *et al.*, 2017). It changes into nitric oxide a powerful neurotransmitter substance that improves blood circulation by causing the blood vessels to relax. When arteries relax it improves blood flow it may improve erectile dysfunction. In addition, arginine leads to wound healing, causes the kidneys to remove waste materials, and maintains immune and hormone function (Albaugh, V.L. *et. al.*, 2017; McNeal, C.J., *et.al.*, 2018). Arginine was the highest in *P. grandiceps* at 6.90g/100g and the lowest in *A. tenax* at 4.15g/100g. Arginine daily requirement is 2 to 3g taken three times per day (McNeal, C.J., *et.al.*, 2018).

Methionine is important in the treatment of individuals who are undergoing depression, those who are recovering from alcoholism, allergies, asthma and Parkinson's disease. Methionine was highest in A. tenax at 6.86g/100g and lowest in P. grandiceps at 0.88g/100g. Crickets have a methionine content of 2.29g/100g and moths have 0.62g/100g (Oibiokpa F.I. *et. al.*, 2018). Therefore, on average, the three termites had a higher content of methionine than the other insects analyzed.

Tryptophan is a precursor in the synthesis of neurotransmitter substances like serotonin (Kaluzna-Czsplinska *et al.*, 2019) and is an important amino acid that should be availed in the foods consumed. It's also important in the synthesis of anti-pellagra vitamins, nicotinic acid and melatonin (kaluzna-Czsplinska J. *et. al.*, 2019). Tryptophan was found to be highest in *A. tenax* at 6.43g/100g and lowest in *P. grandiceps* 2.47g/100g. *Macrotermis nigeriensis* have a tryptophan content of 2.36g/100g; Cir*ina foda* contains 1.84g/100g, *Gryllus assimilis* 2.53g/100g and *Melanoplus foedus* 2.64g/100g (Oibiokpa F.I. *et. al.*, 2018). Among the termites, *A. tenax* has the highest content of tryptophan.

Histidine is responsible for keeping a normal pH of 7 in the body by shutting protons to maintain an acid-base balance in tissues and blood (Kessler & Raja, 2019). It's also important to hemoglobin to shuttle oxygen around the body and is a precursor of histamine among other important roles (Kessler & Raja, 2019) Histidine was found to be 3.09g/100g in *P.grandiceps*, 2.15g/100g in *M. jeanelli* and 2.09g/100g in *A. tenax*. The daily requirement of Histidine in the human body is 1.2g/100g (Kessler & Raja, 2019) therefore the three termite species have the potential to provide the required nutrients to humans.

Valine's daily requirement is 2.6g/100g, Isoleucine 2.0g/100g, Phenylalanine and tyrosine 2.5g/100g, and Threonine is 1.5g/100g (Hoffer, L.J, 2016). The three termite species have the potential to provide the daily requirement. Therefore entomophagy especially on the three termite species should be encouraged being a cheap source of protein that can be found locally when they swarm.

The total essential amino acids (TEAA) are required in different proportions across the age groups according to FAO (1985). The infant requires 46.0g/100g His; pre-school 2-5years require 33.9g/100g, school child 10-12 years 24.1g/100g while adult needs 12.7g/100g. (Without His in g/100g): infant 43.4, pre-school 32.0, school child 22.2 and adult 11.1g/100g. The three termite species have the required amount, 31.75g/100g – 35.89g/100g with His and 29.66 - 33.97g/100g without His, to supply the different age groups except for the infants who require a higher amount.

TAAA was found to be more abundant in the three termite samples (13.39g/100g - 23.37g/100g) as compared to TBAA 10.4 – 16.55g/100g. The TSAA was also lower in the three termite samples 1.48 - 10.78g/100g as compared to TEAlAA 22.74 – 28.1g/100g. The daily TSAA requirement is 1.26- 2.1g/100g in young men (Zehra & Khan, 2016.). The three termites contain the required quantities of TSAA and therefore can be recommended to supply the nutrients to the populace. The same applies to TECAA which are amino acids that are not synthesized in the body under some pathophysiological conditions like prematurity in infants and individuals with severe catabolic distress

(FAO/WHO, 1985). They ranged between 18.42 – 24.09g/100g. The percentage Cys/TSAA was between 36.4 – 43.2g/100g. This ratio is usually low in most animal proteins (Adeyeye and Kenni, 2008). The ratios of Leu/Ile were between 1.51 – 1.83g/100g and this was lower than the required amount of 2.36. Any imbalance between Leu/Ile can lead to pellagra, especially in countries like Kenya in which maize is a stable food (Ekpa, 2020). This study also showed that Lys/Trp ranged between 0.65 - 4.59g/100g while Met/Trp range was 0.36 - 1.46g/100g.

CONCLUSION

The three termite species were found to have the required essential and nonessential amino acids enough to supply all age groups except infants who have a higher protein requirement. The three termite species are therefore recommended to alleviate malnutrition, especially in low-income households which use termites as food.

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