NUTRITIONAL EVALUATION, TOXICOLOGICAL EFFECT AND POSSIBLE UTILIZATION OF *ARECA CATECHU* SEED FLOURS AS AN ADDITIVE IN FEED FORMULATION FOR AFRICAN CATFISH FINGERLINGS (*CLARIAS GARIEPINUS*)

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Abstract
This study was carried out on fingerlings (Clarias gariepinus) with initial fish weight ranging from 4.55±0.25 to 5.53±1.00 g to evaluate the effects of supplementation of Areca catechu flour (ACSF) as an additive in graded level to the diet formulation on: growth performance, nutrient utilization, body composition, histopathology, blood parameters. Four experimental diets with 35 % crude protein were formulated. Diet 1 serves as control diet (without ACSF) and diets 2, 3 and 4 which contain respectively 10 %, 20 % and 30 % addition of ACSF serve as experimental diets. One hundred and eighty fishes were divided into four experimental groups and each group into three replicate of fifteen fishes stocked in 30-litre plastic bowls. Fish were fed with experimental diets twice a day at 3 % of their body weight for 7 days in a week and the experiment lasted for 56 days. The proximate analysis results showed an enhancement in carbohydrate content with a reduction in protein content as the level of incorporation is increasing. No significant differences (P≤0.05) were observed in protein efficiency rate, nitrogen metabolism and mean weight gain among the control and experimental diet. Haemoglobin and Packed cell volume obtained were highest in control group and lowest at 30 % inclusion. Biochemical parameter such as total protein and albumin reduced while globulin increased at 20 % and 30 % inclusion as compared to others. Histopathological reports showed some severe diffuse hepatic vacuolation on the liver of fish fed at 30 % inclusion while a severe congestion of the blood vessels was shown in the kidney of fish at 20 % inclusion. Areca catechu seed flour might not be toxic to catfish fingerling but would rather be a nutritional source and safe at 10 % inclusion level.

Key Words: *Areca catechu*, *C. gariepinus*, growth, toxicology and blood parameter

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1. INTRODUCTION

Fish require diets with proper balance of nutrients for normal growth and development from conventional and non-conventional sources. Available feedstuffs are used by man as food and raw materials for industries. Research is ongoing to source for alternatives with fish feeds generating a lot of interest (El-Dakar *et al*., 2008; Gabriel *et al*., 2007) since fish is an important and less expensive source of protein and minerals than meat. To optimize growth and produce high quality fish are the main goals of aquaculture industry (Bello *et al*., 2012). The rise in globalawareness of fish as a valuable source of protein has led to increased progress in aqua feeds with diets being specifically designed to meet the nutritional requirements of species, life cycle and health condition of fish (Rawling *et al*., 2012). The incorporation of feed additives in diets of aquaculture fish aimed to enhance fish performance, immunity and quality of fish. The search of new feed additives is still a very importantpoint for aquaculture researchers (Cho and Lee 2012). *Areca catechu* seed, regarded as an under-utilized plant product which is rich in carbohydrate, fibre and other phyto-chemical can be used as feed additive in the diet formulation of *Clarias gariepinus* juveniles. Feed additives are substances which are usually included in feeds in trace amounts to preserve its nutritional characteristics prior to feeding (antioxidants and mould inhibitors); facilitate ingredient dispersion or feed pelleting (emulsifiers, binders or stabilizers); promote growth (growth promoters, antibiotics and hormones); facilitate feed intake and

acceptance (feeding stimulants and colourants); or supply some essential nutrients such as minerals and vitamins (Food and Agricultural Organization, 1987).

The essential nutrient requirements of fish are protein, carbohydrate, lipid, minerals and vitamins. These nutrients depend on the age, species, production functions and environmental conditions (Falaye, 1992). In Nigeria today, soy bean, ground nut cake, fish meal among others are the major sources of protein while corn bran, wheat offal and maize are the major sources of carbohydrate in fish feed formulation. The cost of soy bean and mostly maize are relatively high compare to other sources as a result of their high human competing uses and demands especially in most developing African countries. This has made it very important to look for and evaluate other ingredient to replace maize and soy bean as sources of energy in formulating fish feed (Olurin *et al*., 2006). *Areca* nut (*A. catechu*), a slender single trunked palm that can grow up to 30 meters, popularly known as "betel nut" is one of the oldest known masticatories amongst Asia (Trease and Evans, 2009).

Areca catechu L. (*Palmea*, *Arecaceae*) from the *Arecaceae* family is one of the popular traditional herbal medicines grown in India, Malaysia, Taiwan, Far East Asia, and the South Pacific. The main constituents of *Areca catechu* seed are carbohydrate, fibre, polyphenol including flavonoids, tannins, alkaloids and minerals (IARC 2004). The fatty acid constituents of the seed oil include lauric acid, palmitic acid, stearic acid, decanoic acid, oleic and linoleic acids among others (Anonymous 1985). The use of *Areca catechu* seed was recommended in many diseases, such as leucoderma, leprosy, anaemia and obesity. It has been used as a vermifuge (Sharan, 1996) and was cited for antibacterial and antiviral activity (Reena *et al.*, 2009).Azeez *et al*. (2007) showed that the alkaloid andpolyphenols of *Areca catechu*could be used to enhance thehealing of burn wounds, leg ulcers and skin graft surgery.The seed has anthelmintic, antifungal,antibacterial, anti-inflammatory and antioxidant activities(Wetwitayaklung *et al*., 2006). In rural region and local communities where there is food deficit, it is used as an appetite suppressant (Strickland *et al*., 2003). Chewing quid comprising *Areca* nut and tobacco had adverse effects onperiodontal tissues, oral hygiene and incidence of orallesions (Parmar *et al*., 2008). *Areca catechu* seed chewing has alsobeen associated with reduced rates of dental caries andchanges in the oral microbiological flora (Reichart *et al*.,2002) while contributing significantly to oral health-relatedmorbidity and mortality (Trivedy *et al*., 2002), andadditionally, is an independent risk factor forhepatocellular carcinoma (Tsai *et al*., 2004). Hung *et al*. (2006) determined its effects on phagocytisis, chemotoxix and adhesion of human neutrophits. Despite of all researches already been done on *A. catechu* seeds, there are still lack of information on the nutritional composition and utilization of this seed flour in food system.This study therefore aims at evaluating the nutritional and toxicological evaluation of graded levels of *Areca catechu* seed flours as an additive in feed formulation and performance of catfish fingerlings (*Clarias gariepinus*).

