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Research Article

Effects of Different Processing Methods on the Essential Amino Acid Profile of Some Non-Conventional Plant Protein Feedstuffs

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Abstract

Background and Objective: The nutrient quality of feed ingredient is one of the major prerequisites apart from availability for the production of good quality feeds. The basic nutrient that cannot be compromised in the choice of ingredients for feed formulation and preparation is protein. The objective of the study was to analyze the amino acid profile of *Canavalia ensiformis*, *Detarium microcarpum*, *Jatropha curcas* in comparison with *Glycine max* meals. **Materials and Methods:** Four plant protein meals were processed and analyzed for their amino acid profile in comparison with the conventional soybean. Data collected were subjected to ANOVA and LSD was used for the significant difference among the treatments. **Results:** Fermented *Jatropha* meal had the highest arginine value of 6.32 g/16 Ng. Raw *Jatropha* had the greatest values of histidine, isoleucine, leucine, lysine, methionine, tryptophan, valine, total essential amino acid and chemical score ratio to protein score of (4.04, 5.13, 8.86, 5.85, 2.65, 5.63, 7.82, 54.8 g/16 Ng and 3128%). Toasted *Canavalia* meal had the highest phenylalanine value of 9.34 g/16 Ng. Toasted *Detarium* meal had the highest threonine value of 3.52 g/16 Ng. Toasted *Glycine* meal had the highest value of 46.03%. Raw *Detarium* meal had the highest total essential amino acid ratio to crude protein value of 257.6%. **Conclusion:** Based on the results from this study, any of the understudied plant proteins tend to supplement *Glycine max* in fish feed since they all have competitive nutrient values.

Key words: Non-conventional plant proteins, essential amino acids profile, feedstuffs, processed

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Essential or indispensable Amino Acids (EAAs) cannot be synthesized by fish and often remain inadequate but are needed for growth and tissue development[1,2]. Fishmeal is known to contain a complete EAA profile that is needed to meet the protein requirement of most fish species. Since fishmeal is expensive as a feed ingredient, the use of non-conventional feedstuffs has been reported with good growth and better cost-benefit values. Several plants and animal protein sources have been tested for total or partial replacement of fishmeal in aquafeed. Soybean meal is one of the most commonly used plant proteins in fish feed due to global availability, low price, good nutritional value and relatively balanced amino acid profile[3,4].

Non-Conventional Feed Resources (NCFRs) are feeds that are not usually common in the markets and are not the traditional ingredients used for commercial fish feed production[1]. NCFRs is credited for being noncompetitive in terms of human consumption, very cheap to purchase, by-products or waste products from agriculture, farm-made feeds and processing industries and serve as a form of waste management in enhancing good sanitation.

The nutrient quality of feed ingredient is one of the major prerequisites apart from availability (which sometimes is a function of cost and season) for the production of good quality feeds. The basic nutrient that cannot be compromised in the choice of ingredients for feed formulation and preparation is protein[5]. Hence it becomes imperative to research into the nutrient composition of some of the plant protein sources.

The experiment aimed to analyze the amino acid profile of *Canavalia ensiformis*, *Detarium microcarpum*, *Jatropha-curcas* in comparison with *Glycine max* meals to provide information that will help in incorporating any of these non-conventional plants into fish feed ingredients during the feed formulation by fish nutritionist and fish farmers who may want to use them as on-feed ingredients.

MATERIALS AND METHODS

Study area: The legume seeds were collected in the Girei environs of Yola, Adamawa state and the analysis was conducted in the Animal Nutrition Laboratory of Adamawa State University, Mubi in March, 2017.

Preparation of the legume seeds and processing: The fruits were cracked open mechanically to remove the seeds. The seeds were dehulled, clean of debris by handpicking and winnowed. The seeds size was reduced with pestle and mortar and subjected to various processing methods according to Doss *et al.*[5] methods:

- Raw seeds were milled and tag Raw Seed Meal (RSM)
- Raw seeds were soaked in water to the ratio of 1:3 for 72 hrs, oven-dried at 50°C to constant weight then milled and tag Soaked Seed Meal (SSM)
- Raw seeds were boiled for 30 min, oven-dried at 50°C to constant weight then milled and tag Boiled Seed Meal (BSM)
- Raw seeds were toasted at 70°C using an electric hot plate until seeds turn brown then milled and tag Toasted Seed Meal (TSM)
- Raw seeds were moistened with water, kept in a container with a cover to fermented for 72 hrs under laboratory condition, oven-dried at 50°C then milled and tag Fermented Seed Meal (FSM)

The amino acids profiles of the processed meals were carried out at the Animal Nutrition Laboratory Adamawa State University Mubi according to Sogbesan and Ugwumba[1]. The mean of 3 samples for each was recorded.

