

## EFFECTS OF FERMENTATION DURATION ON THE NUTRITIONAL VALUES OF SESBANIA CANNABINA SEEDS

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### ABSTRACT

Fermentation is a well-established bioprocess known to enhance the nutritional quality of plant-based feedstuffs by improving protein content, amino acid profile, and reducing anti-nutritional factors. This study investigated the effect of fermentation duration (0, 24, 48, 72, and 96 hours) on the proximate composition and amino acid profile of *Sesbania cannabina* seeds, with the aim of evaluating their potential as an alternative protein source in fish feed formulation. Seeds were collected from the University of Maiduguri campus, processed, and subjected to fermentation at room temperature. Proximate analysis and amino acid composition were determined using standard procedures and a TSM Amino Acid Analyzer. Results revealed a progressive increase in crude protein (25.72% to 42.59%), ether extract, crude fiber, ash, and nitrogen-free extract with increasing fermentation duration. Total essential amino acids increased from 18.63 g/16g N (0 hours) to 50.97 g/16g N (96 hours), while total non-essential amino acids rose from 26.57 to 70.52 g/16g N across treatment groups. Sulfur-containing amino acids also increased correspondingly with fermentation hours. Statistical analysis using one-way ANOVA and LSD mean separation showed significant differences ( $p < 0.05$ ) across treatments for most parameters. These findings demonstrate that fermentation, particularly for 96 hours, substantially improves the nutritional value of *Sesbania cannabina* seeds, making them a viable and economical plant-based protein substitute in aquaculture feed.

**Key Words:** Amino acid, Aquaculture, Fermentation, Fish feed, Proximate composition, *Sesbania cannabina*

### 1.0 INTRODUCTION

Fermentation is an extremely old method that subjects food to the activity of microorganisms and enzymes. This prolongs shelf life, and it also improves the palatability, digestibility, flavor, and nutritional value of the food. Apart from nutritional improvement, fermentation can increase the functional properties of fermented food by breaking down complex substrates such as phenolic compounds into simpler components (Hui, 2004). Fermented foods play an important role in providing food security, enhancing livelihoods, and improving nutrition and social well-being (Adesulu and Awojobi, 2014). Fermentation leads to improved food preservation (Ross *et al.*, 2002), food quality, and an increase in the range of edible food products. Enhancement of nutritive value through an increase in essential nutrients or reduction of toxicants in food remains a key benefit of the fermentation process (Evans *et al.*, 2013). The process of fermentation destroys many harmful microorganisms and chemicals in foods and introduces beneficial bacteria that produce new enzymes to assist in digestion.

In fish production systems, good nutrition is essential to economical production of a healthy, high-quality product. Feed typically represents approximately 70% of variable production costs (Adeyeye and Fagbohun, 2005). The digestibility of nutrients in feed directly affects aquaculture production efficiency and environmental impact. A wide variety of chemical compounds found in plants have been shown to have beneficial effects on appetite, growth, and the immune status of fish (Ramachandran *et al.*, 2007). One of the primary concerns of fish culture is to provide a diet containing all necessary nutrients in suitable proportions to optimize growth performance while reducing production costs. Increasing demand, unstable supply, and the high price of fishmeal have necessitated the evaluation of alternative protein sources of plant origin in fish diets as partial or total replacements for fishmeal (Gatlin *et al.*, 2007). Primary protein sources used in catfish feeds are oil seed meals, such as soybean meal and cottonseed meal. Compared to animal proteins, most plant proteins, except soybean meal, are deficient in lysine, the most limiting essential amino acid in

catfish feeds (Eze *et al.*, 2005). It is therefore crucial to find cheaper alternative protein sources for fish feed formulation. *Sesbania cannabina* seeds are widely distributed, locally available, and inexpensive. This study aimed to determine the effects of fermentation on the nutritional value of *Sesbania cannabina* seeds, establishing their essential amino acid composition and suitability as a protein source in fish feed production (Amoo, 2004).

## 2.0 MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted in the Fish Nutrition Unit of the Fish Farm, Department of Fisheries, University of Maiduguri, Maiduguri, Borno State, located at Latitude 11°51'N and Longitude 13°05'E (Ministry of Land and Survey Maiduguri, 2008). The area experiences two characteristic seasons: a longer dry season and a rainy season of approximately 3-4 months. Temperatures are low during December and January, and variably high from March to June (Nigeria Meteorological Agency, 2006).

