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Mouldy Groundnut Cake and Hydrated Sodium Calcium Aluminosilicate in Practical Diet for African Catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract

The study was designed to detect fungi and quantify aflatoxins B_1 and B_2 microbial load levels and toxin binding efficacy of hydrated sodium calcium aluminosilicate (HSCAS) on growth and nutrient utilization of *Clarias gariepinus* African catfish fingerlings diet formulated with mouldy groundnut cake (MGNC). MGC containing 185.00 ± 7.07 µg/kg and 137.50 ± 10.00 µg/kg of aflatoxins B_1 and B_2 was incorporated into practical diets. Three practical isoproteinous diets were formulated. Diet 1 contained Groundnut Cake (GNC) with 0.20 ± 0.14 cfu microbial load but no apparent mould contamination. Diet 2 contained MGC 4.38 ± 0.40, 1.99 ± 0.01 µg/kg of aflatoxins B_1, B_2 and 2.20 ± 0.14 cfu microbial load. Diet 3 contained MGC, HSCAS; 3.49 ± 0.20, 1.34 ± 0.01 µg/kg and 1.35 ± 0.20 cfu of aflatoxins B_1, B_2 and microbial load, respectively. The diets were fed twice daily to *Clarias gariepinus* fingerlings mean body weight 1.68 ± 0.01g in triplicate of 20 fish each for 12 weeks. Six fungi; *Aspergillus parasiticus, A. flavus, A. niger, A. tamarii, Penicillium citriinum and P. oxalicum* were isolated from MGNC diet. Addition of HSCAS reduced fungi present in diet 3 to *A. flavus, A. tamarii and P. oxalicum*. Also aflatoxins B_1, B_2 and microbial load in the diet and carcass of *Clarias gariepinus* were reduced with HSCAS inclusion in diet 3. Feeding African catfish fingerlings with mould contaminated diet (2) significantly ($P<0.05$) reduced growth performance and feed utilization but had no significant ($P>0.05$) effect on survival of *Clarias gariepinus* fingerlings. The results of this study show that the inclusion of HSCAS reduced the number of fungi, microbial load and aflatoxins B_1 and B_2 in the carcass and diet of African catfish formulated with mouldy GNC. But was not effective in alleviating the growth depression induced by mould contamination of the diet. Hence the need for identification of toxins present in mould contaminated feedstuffs in order to design effective toxins management strategies.

Keywords: Mouldy groundnut cake; HSCAS; Aflatoxin B_1; Aflatoxin B_2; Microbial load; *Clarias gariepinus*; Feed utilization; Growth performance

Introduction

Feed ingredients contamination with toxins may occur anywhere in the supply chain from the field, manufacturing process, transportation to storage. In the tropics conducive environmental condition has majorly been attributed to production and growth of micro-organisms and toxic substances; particularly fungi. This is exacerbated by the cultural practice and lack of adequate regulatory and control system by government for food and feedstuffs contaminant screening in most developing countries in the humid tropics.

Fungi, *Aspergillus flavus* and A. *parasiticus* contamination are mostly responsible for the production of aflatoxins in the humid tropics [1]. They are found when environmental temperature is above 27°C, humidity levels greater than 65% and moisture levels in feedstuff is above 14%. Also suboptimal handling and storage are considered as important factors favouring the growth of aflatoxin-producing fungi [2]. Aflatoxins are the most potent toxic substances occurring naturally. Large yield of aflatoxins are found in feed ingredients with high carbohydrates concentration as found in cereals and to a lesser extent in oil seeds. But in West Africa aflatoxins are of major concern in groundnut. Four major forms of aflatoxins are found in feedstuffs: B_1, B_2, G_1 and G_2. Aflatoxin B_1 is regarded as carcinogenic, most prevalent and toxic of the four. It has significant economic and health implications in both animal and man. Hence efforts have been focused on reducing the impact of aflatoxin B_1 contamination in livestock and fish, with dearth of information on the other aflatoxins.

Recently studies have reported the co-occurrence or co-contamination of substance(s) with more than one aflatoxins [3,4]. This is because in practical situation contaminants are rarely found individually [5]. Especially when environmental condition is conducive, the probability of multiple or co- contamination is always high. Hence there is need to assess contaminants present naturally in feed raw materials particularly in endemic environment like the humid tropics. Sequel to this, there might be need for the identification of fungi present in such substances which are potential toxins producers. This could provide necessary information for designing strategies in the management of these toxins.

