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Dietary Improvement and Utilization Efficiencies of Maize Gluten Supplemented with Lysine for Nile Tilapia, *Oreochromis niloticus*. (L.)

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Abstract: The effect of supplemental crystalline L-lysine amino acid to improve the nutritive quality of maize gluten meal in diets of tilapia, *Oreochromis niloticus* was investigated in a 56-day experimental period using growth performance, efficiencies of nutrients utilization and whole-body composition as the response criteria. The trial comprised of eight isoproteic (32% crude protein) and isoenergetic (12% lipid) diets containing High Grade Protein (HGP) source from low temperature Norwegian fish meal (LT-94) as major provider of protein in diet 1, which served as a positive control or maize gluten meal as Low Grade Protein (LGP) source in diets 2-8 with fish meal reduced by 65%. Diets 3-8 containing mostly of maize gluten protein were supplemented with crystalline L-lysine at the rate of 1.3, 1.6, 1.9, 2.2. 2.5 and 2.8%, respectively while diet 2 without lysine supplementation served as the negative control. Diets were fed to triplicate groups of hatchery bred juvenile fish at 4% body weight twice daily at 10.00 and 16.00hrs in a freshwater recirculation system consisting of 24 fiber tanks with 15 fish randomly allotted to each tank. The results showed that reducing fish meal protein component in LGP diets (D2-D8) had negative effect on the growth performance and nutrient utilization of the fish. The fish fed on diet 2 (negative control) had the poorest growth, less efficient for feed consumption and nutrient utilization. However, increasing lysine supplementation in the diets (D3-D8) resulted in a progressive increase in growth performance and nutrient utilization indices. Optimum growth performance was however achieved for group of fish fed with diet containing 1.9% lysine supplementation but lower in values ($p \leq 0.05$) compared to the fish fed with fish meal based diet.

Key words: Supplemental, L-lysine, maize gluten, diets, fish, growth, nutrient, utilization

INTRODUCTION

Several research studies on fish nutrition have shown that higher quantity and quality of protein in feeds are essential for increased fish production per hectare in intensive aquaculture[1-3]. However, the cost of feeding has been estimated to account for about 40-60% of the total costs of raising fish in intensive aquaculture in most developing countries[4,5], while fish meal alone accounts for more than 40% of the total cost of finished fish feed[3]. Reducing protein cost in fish diet formulations has necessitated a quest for cheaper and locally available plant and animal protein sources to replace high cost fish meal and soybean that have other competing uses by man[6-9]. The increasing world demand for high grade protein crops and the prospect for cheaper and affordable plant and animal protein sources have enlisted considerable interest in the production and processing of locally grown grain leguminous crops. One of the most widely cultivated and agronomically suitable grain crop in most countries of the world is maize (*Zea mays*) and it is recognized that considerable improvement of this crop in terms of protein quality is feasible[10] for utilization as protein source in fish feed. Maize gluten has been demonstrated to show significantly higher nutritional value for raising salmon and sea bream[11,12] than unprocessed maize meal which is conventionally used as an energy source in practical feed formulation for warm water fish species in the tropics[13]. Maize gluten meal is a high protein by-product of maize processing, during which most of the starch, bran and germ are removed with the crude protein level ranging between 41-43% with low

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Table1: Formulation ($g100g^{-1}$ dry matter) and proximate composition of experimental diets for *Oreochromis niloticus*

Components	D1	D2	D3	D4	D5	D6	D7	D8
Fishmeal[1] (71% cp)	40.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
Maize gluten1 (63.9% cp)	-	28.0	28.0	28.0	28.0	28.0	28.0	28.0
Oil plant[2]	5.6	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Plant oil[3]	1.6	4.7	4.7	4.7	4.7	4.7	4.7	4.7
L-lysine[4]	-	-	1,3	1.6	1.9	2.2	2.5	2.8
L-Glutamic acid[4]	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Vitamins[5]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Minerals[5]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2,0
WheatFeed (Filler)	42.0	39.6	39.6	39.6	39.6	39.6	39.6	39.6
Binder (CBM-C)[4]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Alpha cellulose	-	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Proximate composition (%)								
Moisture	5.53	8.72	7.77	9.27	6.73	7.47	7.61	7.34
Protein	33.78	31.78	32.72	32.01	33.22	34.25	34.11	32.19
Ash	7.15	3.72	3.39	3.36	4.66	4.64	4.14	4.08
Lipid	11.90	12.46	13.07	12.52	12.66	12.06	12.13	11.67

