Dietary African yam bean meal (*Sphenostylis sternocarpa)* as a complementary substitute for soybean meal in African catfish (*Clarias gariepinus)* fingerlings: Effect on intestinal histology and zootechnical performance

Abstract:

Background: *The effects of soybean meal replacement with African yam bean meal on the intestinal histology and zootechnical performance of African catfish, Clarias gariepinus fingerlings was assessed in this study. One hundred and eighty C. gariepinus fingerlings weighing 13.62±0.12g were randomly distributed into plastic tanks at ten fish per tank in triplicate and fed six rations (treatments) representing graded replacement levels of soybean with African yam bean for 70 days. Soybean meal was replaced with African yam bean (AYB) at 0, 20, 40, 60, 80 and 100% denoted as the control T1 (0%), T2 (20%), T3 (40%), T4 (60%), T5 (80%) and T6 (100%) respectively. Weekly weighing of fish was done and data collected was subjected to statistical analysis. Zootechnical performance was based on growth performance and nutrient utilization efficiency.*

Results*: After the feeding trials, tissue samples from the intestines were removed by dissection for histological study. Tissue sections were compared after examination under the microscope for differences in the morphology of the tissues. The histology of the intestine of fish fed African yam bean meal included diets showed apparently normal intestinal villi and enterocytes in T2, T3 and T4. Histology of the intestine of C. gariepinus in T6 had disorganized enterocytes which induced enteritis in the fish. The result on growth performance and nutrient utilisation showed that body weight gain, specific growth rate and feed conversion ratio were significantly different from each other (P< 0.05). The best feed conversion ratio was recorded in the control.*

Conclusions: *This study showed that African yam bean can replace soybean up to 60% in the diet of African catfish, Clarias gariepinus for required growth without any negative effect on the gut histology of the fish.*

Keywords: Underutilised legumes, African catfish; African yam bean, Growth; Histology

Introduction

"There is a shortfall in global fish and fish products supply while the global demand is increasing as a result of increasing populations and public awareness of the health benefits of eating fish and fish products" (FAO, 2020). "The need to offset this shortfall in supply and stagnating global capture fisheries has led to an increasing focus on aquaculture to meet the shortage of fish products and cater for the growing demand of the ever – increasing population. Globally and especially in Africa, the aquaculture sub-sector of agriculture is emerging as an important production sector for high protein food. Aquaculture will continue to be relevant in the future as a source of protein. However, Gbadamosi and Lupatsch (2018) reported that the growth in aquaculture has caused major challenges; one of these challenges is the production of practical feeds for the farming of fish and shellfish. Increased fish farming has resulted in increased production of aquafeeds which depend heavily on fishmeal and legumes especially soybean as the main source of protein, because of its well-balanced nutrients" (IFFO, 2008). "Therefore, it has become imperative to assess different protein sources as cheaper and appropriate replacement for fishmeal and soybean in aquafeeds. Conventional and commonly traded ingredients at the local and international markets such as fish and fishmeal, soybean, fish oil, rice and wheat, in the human food and animal feed production industry are also common feedstuffs used in aquafeed production. There is therefore a stiff competition in the marketplace with the animal husbandry sector as well as with direct human consumption" (Globefish, 2014). Reduction in inclusion level of these conventional feedstuffs especially fishmeal and soybean will therefore be important to reducing feed costs and avoid competition with other users (FAO, 2020). Srenuja et al., (2023) stated that non-conventional phytogenic foods and their products especially grain legumes, which are highly valued for their important and essential macronutrients (protein and fiber) and micronutrients are becoming novel and emerging nutrients in the agricultural and agro-food industry as alternatives to conventional and common foodstuffs.