### 2.2 Collection and Processing of *Sesbania cannabina* Seeds

Ripe *Sesbania cannabina* seeds were collected locally from the University of Maiduguri campus. The seeds were threshed and winnowed to obtain clean seeds, washed with distilled water to remove dirt and debris, and then allowed to dry. The dried seeds were reduced in size using a local pestle and mortar and subjected to various processing methods. The raw seed was milled, stored in an airtight polythene bag, and labelled as raw seed meal (RSM; T<sub>1</sub>, 0 hours). Raw seeds were moistened with water and kept in covered containers to ferment for 24, 48, 72, and 96 hours under laboratory conditions, then oven-dried at 50°C. Fermented seeds were milled and stored in airtight polythene bags labelled as T<sub>2</sub>FSM (24h), T<sub>3</sub>FSM (48h), T<sub>4</sub>FSM (72h), and T<sub>5</sub>FSM (96h).

### 2.3 Proximate Composition

Proximate composition of raw and processed seeds was determined using the methods described by AOAC (2010). Parameters assessed included moisture content, crude protein, ether extract, crude fibre, ash, and nitrogen-free extract.

#### 2.3.1 Moisture content

Five grams of milled sample was weighed in triplicates, placed in an oven, and dried at 105°C for 12 hours. Samples were cooled in a desiccator and reweighed. Percentage moisture was estimated as: % Moisture = (weight of sun-dried sample - weight of oven-dried sample) / weight of sun-dried sample × 100.

#### 2.3.2 Crude protein

Two grams of ground sample were weighed in triplicates into digestion tubes, digested with concentrated H<sub>2</sub>SO<sub>4</sub> until a clear solution was obtained, then distilled and titrated using the

Kjeldahl method. Percentage nitrogen was calculated and multiplied by a factor of 6.25 to obtain crude protein (%).

#### 2.3.3 Ether extract

Two grams of ground sample were placed in thimbles and extracted with petroleum ether in a Soxhlet apparatus for 6 hours. The receiving flask was dried at 60°C, cooled, and weighed. Ether extract (%) = (weight of fat extracted / weight of sample) × 100.

#### 2.3.4 Crude fibre

One gram of ground sample was digested with a mixture of glacial acetic acid, distilled water, concentrated nitric acid, and trichloroacetic acid under reflux for 40 minutes. The residue was filtered, dried overnight at 105°C, then ashed at 600°C. Crude fibre (%) = (weight of oven-dried residue - weight of ash) / weight of sample × 100.

#### 2.3.5 Ash

One gram of milled sample was charred in a muffle furnace at 600°C for 6 hours. The residue was cooled in a desiccator and weighed. Ash (%) = weight of ash / weight of sample × 100.

### 2.4 Amino Acid Analysis

The essential amino acid composition was analysed using the method of AOAC (2012). Samples were defatted by soxhlet extraction with chloroform-methanol, hydrolysed with 6N HCl at 105°C for 22 hours in sealed ampoules under nitrogen, and the filtrates were evaporated under vacuum. Residues were dissolved in acetate buffer (pH 2.0) and loaded into a TSM Amino Acid Analyzer for a 75-minute automated separation and quantification. Amino acid values were calculated from chromatogram peak areas using Norleucine as an internal standard and expressed in g/16g N. Essential amino acid indices including chemical score, chemical score to protein ratio, and total essential amino acid to crude protein ratio were calculated using whole hen egg amino acid composition as reference (Sogbesan, 2004).

### 2.5 Data Analysis

Data obtained from proximate and amino acid analyses were subjected to one-way Analysis of Variance (ANOVA). Means were separated using the Least Significant Difference (LSD) test at p<0.05.