Consequently with the recent trend in increase utilization of plant feedstuffs (which are good substrates for aflatoxins) in warm water fish species, there is need to investigate the implications of natural aflatoxins contamination. Also the practice of inclusion of toxin binder like Hydrated Sodium Calcium Aluminosilicate (HSCAS), which is generally routinely added to suspected contaminated (mouldy) feedstuffs in endemic environment [6].

From the foregoing, this study was designed to detect fungi, quantify microbial load and aflatoxins B_1, B_2 of the diets formulated with mouldy groundnut cake. Also the toxin binding efficacy of HSCAS on growth performance and aflatoxins B_1 and B_2 levels in the diets and

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carcass of the African catfish; *Clarias gariepinus* fingerlings fed diets formulated with mouldy groundnut cake was evaluated.

Materials and Methods

Dietary Design and Feeding

A 50 kg bag of Mouldy Groundnut Cake (MGC) suspected to be naturally contaminated was obtained from a commercial feed ingredients seller at Ojo, Lagos, Nigeria. MGC contained 185.00 ± 7.00 and 137.50 ± 10.00 µg/kg of aflatoxin B_1 and B_2, respectively. Three experimental diets were formulated to contain 35% crude protein each. Diet one contained uncontaminated groundnut cake and other feed ingredients which served as the control, diet two contained contaminated groundnut cake and other feed ingredients while diet three contained contaminated groundnut cake, other feed ingredients and toxin binder (HSCAS) at the manufacturer's recommended inclusion rate of 0.3% of the diet. Percentage gross composition of the experimental diets is shown in Table 1. The feed preparation was done at a reputable commercial fish feed manufacturer: Act livestock consult, Badagry Expressway, Lagos-Nigeria, where all ingredients were weighed, ground into fine powder, thoroughly mixed and pelleted (into 2 mm) with a 350 kg/hr table top pelleting machine. The pellets were crushed into pieces and stored in air tight containers. Fish were fed twice daily at 8.00 and 16.00 hours at the rate of 3% body weight for a period of 12 weeks. Feeding was done by hand and spread evenly across the water surface of each aquarium.

Fish management

The experiment was conducted at the indoor of Lagos State University Hatchery, Ojo, Lagos, Nigeria. Nine (9) plastic aquaria tanks with 80 L water capacity and 0.5 m depth were filled with borehole water to three quarter capacity. The water in the experimental tanks was aerated by electric air pump (Shining model; horsepower 50Hz). *Clarias gariepinus* fingerlings of mean weight of 1.68 ± 0.01 gm were obtained from Lagos State Agricultural Cooperative farm, Ojo, Lagos, Nigeria. The fish were transported early in the morning between the hours of 6.30 and 7.00 from the hatchery and acclimatized for 5 days prior to the start of the experiment. They were randomly distributed to the 9 plastic aquaria at the stocking rate of 60 fingerlings per experimental diet and 20 per replicate making a total of 180 fingerlings. Daily 50% of water in each tank was gently siphoned in exchange for fresh water. This was done to get rid of left over feed and faecal matter.

Ingredients	Diet 1	Diet 2	Diet 3
Maize (CP 9%)	29.50	29.50	29.20
GNC (CP 45%)	21.00	0.00	0.00
MGNC (45%)	0.00	21.00	21.00
SBC (CP 45.30%)	21.00	21.00	21.00
Wheat offal (CP 16%)	8.00	8.00	8.00
Fishmeal (CP 72%)	18.50	18.50	18.50
Soy oil	1.00	1.00	1.00
Premix*	0.50	0.50	0.50
Salt	0.25	0.25	0.25
Vitamin C	0.25	0.25	0.25
HSCAS	0.00	0.00	0.30
Gross energy**	4212.50	4193.80	4207.30
Proximate composition			
CrudeProtein (N×6.25%)	34.97	34.72	35.00
Fibre (%)	3.71	3.68	3.70
Ether extract (%)	4.41	4.30	4.38
Total ash (%)	4.92	4.95	5.02
Dry matter (%)	92.40	92.18	92.39

*Each kg of diet contained 2,000.000 IU vit A; 4,000.000 IU vitD_3; 2,000.000 vit E; 1,200 mg vit K; 10,000.000 mg vitB_1; 30,000 mg vitB_2; 19,000 mg vitB_6; 1000 mg vit.$B1_2$; 5000 mg Panthotenic acid; 200,000 mg Niacin; 5,000 mg Folic acid; 30 Mn; 40 gm Zn; 40 mg Fe; 4 gm Cu; 5gm I_2; 0.2 mg Co; 600 gm calcium; 400 mg choline chloride; 40 mg biotin; 400,000 mg phosphorus; 100,000 m glysine; 400 gm methionine and 125 IU antioxidant. Gross energy** (KCal kg^{-1}); calorific value of protein 5.65; nitrogen free extract 4.1; lipid 9.45.