"Grain legumes belong to the family fabaceae of the Angiospermae and are significantly rich in high-quality proteins. They have high nutritive value and contain, on average, about twice as much protein as cereals" (Kamboj and Nanda, 2017). "They are adaptable under unfavorable ecological conditions, nutritious, and stress tolerant, possessing features for enhancing the sustainability of dry subtropical and tropical agricultural systems" (Adegboyega et al, 2020). "They also possess some significant health protective compounds such as phenolics and are considered to be an inexpensive dietary source of protein, minerals, carbohydrates, and vitamins" (Kamboj and Nanda, 2017). Grain legumes are used as animal feed and in other agricultural activities to generate income for small-holder farmers. Based on plant utility and economy, legumes are categorized into utilised and underutilized species. Utilised or major species are popular and common with well-established domestication and cultivation, agronomic practices, utilisation and domestication. Examples include soybean, *Glycine max*, cowpea, *Vigna unguculata*, and groundnut, *Arachis hypogea* etc. Underutilised or minor legumes are less known, less exploited and neglected. Some species in this category are African yam bean, *Sphenostylis sternocarpa*, winged bean *Psophocarpus tetragonolobus*, Pigeon peas, *Cajanus cajan* and Lima bean, *Phaseolus lunatus* to mention few. "In many developing countries underutilized legumes are found among crops with economic potential constrained by several factors such as limited global economic value, neglect by research and development projects, and agronomic concerns" (Adegboyega et al., 2020).

NRC (2011) reported that among the highly utilised legumes and plant sources of protein soybean meal still remains as most extensively used resulting in up to 60% inclusion in animal and aquafeeds. It is also the most evaluated substitute for fishmeal as a result of its availability, consistent quality, high protein content and relatively well-balanced amino acid. Furthermore, soybean meal is currently utilised routinely as a partial or total substitute for costly protein animal protein ingredients like fishmeal. Many studies have reported that the replacement of fishmeal with soybean meal was a low cost alternative in aquafeeds without impairing on the good growth and health performance of fish (FAO, 2020). However, soybean meal is deficient in methionine, lysine and threonine (NRC, 2011). Furthermore, there are some indications that soybean may induce enteritis in Atlantic salmon (Krogdahl et al, 2015). The need to replace conventional protein sources like fish and soybean meal has led to focus on unconventional feed stuffs especially inder utilised grain legumes like African yam bean.

"African yam bean (*Sphenostylis stenocarpa*) is one of minor lesser-known legumes with significant nutritional potentials. It had its origin in Ethiopia but is now widely cultivated in tropical Africa, especially West Africa, in Nigeria, Ghana and Cameroon" (Uchegbu and Amulu, 2015). "African yam bean contains approximately 30% crude protein in the grain which is lower than soybean which contains 42%" (Uchegbu and Amulu, 2015). However, biotechnological advancement in food technology like encapsualation, fermentation and sonification have been reported to increase the crude protein content of underutilised legumes with encapsulation efficiency (EE) of approximately 43.99%. "There is still low awareness of the productive and nutritional value of underutilised legumes like the African yam bean (*S. stenocarpa*). 'Subsistence production may have been caused by the low acceptability of African yam bean as a valuable crop among middle-aged farmers in Africa" because of the presence of antinutritional factors (ANFs) and longer cooking times" **(**Adegboyega et al., 2020).

"The African catfish, *Clarias gariepinus* is the most important fish species cultured in Nigeria; it grows rapidly, it is disease and stress resistant, sturdy and highly productive in polyculture with many other fish species" (FAO, 2020). "*C. gariepinus,* production is considered to be the fastest growing segment of the Nigeria aquaculture industry over the past decade" (Gbadamosi et al, 2017). The abundance of *C. gariepinus* in Nigeria and acceptability in the Nigerian markets have been attributed to its culturable attributes, good flavour and ability to attain a large size (Olorunyomi et al, 2021). "More investors are entering the African catfish, *C. gariepinus* farming in Nigeria as there exists a large unmet demand and market prices for catfish *C. gariepinus* which is more than those of other species" (Adeparusi, 1998). In the present study, the effects of soybean replacements with African yam bean meal, an underutilised legume on the intestinal histology and zootechnical performance of African catfish, *Clarias gariepinus* fingerlings was assessed to examine the potentials of *S. stenocarpa* as a phytogenic crude protein in aquafeed production.

Methodology

The experiment was conducted at the Department of Fisheries and Aquaculture Research farm, Obakekere, Federal University of Technology Akure, Ondo state, Nigeria. Powdered and defatted African yam bean grains was procured from a local market, in Akure, Ondo state, Nigeria and stored at room temperature prior to use for the fish feed formulation. The experiment consisted of six treatments, with each representing different replacement levels of African yam bean meal. Soybean meal was replaced with African yam bean (AYB) at 0, 20, 40, 60, 80 and 100% denoted as the control Treatment 1(T1), Treatment 2, T2 (20%), Treatment 3, T3 (40%), Treatment 4, T4 (60%), Treatment 5, T5 (80%) and Treatment 6, T6 (100%) respectively.

