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Efficacy of Maize Gluten Supplemented with Crystalline L-Lysine in the Diets for the African Clariid Catfish, Clarias Gariepinus (Burchell, 1822)

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Abstract: The efficacy of maize gluten meal supplemented with crystalline L-lysine as a potential source of protein to enhance productivity rate of the African clariid catfish, *Clarias gariepinus* in a partially substituted fish meal diets was investigated in a trial that lasted seven weeks. Six isonitogenous and isocaloric diets containing 36% crude protein and 13% oil were formulated. Diet 1 containing 100% high grade fish meal protein source (Norwegian, LT-94) served as the control. Maize gluten meal in diets 2 to 6, were improved upon incrementally with crystalline L-lysine supplementation at 0.6, 0.9, 1.2, 1.5 and 1.8% respectively in which fish meal inclusion in the diets were reduced by 75%. Hatchery bred *C. gariepinus* juveniles weighing 5.2±1.3g (weight) were randomly distributed into fiber glass tanks at 34 fish per tank in a triplicate treatments and fed twice daily in a well aerated recirculation system. Biological evaluation of the fish was based on growth performance and nutrient utilization efficiencies. The results showed that the productivity indexes, mean Body Weight Gain (BWG), Specific Growth Rate (SGR), Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Apparent Net Protein Utilization (ANPU) of the fish were good in all the diets with SGR values exceeding 3% day^{-1} and FCR below the value of 1 (for as fed basis). However, fish fed on fish meal based diets (control) had the best overall performance and significantly ($p \leq 0.05$) different from other groups of fish fed maize gluten supplemented with L-lysine. There was, however, an improvement in the performance indexes of the fish with increasing levels of L-lysine supplementation in maize gluten based diets. However, growth performance and nutrient utilization efficiencies did not attain a plateau at 1.8% lysine supplementation of maize gluten in African catfish diets.

Key words: Maize gluten, supplementation, crystalline L-lysine, catfish, fish meal

INTRODUCTION

The African clariid catfish, *Clarias gariepinus*, is a highly valued and heavily exploited animal protein food source in many parts of the world. The current annual world aquaculture production of fish ranked clariid catfish as fourth most widely cultivated fish species after carp, salmon and tilapia[1]. In Africa, its original domain, *C. gariepinus* is of great economic importance and an esteemed fish food with high dressing percentage[2] and commands exceptionally high market preference than most cultured fish species in the tropics[3]. One major constraint to the development and profitability of catfish aquaculture especially in the tropics is inadequate supply of quality feed ingredients to meet the nutritional requirements of fish in aquafeed formulation. Fishmeal which has been conventionally used as the main source of animal protein in catfish feeds is becoming increasingly too expensive for a viable and profitable aquaculture enterprise worldwide due to dwindling supply from capture fishery especially in the tropics[4,5].

Research attempts at finding alternative animal and plant protein sources to replace totally or partially fishmeal in catfish diets have not been fully successful or accepted by catfish farmers due to the nutritional deficiencies of most feedstuffs so far evaluated[6-8]. However, soybean has been used exceptionally in catfish production because of its nutritive quality to support catfish production[9-11] but its competing uses for food by man and livestock has placed strong limitation on

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soybean as cheap and affordable alternative protein source in catfish feed production.

Maize meal has tremendous use in livestock production worldwide[12]. However, its use in fish feed has been limited only as source of energy and filler in diet formulation. Since fish require more protein than terrestrial animals[13], more attention has been focused on good quality protein sources to meet the requirements of fish. Maize gluten meal is an important by- product of maize processing during which most of the starch, bran and germ are removed[14]. It has been reported that maize gluten has considerable potential to replace fish meal in diets up to a certain level without compromising growth and health[15,16] and does not contain anti-nutritional factors[12] like most grains and seed oils currently being used as feed ingredients in catfish diets. However, diets containing maize gluten are known to be deficient in lysine[17,18]. Supplementation of maize gluten meal with L-lysine, an economical crystalline amino acid commonly used in livestock production, can alleviate this deficiency thus enhancing the utilization potential of this valuable plant protein source in fish diets. What is contentious, however, is the delivery of crystalline amino acids to fish at the right proportion without leaching in an aquatic environment unlike its uses in terrestrial animal production. The technology and cost of encapsulation of crystalline amino acids in fish diets as suggested by several workers in fish nutrition[19,20] or covalent binding[21] may be too expensive to meet the need of fish farmers. In recent study, Williams *et al*.,[22] showed that supplementation of crystalline amino acids in low grade protein diets of Asian seabass, *Lates calcarifer* Bloch, enhanced good growth and nutrient utilization efficiencies.

