Amino acid profile and apparent digestibility in hybrid catfish (*Heteroclarias juvenile*) fed ackee apple *(Blighia sapida)* seed meal

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Abstract

The usefulness of protein in the system of fish can be evaluated through the degree of availability of its essential amino acids while the degree of digestion is an indicator of absorption-waste relationship of the diet consumed. Therefore, this study investigated the release of amino acids in a soya bean meal based diet (SMB) and an unconventional test diet using Ackee apple seed meal as well as the digestibility of diets and test ingredient. The test diet was formulated from 30% addition of test ingredient to a 70% reference diet (SMB). Two (2) treatments accommodated 30 Heteroclarias juveniles per treatment in three (3) replications; using six (6) rectangular plastic tanks with dimensions of 0.33m x 0.33m x 0.5m. A complete randomize design was applied during laboratory investigation over a period of 21 days. Each amino acid was determined by using a single column amino acid analyzer (Model 120 C). Availability of each acid and digestibility of diets were obtained through standard formulae. Data were analysed through the use of descriptive statistics (percentage and means) and student t – test for comparison of means of proximate chemical compositions. Lysine and methionine were most limiting in the two isoproteic diets (40% protein). Five (5) essential amino acids (Alanine, Arginine, Tryptophan, Threonine and Aspartic acid) were bountifully released in the reference diet compared to three (Alanine, Phenylalanine and Tryptophan) recorded by test diet. Apparent digestibility was higher in the reference diet (98.41%) compared to that of test diet (82.17%). Test ingredient recorded a digestibility level of 44.26% in the system of Heteroclarias juveniles. Ackee apple seed meal could be used in formulating fish feed in saving cost or due to scarcity of conventional ingredients and serve as a good source of tryptophan and alanine in feed supplementation.

Keywords: Amino acid, Hybrid Catfish, Ackee apple

Running Title: The bio-availability of amino-acids and digestibility in Heteroclarias fed ackee apple seed meal

Profil des acides aminés et digestibilité apparente chez le poisson-chat hybride (*Heteroclarias juvénile*) nourri avec des tourteaux de graines de pomme ackee (*Blighia sapida*)

Résumé

L'utilité des protéines dans le système des poissons peut être évaluée par le degré de disponibilité de leurs acides aminés essentiels, tandis que le degré de digestion est un indicateur de la relation absorption-déchets du régime alimentaire consommé. Par conséquent, cette étude a examiné la libération des acides aminés dans un régime alimentaire à base de tourteaux de soja (SMB) et dans un régime alimentaire test non conventionnel utilisant des tourteaux de graines de pomme ackee, ainsi que la digestibilité des régimes alimentaires et de l'ingrédient test. Le régime test a été formulé en ajoutant 30 % de l'ingrédient test à un régime de référence de 70 % (SMB). Deux (2) traitements ont été réalisés avec 30 juvéniles de Heteroclarias par traitement en trois (3) répétitions ; utilisant six (6) cuves plastiques rectangulaires de dimensions 0,33 m x 0,33 m x 0,5 m. Un plan complètement aléatoire a été appliqué lors de l'investigation en laboratoire pendant une période de 21 jours. Chaque acide aminé a

été déterminé à l'aide d'un analyseur d'acides aminés à colonne unique (Modèle 120 C). La disponibilité de chaque acide et la digestibilité des régimes alimentaires ont été obtenues par des formules standard. Les données ont été analysées à l'aide de statistiques descriptives (pourcentages et moyennes) et du test t de Student pour la comparaison des moyennes des compositions chimiques approximatives. La lysine et la méthionine étaient les plus limitantes dans les deux régimes isoprotéiques (40 % de protéines). Cinq (5) acides aminés essentiels (Alanine, Arginine, Tryptophane, Thréonine et Acide aspartique) ont été abondamment libérés dans le régime de référence par rapport aux trois (Alanine, Phénylalanine et Tryptophane) enregistrés dans le régime test. La digestibilité apparente était plus élevée dans le régime de référence (98,41 %) comparée à celle du régime test (82,17 %). L'ingrédient test a enregistré un niveau de digestibilité de 44,26 % dans le système des juvéniles de Heteroclarias. Les tourteaux de graines de pomme ackee pourraient être utilisés dans la formulation de l'aliment pour poissons afin de réduire les coûts ou en raison de la rareté des ingrédients conventionnels et servir de bonne source de tryptophane et d'alanine dans la supplémentation des aliments.