## 3.0 RESULTS

### 3.1 Proximate Composition of Fermented *Sesbania cannabina* Seeds

The proximate composition of fermented *Sesbania cannabina* seeds across the five treatment groups is presented in Table 1. Moisture content ranged from 6.23% (T<sub>1</sub>, 0 hours) to 6.88% (T<sub>5</sub>, 96 hours). Treatments T<sub>1</sub> and T<sub>2</sub> showed no significant difference (p<0.05) but differed significantly from T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub>, which were not significantly different from each other. Crude protein values increased substantially from 25.72% (T<sub>1</sub>) to 42.59% (T<sub>5</sub>). T<sub>2</sub> and T<sub>3</sub> showed no significant difference from each other, while T<sub>1</sub>, T<sub>4</sub>, and T<sub>5</sub> each differed

significantly ( $p < 0.05$ ). Ether extract was lowest at T<sub>1</sub> (2.32%) and highest at T<sub>5</sub> (7.83%), with significant differences between T<sub>1</sub> and all other treatments, while T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> were not significantly different from each other. Crude fiber increased from 1.86% (T<sub>1</sub>) to 6.86% (T<sub>5</sub>), while ash ranged from 3.92% (T<sub>1</sub>) to 5.96% (T<sub>5</sub>). Nitrogen-free extract increased from 28.00% (T<sub>1</sub>) to 34.00% (T<sub>5</sub>), with significant differences between T<sub>4</sub>, T<sub>5</sub> and the lower treatment groups.

**Table 1: Proximate analysis of *Sesbania cannabina* seeds**

Parameters	T <sub>1</sub> (0hrs)	T <sub>2</sub> (24hrs)	T <sub>3</sub> (48hrs)	T <sub>4</sub> (72hrs)	T <sub>5</sub> (96hrs)	SEM
Moisture	6.23 <sup>b</sup>	6.42 <sup>b</sup>	6.81 <sup>a</sup>	6.84 <sup>a</sup>	6.88 <sup>a</sup>	0.07
Crude Protein	25.72 <sup>c</sup>	35.78 <sup>b</sup>	37.40 <sup>b</sup>	39.62 <sup>ab</sup>	42.59 <sup>a</sup>	1.41
Ether Extract	2.32 <sup>b</sup>	7.52 <sup>a</sup>	7.56 <sup>a</sup>	7.58 <sup>a</sup>	7.83 <sup>a</sup>	0.45
Crude Fiber	1.86 <sup>b</sup>	2.43 <sup>b</sup>	4.30 <sup>ab</sup>	6.00 <sup>a</sup>	6.86 <sup>a</sup>	0.77
Ash	3.92 <sup>b</sup>	5.30 <sup>ab</sup>	5.61 <sup>a</sup>	5.81 <sup>a</sup>	5.96 <sup>a</sup>	0.46
Nitrogen Free Extract	28.00 <sup>b</sup>	28.00 <sup>b</sup>	28.03 <sup>b</sup>	30.16 <sup>ab</sup>	34.00 <sup>a</sup>	1.41
Total	68.05	84.45	89.71	96.01	104.12	4.57

Means with same superscript on the same row are not significantly different ( $p < 0.05$ )

### 3.2 Essential Amino Acid Composition

Table 2 presents the essential amino acid composition of fermented *Sesbania cannabina* seeds. Total essential amino acids (TEAA) increased progressively from 18.63 g/16g N (T<sub>1</sub>) to 50.97 g/16g N (T<sub>5</sub>), indicating a positive correlation between fermentation duration and amino acid content. Individual essential amino acids including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, valine, and threonine all showed significant increases ( $p < 0.05$ ) with increasing fermentation hours. Sulfur-containing amino acid totals (SCAA) increased from 5.65 (T<sub>1</sub>) to 13.74 (T<sub>5</sub>), likewise correlated with fermentation duration.