[1]Skretting Aquaculture (a Nutreco company, Scotland, UK.). [2]Mazola, CPC International. [3]Cod liver oil (Seven seas Ltd. Hull, U.K.) [4]Sigma Chemical Co. Dorset, U.K. [5]Supplying per kg of diet:vitamin A,5000IU; vitamin D3,1000IU; alpha tocopherol, 100IU; menadione, 100mg; thiamin HCL, 30mg; riboflavin, 45mg; pyridoxine HCL,30mg; cyanocobalamin, 12mg; ascorbic acid, 1000mg; D-calcium pantothenate, 105mg; choline chloride, 5000mg; folic acid, 10.5mg; inositol, 500mg; biotin,5,10mg; niacin,150mg

fiber and lipid contents[14,12]. Successful results on the incorporation of maize gluten meal into fish diets have been documented in species such as channel cat fish, milk fish[15], sea bream[12], rainbow trout[5] and Atlantic salmon[16]. However, one major problem associated with maize gluten utilization in feeds is insufficient lysine content[17,11,16] which unfortunately happens to be the first limiting amino acid in fish[18]. Supplementation of maize gluten with lysine in fish diets could be promising especially in countries that have comparative advantage in maize production.

The Nile tilapia, (*Oreochromis niloticus)* is an inexpensive protein source in many rural communities and an excellent table fish in urban centers in the tropics and most Asian countries. It has wider geographical distribution, economic potential, ability to reproduce in captivity, good converter of feed and commands excellent market preference in many tropical countries[19-21]. In spite of the culturable attributes of Nile tilapia and its potential means of alleviating animal protein deficiencies amongst the rural populace, availability of cheap but cost effective feeds to meet the growing needs of aquaculture production of this valuable fish species remains a myriad. This study is therefore aimed at evaluating the effects of maize gluten, a Low Grade Protein (LGP), supplemented with crystalline lysine as a partial replacement for high grade protein (HGP) fish meal (low temperature Norwegian fish meal) on growth performance and nutrient utilization efficiencies of Nile tilapia, *Oreochromis niloticus*.

MATERIALS AND METHODS

Experimental diets: The trial comprised eight isonitrogenous (32 % crude protein) and isocaloric (12% lipid) for juvenile tilapia diets (D1-D8: Table 1) containing Norwegian Low Temperature (LT-94) fish meal with 71% crude protein (high grade protein; HGP-DI) or maize gluten meal with 64% crude protein (low grade protein; LGP-D2-D8). Protein in diets 2 to 8 were furnished by fish meal and maize gluten meal at ratio 1:2 mixture (weight),, respectively. The HGP diet-D1, therefore, served as the positive control while the LGP diet -D2, without lysine supplementation, served as a negative control. In order to minimize amino acid imbalance in the LGP diets, (D3-D8), containing mostly of maize gluten protein, L-lysine in crystalline form were supplemented at the rate 1.3, 1.6, 1.9, 2.2, 2.5 and 2.8% dietary inclusion, respectively. All the diets were mixed mechanically and extruded using a Hobart A-120 pelleting and mixing machine (Hobart Manufacturing Ltd., Bristol, England) before passing through a 3mm die to produce feed pellets. Pelleted diets were dried in a drying cabinet for 48 hrs at 400C to harden and immediately kept in polyethylene bags in freezer at -25°C prior to use.