Table 1: Percentage composition of the experimental diets.

Fungi isolation and quantification of aflatoxins and microbial loads

Mouldy groundnut cake was subjected to extraction and quantification of aflatoxins B_1 and B_2 before the commencement of the study and other major ingredients were screened against aflatoxins. The coarse ingredients were ground, mixed before samples were collected for analyses. Fungi present in the experimental diets were isolated; the diets were also subjected to extraction and quantification of aflatoxins B_1, B_2 and microbial load. Twenty fingerlings were randomly picked before the start of the experiment. The fingerlings were washed, crushed under sterile condition for microbial load determination. At the end of the experiment 20 fingerlings (divide into two groups) from each treatment were randomly picked for microbial load and aflatoxin B_1 and B_2 determination. The isolation of fungi, extraction and quantification of aflatoxins and microbial load were done at the Institute of Agricultural Research and Training (IAR&T), Ibadan, Oyo-Nigeria [7-10].

Growth and feed utilization

Batch weighing of fish in each replicate aquarium was done at the beginning of the experiment, subsequently biweekly, using a MettleR 20110 top-loading balance. The evaluation of experimental diets for growth and feed utilization was carried out as follows.

Weight gain=Final Body Weight - Initial Body Weight

Specific growth rate (SGR% day^{-1})=($\log_e$FBW-logeIBW)×100

Time

Where, FBW represents final body weight,

IBW represents initial body weight.

Time represents difference in days between final and initial body weight.

$$\text{Protein efficiency ratio } (\text{PER}) = \frac{\text{Fish weight gain } (\text{kg})}{\text{Protein fed } (\text{kg})}$$

$$\text{Percentage survival} = \frac{(\text{Initial number of fish stocked} - \text{number of dead fish})}{\text{Initial number of fish stocked}} \times 100$$

Economy analysis

A simple analysis was conducted to assess the cost effectiveness of the experimental diets, only diet cost was used for the calculations with the assumption that all other operating costs remained the same. The average prices of each feedstuff ($) during the study period was used to calculate the amounts required to make the different diets, cost per kilogram of each diet was calculated. The economic conversion ratio (ECR) was determined using this equation: ECR=COST OF DIET×FCR.

Water quality parameters

Daily water temperature was measured using a mercury-in-glass

	Parameters			
Specimen: Diet	AFB_1(µg/kg)	AFB_2(µg/kg)	Microbial load (cfu)	Fungi isolated
1	ND^c	ND^c	0.20 ± 0.14^c	1*
2	4.38 ± 0.40^a	1.99 ± 0.01^a	2.20 ± 0.14^a	6*
3	3.49 ± 0.20^b	1.34 ± 0.01^b	1.35 ± 0.20^b	3*
Specimen: Carcass				
1	ND^c	ND^c	0.25 ± 0.07^c	ND*
2	2.99 ± 0.02^a	1.05 ± 0.01^a	1.70 ± 0.14^a	ND*
3	2.24 ± 0.01^b	0.89 ± 0.03^b	0.95 ± 0.21^b	ND*
Toxin absorbed**	0.75 ± 0.02^a	0.16 ± 0.02^b	0.75 ± 0.20^a	ND*

1*= *Aspergillusparasiticus*6*= *A. parasiticus, A. flavus; A. niger, A. tamarii, Penicillumcitriinum and P.oxalicum*3*=*A. parasiticus, A. niger and P. citriinum*. ND=Not detected; ND*=Not Determined. Toxin absorbed**with same superscript in the same row are not significantly (P>0.05) different. Mean with same superscripts in the same column are not significantly different (P>0.05).
a=highest mean, b=intermediate mean and c=lowest mean.

Table 2: Summary of aflatoxins and microbial load in the diets and carcass of African catfish (Clariasgariepinus) fed the experimental diets.