The present study is therefore aimed at evaluating the optimum growth performance and nutrient utilization efficiencies of African catfish, *Clarias gariepinus* fed on maize gluten supplemented with crystalline L-lysine as partial replacement for high grade fishmeal in a practical diet.

MATERIALS AND METHODS

Experimental diets: Six isocaloric and isonitrogenous diets containing 36% crude protein and 13% oil were formulated for juvenile clariid catfish, *Clarias gariepinus* in a seven-week trial experiment (Table 1). Maize gluten obtained from Skretting Aquaculture (a Nutreco Company, United Kingdom) was used to partially replace high grade Norwegian low- temperature fish meal (LT-94). Diet containing 100% fish meal protein source served as the control. Maize gluten meal in diets 2 to 6 was improved upon incrementally with crystalline L- lysine supplementation at 0.6, 0.9, 1.2, 1.5 and 1.8%, respectively.

Alpha cellulose, a binder (CBMC-C), obtained from Sigma, Chemical Company, Poole, Dorset, UK was used as binder in the diets to ensure water stability and leaching of nutrients from the diets. All dietary ingredients were first milled to small particle sizes using laboratory mill; ingredients were thoroughly mixed in a Hobart A120 pelleting and extruding machine (Hobart Manufacturing Ltd., London, England) to obtain a homogeneous mixture. Extruded diets were passed through a 3 mm die to obtain fine strands which were dried immediately in a drying cabinet for 48hrs at 40^{oc} to harden. Dried strands of feed were later broken down into convenience sizes suitable for the experimental fish. In order to prevent any loss and deterioration of crystalline amino acids in the diets, the pellet diets were freeze- dried at -20°C until feeding (Table 2 and Fig. 1).

Experimental fish and management: *C. gariepinus* juveniles weighing 5.2±1.3g on the average were randomly distributed into 60-l rectangular fibre glass tanks at 34 fish per tank. Each treatment was in triplicates group of fish. Tanks were supplied with water through a spray bar after filtration and sufficient aeration from a sump compartment in a circulatory system. A photoperiod of 12 hrs light and 12 hrs darkness was maintained throughout the experimental period with water temperature maintained at 26±1°C dissolved oxygen was kept at a saturation level of 0.8 mg L^{-1}. The fish were acclimated to the experimental conditions and their respective diets for one week prior to the start of the feeding trial. The fish were fed with their respective diets at 4% body weight twice daily at 10.00 and 16,00 h throughout the duration of the experiment. Fish weights were determined at the 7th day of each week and the quantity of feed adjusted based on the changes in body weight of fish for subsequent feeding.

Proximate composition: Proximate composition of diets and fish carcasses before and after experiment, were performed according to AOAC[23] for moisture content and ash. Crude protein (%Nx6.25) was determined by micro Kjeldahl method. Total lipid was determined by a modification of the Folch method[24].

Performance evaluation: Fish performances during the experiment were based on productivity indices on growth performance and nutrient utilization efficiencies as described by Fasakin, Balogun and Ajayi.,[2] as follows;

- **Mean Weight Gain (MWG):** Mean final weight-initial weight.