Preparation of experimental diet

Six experimental diets were formulated to provide 40% crude protein based on the formulation defined for African catfish, *C. gariepinus* fingerlings as defined by NRC (2011) as stated in Table 1. The ingredients were grinded to small particle size. All dietary ingredient were weighed with a weighing top balance (Metler Toledo, PB8001 London). Ingredients including were thoroughly mixed in a Hobart A-200 T mixing machine (Hobart Ltd, England) to obtain a homogenous mass. Cassava starch was added as a binder. The resultant mash was then pressed without steam through a mincer with a 4mm die attached to the Hobart pelleting machine. Diets were immediately sun-dried after pelleting and stored at 18^{0}C prior to the feeding trial.

Table 1. Composition of the experimental diet in g/100g dry matter containing various replacement level of African yam bean for *Clarias gariepinus*

INGREDIENT	T1 (control)	T2	T3	T4	T5	T6
Fishmeal (70%)	30.00	30.00	30.00	30.00	30.00	30.00
Soybean meal (42%)	44.00	35.20	26.40	17.60	8.80	0.00
African yam bean (30%)	0.00	8.80	17.60	26.40	35.20	44.00
Yellow maize (10%)	15.00	15.00	15.00	15.00	15.00	15.00
Vegetable oil	6.00	6.00	6.00	6.00	6.00	6.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00
Binder	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00
Proximate composition of experimental diets (% DM)						
Crude Protein	40.32	40.30	40.27	40.22	40.16	40.12
Lipid	9.26	9.08	9.09	9.10	9.09	8.93
Crude fibre	3.22	3.22	3.23	3.23	3.21	3.17
Ash	10.53	10.23	10.48	10.26	10.41	9.43
Moisture content	11.71	11.66	11.56	11.59	11.49	12.63
Nitrogen Free Extract	24.97	25.11	25.37	25.31	25.64	25.72

Vitamins and minerals supplied mg/100g: vitamin A 20,000,000 I.U. Chol.Chloride. 400GR, vitamin D3 4,000,000 I.U. Manganese 30GR, vitamin E 200,000 I.U. Zinc 40GR, vitamin K 1,200Mgr Iron 40GR, vitamin B1 10,000Mgr Copper 4GR, vitamin B2 30,00 Mgr Iodine 5GR, vitamin B6 19,000Mgr Selenium 0.2Mgr, vitaminB12 1,000Mgr Cobalt 0.2Mgr, Niacin 200,000Mgr Calcium 600GR, Folic acid 5,000Mgr Lysine 100,000 Mgr, Panth.acid 50,000 Mgr, Phosphorus 400,000 Mgr, Biotin 400 Mgr, Methionine 1,000Gr.

Experimental fish

One hundred and eighty African catfish, *Clarias gariepinus* fingerlings with initial weight of 13.62±0.12 were acclimatized for two weeks prior to the commencement of the experiment and they were fed on commercial diets Nutreco® (40% crude protein). The fingerlings were not fed for 24 hours before they were started on the experimental diet to maintain a uniform stomach condition of fish and to induce their appetite for the commencement of the feeding trial. The fish were weighed and the average weight recorded. The experiment lasted for 70 days.

Experimental system and procedure

A total of eighteen plastic tanks of 60cm ×45cm×45cm dimension were used for the experiment. Ten fingerlings were randomly stocked into each plastic tank, with three replications per treatment. Experimental diets were assigned randomly to the plastic tank and each group of fish was fed at 5% body weight per day in two equal portions at 9.00-10.00h and 16.00-17.00h GMT. All fish were removed from each glass tanks every two weeks and weighed respectively.

Monitoring of water quality parameters

Dissolved oxygen was monitored daily using HANNA 98103SE (HANNA instruments, Rhode Island). Temperature and pH were monitored also monitored daily using YSI-IODO 700 digital probe (IFI Olsztyn, Poland). The physical assessment of culture water was carried out weekly and included: temperature, pH, and dissolved oxygen (DO). The water was maintained at 27 - 30 ^{o}C, dissolved oxygen at 6.5-8.3 mg/L and pH 6.0 - 8.5.