Mots-clés : Acide aminé, Poisson-chat hybride, Pomme ackee

Introduction

Recently, there has been an increased competition for feed ingredients that are used in manufacturing animal feed because some of these ingredients are used by humans as food (Ajibade *et al.,* 2021; Gadzirayi *et al.,* 2012). Also, the population of the world is expected to increase by about 29% from the current 7 billion to 9 billion in 2050 (Meissner *et al.,* 2013). Therefore, there is a need to search for alternative source of nutrients that can marry the high demands in aqua-culture and livestock production (Meissner *et al.,* 2013; Adeosun *et al.,* 2019).

Protein is needed by animals for maintenance and growth. However, it is broken down in the process of digestion into simpler and absorbable form called amino acids. Therefore, amino acids are described as the building blocks of proteins. These amino acids are classified into two broad groups: essential and non – essential amino acids. There are ten (10) essential amino acids. They are essential because they cannot be produced directly by the body of an animal but are obtained from diets. However, a single source of protein, usually, does not produce all essential amino acids in adequate quantity. This calls for a mixture of proteins in the diet of any concern animal; such as *Heteroclarias*

Heteroclarias are hybrid products of cross between male *Heterobranchus longifilis* and female *Clarias gariepinus*, (*Clarias gariepinus* x *Heterobranchus longifilis*). It is known among the fish aqua-culturists as 'Heteroclarias' and very popular in Nigeria and Africa because of its fast growth rate, hardiness, high demand and excellent taste (Owolabi *et al*., 2021). The hybrid fish is also very rugged, diseases resistant and usually consume less feed compared to other *Clarias species*. The average weight and size are better than that of *Clarias gariepinus* (Merchie *et al*., 1996).

Digestibility is a factor that determines the ease with which an ingredient can be digested by an animal's system, i.e. an ingredient with high digestibility will be better exploited by the digestive system, having its nutrients and amino acids absorbed and used to the benefit of the health and nutrition of the animal. Most studies on catfish protein and amino acid digestibility were performed in the 1970s and 1980s (Cruz, 1975; Wilson *and Poe*, 1981; Brown *et al*., 1985; Wilson and Poe, 1985).

Ackee (*Blighia sapida*, Koenig) is an underutilized tree crop native to West Africa, and widespread in tropical and subtropical environments. It belongs to the plant family called Sapindaceae (soapberry) (Hecht, 2006). The fruit is a capsule consisting of three (3) fleshy valves which split open at maturity and ripeness (Olorode, 1984). A hard, shiny, round and black seed is located within the pod (capsule) with a characterized fleshy par (aril).

The aril is highly nutritive and eaten in Haita, Jamaica and West African countries (Gordon *et al.*, 2015). However, the anti-nutrients properties in the seed include phytates (0.93 mg/100g), oxalates (5.42 mg/100g) and Tannins (0.37 mg/100g) which limit the nutritional value of the seed (Hoba, *et al.,* 2018)..This non-bioavailability can be reduced through processing, such as water and microbial fermentation, milling, soaking, removal of seed coat, autoclave and microwave treatment, cooking and soaking to reduce the natural plant inhibitors (Samitiya *et al.,* 2020). Hence, the black seed has not found maximum use or application in many countries. Therefore, this present student investigated the digestibility of the wasted nuts in the seed as a plant-protein source in the feed of Heteroclarias juveniles and availability of the constituent amino acids.