Experimental fish and their management: 200 male juveniles, *Oreochomis niloticus* of uniform sizes (6.45±0.4g) obtained from a stock held at the Department of Genetics, University of Swansea, Swansea, England were fed on standard tilapia diet in fiber tanks pre-experimental period. The acclimated period was seven days after which 15 fish per tank were assigned to each of the eight dietary treatments in three replicates. The selection and allocation of the fish were such that the initial mean group weights were identical. Water was allowed into the tanks through a spray bar at the rate of 3.2 liters per minute after filtration. Water supply was well aerated using a sump compartment with a flow through system which was maintained through out the experiment. Fish were provided with a photoperiod of 12 hrs light and

Table 2: Calculated values of lysine content of experimental diets (% dietary protein)

Components	D1	D2	D3	D4	D5	D6	D7	D8
Fish meal	2.2	0.77	0.77	0.77	0.77	0.77	0.77	0.77
Maize gluten	-	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Wheat Feed	0.26	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Suppl.lysine	-	-	1.30	1.60	1.90	2.20	2.50	2.80
Total lysine in diet (%)	2.46	1.33	2.63	2.93	3.23	3.53	3.83	4.13
Total lysine in protein(%)	7.08	3.74	7.40	8.24	9.09	9.93	10.77	11.62

12hrs darkness. Water temperature was maintained at 27°C while dissolved oxygen at saturation level was 0.8 mg L^{-1}. The fish were fed the test diets at 3% body weight per day in two equal portions between 9.00-10.00h and 16.00-17.00h for 49days. All fish were removed from each tank every 7days, batch weighed and the quantity of feed for subsequent feeding adjusted accordingly.

At the beginning of the feeding trial, 50 fish representing the initial stock and five sample fish from each of the treatment tanks at the end of the experiment were sacrificed by anesthesia using MS222 while avoiding loss of blood. Sampled fish were put into polyethylene bags which was carefully labeled according to groups and kept deep-frozen at-25°C prior to chemical analysis.

Analytical procedures: Prior to dietary formulation, the crude protein of fish meal and maize gluten meal were analyzed by the automated kjeldahl method[22]. Formulated diets and fish carcasses used for the experiment were analyzed for proximate composition using standard methods according to[22].

Diet performance evaluation: The effects of maize gluten supplemented with L-lysine in diets of Nile tilapia were evaluated based on growth and nutrients utilization indices as described by[23] as follows:

- Weight gain (%) = 100 (final body weight -initial body weight/initial body weight
- Specific growth rate (SGR %/ day) = 100 {loge final body weight-loge initial body weight.}/Time (days)

Food Conversion Ratio (FCR) = (dry weight of feed fed (g)/fish weight gain (g)

Protein Efficient Ratio (PER) = {fish weight gain (g)}/ {protein fed (g) }

Apparent Net Protein Utilization (ANPU) = Final body protein -Initial body protein x 100/total protein fed (g).

Statistical analysis: Statistical comparisons were made using one-way analysis of variance. Mean differences were obtained using Duncan Multiple Range Test[24] while standard errors were calculated to identify the range of the means.

RESULTS

Table 2 presents calculated L-lysine content of the experimental diets. The values showed an increase in lysine level of the diets with corresponding increase in lysine supplementation. Diet 2 (i.e., negative control) had the least lysine content of 3.74% in the diet as against 5.12% dietary lysine requirement for *O. niloticus.*

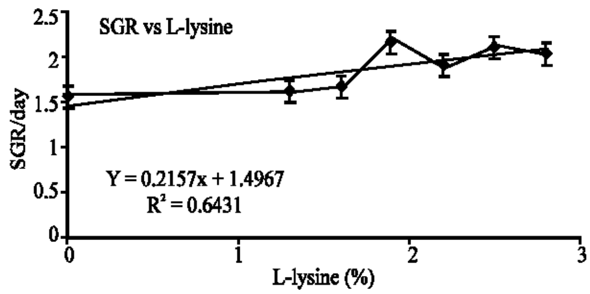

Fig. 1: Relationship between the specific growth rate and the supplemental crystalline L-lysine in the diets of *O. niloticus*