Essential amino acid indices: The essential amino acids indices were calculated using whole hen egg crude protein and essential amino acids composition documented in Sogbesan and Ugwumba[1]. The essential amino acids indices were determined as:

$$\text{Chemical score (\%)} = \frac{\text{Essential amino acid of the sample}}{\text{Essential amino acid of the whole hen egg}} \times 100$$

$$\text{Chemical score to protein score ratio (C.S.P.S) (\%)} = \frac{\text{Total essential amino acid of the sample}}{\text{Total essential amino acid of whole hen egg}} \times 100$$

$$\text{Total essential amino acid to crude protein content ratio (EAA:CP) (\%)} = \frac{\text{Total essential amino acid}}{\text{Crude protein of the animal meal/100 g of diet}} \times 100$$

Statistical analysis: Data collected were subjected to Analysis of Variance (ANOVA). Comparisons among treatment means were carried out by one-way analysis of variance followed by Turkey's multiple tests and Dunnett's test. Standard deviation and standard error were calculated to identify the range of means and error, respectively. The Least Significant Differences (LSD) was used to determine the level of significance among treatments.

RESULTS

Table 1 shows the essential amino acids compositions of all the meals. They all have 10 essential amino acids. Fermented *Jatropha* meal had the highest arginine value of 6.32 g/16 Ng while toasted *Glycine* meal had the lowest 4.67 g/16 Ng. Isoleucine values of 5.13 g/16 Ng were the highest from raw *Jatropha* meal while the lowest value of 3.11g/16 Ng in boiled *Canavalia* meal. The highest tryptophan value of 5.63 g/16 Ng was from raw *Jatropha* meal while the lowest of 4.32 g/16 Ng was from toasted *Detarium* meal. The highest histidine value of 4.04 g/16 Ng was in raw *Jatropha* meal while the lowest value of 2.16 g/16 Ng was recorded from the toasted *Glycine* meal. The highest leucine and valine of 9.01 g/16 Ng and 7.82 g/16 Ng, respectively were recorded from the raw *Jatropha* meal while the lower value of leucine 8.12 g/16 Ng was from toasted *Glycine* meal and valine 5.04 g/16 Ng from both toasted *Detarium* and *Glycine* meals. The highest methionine and lysine were recorded from raw *Jatropha* meal 2.65 g/16 Ng and 5.85 g/16 Ng while the lowest methionine 1.24 g/16 Ng was from toasted *Glycine*, followed by toasted *Detarium* meal with 3.25 g/16 Ng. The highest threonine 3.52 g/16 Ng was recorded from the toasted *Detarium* meal while the lowest value of 2.65 g/16 Ng was from the toasted *Glycine* meal. The highest phenylalanine value of 10.23 g/16 Ng was recorded from fermented *Canavalia* meal while the lowest value of 4.64 g/16 Ng was from toasted *Glycine* meal. The highest total essential amino acids value of 54.8 g/16 Ng was from raw *Jatropha* meal while the lowest 41.07 g/16 Ng was from toasted *Glycine* meal. The highest chemical score for the 10 essential amino acids value of 2016% was from raw *Jatropha* meal while the lowest 1092% was from raw *Canavalia* meal. The highest percentage ratio of the chemical scores to protein scores value of 3128% was recorded from raw *Jatropha* meal while the lowest 1694% was from raw *Canavalia* meal. The highest essential amino acids ratio to crude protein (EAA: CP) 257.6% was recorded from raw *Detarium* meal while the lowest 88% was from soaked *Canavalia* meal. There was a significant difference (p<0.001) between the highest and lowest EAA: CP. The result showed that processing methods had significant (p<0.001, p<0.01) effects on all the parameters determined. The overall highest chemical score value of essential amino acids 605% tryptophan was from raw *Jatropha* meal while the lowest 311% was from raw *Canavalia* meal as presented in Fig. 1. The overall highest lysine 134% was from raw *Jatropha* meal while the lowest 55% was from soaked *Detarium* meal. The highest methionine value of 126% was recorded from raw *Jatropha* meal while the lowest value of 28% was from toasted *Glycine* meal and are significantly different (p<0.001).

