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AMINO ACIDS PROFILE AND NUTRITIONAL POTENTIALS OF CASHEW NOT WASTE ON *Clarias gariepinus* CULTURE

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ABSTRACT

A 98-day feeding trial was conducted to investigate the Amino acids profile and Nutritional potentials of cashew nut waste on *Clarias gariepinus* juveniles Culture. Four hundred and eight juveniles 14.10±0.06g mean weight and mean length of 10.87±0.32cm were randomly distributed into four treatment tanks in triplicate groups of thirty four (34) fish per replicate in a completely randomized design. Four iso-nitrogenous diets containing 40% crude protein were prepared with varying ratios of 0%, 25%, 50% and 75% of the formulated diets respectfully contributing $0.0g100g^{-1}$, $13.50g100g^{-1}$, $17.50g100^{-1}$, and $25.00g100g^{-1}$ of the cashew nut waste. The fish in each dietary treatment were fed the allotted experimental diet at 5% body weight twice daily. Statistical analyses for all the parameters in the research were made. Nutrient composition analysis of the test diets showed significant differences among the treatments and the control. Water quality parameters measured were within the range (40-45%) for catfish culture. The result of amino acid profile showed that cashew nut waste contains all the essential amino acids and some non-essential amino acids. This investigation reveals that cashew nut waste could be a wealth-spinning venture if harnessed well and will be an alternative replacer of fishmeal in fish diets.

Keywords: *Cashew nut waste, Clarias gariepinus, Amino acid*

INTRODUCTION

Globally, marine and inland waters had made many countries potentially good in fish farming. However, a gap still exists because of unavailability and high cost of fish feeds for different stages of fish farming. Currently, the concern of fish nutritionists about the high cost and inadequate fishmeal is to explore the possibility of using agricultural by-products and wastes such as discarded cashew nut-waste in rearing of fish . The use of plant-based protein source to replace the high cost and limited fishmeal in fish farming is increasing on daily bases, which will reduce the high cost of fish farming and make it feasible globally. The high demand for fishmeal for the production of aquafeed by fish feed producing industries is increasing. The zeal to formulate cheap and affordable fish feeds for aquaculture has led to the emergence of many feed industries producing all sorts of fish feeds. Fish farmers embrace these feeds without knowing the proximate make up of such feed. this high demand has likely contributed to overfishing and exploitation of capture fisheries all over the world (Türkman, 2019). This has led to the forecast of Hua *et al.* (2019) who reported that by the year 2025; about 37.4 million tons of fish would be required to meet the manufacturing of aquafeed needed for fish farming all over the world. Many trial studies have been done on many plant-based ingredients, animal protein source, and their by-products globally. This current situation requires a continuous search for other alternative feedstuffs from plant protein sources that are cheap and readily available. Plant products and their by-products have nutritionally balanced essential and non-essential amino acids that improve nutrient availability and digestibility thereby aiding good conversion ratio (Jahazi *et al.,* 2020). Feeds needed by catfish must be durable, palatable, and acceptable by the fish and must contain all the essential nutrients such as amino acids, vitamins and minerals in good proportion for good growth and health status of the fish. Most of the plant-base sources and their by-products used as fish feed are restricted by the presence of anti-nutritional factors.

African catfish (*C. gariepinus*) is an important aquaculture species that is cultured in various regions of the world. It is a species of the family clariidae, the breathing catfish. It is an indigenous species to Africa and spread widely too far away Middle East and Turkey and```` different parts of the world. Nigeria is the highest producer of catfish, followed by Hungary, Brazil, Netherland, Kenya, Cameroun South Africa, and Mali (FAO, 2016) and many Asian countries such as China, Indonesia, Thailand and Malaysia. It is one of the commercially fish species that are very important and have enjoyed development in aquaculture (Nazia *et al.,* 2014 and Sadauki *et al.,* 2022a). African catfish is rated high in warm water aquaculture, and accepted by many fish farmers, because they grow fast, have large size, have low bone content; can tolerate poor water quality parameters, (temperature, low dissolved oxygen and salinity) (Sadauki *et al.,* 2022b). Catfish are carnivorous fish use to control much breeding of tilapia in a mix-sex tilapia culture. African catfish is an important fish species in Africa because of the high market value, and economic importance (Ude, 2011; and Afolabi *et al.,* 2020). Their fry and fingerlings are found easily because of artificial propagation (Asraf *et al.,* 2013; Muchilisin *et al.,* 2015 and Marimuthu *et al.,* 2019).