**Table 2: Essential amino acid composition of fermented *Sesbania cannabina* seeds (g/16g N)**

Parameters	T <sub>1</sub> (0hrs)	T <sub>2</sub> (24hrs)	T <sub>3</sub> (48hrs)	T <sub>4</sub> (72hrs)	T <sub>5</sub> (96hrs)	SEM
Histidine	0.79 <sup>c</sup>	1.65 <sup>d</sup>	3.23 <sup>c</sup>	3.32 <sup>b</sup>	3.54 <sup>a</sup>	0.01
Isoleucine	1.65 <sup>c</sup>	3.13 <sup>d</sup>	3.97 <sup>c</sup>	4.04 <sup>b</sup>	5.56 <sup>a</sup>	0.01
Leucine	1.55 <sup>c</sup>	2.43 <sup>d</sup>	3.41 <sup>c</sup>	3.65 <sup>b</sup>	4.75 <sup>a</sup>	9.48
Lysine	1.85 <sup>c</sup>	3.51 <sup>d</sup>	4.17 <sup>c</sup>	4.26 <sup>b</sup>	6.62 <sup>a</sup>	8.36
Methionine	2.65 <sup>c</sup>	3.63 <sup>d</sup>	4.11 <sup>c</sup>	5.26 <sup>b</sup>	6.62 <sup>a</sup>	8.36
Phenylalanine	4.88 <sup>d</sup>	6.41 <sup>c</sup>	8.13 <sup>b</sup>	8.93 <sup>ab</sup>	9.42 <sup>a</sup>	0.22
Tryptophan	1.05 <sup>c</sup>	1.83 <sup>d</sup>	3.16 <sup>c</sup>	4.17 <sup>b</sup>	4.36 <sup>a</sup>	9.22
Valine	2.71 <sup>c</sup>	3.00 <sup>d</sup>	4.34 <sup>c</sup>	4.34 <sup>c</sup>	4.87 <sup>b</sup>	8.06
Threonine	1.50 <sup>c</sup>	4.04 <sup>d</sup>	4.45 <sup>c</sup>	4.85 <sup>b</sup>	5.16 <sup>a</sup>	7.07
TEAA	18.63	29.63	38.97	43.35	50.97	
SCAA	5.65	7.62	9.52	11.29	13.74	

Means with same superscript on the same row are not significantly different ( $p < 0.05$ ); TEAA = Total Essential Amino Acids; SCAA = Sulphur-Containing Amino Acids

### 3.3 Non-Essential Amino Acid Composition

Table 3 shows the non-essential amino acid composition of *Sesbania cannabina* seeds across treatment groups. Total non-essential amino acids (TNEAA) increased progressively from 26.57 g/16g N (T<sub>1</sub>) to 70.52 g/16g N (T<sub>5</sub>), demonstrating a significant increase in all measured non-essential amino acids with increasing fermentation duration.

**Table 3: Non-essential amino acid composition of *Sesbania cannabina* seeds (g/16g N)**

Parameters	T <sub>1</sub> (0hrs)	T <sub>2</sub> (24hrs)	T <sub>3</sub> (48hrs)	T <sub>4</sub> (72hrs)	T <sub>5</sub> (96hrs)	SEM
Alanine	0.55 <sup>d</sup>	1.77 <sup>c</sup>	3.97 <sup>b</sup>	3.97 <sup>b</sup>	4.65 <sup>a</sup>	9.22
Arginine	2.71 <sup>c</sup>	3.28 <sup>d</sup>	5.78 <sup>c</sup>	6.45 <sup>b</sup>	6.98 <sup>a</sup>	8.06
Asparagine	3.60 <sup>c</sup>	4.54 <sup>d</sup>	5.96 <sup>c</sup>	6.32 <sup>b</sup>	6.67 <sup>a</sup>	8.94
Aspartate	3.21 <sup>c</sup>	5.37 <sup>d</sup>	6.64 <sup>c</sup>	6.81 <sup>b</sup>	6.92 <sup>a</sup>	7.41
Glutamate	1.45 <sup>c</sup>	3.54 <sup>d</sup>	4.71 <sup>c</sup>	5.15 <sup>b</sup>	6.36 <sup>a</sup>	7.74
Glycine	3.65 <sup>c</sup>	7.14 <sup>d</sup>	8.21 <sup>c</sup>	9.34 <sup>b</sup>	9.62 <sup>a</sup>	5.91
Tyrosine	4.10 <sup>c</sup>	4.79 <sup>d</sup>	7.46 <sup>c</sup>	8.11 <sup>b</sup>	9.18 <sup>a</sup>	8.06
Cysteine	3.00 <sup>c</sup>	3.99 <sup>d</sup>	5.41 <sup>c</sup>	6.05 <sup>b</sup>	7.12 <sup>a</sup>	6.70
Proline	1.70 <sup>d</sup>	4.35 <sup>c</sup>	6.27 <sup>b</sup>	6.29 <sup>b</sup>	6.66 <sup>a</sup>	6.32
Serine	2.60 <sup>c</sup>	3.22 <sup>d</sup>	3.83 <sup>c</sup>	4.84 <sup>b</sup>	6.36 <sup>a</sup>	9.48
TNEAA	26.57	41.99	58.24	63.33	70.52	