Parameters	Diet 1	Diet 2	Diet 3
Initial weight/fish (g)	1.68 ± 0.01^a	1.68 ± 0.01^a	1.68 ± 0.00^a
Final weight/fish (g)	9.92 ± 1.06^a	5.22 ± 0.64^b	6.12 ± 1.00^b
Weight gain/fish (g)	8.24 ± 1.07^a	3.54 ± 0.64^b	4.44 ± 0.97^b
Specific growth rate	1.58 ± 0.10^a	1.01 ± 0.11^b	1.15 ± 0.14^b
Feed intake/fish (g)	14.64 ± 1.14^a	9.93 ± 0.64^b	10.84 ± 0.96^b
Feed conversion ratio	1.78 ± 0.0^b	2.85 ± 0.41^a	2.50 ± 0.46^a
Protein efficiency ratio	1.61 ± 0.08^a	1.01 ± 0.33^b	1.20 ± 0.13^b
Survival percentage	71.67 ± 3.00^a	74.00 ± 1.01^a	70.00 ± 4.04^a
Economic conversion analysis			
Feed cost/kg ($)	0.79 ± 0.07	0.78 ± 0.06	0.78 ± 0.07
Fish cost/kg ($)	1.41 ± 0.06	2.21 ± 0.06	1.94 ± 0.06

Means with the same superscripts along each row are not significantly different (P>0.05).

Table 3: Growth, feed utilization, survival and economic analysis of *Clariasgariepinus fingerlings* fed the experimental diets.

Parameters	Diet 1	Diet 2	Diet 3	
pH		6.56 ± 0.43	6.49 ± 0.10	6.54 ± 0.32
Temperature (oC)	27.67 ± 0.01	27.40 ± 0.40	27.60 ± 0.20	
DO* (mg/l)	5.77 ± 0.10^a	3.59 ± 0.30^b	3.67 ± 0.50^b	

Means with the same superscripts along each row are not significantly (P>0.05) different. DO* represents dissolved oxygen.

Table 4: Water quality analysis of *Clariasgariepinus* fingerlings fed the experimental diets.

thermometer, hydrogen ions (p^H) concentration measured using p^H meter (Jenway Model 9060) and weekly dissolved oxygen (DO) concentration was measured by oxygen meter (Hanna Model HI-9142).

Observation

The fish were observed for behavioural and physical abnormalities that could suggest possibility of disease situation.

Statistical and chemical analyses

Feed proximate composition was done according to the method of Association of Analytical Chemists [11]. Data collected from feeding trial were subjected to one-way analysis of variance (ANOVA). Significance difference in means was evaluated by Duncan's Multiple Range Test using SPSS for windows (version 11). Values are expressed as Means ± SD.

Results

Fungi, aflatoxins content and microbial load of the experimental materials:

The results of the fungi isolated, aflatoxins and microbial load of the experimental diets, fish and carcass are presented in Table 2. Summary of growth performance characteristics and water quality results are presented in Tables 3 and 4, respectively.

Six fungi from two genera comprising of *Aspergillus* and *Penicillum* were isolated from the three diets. *Aspergillus parasiticus* was found in all the experimental diets. In addition diet 2 contained five other fungi; *A. niger, A. flavus, A. tamarii, Penicillum citriinum* and *P. oxalicum*. Inclusion of HCSAS to diet 3 reduced the number of fungi isolated to two; *A. niger* and *P. citriinum*.

Aflatoxins B_1 and B_2 were not detected in the diet and carcass of African catfish fed diet 1 (uncontaminated). Generally addition of HCSAS significantly (P<0.05) reduced the concentrations of aflatoxins B_1, B_2 and microbial load in the diets and the carcass of African catfish (*Clarias gariepinus*) in this study.

Feed utilization, growth and economic analyses:

Inclusion of mouldy groundnut cake in the diet of *Clarias gariepinus* had no significant (P>0.05) effect besides survival, but significantly (P<0.05) reduced final weight, weight gain, specific growth rate, feed intake, feed conversion ratio and protein efficiency ratio. However, addition of HCSAS to diet 3 slightly but not significantly (P>0.05) improved all growth and feed utilization parameters assessed.

Economic analysis showed that mould contaminated diet (2) had the least cost of production ($ 0.791), but most expensive ($ 2.21) for African catfish to attain a kilogram body weight when fed with this diet.

Water quality analysis:

Inclusion of mouldy groundnut cake to the diet of *Clarias gariepinus* significantly (P<0.05) reduced dissolved oxygen concentration of catfish fed the contaminated diet.

Observation:

No external changes or unusual behaviour was observed in any of the fish fed the experimental diets throughout the study. Fish in all experimental diets appeared healthy and normal throughout the period of study.