2. MATERIALS AND METHODS

Sampling and sample treatment

Mature*Areca catechu*seeds were picked from the trees within the University of Ibadan, Ibadan in Oyo State, Nigeria. The sample was collected in black polythene bag and transported to the laboratory. Prior to the analyses, the sample was authenticated at the Herbarium unit, Botany department, University of Ibadan, Nigeria. The sample was thoroughly washed with distilled water and air dried. The thin layers covering the seeds were manually decorticated to remove the kernels. Only healthy looking kernels, without infection or damage, were chosen for the analyses. The kernels of *Areca catechu* seeds were air dried, grounded using a mechanical grinder and to give the coarse seed flour. The coarse seed flour obtained was further dried, pulverized to fine powder and stored at room temperature for the analyses.

Proximate analysis

At the end of feeding experiment, five fishes were selected from each plastic bowls. They were then pooled together, oven dried and homogenized for proximate composition (total of 15 fish per treatment). Moisture, crude fat, crude protein, ash and crude fibre contents of *Areca catechu* seed flour and the compounded feeds were all determined using Standard methodsdescribed by Association of Official Analytical Chemist, AOAC (2000). The ash content was determined by the incineration of 2 g dried sample in a muffle furnace at 550 ^{0}C for 2 hrs. Crude fat was obtained by soxhlet extraction of 2 g of the dried sample for 8hrs using n-hexane. The nitrogen (N) content was estimated by micro-Kjeldahl method and crude protein content was calculated as N % x 6.25. The available carbohydrate content was determined by difference. . Triplicates of diet samples were used for proximate analyses.

Mineral analysis

Mineral determined in *Areca catechu* seed flour, compounded feed and the fish under this studied were magnesium, calcium, sodium and potassium. The mineral analyses were carried out after digestion of 2 g of dried sample with 20 ml mixture of nitric acid (70% v/v) andperchloric acid (90% v/v) in the ratio 2:1 respectively (Onyeike and Acheru,2002 & Ajayi, 2015).The digests were carefully filtered into 100 ml standard bottle and made up to mark with deionized water.Calcium and magnesium were analyzed by atomic absorptionspectrophotometry (Perkin - Elmer Model 703, Norwalk CT, USA) while sodium and potassium were determined using a flame photometer (Model, 405, Corning, UK). All determinations were done intriplicate.

Experimental conditions for the fish

One hundred and eighty fingerlings of *C. gariepinus* (mean body weight: 5.75±0.70 – 5.79±0.95 g) were obtained from the Teaching and Research Farm of Department of Aquaculture andFisheries Management, University of Ibadan, Nigeria. They were kept in twelve circular 40-litres plastic bowls and allowed to acclimatize for 7 days while feeding with an imported commercial feed at 3 % body weight. After acclimatization, the fishes were divided into groups of 15 fish per bowl to the 12 circular bowls (three bowls of replicates per treatment). Each bowl was filled with 35 liters of de-chlorinated water and covered using synthetic nets toprevent the fish from jumping out of the bowl and protect them from foreign materials and invasion of insects. Water quality parameters i.e. temperature and pH were monitored weekly at regular interval using thermometer and pH meter respectively.

Fish feed formulation, preparation and feeding

The feed ingredients purchased for this study included fishmeal, soybean meal, maize, wheat offal,vitamin/mineral premix, millet, starch, calcium phosphate, salt, vegetable oil andgroundnut cake (Table 1).These ingredients were mixed together to produce a 35 % crude protein diet.ASCF was added to the total feed formulated as an additiveat 0 % (control), 10 %, 20 % and 30 % experimental diets. Each diet mixture was treated separately, extruded through a 4mm die mincer ofHobart A-200T pelleting machine (Hobart GmbH, Rben-Bosch, and Offenburg, Germany). The diets were sun-dried,brokenmechanically into suitable sizes for the fish, packaged in labeled polythene bags and stored at room temperature.Fishes were fed by hand twice daily at 3 % body. The mean weight and length of fish in each treatment were recorded weeklyusing a digital scale (model EHA 251) and a 30 cm ruler respectively. The experiment lasted for 56 days.