Proximate composition of experimental feed and fish

The formulated feeds and fish were sampled at the beginning of the trial and analysed for their proximate components fish samples from each treatment were also analysed at the end of the experiment. Before the analysis, feeds and fish samples were blended to a homogeneous mince using a meat grinder with a 4 mm diameter orifice plate. A sub-sample of this mince from each tank was taken and stored for estimation of dry matter which was determined after drying in the oven (Gallenkamp, UK) at 105°C for 24 hrs. The remaining feed and fish homogenate were dried in the oven and used for all subsequent analyses. Ash content was calculated by weight loss after incineration in a muffle furnace (Carbolite, UK) for 12 hrs at 550ºC. The Kjeldahl technique was used to measure crude protein. In this technique, the nitrogen (N) content is determined and multiplied by a conversion factor of 6.25.

Zootechnical performance of fish fed experimental diet

Growth performance and nutritional utilisation

Zootechnical performance was performed using growth performance and nutritional utilisation parameters according to Gbadamosi and Lupatsch, (2018) as follows:

Daily weight gain (g/fish/day) = (final BW (g) - initial BW (g)) / days

Feed conversion ratio (FCR) = feed consumed (g)/ (final BW (g) - initial BW (g))

Specific growth rate (%) = 100 × (ln[final body weight]- ln[initial body weight])/no. of days

Survival (%) = Number of fish at the end of the experiment / Number of fish stocked at the onset of the experiment x 100.

Histology of the intestine

At the end of the experiment, 2 fish from each treatment were used for histological analysis. The intestine was removed carefully and fixed in 10 % formalin for three days to preserve the organs. The fixed organs were dehydrated in graded levels of alcohol (50 %, 70 %, 90%, 100 %) after which they were immersed in 50/50 mixture of alcohol and xylene for three hours, followed by cleaning in 100% xylene for three hours after which they were embedded in petri dishes with wax. The specimens were later mounted on wooden blocks and sectioned with the aid of a microtome to 5 µm sections before staining in haematoxylin and eosin. The stained specimens were observed under a light microscope. The examined sections were photographed using an Olympus BH2 microscope fitted with photographic attachment and an automatic light exposure unit (Olympus C35 AD4).

Statistical analyses

All data collected during the trial were tested for normality using Levene's homogeneity of variance test and subjected to one way analysis of variance (ANOVA). Differences between mean of treatments were considered significant at $P < 0.05$ using Tukey post hoc tests. All analyses were performed using SPSS software version 22 (SPSS Inc.).

Results

Proximate composition of feed, fish fed experimental diets and water quality parameters during the feeding trial

In the present study, there was no significant difference in the crude protein, lipid and ash contents of fish fed the experimental diets up to 60% inclusion of the African yam bean in the diet as shown in Table 2. However, in the present study significant decrease was reported in the carcass CP, ash and lipid of experimental fish when the content of African yam beans was increased to 80% in the fish diet.

Growth parameters and nutrient utilisation of C. gariepinus fed varying replacement level of African yam bean

After eight (8) weeks of feeding, it was observed that the inclusion of African yam bean in the feed of *C. gariepinus* at different levels influenced the growth performance of experimental fish. As shown in Figure 1, fish fed diets with 80 and 100% replacement of soybean with African yam bean showed reduction in growth as early as the third week of the feeding trial till the end of the experiment. However, fish fed the control diets and replacement of soybean up to 60% with African yam bean continued to grow during the 10-week feeding period and reached a significantly (P<0.05) greater weight than those in T_4 and T_5. Growth performance in term of weight gain and specific growth rate of fish fed the control diet containing fishmeal and soybean meal as the crude protein source, were significant better (P<0.05) compared with the other diets as shown in Table 3. Fish fed with the control also recorded the highest specific growth rate and the best feed conversion ratio but was not significantly different from fish fed 20 and 40% replacement with African yam bean. There were no significant differences (P>0.05) in the feed intake of fish during the feeding trial. The results of this study showed high feed intake for the *S. stenocarpa* based diet in fish fed diet 2 and 3, in contrast feed consumption was reduced in treatment 4,5 and 6.Fish in the control treatment in the present study produced the best growth despite having lower feed intake than fish in treatment 2 and 3.

In the current study, the replacement of soybean meal with African yam bean at 100%, significantly reduced survival in experimental fish (Table 2). Likewise, 80 and 100% replacement of soybean meal with African yam bean induced enteritis in the intestine of African catfish (Figure 2).