Materials and Methods

The experiment was conducted in the laboratory of the Department of Fishery Technology, Oyo State College of Agriculture and Technology, Igboora. 100 fingerlings of *Heteroclarias juvenile* with average weight of 107.5g ± 2.5 and length of 10.0cm ± 0.6 were purchased from a commercial farm at Ibadan and were randomly distributed in a group of twenty into six plastic tanks (0.33m X 0.33m X 0.5m) in plastic aquaria before trial. Test organisms were conditioned for one week in the laboratory by using fresh water obtained from the bore – hole (underground water) at the Fisheries Technology Department. A storage cylindrical tank was first used in storing the water for at least eight (8) hours for proper oxygenation and later channeled by gravity into the experimental tanks. Fish were acclimated and fed with 40% crude protein using commercial feed (Coppens). Fish were generally fed with control diet containing 40% crude protein at 5% of their body weight for the period of acclimation. Feed left over and feed discharged were removed by changing the water with fresh one every 48 hours. Faecal collections were done by simple siphoning every 2 hours after feeding following a modified method described by (Zhou *et al*., 2004).

Physicochemical analysis of water was determined by using Philip's pH meter. Water temperature was obtained using mercury glass thermometer. Dissolved oxygen was determined by Boyd (1981) in the laboratory of the department of Fisheries Technology, Oyo State College of Agriculture and Technology.

Twelve (12) pods of Ackee apple seed were obtained from the College premises and then processed in the laboratory. The seeds were removed from each pod and then sun-dried for 15 hours (5 hours per day for 3 days. The seed-coat was then removed manually by using sharp razor-blades and scapula from the dissecting sets to obtain white-brown-yellow nuts followed by grinding before incorporation into formulated diets. Chromic Oxide (1%) was then added to the two diets (Reference and test) as a monitoring agent. 150 fish were randomly allocated into 6 plastic tanks (0.33m×0.33m×0.5m) for the two treatments; 25 fishes per plastic tank and three tanks per treatment.

Reference diet targeted 40% CP. 70% of each ingredient of reference diet was then determined to achieve 30% total reduction. The balance was then made up with 30% Ackee apple seed meal (ASM) substitution as described by Cho and Slinger (1979) in digestibility study. Diet was manually mixed with distilled water ($40cm^3$/100g diet mix) pressure-pelleted using sieve and subsequently dried in a current of air at room temperature for 24 hours.

Statistical analysis

The experimental layout was a complete randomized design (CRP) with the following model.

Yij = μ+ Eij (Where yij= individual observation, μ= overall mean, Ti= Treatment. and Eij= Random error). Student t-test was used to compare the difference between proximate chemical composition of each diet and faecal discharge. Also, descriptive statistics (means and percentages) were applied for the evaluation of digestibility coefficients.

Availability of amino acids and apparent digestibility were mathematically defined as follows:

- Percentage apparent amino acid was calculated using standard formula as described by NRC (1993) and Bureau *et al.* (1999) as follows:

$$100 - \frac{(100 \times \%Cr_2O_3 \text{ in feed} \times \%\text{amino acid in faeces})}{\%\ Cr_2O_3 \text{ in faeces} \quad \%\text{ amino acid in feed}}$$

- Apparent digestibility coefficient (ADC) was calculated using the equation below:

$$ADC_{test\ ingredient} = ADC_{test\ diet} + (ADC_{test\ diet} - ADC_{ref\ diet}) \times (D_{ref} / 0.3 \times 3_{ingr}).$$

Results and Discussion

The mean values of selected water quality parameter of the experimental unit is shown in Table 1. Water temperature recorded an average value of 26.5. This agreed with the standard requirement (25^0C to 30^0C) favourable to tropical fresh water special in aquaculture. Furthermore, dissolved oxygen value was 6.6mg/l during the laboratory investigation which agree with the minimum requirement of 4.0mg/l reported by Boyde (1981) and Kumar (1992). Water acidity (pH) was averagely 7.4 and within the standard range of 6.0 to 9.0. Statistically, all the chosen water parameters (dissolved oxygen, water acidity and temperature) were insignificantly different from recommended standards during the 21 days of feeding trials. This means that high stress factors from the water environment were prevented during investigation. This good water quality was made possible by the act of changing water partially every day before feeding in the current study.