Evaluation of growth performance and feed utilization efficiency

The growth performance parameters were measured according to the methods described by Oleva- Novoa *et al.* (1990). Mean weight gain (MWG) was calculated as the difference between the initial and final weight divided by the number of the surviving fish at the end of the feeding period. Specific growth rate (SGR)is the relationship of the difference in the weight of the fish within the experimental period. Feed conversion ratio (FCR) was determined by dividing the total weight of the food given by the total weight gained by the

fish over a period of time while feed intake (FI) was calculated as the addition of daily mean feed intake of the fish during the period. Average daily growth (ADG) was calculated as the difference between the final weight and the initial weight divided by the number of days for the experiment.

Mean Weight Gain (MWG): = Initial mean weight - Final mean weight

Specific growth rate (SGR):

$$SGR = \frac{(\ln W_2 - \ln W_1) \times 100}{T}$$

Where: Ln = Natural log, W_1= initial mean weight, W_2 = final mean weight and T = time interval

Relative growth rate (RGR):

$$RGR = \frac{\text{Weight gain by fish(g)} \times 100}{\text{Initial body wieght (g)}}$$

Survival rate % (s):

$$S = \frac{\text{final number of fish at the end of experiment} \times 100}{\text{Initial number of fish at the begining of experimrnt}}$$

Nitrogen metabolism (NM):

$$NM = \frac{(0.549)\ (a + b)h}{2}$$

Where: a = initial mean weight of fish (g), b= final mean weight of fish (g), h= experimental period in days

Protein efficiency ratio (PER):

$$PER = \frac{\text{Wet body weight gain (g)}}{\text{Crude protein fed}}$$

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Condition factor (K): $K = \frac{W(g)}{L^3}$ **7.**

Where W is weight of the fish and L is standard length.

Haematological and biochemical analysis

The haemtological and biochemical analyses of the fish were evaluated at the beginning (0 day) and the end of the fifty six days of the feeding. Three fishes were randomly selected for each treatment for haematological and biochemical analyses. Blood samples were collected through the lateral line of fish for each treatment into ethylenediamine tetra acetic acid (EDTA) bottles by the use of needle and syringe. Packed cell volume (PVC), red blood cell (RBC), white blood cell (WBC), haemoglobine (Hb)and other haematological parameters were assessed including platelets, monocytes using the method described by Jain (1986) and Kelly (1975). Biochemical parameters such total blood protein, alkaline aminotransferase (ATP), aspartate aminotransferase (AST), albumin (ALB), creatinine and urea were all determined using the method described by Henry (1974). All analyses were done in triplicate.

Tissue pathology

The histopathology of selected organs such as heart, gill, kidney and liver collected from the fish randomly picked from the bowl was examined. These organs were collected, fixed in 10 % formalin and then passed through a series of dehydration in graded concentrations of xylene. Sections of the tissues were taken out and assessed using the methods of Jain (1986).

Statistical analysis

The data obtained during the feedingtrial were expressed as mean values±standard deviation.Organ weights, biochemical and haematological determinations and others were subjected to the statistical analysis of variance (ANOVA) using SPSS(Statistical Package for Social Sciences)while the difference among individual means was determined by the use of Duncan Multiple Range Test. A probability level less than 5 % ($p \leq 0.05$) was considered significant.

3.RESULTS AND DISCUSSION

Proximate analysis of ACSF, formulated diets and fish after experiment

The results of the proximate composition of ACSFand the compounded feeds examined are shown on Table 2 while those of the fishes after experiment are given in Table 3. It could be observed from Table 2 that there were significant ($p \leq 0.05$) differences among the various feed compounded for this experiment in their protein, fat, crude fibre, ash, moisture and carbohydrate contents. Proteins are essential component of the diet that is needed for survival of animals, humans and of which basic function in nutrition is to supply adequate amounts of required amino acids(Pugalenthi *et al*., 2004).

Table 1. Gross composition of experimental diets prepared for fish (%)

Ingredients	0%	10%	20%	30%
Fish meal	24.2	24.2	24.2	24.2
Soy beans	24.5	24.5	24.5	24.5
GNC	24.3	24.3	24.3	24.3
Corn meal	19	19	19	19
Fish oil	1.5	1.5	1.5	1.5
Chromic oxide	0.5	0.5	0.5	0.5
Vitamin premium	2	2	2	2
DCP	1	1	1	1
Starch	3	3	3	3
Total	100	100	100	100
Areca seed flour	0%	10%	20%	30%

Table 2. Proximate composition of formulated diets used in the experiment (%)

Parameters	ACSF	0%	10%	20%	30%
Carbohydrate	57.55± 0.01^{a}	35.67 ± 0.12^{b}	36.42 ± 0.06^{b}	43.26 ± 0.12^{c}	45.08 ± 0.24^{c}
Crude protein	6.42± 0.13^{d}	37.94 ± 0.13^{a}	36.48 ±0.127^{a}	32.03 ± 0.16^{b}	30.28 ± 0.13^{b}
Moisture	14.54± 0.04^{a}	10.07 ± 0.01^{c}	10.57 ±0.035^{b}	10.50 ± 0.02^{b}	10.86 ±0.05^{b}
Crude fiber	18.28±0.02	4.53 ±0.03	7.28 ± 0.03^{d}	9.10 ±0.02^{c}	10.47 ±0.01^{b}
Ash	3.22± 0.00^{d}	10.63 ± 0.06^{a}	10.93 ± 0.16^{a}	9.28 ± 0.03^{b}	9.07 ±0.07^{c}
Crude fat	0.27±0.01^{d}	5.68 ± 0.03^{a}	5.59 ±0.03^{a}	4.92 ±0.03^{b}	4.71 ± 0.03^{c}

Means of three determinations ± SD, mean values in columns with different letters are significantly different at ($P \leq 0.05$).