Table 2: Analysed composition of *Clarias gariepinus* (per g dry weight) fed the experimental diets

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment6
Protein (g)	15.52 ± 0.12[a]	15.62 ± 0.10[a]	15.64 ± 0.21[a]	15.66 ± 0.16[a]	15.49 ± 0.11[a]	15.32 ± 0.25[b]
Lipid (g)	6.11 ± 0.02[a]	5.58± 0.52[a]	6.16 ± 0.17[a]	5.98 ± 0.06[a]	6.09 ± 0.07[a]	6.01 ± 0.01[a]

Ash (g)	4.30 ± 0.01[a]	4.45 ± 0.07[a]	4.38 ± 0.09[a]	4.42 ± 0.15[a]	5.86 ± 0.15[b]	5.95 ± 0.15[b]

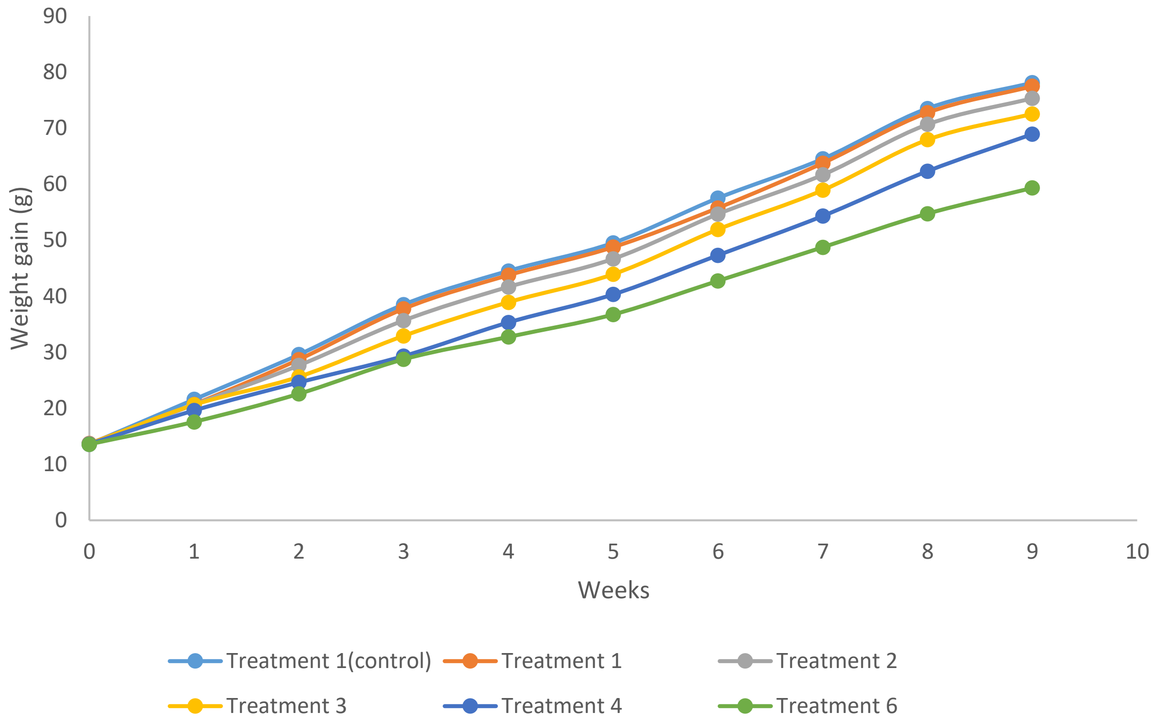


Figure 1: Weight gain pattern of *C. gariepinus* fed varying inclusion levels of African yam beans diets

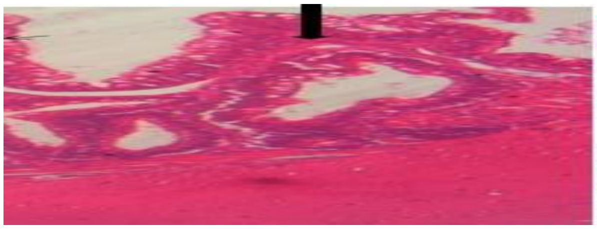
Table 3. Growth parameters and nutrient ultilisation of *C. gariepinus* fed varying replacement level of African yam bean