The degree of utilization of each nutrient was first measured ,in the present study, by a direct comparison; using initial level of nutrient in a specific diet and final value in the faeces of experimental fish. Therefore, a decreasing order of utilization (change) of nutrients in the control diet (CD) consumed by Heteroclarias juvenile recorded crude protein (23.64%), moisture (11.43%), Ash (9.91%), Ether extract (3.45%) and crude fiber (0.45%). Similarly, the test diet recorded Ash (15.13%), crude protein (11.67%), Ether extract (5.91%), Moisture (5.62%) and crude fiber (1.90%), as shown in Table 2. This means that the ash (minerals) and crude protein fractions of the diet were better utilized after consumption by the experimental fish (Heteroclarias juveniles) while the crude fiber was least affected during absorption.

Table 1: Water quality parameters of experimental set up

Parameter (s)	Value (s)
Water temperature (^{0}C)	26.6 ± 0.5
pH (Water Acidity)	7.4 ± 0.20
Dissolved oxygen (mg/l)	6.6 ± 0.31

Table 2: Proximate chemical composition of reference diet and faeces of Heteroclarias Juveniles (x ±SD)

Diet/faeces	Nutrient (%)					
	CP	Ash	EE	CF	Moisture	CR
Control Diet (CD)	36.84±0.51^a	23.30±0.54^a	2.90±0.06^a	6.72±0.04^a	7.0±0.16^a	11.50±0.22^a
Faeces	28.13±0.82^b	20.35±0.50^a	2.80±0.03^a	6.75±0.31^a	7.8±0.08^a	13.60±0.31^a

Means with the same superscript along the same column are not significantly different at P≥0.5.

Keys: CP = Crude Protein; EE = Ether extract; CF = Crude Fibre; CR = Chromium

Twenty amino acids were analyzed in the sample of diet and faeces. The reference diet was generally richer in the release of amino acids than the test diet to the *Heteroclarias juveniles* in this study (Table 3). Specifically, the reference diet showed a higher true amino acid availability (TAAA) in the juvenile of Heteroclarias (≥ 40.0%) in relation to Alanine (56.41%), Arginine (50.64%), Tryptophan (51.42%), Threonine (60.22%), Aspartic acid (47.0%), Phenylalanine (41.91%) and Ornithine (41.69%). However, the test diet richly supplied the chosen fish with Tryptophan (75.68%), Alanine (63.79%), Phenylalanine (50.0%) and Threonine (40.14%) compared to the lower values recorded in reference diet.

Nutritionally, the ten (10) essential amino acids are needed in the diet of fish, out of twenty required for protein syntheses (Smith, 1995). This requirement has led to the need to add commercially prepared lysine and methionine to formulated feeds (Obado *et al.,* 2018). Similarly, the present study recorded limited availability of valine (22.24%; 13.39), methionine (09.27%; 03.34%), lysine (02.43%; 11.81%), Leucine (20.96%; 09%), histidine (23.96%; 15.62%), isoleucine (32.45%, 23.10%) in the reference and test diet, respectively. Consequently, lysine and methionine were the most limited amino acids because of the poor release (availability) from the two feeds to the system of the experimental fish (*Heteroclarias juveniles*). This agreed with the report of Bilial *et al.* (2017) that lysine supplementation was gaining popularity in growing aquaculture in Pakistan because of shortage in fish feeds. Also, Yaghoubi *et al.* (2019) reported a need to supplement lysine and methionine in soybean diets for silvery black porgy juveniles (*Spar hasta*).

Table 3: Mean values of true amino acids availability (TAAA) in catfish (*Heteroclarias)*