Table 3. Proximate analysis of the fish after two months treatments

Parameters	0%	10%	20%	30%
Crude protein	80.43 ± 0.08^{a}	75.67 ±0.13^{d}	78.22 ±0.12^{c}	79.55 ±0.05^{b}
Ash	06.20 ± 0.03^{d}	09.70 ± 0.02^{b}	07.92 ± 0.03^{c}	10.24 ± 0.02^{a}
Moisture content	04.22 ± 0.01^{b}	05.37 ± 0.03^{a}	03.88 ± 0.03^{d}	04.13 ± 0.03^{c}
Crude fat	09.05 ± 0.03^{a}	09.07 ± 0.03^{c}	09.24 ± 0.01^{b}	06.07 ±0.02^{d}
Crude fiber	0.00	0.00	0.00	0.00

Means of three determinations ± SD, mean values in columns with different letters are significantly different at ($P \leq 0.05$).

The ACSF crude protein content is 6.42±0.13%. This is lower than 23.24 ± 1.45 % in the seed of *Neocarya macrophyla* reported by *Muhammad et al*. (2015) but higher than 4.5±0.05 % reported for African star apple kernel (Akubor *et al*., 2013). The main function of carbohydrate is for energy supply in the food system. The ACSF, carbohydrate content is 57.55±0.01 %. This value is higher than 6.04±1.2 % reported for *Neocarya macrophyla*(Muhammad *et al*. (2015). It is also higher than 26.79±0.02 reported on *Sesamum indicum* seed flour by Makinde *et al*. (2013). The high carbohydrate content of 57.55±0.01% suggests that ACSF could supplement the energy requirement of some of our daily activity or supply energy in livestock rations. The moisture content of ACSF is 14.54±0.04 %. The value obtained is higher than 7.43±0.01% and 6.79±0.02% reported on*T. occidentalis* and *A. catechu* seed flour (Ajayi *et al*., 2013 and 2015). The moisture content obtained is higher than 10.57% and 12.55±1.11 recorded for *N. macrophyla* seed flour (Muhammad *et al*., 2015 and Tidjani, 2010). It is reported that moisture content might be associated with rise in microbial activities during storage (Hassan *et al*., 2013) and even the storage condition. Ash represents the mineral matter left after feeds are burnt in oxygen; it is used as a measure of the mineral content in any sample (Pearson, 1981). The ash content of ACSF is 3.22±0.00 %; it is lower than 4.5±0.2 % and 4.4±0.1 % obtained respectively for Kenaf seed (Mario *et al*., 2010) and *P. africanum* (Aremu *et al*., 2007), lower than 4.75±0.04 % and 4.61±0.12 % obtained for *T. tetraptera* and *M. monodora* seeds (Aguomo *et al*., 2011). The ash content obtained for ASCF is higher than 2.55±0.12% and 1.6±0.02 % reported respectively for Nicker bean seed flour (Ogungbele *et al*., 2015) and cashew seed flour (Emelike *et al*., 2015).

The proximate compositions of the diets were presented in tables 1. All diets were formulated with similar gross compositions except for the addition of 10 %, 20 % and 30 %of the areca seed flour to the experimental diets.The inclusion of ACSF into the diet led to a significant difference in the carbohydrate contents of the formulated diet. The carbohydrate content increased from 35.67 % at control (%) through 36.42 %, 43.26 % for both 10 % and 20 % with the highest value of 45.08 % for 30 % diet. The inclusion of ACSF also reduced the protein content of the diet formulated. The protein content decreased from 37.94 % in the control diet trough 36.48 % and 32.03 % respectively for 10 % and 20 % formulated diets with the lowest value of 30.38 % for 30 % diets. A gradual decrease was obtained for the ash contents while gradual decrease was observed for the crude fibre in the various concentrations. ACSF seemsto be a very good source of carbohydrate.

The proximate composition of the fish after the experiment periods is shown in Table 3. And revealed a high protein content in all the fish fed with all the diets. The protein content obtained is higher in the control (0 %) and 30 % than the other experiment diets. Moisture content was found to be highest at 10 % diet while ash content was also highest at 30 % diet.

Mineral element of Areca seed flour, compounded diet and the fish after experiment

The mineral composition of ACSF formulated diets and the fish after experiment is shown in table 4. ACSF seed flour has high concentration of calcium (249.84 mg/100g), potassium (265.17 md/100g) and sodium (239.81 mg/100g). Values of 78.76mg/100g, 489.12 mg/100g, and 312.55 mg/100g were reported respectively for sodium, potassium and calcium in *N. macrophylla* seed flour (Muhammad *et al*., 2015). These values obtained are in accordance with those reported earlier for ACSF (Ajayi *et al*., 2015). With the incorporation of the ACSF in the various compounded diets, there is significant difference in the minerals obtained. Calcium, magnesium and potassium obtained are higher in all the diets than the control group. Calcium is the highest in 10 % diet (286.52 mg/100g), magnesium is the highest in the 20 % diet (65.58 mg/100g) in the 20 % diet followed by 63.04 mg/100g in the 10 % diets. Potassium is the highest in the 20 % diet (316.24 mg/100g)

and sodium is the highest in 10 % diet (253.08 mg/100g). The mineral composition of the fish fed with different dietsalso revealed a gradual increase in all these nutrients as compared to the control diet. Potassium was highest in 10% diet (300.52 mg/100g), calcium in 20% diet (160.54 mg/100g), both magnesium (40.14 mg/100g) and sodium were highest in 30% diet (162.23 mg/100g).