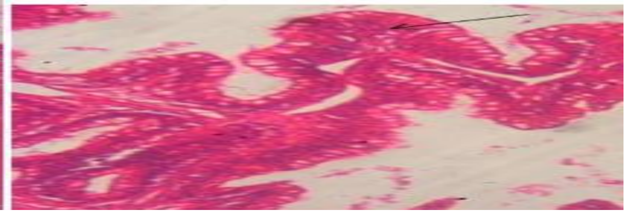
PARAMETERS	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment6
Final weight (g)	78.10 ± 0.75^a	77.47 ± 0.49^a	75.32 ± 0.77^b	72.55 ± 0.6^{bc}	68.93 ± 0.94^c	59.32 ± 1.75^d
Initial weight (g)	13.58 ± 0.18^a	13.71± 0.12^a	13.65 ± 0.30^a	13.62 ± 0.12^a	13.61 ± 0.76^a	13.58 $\pm 0.87^a$
Weight gain (g)	64.52 ± 0.58^a	63.76 ± 0.38^a	61.67 ± 0.80^b	58.93 $\pm 0.63^{bc}$	55.32 ± 0.99^c	45.74 ± 1.75^{d}
Feed fed (g)	97.70 ± 0.31^a	97.20 ± 0.26^a	98.00 ± 0.17^a	96.33 ± 0.70^a	97.63 ± 0.42^a	92.53 ± 0.60^b
FCR	1.51 ± 0.27^a	1.52 ± 0.14^a	1.58 ± 0.13^b	1.63 ± 0.10^{bc}	1.76 ± 0.13^c	2.02 ± 0.05^d
SGR (%)	3.09 ± 0.64^a	3.05 ± 0.26^a	2.98 ± 0.8^b	2.90 ± 0.71^{bc}	2.63 ± 0.92^c	2.05 ± 0.95^{d}
Survival (%)	100 ± 0.00^b	100 ± 0.00^b	100 ± 0.00^b	100 ± 0.00^b	97.90 ± 0.20^b	90.83 ± 0.29^a

a, b, c, d, e value in each row with the different superscript are significantly different ($p<0.05$) using ANOVA Post Hoc (Tukey test). Keys: FCR: Feed Conversion Ratio. SGR: Specific Growth rate.

Histology of the intestine of C. gariepinus fed varying replacement level of African yam bean

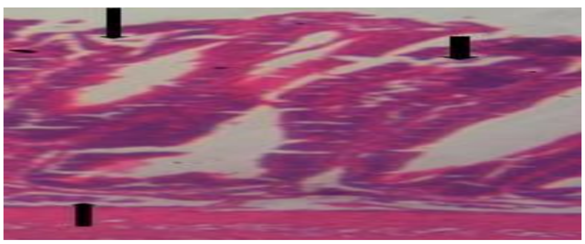
The histological plates of the intestine of experimental fish is shown in Figure 2. Plate A, B and C revealed normal enterocytes with microvilli and goblet cells in the intestine of fish in the T1 (control), T2, T3 and T4. Mild erosion of the enterocytes was noticed in T5 and severe erosion of the enterocyte and intestinal villi was observed in T6. Histology of the intestine showing mild erosion of enterocytes with microvilli and reduced goblet cells in the intestine of fish in T5.

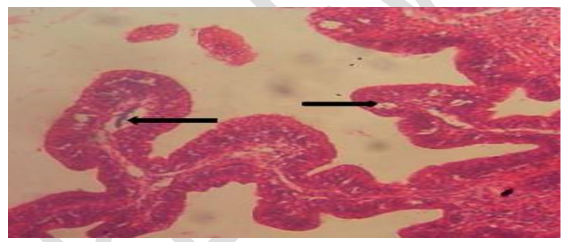




(A) Histology of the intestine showing normal enterocytes (arrow) with microvilli and goblet cells in the intestine of fish the control diet (T1).

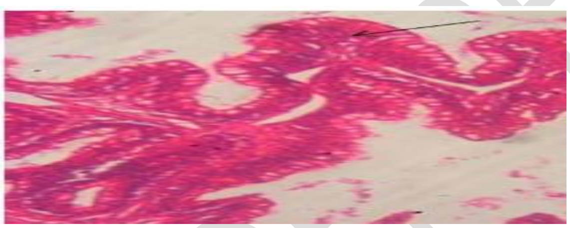
(B) Histology of the intestine showing normal enterocytes with microvilli and goblet cells (arrow) in the intestine of fish in T2.