Means with same superscript on the same row are not significantly different ( $p < 0.05$ ); TNEAA = Total Non-Essential Amino Acids

## 4.0 DISCUSSION

The crude protein value of fermented *Sesbania cannabina* seeds (42.59% at 96 hours) is higher than the 17.63% reported by Nwachukwu et al. (2018) after fermenting *Pentaclethra macrophylla* seeds for 72 hours, attributable to the longer fermentation period applied in the present study. However, Agblemanyo and Abrokwah (2019) recorded a higher crude protein (52.33%) after fermenting *Parkia biglobosa* for 72 hours, likely due to interspecific differences in seed composition. Crude fiber values of fermented *Sesbania cannabina* seeds exceeded the best result (6.77%) obtained from fermented *Parkia biglobosa* by Agblemanyo and Abrokwah (2019), but were lower than those reported by Suleiman (2019) for *Crescentia cujete* seeds fermented for 48 hours, reflecting differences in species and fermentation duration. Ash content in this study was higher than the 2.64% reported for fermented *Pentaclethra macrophylla* seeds (Nwachukwu et al., 2018) but lower than the 8.86% recorded for fermented *Crescentia cujete* seeds (Suleiman, 2019). Ether extract was higher than the 1.53% reported for fermented *Senna occidentalis* seeds (Augustine et al., 2016) but lower than the 47.13% recorded for *Jatropha carthatica* (Oladele & Oshodi, 2008), reflecting species-specific lipid content.

Nitrogen-free extract of fermented *Sesbania cannabina* seeds was higher than the values (6.45%, 6.33%) reported for fermented *Pentaclethra macrophylla* and *Jatropha carthatica* (Oladele and Oshodi, 2008; Nwachukwu et al., 2018), but lower than those for fermented *Senna occidentalis* and *Crescentia cujete* seeds (Augustine et al., 2016; Suleiman, 2019). The total essential amino acid of raw *Sesbania cannabina* seeds (18.63 g/16g N) was lower than values reported for other fermented seeds (Chidi et al., 2012; Muhammad et al., 2013; Makinde and Akinoso, 2014; Ogbuagu et al., 2018). However, the fermented *Sesbania cannabina* seeds considerably exceeded these values after 96 hours of fermentation, attributed to the extended fermentation period and species-specific differences. Similarly, total non-essential amino acid composition of raw seeds was lower than values reported by Chidi et al. (2012), Muhammad et al. (2013), Makinde and Akinoso (2014), and Ogbuagu et al. (2018). Following fermentation for 96 hours, however, total

non-essential amino acid values of *Sesbania cannabina* seeds surpassed these comparative studies, again reflecting the advantage of extended fermentation. Sulfur-containing amino acid values also increased with fermentation hours, although Chidi *et al.* (2012) reported a higher sulphur amino acid value (17.29%) than the present study's best result, while values were higher than those reported by Muhammad *et al.* (2013), Makinde and Akinoso (2014), and Ogbuagu *et al.* (2018) for *Aeschynomene indica* seeds.

## 5.0 CONCLUSION

Fermentation of *Sesbania cannabina* seeds for up to 96 hours substantially increased their proximate composition and total amino acid content, with the best crude protein and sulphur-containing amino acid values recorded at 96 hours of fermentation. These findings demonstrate that fermented *Sesbania cannabina* seeds possess adequate proximate and amino acid profiles to serve as a viable, economical plant-based protein substitute for conventional feed sources in fish feed formulation. Future studies should investigate the anti-nutritional properties of *Sesbania cannabina* seeds to complement the present findings. Comparative growth performance studies using *Sesbania cannabina* seeds as a protein source relative to conventional protein sources such as fishmeal and soybean meal are also recommended.

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