Water quality parameter

Water temperature in the experimental systems ranged from 28.0-29.0 ^{o}C with an average value of 28.5 ^{0}C while pH ranged from 7.4 and 7.6 (Table 4). The water quality parameters in all the treatments were within the recommended limits for warm-water fishes (Boyd andGross, 2000; Ajani, 2006).Moreover, fish responded very well to the diets in all treatments from the initial time of the experiment to the end.

Growth response and protein utilization efficiency

The growth response and protein utilization efficiency by catfish fed on ACSFinclusions are summarized on Table 6. It reveals that the best overall body weight gain was obtained in fish fed with 0 % diet (control) diet (8.14±1.79),followed by (7.88±1.05) in 30 % diet and theleast weight gain was recorded in fish fed with 10 %.(7.20±1.12). These responses of fish to the different diets showed thatgrowth and nutrient utilization differed significantly ($p \leq 0.05$) among the treatments. Feed conversion ratio, specific growth rate, percentage weight gain, daily growth rate, gross feed conversion efficiency and survival rate in fish did not differ significantly among the diets. The nitrogen metabolism was found to be highest (307.23±53.8) in 30 % diet followed by 278.70±22.68 in the control diet. The condition factor increase gradually from 0.80 at 10 % inclusion to 0.95 at 30 % inclusion but 0.80 at 10 % compare favorably to 0.82 at 0 % inclusion. These values reveal that ACSF could really be used as an additive in fish feed formulation as the results obtained in all the experimental diets are comparable to those of the control diet.

Heamatology analysis of fish

The heamatological analyses were also represented in Table 7. The result shows no significant differences in most of the blood parameters of the fish fed with control and other experimental diets. The values obtained in the experimental fish are higher than those taking before the experimental period. Evaluation of hematological and blood chemistry analyses will enhance the culture of fish by facilitating early detection of infectious diseases and identification of sub-lethal conditions that may affect the production performance. MCV, heterophils and eocinophils decreased while other parameters increased as compared to the initial values. PVC decrease gradually from the control diet (29.33%) followed by 25.67% at 10% diet to the lowest (23.33%) at 30 % diet. Haemoglobin and red blood cell had the least values at 30% diet. The slight changes observed in some of the blood parameters of the fish during the experiment might be due to the presence of anti-nutritional factors in the feeds (Ajayi *et al*., 2013).

Table 4. Metal composition of *Areca catechu* seed flour and the feed used (mg/100g)

	Seed flour	Control (0%)	10%	20%	30%
	Compounded	**Feed**			
Ca	249.84 ± 1.15^{b}	148.24 ± 1.78^{e}	286.52 ± 0.280^{a}	205.76 ± 0.733^{c}	191.52 ± 1.143^{d}
Mg	68.19 ± 2.32^{a}	44.17 ± 0.07^{e}	63.04 ± 0.20^{c}	65.58 ± 0.42^{b}	59.03 ± 0.91^{d}
K	265.17 ± 2.63^{c}	180.24 ± 0.04^{d}	267.27 ± 0.17^{c}	316.24 ± 0.16^{a}	277.57 ± 1.20^{b}
Na	239.81 ± 1.22^{b}	217.29 ± 0.09^{c}	253.08 ± 0.07^{a}	198.59 ± 0.30^{d}	192.35 ± 1.81^{e}
	Fish used after	**eight weeks**	**of experiment**		
Ca	249.84 ± 1.15^{a}	26.35 ± 0.05^{e}	72.59 ± 0.09^{d}	160.54 ± 0.23^{b}	137.22 ± 0.58^{c}
Mg	68.19 ± 2.32^{a}	28.59 ± 0.09^{d}	34.58 ± 0.08^{c}	39.17 ± 0.13^{b}	40.14 ± 0.03^{b}
K	265.17 ± 2.63^{c}	241.52 ± 0.12^{d}	300.52 ± 0.02^{a}	291.54 ± 0.22^{b}	198.47 ± 0.06^{e}
Na	239.81 ± 1.22^{a}	41.11 ± 0.10^{d}	84.76 ± 0.16^{c}	85.36 ± 0.24^{c}	162.23 ± 0.20^{b}

Means of three determinations ± SD, mean values in columns with different letters are significantly different at ($P \leq 0.05$).

Table 5. Mean weekly values of water quality parameters of the experimental plastic bowl

Week	1	2	3	4	5	6	7	8	Average
Temperature	28	27	29	28	28	29	28	29	28.25
pH	7.4	7.6	7.6	7.6	7.9	7.6	7.7	7.6	7.63

Table 6. Growth, feed utilization and % survival rate of *Clarias gariepinus* fed with different concentration of *A. catechu* seed flour.