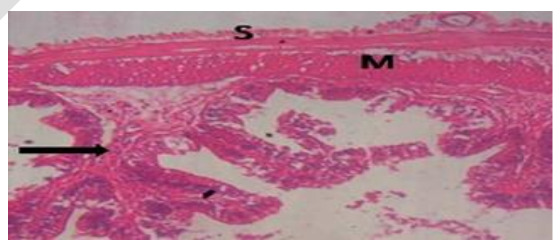




. **(C)** Histology of the intestine showing normal enterocytes with microvilli and goblet cells (arrow) in the intestine of fish in T3.

(D) Histology of the intestine showing normal enterocytes with microvilli (arrow) and goblet cells in the intestine of fish in T4.





(E) Histology of the intestine showing mild erosion of enterocytes with microvilli and reduced goblet cells (arrow) in the intestine of fish in T5.

(F) Histology of the intestine showing severe erosion of enterocytes (arrow) with microvilli and reduced goblet cells in the intestine of fish in T6.

Figure 2 : Histology of the intestine of *C. gariepinus* fed varying replacement level of African yam bean
Mag X100

Discussion

Proximate composition of feed, fish fed experimental diets and water quality parameters during the feeding trial

Similar results on the proximate composition of feed and fish fed experimental diets in the current study were reported by Ibrahim *et al*. (2017) and Abdulhamid et al (2020) which stated that body protein and fat content were not significantly affected with increasing Azolla meal and Curcumin percentage respectively in the diets of Nile tilapia and rats. They further found that animal diet containing phycogenes up to 45% did not cause a significant variation in carcass CP, ash and dry matter compared with control diet for Nile tilapia (*O. niloticus*) and rats.

Differences in result may be related to the accumulation of anti-nutritional factors inherent in the African yam bean at high inclusion levels which the experimental fish responded to in terms of utilization and carcass deposits. The proximate composition of experimental diets in the present study revealed that the formulated diets were isonitrogenous, this agreed with obtained values by Adeparusi et al (1998) for African catfish, *C. gariepinus* fed *S. stenocarpa,* in a study that assessed the digestive capability of *C. gariepinus* for the legume. They concluded that *S. stenocarpa* has the potential for inclusion in practical diets for Clariid catfish.

The water quality parameters monitored during the present study were adequate for *C. gariepinus* growth and it is in agreement with the report of Lim and Webster (2006) for the production of *C. gariepinus.* Furthermore, the water quality parameters measured during the experimental period for *C. gariepinus* fall within the normal range as reported by Viveen et al (2005) for the production of *C. gariepinus.* Therefore, the differences reported in the zootechnical parameters and physiology of fish in the present study could not be traced to the water quality parameters.

Growth parameters and nutrient utilisation of C. gariepinus fed varying replacement level of African yam bean

The results on the zootechnical parameters as shown by the growth parameters and nutrient utilisation of fish in the present study agreed with major findings in fish nutrition, which stated that fishmeal and soybean meal are still the major protein sources in aquaculture feeds, because of their palatable, highly digestible and rich essential amino acids, which closely matches the amino acid requirement for fish and also a good source of fatty acids, energy and minerals (Gbadamosi and Lupatsch, 2022). Moreover, the growth performance differences in this study was consistent with the findings of Blake and Lupatsch (2012) which reported that growth performance and feed efficiency of *O. niloticus* was best when fed the fishmeal diet and gradually decreased with increasing algae inclusion at the expense of fishmeal.

In this study, the FCR of fish diets with African yam bean replacement of soybean meal up to 60% improved in the same proportion as the control. According to Okeke et al *(*2017) African yam bean *S. stenocarpa* is an underutilised legume that can replace fishmeal for fish normal growth and development of tropical fish species. Similarly, Adeparusi et al (1998) reported enhanced feed utilization and digestibility coefficient in African catfish when *S. stenocarpa* was used as a protein dietary source. African yam bean contains a good proportion of high protein making it a promising food crop that can be useful in combating protein malnutrition in fish and animals (Adegboyega et al., 2020). However, inclusion of legumes at high percentage may be detrimental to the growth performance and health of fish because legumes have been reported to possess antinutritional factors (ANFs) which affect their nutritional quality. The ANFs are capable of changing the taste, protein digestibility, and bioavailability of nutrients (Jain et al., 2019; Srenuja et al., 2023).