Cashew nut (*Anacardium occidentale)* belongs to the family Anacardiaceae. It is native to Brazil and grown in other places such as West Indies, India, Vietnam, and Africa. Cashew is a supple plant that grows well on poor sandy soils under different climate condition (Runjala and Kella, 2017). It can tolerate draught and soil that is deficient in nutrients. It grows well in a climate of about 27º North and 28º south of the equator (Akinhanmi *et al.,* 2008). Cashew is the most important edible nut in the world with soaring nutrient value. It is one of the most famous plantation crops, marketed because of their nutritional value (John *et al.,* 2017). The plants of cashew produces cashew nut which is its actual fruit affixed at the bottom of a cashew apple which is pseudo fruit formed from an enlarged fruit stalk, with only one cashew nut, attached to each apple, and enclosed in a stiff shell (Balandran-Quitana *et al.,* 2019) . Cashew nut constitute only 10% of the total nut weight (Oliveira *et al.,* 2020). Aidoo *et al.* (2020) opined that if cashew nut is not disposed as waste, it can be used as animal feeds while so many researchers such as Prabhudessai *et al.* (2013) and Setyobudi *et al.* (2021) suggested using this waste for biogas. The value of cashew nuts as foreign exchange earner is very high in comparison to most edible nuts in the world markets. Globally, cashew nut are consumed because of their desirable nutrient, sensory attributes and source of vitamin, proteins, fats and minerals (Das *et al.*, 2014 and Akande *et al.*, 2015). Tanzania, India, Mozambique, Sri Lanka, Kenya, Madagascar, Thailand and Angola are the major producing countries of cashew in the whole world. African countries produce about 100,000 metric tons of cashews yearly. Despite their huge production, they gain little from the products because they export the products unprocessed to countries like Netherlands, USA and other European countries. World Bank estimated data show that about 97% of cashew production in Africa came from the wild while only 3% is from well established plantations (Rosengarten, 1984). Cashew is available globally and can be assessed all year round (Akande *et al.,* 2015).

MATERIALS AND METHODS

Study Area

The research was conducted at the Department of Fisheries and Aquaculture Ebonyi State University Abakaliki, South East, Nigeria.

Collection of Research Materials

Cashew nut waste

Cashew nuts waste (*Anacardium occidentale*) used for the research was collected from Embik Cashew Nut Processing Industry at Umuori Urata in, Imo State, South East Nigeria.

Procurement of other feed materials:

Other ingredients such as fishmeal, soybean, wheat, yellow maize, fish premix, methionine, lysine, salt, vitamin C, and oil were bought from International Market, Abakaliki, Ebonyi State Nigeria. The soybean was toasted until the colour changed to orange to reduce the anti-nutritional factors. All the ingredients (Soybean, yellow corn, wheat and fishmeal) were ground separately to fine powders with R175A made in China hammer mill. They were packaged differently in sealed containers for use.