Table 1: Essential amino acid (g/16Ng) compositions of the tested plant protein sources

	Raw			Boiled			Toasted				Soaked			Fermented			
	C	D	J	C	D	J	C	D	J	G	C	D	J	C	D	J	SEM
Arginine	3.81^{f}	4.23^{def}	6.24^{a}	4.0^{ef}	4.24^{def}	4.62^{cdef}	5.32^{abcd}	5.12^{abcde}	6.00^{ab}	4.67^{cdef}	3.83^{f}	4.05^{ef}	4.21^{def}	5.72^{abc}	4.96^{bcdef}	6.32^{a}	0.43***
Histidine	1.76^{d}	1.85^{d}	4.04^{a}	1.92^{d}	1.87^{d}	2.54^{abcd}	2.23^{cd}	2.67^{abcd}	3.64^{abc}	2.16^{cd}	1.85 ^{d}	1.79 ^{d}	2.05^{d}	3.09^{abcd}	2.45^{bcd}	3.86^{ab}	0.53**
Isoleucine	2.94^{bc}	2.65^{c}	5.13^{a}	3.11^{bc}	2.85^{bc}	3.01^{bc}	2.76^{c}	3.30^{bc}	4.53^{ab}	3.56^{abc}	2.87^{bc}	2.56^{c}	2.86^{bc}	3.05^{bc}	2.97^{bc}	4.04^{abc}	0.58***
Leucine	6.53^{d}	6.84^{cd}	8.86^{a}	7.62^{abcd}	7.34^{bcd}	7.96^{abcd}	8.36^{ab}	8.23^{abc}	7.91^{abcd}	8.12^{abc}	6.78^{cd}	6.94^{bcd}	6.89^{bcd}	9.01^{a}	8.13^{abcd}	7.93^{abcd}	0.52**
Lysine	3.23^{cde}	2.46^{e}	5.85^{a}	3.41^{cde}	2.62^{de}	4.54^{abc}	3.64^{bcde}	3.25^{cde}	5.68^{a}	4.36^{abc}	3.26^{cde}	2.38^{e}	4.24^{abcd}	4.73^{abc}	3.16cde	5.17^{ab}	0.58***
Methionine	0.81^{d}	0.87^{d}	2.65^{a}	0.91^{d}	1.13^{cd}	1.35^{bcd}	0.88^{d}	1.66^{abcd}	2.34^{ab}	1.24^{bcd}	0.82^{d}	0.93^{d}	1.71^{abcd}	2.12^{abc}	1.53^{bcd}	2.12^{abc}	0.38**
Phenylalanine	3.76^{bc}	3.72^{bc}	5.00^{b}	4.02^{bc}	3.85^{bc}	3.75^{bc}	9.34^{a}	4.76^{bc}	4.63^{bc}	4.64^{bc}	3.84^{bc}	3.68^{c}	3.52^{c}	10.2^{a}	4.34^{bc}	3.87^{bc}	0.45***
Threonine	1.91^{de}	2.13^{cd}	3.13^{ab}	1.21^{e}	2.25^{bcd}	2.46^{bcd}	2.11^{cde}	3.52^{a}	2.95^{abc}	2.65^{abcd}	1.96^{de}	2.07^{cde}	2.32^{bcd}	3.43^{a}	3.41^{a}	2.66^{abcd}	0.31***
Tryptophan	2.89^{h}	3.53^{fgh}	5.63^{a}	3.01^{gh}	3.78^{efg}	4.72^{bcd}	4.02^{def}	4.32^{cdef}	5.33^{ab}	4.63^{bcde}	2.97^{gh}	3.65^{fgh}	4.16^{cdef}	5.01^{abc}	4.11^{def}	5.26^{ab}	0.30***
Valine	3.98^{e}	4.04^{e}	7.82^{a}	4.11^{de}	4.01^{e}	4.87^{de}	5.32^{bcd}	5.04^{cde}	6.29^{b}	5.04^{cde}	3.98^{e}	3.93^{e}	4.42^{e}	6.08^{bc}	4.87^{cde}	7.66^{a}	0.43***
TEAA	31.7^{h}	32.3^{gh}	54.8^{a}	33.3^{gh}	33.9^{fg}	39.1^{e}	44.4^{c}	41.9^{d}	49.5^{b}	41.07^{de}	32.2^{gh}	32^{g}	35.8^{f}	49.5^{b}	39.7^{e}	48.9^{b}	0.73***
Crude protein (%)	30.32^{e}	12.54^{h}	37.68^{b}	32.42^{d}	14.3^{h}	28.33^{f}	35.23^{c}	16.43^{g}	30.46^{de}	46.03^{a}	36.60^{bc}	13.25^{h}	29.65^{ef}	34.73^{c}	16.83^{g}	31.46^{de}	0.68***
TEAA:CP (%)	104.6^{l}	257.6^{a}	145.4^{g}	102.7^{l}	236.1^{d}	138^{i}	126^{j}	255^{b}	162.5^{e}	89.2^{m}	88^{m}	241.5^{c}	120.7^{k}	142.5^{h}	235.9^{d}	155.4^{f}	0.73***
CS:PS (%)	1694^{p}	1809^{l}	3128^{a}	1767^{o}	1911^{k}	2279^{g}	2353^{f}	2363^{e}	2854^{c}	1786^{n}	1807^{m}	2034^{j}	2913^{b}	2240^{i}	2828^{d}	2259^{h}	0.54***