Parameter	0%	10%	20%	30%
Initial body weight (g)	4.99 ± 0.62^{a}	4.66 ± 0.43^{a}	4.55 ± 0.25^{a}	5.53 ± 1.00^{a}
Final body weight (g)	13.14 ± 1.52^{a}	11.87 ± 0.83^{a}	11.77 ± 0.80^{a}	13.45 ± 1.46^{a}
Body weight gain (g)	8.14 ± 1. 79^{a}	7.20 ± 1.12^{b}	7.39 ± 0.72^{b}	7.88 ± 1.05^{a}
Relative growth rate (%)	166.22 ± 49.05^{a}	156.66 ± 38.74^{a}	164.39 ± 12.96^{a}	145.32 ± 29.45^{b}
Mean weight gain	54.27 ± 11.96^{a}	48.03 ±7.76^{a}	49.50 ± 4.96^{a}	52.53 ± 6.98^{a}
Daily growth rate (g/day)	0.15 ± 0.03^{a}	0.13 ± 0.02^{a}	0.13 ± 0.01^{a}	0.14 ± 0.01^{a}
Specific growth rate (%/day)	0.75 ± 0.14^{a}	0.73 ± 0.11^{a}	0.74 ± 0.05^{a}	0.69 ± 0.01^{a}
Feed conversion ratio (FCR)	1.32 ± 0.27^{a}	1.38 ± 0.12^{a}	1.32 ± 0.12^{a}	1.41 ± 0.01^{a}
Protein efficiency ratio	0.27 ± 0.06^{a}	0.24 ± 0.03^{a}	0.25 ± 0.02^{a}	0.26 ± 0.03^{a}
Nitrogen metabolism	278.70 ± 22.68^{a}	254.20 ± 9.92^{a}	251.02 ± 14.53^{a}	307.23 ± 53.84^{a}
GFCE	77.85 ± 7.86^{a}	72.83 ± 6.35^{a}	75.97 ± 1.64^{a}	71.05 ± 3.77^{a}
Condition factor	0.82 ± 0.05^{a}	0.80 ± 0.14^{a}	0.92 ± 0.20^{a}	0.95 ± 0.28^{a}
Mean length gain (cm)	3.51 ± 0.29^{a}	3.37 ± 0.43^{a}	2.83 ± 0.36^{a}	3.57 ± 1.28^{a}
Survival rate (%)	93.33	91.43	88.02	92.50

Means of three determinations ± SD, mean values in columns with different letters are significantly different at ($P \leq 0.05$). GFCE = Gross feed conversion efficiency

Table 7. Haematological parameters of the A. catfish (*Clarias gariepinus*) juveniles fed with graded level of *A. catechu* seed flour

	Initial value	Control (0%)	10 %	20%	30%
PVC (%)	14.00 ± 0.00^{c}	29.33 ± 0.58^{a}	25.67 ± 0.58^{b}	25.33 ± 0.58^{b}	23.33 ± 2.88^{b}
HB (g/dl)	4.67 ± 0.23^{c}	9.20 ± 0.69^{a}	8.60 ± 0.10^{ab}	8.50 ± 0.00^{ab}	7.93 ± 0.98^{b}
RBC (x10^{12}/L)	1.29 ± 0.12^{c}	3.49 ± 0.06^{a}	2.55 ± 0.13^{b}	2.54 ± 0.06^{b}	2.26 ± 0.37^{b}
WBC (x10^{12}/L)	19.88 ± 0.08^{a}	17.52 ± 0.81^{c}	18.12 ± 0.39^{bc}	17.48 ± 0.20^{c}	18.60 ± 0.43^{b}
PLAT (x 10^{12}/L)	170.33 ± 0. 58^{a}	172.67 ± 56.580^{b}	189.00 ± 1.73^{a}	173.50 ± 1.17^{ab}	221.33 ± 28.87^{a}
MCV (fl)	108.90 ± 10.12^{a}	84.04 ± 2.96^{a}	101.28 ± 2.90^{a}	99.48 ± 0.20^{a}	103.62 ± 4.48^{b}
MCH	36.61 ± 1.88^{a}	36.38 ± 2.40^{a}	33.55 ± 1.38^{a}	33.75 ± 0.84^{a}	35.23 ± 1.76^{b}
MCHC	31.96 ± 1.65^{a}	32.26 ± 1.70^{a}	33.39 ± 0.89^{a}	33.96 ± 076^{a}	34.00 ± 0.00^{a}
Lymphocyte (%)	66.00 ± 1.00^{a}	66.68 ± 6.35^{a}	68.33 ± 2.89^{a}	75.33 ± 2.89^{a}	66.00 ± 10.39^{a}
Heterophils (%)	28.33 ± 2.08^{a}	30.67 ± 1.15^{a}	18.67 ± 14.74^{a}	21.33 ± 4.61^{a}	26.67 ± 11.54^{a}
Monocyte (%)	2.33 ± 0.57^{a}	3.68 ± 0.58^{ab}	2.33 ± 1.15^{b}	3.33 ± 0.58^{ab}	4.33 ± 1.15^{a}
Eosinophil (%)	3.00 ± 0.00^{a}	1.67 ± 1.15^{a}	2.67 ± 1.15^{a}	3.00 ± 0.00^{a}	2.67 ± 0.58^{a}
Basophils	0.00 ± 0.00^{a}	0.33 ± 0.57^{a}	0.33 ± 0.57^{a}	0.33 ± 0.57^{a}	0.33 ± 0.57^{a}

Means of three determinations ± SD, mean values in columns with different letters are significantly different at ($P \leq 0.05$). HB= Haemoglobin concentration PCV = Packed cell volume, RBC = Red Blood Cells Counts, WBC = White Blood cells counts. MCV = mean corpuscular volume, MCHC = mean corpuscular haemoglobin concentration, MCH = mean corpuscular haemoglobine and PLAT = Platelets

PVC, RBC and HB decreased gradually from control diet to 30 % diet with the highest in the control and the lowest in 30 % diet.