Procurement of experimental fish and Experimental set-up

Four hundred and twenty two (422) African catfish (*Clarias gariepinus* Burchell 1822) Juveniles were purchased from Mercynuel fish Farm in Abakaliki Metropolis, Ebonyi State, Nigeria. They were conditioned for fourteen days. During this time, the fish were fed 2mm coppens feed at 5% body weight two times daily. The fish were starved a day before the commencement of the feeding trial to empty their gastro-intestinal tract and prepare them for the experimental diets. Four hundred and eight fish were selected, measured, weighed and randomly distributed, into Four treatment tanks (1m x 1m x 0.7m) in triplicate in the ratio of thirty four (34) juveniles for each replicate. The fish, which were stocked in triplicate for each dietary treatment, were fed the assigned experimental diet at 5% of their body weight two times per day between the hours of 8.30am to 9.30am in the morning and 4.30pm to 5.30pm in the evening for a period of 98 days the research lasted. Weighing and measuring of the fish and other routine duties (changing of water, collection of water samples, measuring of the water temperature) were done at two weeks intervals and the feed intake adjusted to reflect their new weights and lengths.

Formulation of Experimental Diets.

Four iso-nitrogenous diets with average protein content of 40% were formulated (Table 1). They were formulated to have varying levels of 0%, 25%, 50%, and 75% of the diets containing inclusion levels per treatment of $0.00g100^{-1}$, $13.5g100^{-1}$, $17.5g100^{-1}$ and $25.0g100g^{-1}$ of the cashew nut waste in CNW0 (control), CNW1, CNW2 and CNW3 respectively. The quantity of the ingredients was weighed according to treatment. All ingredients were weighed with Electronic digital Kitchen Scale CAMYR EK5505E Ohaus Corporation NJ, USA. The cashew nut wastes that were ground to a fine powder, was mixed together with other ground ingredients - soybean, maize, fishmeal, wheat; and vitamin premix, methionine, lysine, vitamin C, iodized salt (NaCl) and oil, with hot water for about ten to fifteen minutes to make sure the ingredients were thoroughly mixed together, cornstarch was used as a binder to form a homogenous mesh. The resultant pellets that were thoroughly mixed together for each treatment group was pressed and extruded through a 2mm die attached to a Viking Exclusive Jancod pelletizing machine. The resultant pellets were sun-dried for two days and analyzed for proximate composition, which include percentage crude protein, crude fibre, crude lipids, moisture, ash and nitrogen free extract, according to AOAC (2005) Table 1.

Water Quality Parameters

The physico-chemical parameters measured were- Temperature, Dissolved Oxygen (DO), pH and Conductivity as described by APHA (1980). Good water quality is ideal for successful fish farming. If the water quality is bad due to disparity in one or more parameters, fish health and growth will be affected (Reid *et al.*, 2019). The water temperature was measured with Mercury-in-Glass Thermometer and recorded in ºC. Dissolved oxygen was monitored every two weeks with Multi- Analyzer water parameter kit model EZ-9909-SP. pH was measured with Multi-Parameter Analyzer Model COM-600. HACH Conductivity Meter 16300 Model was used to measure the Conductivity of the water in the treatment tanks. Water was collected bi-weekly at 8.30am in the morning with a 2–1 Van Dorn bottle for the examination. using APHA (1912) method.

Proximate Composition of Feed:

Proximate composition for percentage Moisture, Protein, Fat, Fibre, Ash, Nitrogen Free Extract (NFE) and Dry Matter (DM) contents were done in duplicates as recommended by AOAC (2005). Percentage Moisture content was determined by drying the samples in the oven for four hours in a temperature range of 105ºC to a constant weight. The crude protein content was determined by the use of Kjeldahl method by multiplying a constant factor 6.25 by Nitrogen content. Crude Fat was determined through petroleum ether extraction using Soxhlet method. Crude Fibre was determined by acid digestion method; this was followed by combusting the samples in a muffle furnace for 6 hours at a temperature of 550ºC to obtain the ash content. Dry Matter was determined by oven drying the samples at 105ºC for 24 hours. NFE was determined by subtracting the sum of the values of CP, CFat, CFibre, Ash, and Moisture from 100.