Table 1: Formulation of experimental diets (g kg^{-1} dry matter) for *C. gariepinus*

	Diets					
Ingredients	1(Control)	2	3	4	5	6
Fishmeal[1]-LT94 (72% cp)	396	132	132	132	132	132
Maize gluten[1](65% cp)	-	275	275	275	275	275
Wheat feed[1](13% cp)	466	450	450	450	450	450
Suppl. Oil[2]	37	42	42	42	42	42
Vit./Min. Premix[3]	40	40	40	40	40	40
L- lysine[4]	-	0.6	0.9	1.2	1.5	1.8
Binder(CBMC)4	20	20	20	20	20	20
Alpha cellulose[4]	41	35	35	35	35	35
Proximate Composition(%) Dry matter	94.76	94.88	94.91	95.07	95.67	94.89
Crude Protein	38,12	38.75	37.01	37.94	37.44	37.41
Lipid	12.68	12.89	13.52	13.37	13.22	13.63
Ash	8.98	5.31	5.55	5.18	4.24	5.12

[1]Skrelting Aquaculture (a Nutreco Company, U.K.) [2]Supplement oil contained cod liver oil (seven seas Ltd, Marfleet, Hill, UK.). [3]Supplying per kg of diet; vitamin A, 5000IU; Vitamin D3, 1000IU; alpha tocopherol,100IU; menadine, 100mg; thiamin HCL,30mg; riboflavin, 45mg; pyridoxine HCL,30mg; cyanobalamin,12mg; ascorbic acid, 1000mg; D-calcium pantothonate, 105mg; choline chloride, 5000mg; folic acid, 10.5mg; inositol, 500mg; biotin, 5.10mg; niacin,150mg. [4]Sigma chemical Co. Poole, Dorset, UK. Carboxy methyl cellulose

Table 2: Essential amino acids[1] (EAAs) profile (% dietary protein, g /100gN) of major protein sources used in the diet formulation for *C. gariepinus*

	Protein sources		
EAAs	Fish meal(LT-94)	Maize gluten	Fishmeal and maize gluten Mix
Lysine	7.74	1.84	3.32
Threonine	4.41	3.43	3.68
Leucine	7.45	15.99	13.86
Methionine	2.55	1.98	2.12
Tryptophan	1.13	0.52	0.67
Arginine	6.12	3.11	3.87
Histidine	2.12	2.10	2.11
Phenylalanine	3.80	5.04	4.73
Valine	4.48	3.78	4.90
Isoleucine	3.98	3.74	3.83
Total %EAA	41.66	41.58	43.09

[1]Amino acids analysis was performed by Eurolysine Ltd, Amiens, France

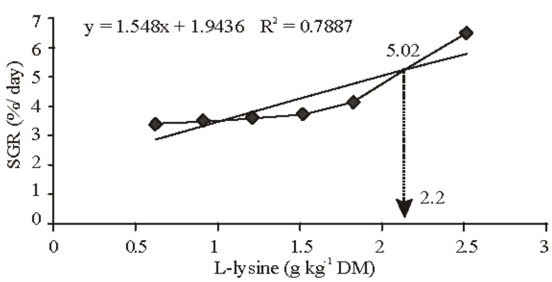

Fig. 1: Relationship between crystalline L-lysine supplementation and SGR of fish fed dietary treatments

- Average Daily weight Gain (ADG): Mean weight gain (MWG) / rearing period (56days)
- Percentage Body Weight Increase (BWI %): Mean weight gain / mean initial weight x 100
- Specific Growth Rate (SGR); ($\log_e$ Wt - $\log_e$ Wi) /T X100
- Where Wt = mean final weight (g), Wi = mean initial weight (g) and T= rearing period (days)
- Feed Conversion Ratio (FCR) = [dry weight of diet fed (g)] / [fish weight gain (g)]
- Protein Efficiency Ratio (PER) = [fish weight gain (g) / [protein fed (g)]

Apparent Net Protein Utilization (ANPU) = Pb-Pa / Pi where Pb is the total protein of the body at the end of the feeding trial, Pa at the beginning of the trial and Pi is the amount of protein consumed over the experimental period. Net Energy Retention (NER) = [energy retained (kcal g^{-1})] / [energy consumed (kcal g^{-1})

Data Analysis: Biological data generated were subjected to one-way analysis of variance (ANOVA) of the General Linear Model (GLM) using SAS[25] statistical package. Where means were significantly different, they were compared with Student-Neuman-Keul`s Test (SNK).