Mean on the same row with different superscripts are significantly different (p<0.01), (p<0.001), SEM: Standard error of the mean, (p<0.01), (p<0.001), C: *Canavalia ensiformis*, D: *Detarium microcarpum*, J: *Jatropha curcas*, G: *Glycine max* seeds, TEAA: Total essential amino acids, TEAA:CP: Total essential amino acids ratio to Crude protein, CS:PS: Chemical scores ratio to Protein score

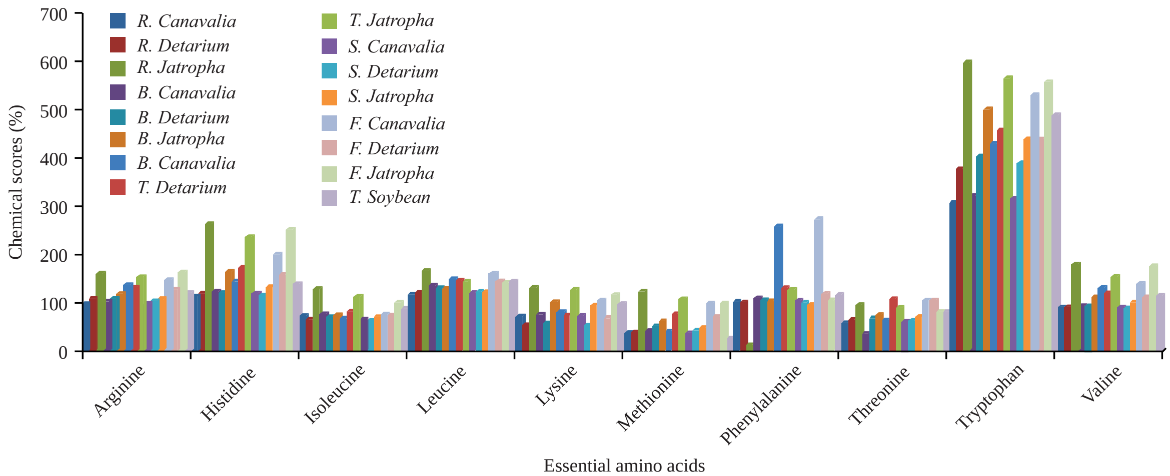

Fig. 1: Chemical scores value of the essential amino acid of the plant protein meals