The major function of red blood cells in the body is to transport haemoglobin, which in turn carries oxygen from lungs to the tissues (Waugh and Grant, 2001). A very low reading of RBC, haemoglobin and PVC will indicate anemia. Observations from this experiment suggest that ACSF might be a very good additive in fish at 10 %.

Table 8. Plasma protein and enzymes of the fish on *A. Catechu* seed flour-based diets at the end of the experiment

	Initial Value	Control (0 %)	10 %	20 %	30 %
Total Protein	4.67 ±0.40^{a}	4.23 ± 0.32^{a}	4.40 ± 0.53^{a}	4.10 ± 0.20^{a}	4.29 ± 0.31^{a}
Albumin	1.63 ± 0.58^{ab}	1.83 ± 0.58^{a}	1.90 ± 0.43^{a}	0.96 ± 0.84^{c}	1.46 ± 1.52^{ab}
Globulin	2.23 ± 0.15^{c}	2.40 ± 0.26^{ab}	2.5 ± 0.17^{ab}	2.70 ± 0.20^{a}	2.63 ± 0.29^{ab}
A/G ration	0.73 ± 0.25^{ab}	0.76 ± 0.06^{a}	0.76 ± 0.15^{a}	0.54 ± 0.60^{c}	0.57 ± 0.12^{bc}
AST	177.00 ± 1.73^{ab}	167.67 ± 51.28^{ab}	208.00 ± 47.28^{a}	137.33 ± 27.39^{c}	199.00 ± 7.21^{ab}
ALT	32.33 ± 0.57^{ab}	34.33 ± 1.5^{a}	21.33 ± 3.51^{c}	23.33 ± 4.16b^{c}	19.67 ± 0.57^{c}
ALP	90.00 ± 1.73^{b}	227.33 ± 12.22^{a}	242.67 ± 38.7^{a}	240.00 ± 44.03^{a}	206.80 ± 65.25^{a}
Bun	6.67 ± 0.57^{a}	5.00 ± 1.00^{a}	6.33 ± 1.52^{a}	6.33 ± 0.57^{a}	6.67 ± 1.52^{a}
Creatinine	0.53 ± 0.57^{a}	0.5 ± 0.1^{a}	0.6 ± 0.1^{a}	0.46 ± 0.11^{a}	0.50 ± 0.10^{a}

Values are expressed as mean ± standard deviation (n=3). Values in the same row with different superscripts are significantly different at P≤0.05. AST- Aspartate aminotransferases, ALT- Alanine aminotransferases, ALP = Alkaline phosphatase; ALB = Albumin; GLB = Globulin; ALB/GLB = Albumin – Globulin ratio; TP = Total Protein.

Table 9. Summary of histopathology of tissues of the control and experimental fish

	Control (0 %)	10 %	20 %	30 %
Heart	No visible lesions seen	No visible lesions seen	No visible lesions seen	No visible lesions seen
Liver	No visible lesions seen	There is mild diffuse hepatic vacuolation. Not pathologic as the nuclei are centrally placed and not displaced by the vacuoles.	There is a severe diffuse vacuolation. There is also a mild portal congestion. There is also a moderate proliferation of the portal ducts.	There is a severe diffuse vacuolation. There is also a mild portal congestion. There is also a moderate cellular infiltration of the portal area
Kidney	The interstitial spaces appear filled with pink staining fluid. No lesion	No visible lesions seen	There is a severe congestion of the blood vessels	The blood vessel are congested
Gills	No visible lesions seen	No visible lesions seen	No visible lesions seen.	No visible lesions seen

Result of the blood biochemistry of the fish samples

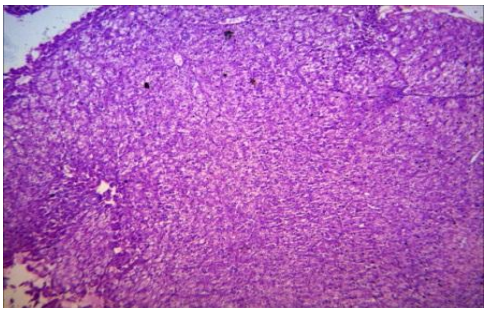
Theserum proteins (albumin and globulin) and blood serum enzymes (aspartate amino transferase and alanine amino transferase) were investigated and the results are summarized in Table 8. The mean values of total protein obtained in all the experimental diets are lower than those of the control and initial observations with the highest value of 4.40±0.53.

The albumin values of 1.83±0.58 and 1.90±0.43 were observed respectively for the control and 10 % diets. These values compared to each other but lower than those in of 20 % and 30 % diets. The enzymes AST and ALP (208.00±47.28and 242.67±38.7) obtained are the highest among the experimental diets. ALT values observe are 21.33±3.51, 23.33±4.16, and 19.67±0.57 for the three experimental diets as against 34.33±1.5 and 32.33±0.57 in the control diet and initial observation. ALT is a cytotoxic enzyme foundin very high concentration in the liver (Aliyu *et al*., 2006), and an increase ofthis specific enzyme indicates hepatocellular damage. Increase in ALT and AST are also clinical indication of diagnosing state of damage doneto visceral organ by toxic substance or infection (Ewuola and Egbunife 2008).