RESULTS

Fish were observed to be in good condition of health, feeding voraciously on all the diets throughout the period of the experiment. Fish survival was 100% in all the group treatments. Table 3 shows the performance evaluation indices of *C. gariepinus* fed on the test diets. The results showed that the productivity rates of the fish (MWG, SGR, FCR, PER and ANUP) were very good in all the diets with Specific Growth Rate (SGR) exceeding 3% and Feed Conversion Ratio (FCR) below 1 (as fed basis) in all the diets. Daily feed intake of fish were similar

Table 3: Performance evaluation of *C. gariepinus* fed on experimental diets

	Diets						
Productive index	1 (control)	2	3	4	5	6	± SEM
Initial weight (g)	6.19	6.30	5.99	6.23	6.25	6.28	0.05
Final weight (g)	56.57^{d}	33.87^{a}	33.18^{a}	36.10^{a}	38.87^{b}	46.93^{c}	3.73
MWG (g)	50.57^{d}	27.57^{a}	27.19^{a}	29.87^{a}	32.62^{b}	40.65^{c}	3.75
ADG (g)	1.03^{d}	0.56^{a}	0.55^{a}	0.61^{a}	0.67^{b}	0.83^{c}	0.08
BWI (%)	813.89^{d}	437.62^{a}	453.92^{a}	479.45^{a}	522.92^{b}	647.29^{c}	59.39
SGR (%day^{-1})	4.51^{d}	3.39^{a}	3.50^{a}	3.59^{a}	3.73^{b}	4.11^{c}	0.18
FCR	0.70^{a}	0.95^{d}	0.94^{d}	0.89^{c}	0.88^{c}	0.75^{b}	0.04
PER	2.78^{a}	2.17^{d}	2.64^{c}	2.67^{c}	2.69^{bc}	2.70^{b}	0.09
ANPU(%)	55.65^{a}	42.19^{c}	45.19^{bc}	46.50^{bc}	48.62^{b}	54.00^{a}	6.11
NER (Kcal g^{-1})	0.49^{b}	0.68^{a}	0.68^{a}	0.64^{a}	0.63^{a}	0.50^{b}	0.08

Means within the same row having different superscripts are significantly different (p=0.05) at the 95% confident level

Table 4: Mean (±SEM)[1] proximate composition of the carcass of *C. gariepinus* fed experimental diets

	Diets							
	Lysine supplt.(%)	0	0.6	0.9	1.2	1.5	1.8	
Composition (%)	Initial	1	2	3	4	5	6	±SEM
Moisture	77.62	77.80	71.02	71.63	71.51	72.02	71.35	0.14
Ash	2.07	2.74	1.83	1.96	1.94	1.94	1.90	0.17
Protein	11.95	15.22	15.43	15.25	15.70	15.80	16.29	0.36
Lipid	6.77	9.66	12.00	12.03	11.13	11.16	11.68	0.25

[1]Standard error of pooled means excluding the values obtained for the initial carcass analysis

($p \leq 0.05$) in all the treatments. Comparatively, fish fed on 0.96 fish meal based diet (control) had the best overall performance and significantly ($p \leq 0.05$) different from other groups of fish fed maize gluten supplemented with crystalline L-lysine based diets. There was however, a progressive increase in fish performance and nutrient utilization efficiencies with increase in L-lysine supplementation in diets 2 to 6. Fish fed diets containing 0.9 to 1.5% L-lysine supplementation did not show any significant different ($p \leq 0.05$) in productivity indexes. However, fish fed on 1.8% dietary inclusion of L-lysine had superior ($p \leq 0.05$) performances than other groups of fish except for the fish fed on fish meal based diet. The ANPU value of diet 6 (1.8% lysine supplementation) was higher than the diets containing maize gluten but similar to the value obtained for the control diet.

The retention (accretion) of dietary energy (NER) in fish during the experiment were similar ($p \leq 0.05$) for all the levels of lysine supplementation but differ significantly ($p \leq 0.05$) from the value obtained for fish fed on basal diet (control).The results of the whole body proximate composition of the fish at the beginning and end of the experimental period is presented in Table 4. The protein and lipid contents of the fish showed a marked increase over the initial whole body composition, although, the protein values of the fish were similar in all the treatments.

The lipid values of fish fed with maize gluten supplemented with lysine showed higher values of fat deposition than the fish fed on control diet.