Discussion

There is dearth of information on natural aflatoxins contamination of feedstuffs and diets of the African catfish in spite of their prevalence and the economic importance of the fish in this part of the world. The results of fungi isolated in the three experimental diets showed the presence of two of the three fungi genera (*Aspergillus*, *Penicillum and fusarium*) associated with mycotoxins production of agriculture and human health significance. The presence of *Aspergillus parasiticus* in the three diets suggests potentials for the production of the four forms of aflatoxins. Although aflatoxins B_1 and B_2 were not detected in diet 1; which may be due to insufficient availability of conditions necessary for their production in this study [12,13]. The co- occurrence of *A. flavus, A. niger and Penicillium* in the contaminated diet could probably encourage the production of ochratoxins and cyclopiazonic acid (CPA), which have been reported to be commonly found with aflatoxins [13-15]. Also possibilities exist for the production of citrinin, secalonic and

oxalic acid *by P. citrinin* and *P. oxalicum* [16,17]. Their co-occurrence may encourage additive, synergy and or antagonistic reactions among these toxins. The addition of HSCAS (diet 3) reduced the number of fungi isolated, however potentials exit for the production of all toxins listed against MGC diet, except secalonic acid which is produced by *P. oxalicum* [18]. The increased microbial load observed in the mould contaminated diet could have resulted from the high number of fungi present in the MGC diet compared to other diets. Therefore from the fungi isolated, there might be need to investigate the presence of other mycotoxins when mouldy groundnut cake is used in diet formulation.

The reduced levels of aflatoxins B_1 and B_2 in HSCAS diet further attest to the ability of HSCAS in reducing aflatoxins levels particularly B_1 [19,20]. The reduced aflatoxins levels may have contributed to lower microbial load observed in the diet and carcass of African catfish fingerlings fed diet containing HSCAS. It is however imperative to note that the level of aflatoxin B_1 (2.24 ± 0.01) found in fish carcass was more than 0.05 µg/kg [21]. Consequently, the addition of HSCAS alone may not be effective in combating mould contamination in the diets of African catfish.

The feed utilization parameters measured showed that African catfish fed mouldy GNC diets had lower weight gain compared to uncontaminated diet. The reduced weight gain was slightly but not significantly improved by the addition of HSCAS to the diet. This is in agreement with the findings [20,22,23]. The lower weight attained may have emanated from low feed consumed by fingerlings on these diets, occasioned by mould contamination which is known to have negative effect on feed taste and nutritive quality [6,24,25]. The low feed intake and nutritive quality may have contributed to the lower growth and feed utilization; feed conversion and protein efficiency ratio observed with the aflatoxins contaminated diets. However, the results of this study disagree with the findings of [26,27] who reported no significant change in growth when tilapia and channel catfish were fed 250 and 2150 ppb purified aflatoxin B_1. The discrepancy might be due to the different species of fish, as well as the nature of toxins used (purified toxin was used in their study as against natural toxins present in feedstuff used in this study). This is besides the fact that more than one toxin was assessed in this this study. These might have aggravated the observed adverse effects. Also the inability of HSCAS to significantly improve the growth, feed utilization and economy of production is in agreement with the findings of [20,28,29] who reported ineffectiveness of HSCAS in diets containing multiple mycotoxins. Consequently supporting the need to determine fungi and all toxins present when mouldy feedstuffs are used in diet formulation. The low dissolved oxygen recorded with contaminated diets could have arisen from the quality of the feed fed. This assumption is based on the fact that aflatoxin is known to reduce nutritive quality of feed [26,30]. The reduced nutritive quality could have reduced fish's appetite resulting in reduced feed intake and increase feed or solid suspension in the culture medium, hence the reduced dissolved oxygen. However, the low dissolved oxygen values were within tolerable levels for catfish production.

Conclusion

The results of this study showed the co-occurrence of six fungi; *A. parasiticus, A. niger A. flavus, A. tamarii, P. citriinum and oxalicum* in diets formulated with mouldy GNC. These fungi have potentials for production of the four forms of aflatoxins, ochratoxins, CPA, citrinin, secalonic and oxalic acid. The co-occurrence of aflatoxins B_1 and B_2 evaluated in this study adversely affected growth, feed utilization and resulted in deleterious impact on production cost of African catfish (*Clarias gariepinus*) fingerlings. The addition of HSCAS slightly improved the adverse effects of the contamination with the elimination of potential for secalonic acid production. However, aflatoxin B_1 levels in the carcass of African catfish; *Clarias gariepinus* is beyond the 21 acceptable level. Consequently, there might be need for identification and quantification of all toxins present in diets formulated with mould contaminated feedstuff (in this study MGC) to proffer needed strategies for the toxins management in catfish diets.

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