Determination of Amino Acid Profile of Cashew Nut Waste

The method Ren *et al.* (2013) was used to analyze the Amino Acids of the cashew nut waste. To protect Methionine and Cystein from degrading during analysis, the use of performic acid oxidation was done. Hydrolyzing the sample was done by the addition of 4ml of 6N HCl with 1% phenol to the sample of cashew nut waste for 24 hours at 120°C for digestion. The sample was diluted to 25ml with 0.2 N HCl after cooling. Filtration was done with nylon syringe filters and analyzed with Amino acid analyzer Hitachi L-8900 model (Hitachi High Technologies, Dallas, TX). Analysis of Tryptophan was separately done using the method as described by Kuminek *et al.* (2011). 2ml of sodium hydroxide in water, 50ml dried hydrolyzed starch and one drop of ethanol were added to the sample of the cashew nut waste. The sample was digested and cooled at room temperature after adding 1.4 ml of 6 N HCl and 5ml of 95% ethanol. it was diluted to 25ml by using 0.2 M phosphate buffer (pH 7).and analyzed with High Performance Liquid Chromatography (HPLC).

Data Presentation and Statistical Analysis: Data collected from the parameters were subjected to one-way analysis of variance (ANOVA), to test the significant differences (P<0.05) using Tukey's post-hoc test analyzed by SPSS IBM version 21 package and Excel 16.0 package.

RESULTS

In this research, the most abundant essential amino acid is Lysine (31.07%) while the most abundant non-essential amino acid is Glutamic acid (14.07%). The least essential and non essential amino acids are Histidine (2.44%) and Omithine (0.06%) Table 2. The analysis showed that there was no deleterious effect on the amino acid profile. The values of most amino acids recorded for cashew nut waste in this research revealed that Lysine (31.07%), Gutarmic acid (13.07%), Aspartic acid (19.03%), Leucine (9.14%),, Arginine (7.00%), Alanine (5.05%), Valine (5.99%), Isoleucine (4.16%), Threonine (4.09%), Sierine (4.6^), Phenylalamine (4.91%), Proline (4.86%), Methionine (2.69%), Histidine (2.44%), Tyrosine (3,9%), Tryptophan (1.4%) and Omithine (0.06) were higher than most feedstuffs 0.26% (maize), 1.73% (Groundnut cake), 0.9% (Brewers dried grain), 0.23% (Millet), 1.52% (Sun flower meal), 0.14% (Guinea corn), 0.03% (wheat) as reported by Aduku, (2005) and 1.6% (maize), 0.24% (Groundnut cake), 0.7% (Brewers dried grain), 0.76% (millet), 0.35% (Sunflower meal) and 0.28% (Wheat)reported by Ohioha (1992).

Table 1: Percentage Composition of Experimental Diets (%)

Parameter	CNW1(Control)	CNW1	CNW2	CNW3
Soy bean meal	25.00	17.50	13.50	10.00
Cashew nut waste meal	0.00	13.50	17.50	25.00
Fishmeal	40.00	37.50	34.00	30.00
Yellow corn meal	12.11	12.11	12.11	12.11
Wheat meal	11.89	11.89	11.89	11.89
Vitamin premix*	02.75	02.75	02.75	02.75
Methionine	02.25	02.25	02.25	02.25
Lysine	01.85	01.85	01.85	01.85
Corn starch	02.15	02.15	02.15	02.15
Oil	01.00	01.00	01.00	01.00
Vitamin C	00.50	00.50	00.50	00.50
Iodized salt	00.50	00.50	00.50	00.50
Crude protein	40.84±0.04[a]	39.13±0.01[b]	34.44±0.01[c]	40.62±0.01[a]
Crude fat	17.96±0.01[a]	18.22±0.01[a]	16.82±1.02[b]	19.44±2.07[a]
Crude fibre	2.52±0,33[b]	2.67±0.02[a]	2.63±0.21 [a]	2.54±0.01 [b]
Ash	6.55±1.03[b]	6.71±1.21[a]	6.64±1.00 [a]	6.49±1.33[b]
Moisture	7.85±1.05[b]	8.10±0.00 [a]	8.03±1.06[a]	7.83±0.03[b]
Nitrogen free extract	24.25±2.1.02[c]	25.17±1.04[b]	27.48±1.13[a]	23.06±1.40[c]
Dry matter	91.15±2.71[a]	91.90±2.51[b]	91.97±2.42 [b]	92.17±2.34 [a]