DISCUSSION

The result of this study shows the efficacy of maize gluten supplemented with crystalline L-lysine as a potential source of amino acid for enhancing productivity index in a partially substituted fish meal diets for the African clariid catfish, *C. gariepinus.* All the fish fed on the experimental diets showed no dietary related mortality or morphological symptoms of nutrient deficiencies such as dorsal or caudal fin erosion and colour pigmentations which are symptoms of lysine deficiency and effects of dietary inclusion of maize gluten in fish diets[26]. The temperature (26±1°C) and dissolved oxygen (0.8 mg L^{-1}) values were within the range recommended for African catfish culture[27,28]. The efficiencies of protein utilization of fish fed diets 2 to 6 increased correspondingly with increasing levels of lysine supplementation resulting in an improved growth performance of the catfish. Thus, fish fed on 1.8% dietary inclusion of lysine had the best growth performance index with ANPU value that compared favourably with fish fed on the control diet. This trend of fish performance corroborates the findings of Williams *et al.*,[22] that supplementation of crystalline amino acids especially lysine, improves the growth performance of fish. Similar observation was made by Davies *et al.*,[29] using wheat gluten supplemented with lysine to enhance the performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Cheng *et al.*,[30],Mente and Deguara[16] also showed that more than 50% fish meal in rainbow trout and Atlantic salmon (*Salmo salar*) diets could be replaced by plant protein supplemented by crystalline lysine.

In spite of the high productivity indexes, BWG, SGR, FRC, PER and ANPU, of the fish fed maize gluten supplemented with crystalline L-lysine in diets 2 to 6, superior growth performance and nutrient utilization efficiencies were obtained in fish fed on a fish meal based diet. This superlative performance may be attributed to the high quality of the fish meal used in the diet formulation. Several research findings[5,9,31,32] attested to the complete nutritional profile of good quality fish meal as preference for fish in meeting their requirements for physiological growth. However, the use of wild fish from capture fishery to feed farm fish species places direct pressure on fisheries resources[4]. Reduction of fish meal by 75% in diets containing maize gluten supplementation as shown in this study brought about a significant decrease in fish growth responses. This may be attributed to differences in the nutrient density, digestibility and sub-optimal amino acid balance of the maize gluten based diets compared to the fish meal basal diet in spite of lysine supplementation. This also suggests that optimum lysine requirement in the diets containing maize gluten has not been attained even at 1.8% supplementation.

The efficiencies of protein utilization and growth performance increased with increasing lysine supplementation, indicating that free (non-bound) crystalline amino acids are available for absorption and utilization by the catfish. Since the requirement for a specific amino acid is to some extent proportional to the dietary protein level, then, more than 1.8% supplementary lysine would be required to meet an optimal balance in the diets containing maize gluten. This could have given better growth performance and efficiency of protein utilization by the fish. This lysine level is appreciably lower than the estimated requirement values of 2.3%[11] for catfish. Growth of rainbow trout fed diets containing 0.9, 1.3 and 1.7% dietary lysine did not give optimum performance when diets contained either 35% or 55% crude protein. However, excess supplemental amino acids in fish diets has been reported to cause lower growth rates in some species of fish due to an imbalance of amino acids at the sites of protein synthesis[33] which often cause poor growth performance in fish compared to naturally bound amino acid protein sources. Similar observation was made by Zarate *et al.*,[34] that utilization of free lysine did not improve growth in channel catfish even with increasing feeding rate. Williams *et al.*,[22] reported that the utilization of free amino acids is dose dependent and greater fish performance is achieved similar to bound amino acids at the lower level of supplementation. It has been reported that excess lysine in fish diets can cause amino acid antagonism[35,36]. Similar investigation by Mente and Deguara[16] showed that there was an overall reduction in essential free amino acids concentrations (threonine and lysine) as dietary maize gluten content increased and an increase in histidine and leucine.

Our investigation shows that fish productivity index was improved by an increase in lysine supplementation in maize gluten protein based diets. However, a plateau level of supplementation was not attained at 1.8% dietary lysine to give an optimum growth performance for clariid catfish. There is need for further study to establish the optimum dietary supplementation of lysine in maize gluten for *C. gariepinus* before reliable information is available for application in practical diet formulations for this species.

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