S/N	Amino acid	Reference diet	Test diet
1	Alanine	63.79 ± 0.71^{a}	56.4 ± 0.55^{a}
2	Arginine	14.08 ± 2.71^{b}	50.64 ± 5.46^{a}
3	Aspartic acid	25.04 ± 2.41^{b}	47.0 ± 3.64^{a}
4	Cysteine	22.13 ± 4.11^{a}	30.29 ± 2.14^{a}
5	Glutamic acid	13.43 ± 1.66^{b}	01.37 ± 0.04^{a}
6	Glycine	12.47 ± 0.53^{a}	15.25 ± 0.77^{a}
7	Histidine	15.62 ± 2.20^{b}	23.96 ± 6.56^{a}
8	Isoleucine	32.45 ± 5.11^{b}	23.10 ± 2.72^{a}
9	Leucine	09.07 ± 0.67^{b}	20.96 ± 1.53^{a}
10	Lysine	11.81 ± 0.91^{b}	02.43 ± 1.56^{a}
11	Methionine	03.34 ± 0.35^{b}	09.27 ± 1.22^{a}
12	Ornithine	19.52 ± 2.6^{b}	41.69 ± 4.52^{a}
13	Phenylalanine	50.00 ± 2.42^{a}	41.91 ± 4.61^{a}
14	Pyro lysine	21.61 ± 5.50^{a}	35.76 ± 2.50^{a}
15	Proline	03.01 ± 0.70^{a}	01.95 ± 0.06^{a}
16	Serine	25.22 ± 1.66^{a}	17.21 ± 2.20^{a}
17	Threonine	40.14 ± 4.42^{b}	60.22 ± 3.95^{a}
18	Tyrosine	13.25 ± 2.11^{b}	23.10 ± 5.40^{a}
19	Tryptophan	$75.68 \pm 3.04b$	51.42 ± 2.01^{a}
20	Valine	13.69 ± 2.45^{b}	22.24 ± 4.21^{a}

Also, Table 4 showed a better ranking of utilization by giving the apparent digestibility coefficients (ADCs) of nutrients in experimental diets through the use of a standard formula reported by Cho *et al* (1982) and Foster (1999). Coefficient of apparent digestibility provide estimates of nutrient availability in feedstuffs and are used to select ingredients that optimize nutritional value and cost of formulated diets (Fagbenro, 1999). Therefore, the current study shows that most nutrients in the control diet were better released for absorption by Heteroclarias juveniles compared to the test-diet; as indicated by the higher percentages of digestibility coefficients in Table 4. Specifically, higher digestibility coefficient was recorded for crude protein, ash, ether extracts (fat) and crude fibre in the control diet compared to lower values observed in corresponding nutrients in test-diet. These results, in the present study, tend to support the superiority of the control diet in terms of release of the nutrients for absorption in the system of the young fish (Heteroclarias juvenile);

especially in relation to crude protein and ash supplied. This implies that the experimental fish maximally benefit from feed-protein and minerals (ash fraction) contained in the feedstuff despite variations in the present study. These variation in availability reflects the quality and quantity of dietary nutrients and influence the digestibility and growth performance in fish (Montoya-Mejia *et al*., 2017; Mmanda *et al*., 2020).

Table 4: Apparent digestibility coefficients (ADCs) of nutrients in experimental diets (%)

Nutrient	Control-Diet	Test-Diet
Crude Protein	35.43	24.21
Ash	26.15	22.33
Ether-extract	18.36	17.19
Crude Fibre	15.12	13.55
Moisture	03.35	04.89

Therefore**,** the test diet requires about six (6) additional or supplemental amino acids while the reference diet (soya bean meal diet) requires about five (5), if given to the fish as the only source of plant protein. However, the test diet could be used as a better source of Tryptophan and Alanine in feed supplementation because of their richness in the test-ingredient. Also, the reference diet, containing sorely soya bean meal as plant protein was better digested and more available compared to test diet.

The crude protein, ash and ether- extract fractions of the two diets were mostly digested by experimental fish (Heteroclarias juveniles). The test diet can serve as a competitor or substitute to reference diet because margins in corresponding ADCs were not too wide; especially in saving cost of production of aquaculture feeds or due to scarcity. The seed of *Blighia sapida* needs an improved processing technique to maximize digestion in fish because only about half (44.26%) of a unit is available for useful functions in Heteroclarias juveniles system after consumption. Partial heat treatment after seed coat removal, such as boiling or roasting, is recommended to reduce the anti-nutrients in the seed of Ackee-apple in further studies. Although, most of the previous studies recommended autoclaving as the best method of reducing levels of anti-nutritional compounds (Doss *et al.,* 2011).

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