*Vitamin premix. Vitalyte-extra containing the following per kg of feed. Vit. A 15,000,000IU, Vit. D3 4,400,000IU, Vit. E 2,500IU, Vit. K 4,350mg, Vit. B2 4,350mg, Vit. B6 2,350mg, Vit.

B12 11,350mg, Vit, C 1,000mg, Nicotinamide 16,700mg, Calcium pantothenate 5,350mg, Potassium chloride 87,000mg, Sodium Sulphate 212,000mg, Sodium Chloride 50,000mg.
Means with different letters (a, b, c) within the same row differ significantly ($p<0.05$)

Table 2: Water Quality Parameters

Parameter	CNW0 (Control) (0%)	CNW1 (25%)	CNW2 (50%)	CNW3 (75%)
Temperature ºC	27.72 ± 2.00^{a}	28.05 ± 2.11^{a}	27.72 ± 2.114^{a}	28.02 ± 1.03^{a}
DO mg/L	5.04 ± 0.06^{a}	5.00 ± 0.02^{a}	5.10 ± 0.02^{a}	5.02 ± 0.06^{a}
pH	6.65 ± 0.22^{b}	6.04 ± 0.43^{b}	6.85 ± 0.36^{b}	7.35 ± 0.78^{a}
NH_3 mg/L	0.05 ± 0.02^{a}	0.02 ± 0.04^{b}	0.03 ± 0.00^{b}	0.02 ± 0.04^{b}
NO_3 mg/L	0.04 ± 1.20^{d}	0.06 ± 0.33^{b}	0.05 ± 0.41^{c}	0.07 ± 0.04^{a}
NO_2 mg/L	0.02 ± 0.81^{a}	0.03 ± 1.00^{a}	0.04 ± 0.23^{a}	$0,03\pm0.00^{a}$
Conductivity µ/cm	120.60 ± 3.0^{d}	127.50 ± 3.45^{b}	125.40 ± 3.06^{c}	129.21 ± 3.64^{a}
Total Dissolved Solids mg/L	67.41 ± 2.07^{a}	63.76 ± 2.11^{c}	62.92 ± 1.34^{d}	65.27 ± 1.33^{b}
Salinity mg/L	0.06 ± 0.01^{a}	0.07 ± 0.45^{a}	0.05 ± 0.28^{a}	0.06 ± 0.77^{a}

Note: NH_3 = Ammonia, NO_3 = Nitrate, NO_2 = Nitrite. Values represent mean standard error of mean (SEM) of 12 replicates of the feeding trial Different letters (a, b, c, d) in each row signifies significant difference ($p<0.05$)

Table 3: Amino Acid Profile of Cashew Nut Waste Used in the Feed Formulation

Amino Acid	Percentage Composition
Glutamic Acid	14.07*
Aspartic Acid	19.13*
Lysine	31.07**
Leucine	9.14**
Arginine	7.00**
Alanine	5.05*
Valine	5.99**
Isoleucine	4.16**
Thronine	4.09**
Serine	4.60*
Phenylalamine	4.91*
Proline	4.86*
Methionine	2.69**
Histidine	2.44**
Cystiene	2.00*
Tyrosine	3.90**
Omithine	0.06*
Tryptophan	1.40*
Total	**117.5**