The best feed intake was recorded in treatment 3, similar to what Hussein et al, (2012) recorded that the best feed intake in phycogen based diets may have been due to increased fish appetites; high acceptability for the diet among cultured fish. Significantly higher feed consumption in the *S. stenocarpa* diet may therefore be as a result of the presence of the group of nutrients and secondary metabolites with orexin capacity present in the legumes. For instance, African yam bean has been reported to contain abundant concentration of calcium, iron, phosphorus, zinc, and copper (Okeke et al, 2017).

In earlier studies, Banday et al, (2012) had surmised that soybean is unpalatable for some fishes such as Chinook salmon and catfish, however herbivorous and omnivorous species have been known to be less choosy in feed preference. According to Lupatsch (2008), too much feeding does not necessarily result in higher growth, beyond a certain level excessive feeding has no influence on the growth, the actual feed intake, which is the amount of feed that the fish is physically able to consume will produce the feeding level at which the fish grows best and the FCR is minimal.

This is supported by the report of Okeke et al (2017) which reported that inclusion of *S. stenocarpa* in the feed of Heteroclarias at a percentage level of 100% did not produce good growth and was attributed to gut incompatibility. *S. stenocarpa* can be toxic and may negatively affect the nutrient value of the seeds by impairing protein digestibility and mineral availability. However, it is heat-labile and hence may be inactivated by processing methods involving heat generation. "On the other hand, a number of the compounds, such as phytic acid, phenols, and tannins, usually considered antinutritional compounds, are currently considered potential antioxidants containing health-promoting effects. For example, phytic acid has now been revealed to have rich antioxidant, hypoglycaemic activities and also to possess anticarcinogenic properties" (Adegboyega et al., 2020). Therefore, elimination of these compounds depends upon the consumer's preferences (Jain *et al.,* 2019).

Histology of the intestine of C. gariepinus fed varying replacement level of African yam bean

The histology of the intestine of fish observed in the present study suggested that the inclusion of African yam bean, *S. stenocarpa* enhanced the function of the enterocytes up to the level of 60% replacement of soybean in *C. gariepinus*. Similarly, positive effects of dietary African yam bean at low inclusion levels on the gonad morphology of *Heteroclarias* was reported by Okeke et al (2017). The population of goblet cells in the intestine increased with increasing levels of African yam bean, while there were severe erosion of the enterocytes with 100% inclusion of African yam bean. Goblet cells and enterocytes in the gastro intestinal tracts of fish have been reported to be influenced by anti-nutritional factors, stress and disease which may have a subsequent effect on the digestion and health of fish (Jain et al, 2019). For instance, increased erosion of the intestine causing enteritis of fish in T5 and T6 of the current study may be due to anti-nutrients present in African yam been at high inclusion levels. This result is in concordance with reduced growth performance observed in fish fed 80 and 100% replacement of soybean meal with African yam bean meal in this study. Similarly, the inclusion of Bambara nut at high levels was reported to negatively affect the zootechnical performance of African catfish (Osho et al, 2013).

Conclusions

This study provided data suggesting that African yam bean, *Sphenostylis stenocarpa* can be considered as a dietary replacement for soybean meal up to 60% in the diets of African catfish, *Clarias gariepinus* for adequate growth performance and nutrient utilization without any negative effect on the intestine histology of the fish. However, care should be taken on replacement of soybean meal at higher levels to prevent enteritis in the African catfish and effective processing techniques like fermentation, sonication and encapsulation should be employed to neutralize the presence of antinutritional factors (ANFs) and longer cooking time which are be responsible for the low consumption rate in African yam bean, *S. stenocarpa.*

List of abbreviations

FCR: Feed Conversion Ratio.

SGR: Specific Growth rate.

Ethics approval

The authors wish to declare that there was no conflicts, informed consent, human or animal rights applicable. Fish were handled according to standard procedure of the Home Office Regulations of the United Kingdom as stipulated by the Animal Scientific Procedures Acts of 1986 with Amendment Regulations (SI 2012/3039).

-Availability of data and material

Data and material are not restricted

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Details of the AI usage are given below:

1.

2.

3.

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