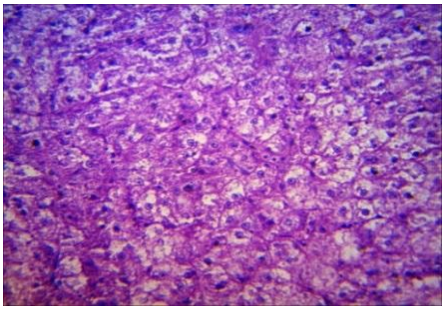
Result of the histological analyses of fish

The histopathology of the fish after the experiment is summarized on Table 9 while the photomicrographs observed with microscopy (x550) are shown in fig (1&2). The fish fed with the control diet showed no lesions in the kidney, liver, heart and gills (fig 1a & fig 2e).

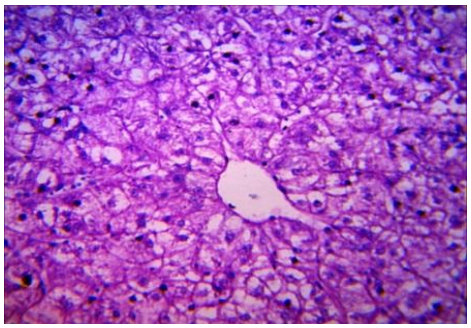
The fish on 10% diet inclusionexhibited mild diffuse hepatic vacuolation in the liver (fig 1b) but no lesions in the kidney, heart and gills. No lesions were observed in the heart and gills of the fish fed with 20% diet but a severe diffuse vacuolation, a mild portal congestion and a moderate proliferation of the portal ducts were seen in the liver (fig 1c) while a severe congestion of the blood vessels was also observed in the kidney (fig 2f). On 30% diet inclusion of ACSF, fish showed no lesions in the heart and gills but the liver exhibited severe diffuse vacuolation, mild portal congestion and moderate cellular infiltration of the portal area (fig 1d). The blood vessels are also congested in the kidney. Most visible changes were exhibited by the liver and the kidney. This is because the liver is responsible for dealing with all chemicals within the body. The liver is the organ involved in the metabolism, detoxification and excretion of chemicals and xenobiotics in the body (Pathan *et al.*, 2010). Vacuolation has been observed to be a common response to the presence of chemicals in fish (Clearwater *et al*., 2002). Ajayi *et al*.(2015) reported in a previous work on ACSF fed to albino rats that severe portal and central venous congestion was exhibited in the kidney of the 20% group while there were moderate periportal and diffuse cellularinfiltration by macrophages and few lymphocytes inthe liver of the 30% experimental group.This suggests that 10 % diet inclusion of ACSF among other diets might be very good since no lesions were observed in the tissues studied.



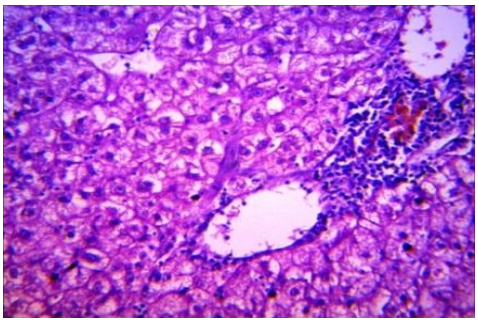
(a) Liver of the control (0 %) showing no visible vacuolation and mild central venous



(b) Liver of 10 % fish showing a very mild diffuse lesion portal congestion

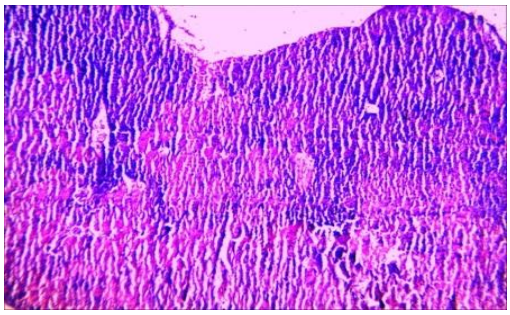


(c) Liver of 20 % fish showing severe diffuse vacuolation, mild portal congestion and moderate cellular infiltration of the portalarea

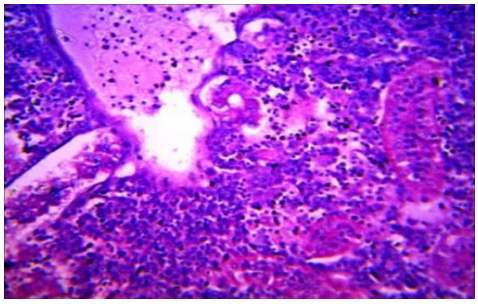


(d) Liver of 30 % fish showing severe diffuse vacuolation, mild portal congestion and moderate proliferation of the portal ducts

Fig 1. Photomicrograph of the liver of 0 %; 10 %, 20 % and 30 % groups of fish fed with *Areca catechu* seed flour (x550)



(e). Kidney of control (0 %) fish showing no visible lession



(f.) Kidney of 20 % fish showing severe congestion of the blood vessels

Fig. 2: Photomicrograph of the kidney of 0 % and 20 % groups of fish fed with *Areca catechu* seed flour (x550)

4. CONCLUSION

This study showed that *Areca catechu* seed flour might find usefulness as an additive in the fish feed due to its high carbohydrate content. The feed however needs to be supplemented with other highprotein residue such as groundnut cake, balanite and soy bean cake because of its low protein content. The higher the inclusion of ACSF in the diets formulated, the higher the carbohydrate content found in the diets. The minerals that are found in ACSF and compounded diets are all useful in making the body strong. Theincorporation of ACSF at 10% level into fish diets didnot produce any significant changes in haematologicalparameters as well as in the heart, kidney, gills and liver of the fishes. It can therefore be suggested that ACSF might be a good additive in fish feed at 10% level of inclusion.

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