Note: ** Essential amino acids, * Non Essential amino acids

DISCUSSION

The analysis in this study revealed that the value of crude protein recorded agreed with the maximum protein requirements of for catfish culture as reported by Bake *et al.* (2021). The values were similar to the findings recorded by Falaye *et al.* (2011). Iheanacho *et al.* (2019), recorded values ranging from 31.15 - 41.05%, for *C. gariepinus* fed discarded cashew nut meal, and Anyanwu *et al.* (2021) who reported a crude protein range of 39.30 – 41.97% for *C. gariepinuis* fed graded levels of *Enterococcus faecium* meal. However, the mean percentage crude protein recorded in this study was lower than the 42.01%

recorded by Olukunle (2009). The principal nutrient of cashew nut is fat, which acts as energy for seeds of plants, and plays a crucial role in the growth, continued existence and maturity of fish (Lim, 2012). Crude fat avert the use of diet protein as energy in feedstuffs (Kyoung *et al.,* 2012, and Sotolu, 2010). Crude fibre plays a vital role in digestion. The percentage crude fibre recorded in this experiment were similar to the values obtained by (Bake *et al.*, 2021; Sotolu, 2008, and Fayele *et al.,* 2011) respectively but differ from the result obtained by Oyelese (2007). The Nitrogen free extract (NFE) obtained from this research was lower than the results of 32.12% obtained by Sotolu (2008), Falaye *et al.* (2011) and Ibrahim *et al.* (2021).

The importance of water to fish is numerous. Fish use water for their biological functions, such as respiration, feeding, reproduction, movements, growth, and osmoregulation. Any defect in the water quality will have adverse effect on the fish. The results of the physico-chemical parameters in this study showed that all the parameters were within the range for catfish culture. There was no harmful effect on the water quality because of the cashew nut waste meal. The values of all the water quality parameters measured were within the ranges recommended by (Boyd, 1982; Solomon *et al.*, 2013 and Ekubo and Abowei, 2011). This finding agrees with the reports of Olukunle (2009), Boyd (1979), Ehiagbonare and Ogunrinde (2010), and Bhatnagar *et al.* (2013).

The results of amino acid in the cashew nut waste show that the sample contains all the essential amino acid and some non-essential amino acids. Lysine (31.07%) ranked highest and omithine as the least value (0.06%). However, it was clear in this study that the most abundance were amino acids with acidic side groups which followed by aliphatic side groups amino acids. These findings corroborate the findings of Venkatachalam and Sathe (2006). The percentage amino acid recorded in this research was within the accepted values recorded by NRC (1998), OECD (2001) and poultry feeding standard (2005). The low methionine and tryptophan could be that the toasted soybean, one of the ingredients used in the diets formulation was over heated during toasting. This brought a reaction from the amino group of the amino acids and carbonyl group of reducing sugar as reported by Lokuruka (2011), Anderson and Wolf (1995). Cashew nut waste in this research contain appreciable levels of lysine, glutamic acld, aspartic acid and leucine, which could be included in fish diets to reduce the cost of producing fish diets. Incorporating cashew nut waste meal in the formulation of fish diets will add to the protein intake of the fish. This will eventually assist in meeting the amino acids needed, which will maximally maintain the functions and structure of the fish by boosting the metabolic pathways (Standlmayr *et al.* (2013).

Conclusion

Non-availability and affordability of some orthodox protein based ingredients have reduced the levels of animal source food consumption in Nigeria. Nigeria experiences high level of food insecurity and malnutrition because of lack of animal protein. Scarcity and high cost of these feed ingredient have made fish nutritionists to continue the search for alternative plant protein source to replace fishmeal. The findings in this research showed that cashew nut waste have the potential to replace fishmeal either partly or wholly and will considerably reduce spending on fishmeal without compromising growth performance of African catfish. Cashew nut waste contains all the essential amino acids and some non-essential amino acids compared to most edible nuts and could be a wealth-spinning venture if well harnessed in aquaculture. Based on the findings of this research, cashew nut wastes can be included to the diets of African catfish at the rate of 13.50 – 17.50g/100g (25 – 50%)

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