DISCUSSION

The results obtained in this study in respect of *Canavalia ensiformis* revealed that processing increased most of the essential amino acids content of the processed seed (Table 1), which is in total disagreement with the work of Okomoda *et al.*[6] and Tiamiyu *et al.*[7], who reported that all the essential amino acids reduced significantly with increasing time of hydrothermal processing. Generally, the reduction in the essential amino acids recorded is likely due to denaturation of the amino acids as boiling time increased. Cereal grain-based diets for fish have been reported to be deficient in lysine, leading to growth reduction[8]. The relatively high concentration of lysine in *C. ensiformis* seed makes it a potential supplement in cereal-based fish diets. One of the most important factors that limit large inclusions of conventional and unconventional feedstuffs in the diet of fish is the leucine/isoleucine ratio[7]. Feed ingredients that are higher in leucine but lower in isoleucine have been reported to result in an antagonistic response caused by acute deficiency of isoleucine reported by Cheng *et al.*[8]. The leucine and isoleucine for all hydrothermally processed *C. ensiformis* observed in this study were higher in value but similar in ratio to reported values in fishmeal[9] and *Agama* meal[10]. Values of sulphur-containing amino acids such as methionine and cystine observed for *C. ensiformis* in this study are relatively lower when compared to the work of Okomoda *et al.*[6] and Tiamiyu *et al.*[9].

The essential amino acid content of *D. microcarpum* seed is generally higher than the values reported by Anhwange *et al.*[11]. Among the essential amino acid, leucine was the highest and processing increased the content (Table 1). Leucine is responsible for regulating the blood sugar concentrations, growth and repairs of muscles/tissues, hormone production, wound healing and energy production. Its deficiency causes dizziness, headaches, fatigue, depression, confusion, irritability and hypoglycemia in infants. Anhwange *et al.*[11] observed that Phenylalanine is another essential amino acid that is used by the brain to produce nor epinephrine (a chemical that transmits signals between the nerve cells and the brain). It keeps the body alert and reduces hunger pains. It is an antidepressant and helps in improving memory and its deficiency could result in slow growth, liver damage and skin lesions. Isoleucine amino acid helps in the development and repair of muscles, development of haemoglobin and acts as an energy regulator. Its deficiency results in ailments similar to leucine deficiency. Anhwange *et al.*[11] stated that lysine insures the adequate absorption of calcium, help the formation of collagen, in addition, it aids the production of antibodies, hormones and enzymes. Lysine deficiency may result in tiredness, inability to concentrate, irritability, bloodshot eyes, retarded growth, hair loss, anaemia and reproductive problems. These values are considered to be high when compared to the World Health Organization protein standard[11]. Threonine is necessary for the body because, it produces antibodies, prevent fat buildup in the liver and assist metabolism and assimilation. It is an important constituent of collagen, elastin and enamel protein. Its deficiency has been associated with skin disorders and weakness[11]. Valine promotes mental vigour, muscle coordination and calm emotions as reported by Antyev *et al.*[12]. Methionine is a sulphur-containing amino acid, it functions as a supplier of sulphur, which prevents disorders of hair, skin and nails. It prevents arterial fat buildup, regulates ammonia formation and creates ammonia-free urine which reduces bladder irritations, its deficiency results in a similar symptom like phenylalanine. Histidine is essential especially in children, it is used for growth, tissue repairs and histamine development[12]. Cystine is a sulphur-containing amino acid that acts as an antioxidant and protects the body from radiation and pollution. It also aids protein synthesis and prevents cellular changes. In addition, it deactivates free radicals and neutralizes.

The result shows that the values for lysine and methionine were good enough to enhance the performance of fish. The values obtained for indispensable and dispensable amino acids for *Jatropha-curcas* in the present study were lower than those reported by Antyev *et al.*[12], Pirgozliev *et al.*[13], Kumar *et al.*[14], Antyev *et al.*[12] and Makkar *et al.*[15] for both toxic and non-toxic genotypes of *Jatropha-curcas* seed meal. The values agreed with the assertion of Antyev[12] that with high content of amino acid, jatropha seed meal can be used as a good quality protein source in animal nutrition. However, some of the values were reduced in the processed groups which are evidence that heat damage these sensitive amino acids after heat treatment. It was observed that heating affected the amino acid profile through leaching and destruction (losses) while soaking enhanced a slight increase as reported by Anhwange *et al.*[11] for raw legumes seeds.

CONCLUSION

From the experiment, any of the under-studied plant proteins tend to supplement *Glycine max* in fish feed since they all have competitive nutrient values. The quality of dietary protein is assessed from essential to nonessential amino acid ratio.

SIGNIFICANCE STATEMENT

This study discovered the protein qualities that can be beneficial for fish diets that many researchers were not able to explore.

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