The results of growth performance and efficiencies of nutrients utilization by *O. niloticus* fed maize gluten supplemented with graded levels of L-lysine is presented in Table 3. Reducing fish meal protein component in LGP diets (D2 - D8) by 65% (Table 1) had significant effect on the performances of the fish as fish fed on control diet (i.e.,HGP-D1) had the best ($P \le 0.05$) overall growth and nutrient utilization indices while fish fed on the negative control diet (Diet 2) had the poorest growth, less efficient for feed consumption and nutrient utilization compared to other diets. However, increasing lysine supplementation in the diets (D3-D8) resulted in a progressive increase in fish performance. There was, however, a relative stabilization of growth performance of fish at 2.5% level of lysine supplementation (Fig. 1) while depression in fish performance was obtained in diet supplemented with 2.8% lysine (D8). Although, there were no significant differences ($p \le 0.05$) in growth responses among fish fed on LGP diets containing 1.9 to 2.8% lysine supplementation; optimum growth was, however, achieved for fish fed at 1.9% lysine supplementation (D5) based on WG, SGR, PER and ANPU values. Feed consumption of fish fed on 2.5% lysine dietary supplementation was highest (29.16g day-1) among the LGP diets with corresponding high food conversion efficiency (1.38). In all, fish fed on diet without lysine supplementation (D2) had the poorest ($p \le 0.05$) growth performance and nutrient utilization indices.

Table 3: Growth performance and nutrient utilization efficiencies of *O.niloticus* fed experimental diets

Parameter	D1 Control	D2 Control	D3	D4	D5	D6	D7	D8
L-lysine Supplement(%)	-	-	1.3	1.6	1.9	2.2	2.5	2.8
Initial Weight(g)	6.38	5.97	6.03	6.64	5.63	6,35	5.77	5.96
Final Weight(g)	24.19^{a}	12.64^{d}	13.18^{cd}	14.89^{c}	15.94^{b}	16.03^{b}	15.94^{b}	15.83^{b}
Weight gain(%)	279.15^{a}	111.73^{d}	118.57^{cd}	124.25^{c}	183.13^{b}	152.44^{b}	176.26^{b}	165.60^{b}
Feed Intake(g/day^{-1})	36.76^{a}	25.32^{c}	26.21^{c}	28.80^{b}	28.64^{b}	30.58^{b}	29.16^{b}	28.63^{b}
SGR (%day^{-1}	2.78^{a}	1.56^{d}	1.63^{cd}	1.68^{cd}	2.17^{b}	1.93^{b}	2.12^{b}	2.04^{b}
FCR	0.99^{d}	1.82^{a}	1.76^{ab}	1.67^{b}	1.67^{b}	1.52^{bc}	1.38^{c}	1.39^{c}
PER	2.9^{a}	1.5^{c}	1.6^{c}	$1.7b^{c}$	2.1^{b}	1.9^{b}	2.0^{b}	2.0^{b}
ANPU (%)	41.1^{a}	23.8^{c}	20.2^{cd}	23.3^{c}	28.7^{b}	26.4^{b}	27.7^{b}	28.6^{b}

Values in each row with the same superscripts are not significantly difference ($p \leq 0.05$)

Table 4: Whole- body composition (% dry matter basis) of *O. niloticus* fed different levels of L-lysine supplementation

Carcass	Initial	D1	D2	D3	D4	D5	D6	D7	D8
Moisture (%)	73.23	71.06	70.70	73.18	72.34	71.67	72.27	72.48	72.22
Protein (%)	13.65	14.21	14.77	13.94	13.96	13.80	14.18	13.77	14.16
Ash (%)	3.05	3.13	2.89	3.03	2.94	2.59	2.54	2.31	2.35
Lipid (%)	3.58	8.25	8.90	7.62	8.00	7.08	7.84	7.31	8.01
Protein : Fat Ratio	3.8	1.7	1.7	1.8	1.7	1.9	1.8	1.9	1.8

Whole body composition (% dry matter basis) of fish is presented in Table 4. Reducing fish meal dietary component in LGP (D2-D8) and increasing L-lysine supplementation in maize gluten diets had no significant effect on dry matter, total body protein or total ash and fat. However, there was an average increase of 75% in body fat of fish fed on experimental diets compared to the initial body fat of the fish. Protein: Fat ratio of the fish at the end of the feeding trial were significantly lower ($p \leq 0.05$) than value obtained for fish at the beginning of the experiment.

DISCUSSION

This investigation provides some basic information on the utilization of maize gluten supplemented with crystalline L- lysine in the diets containing low level (14% by weight) dietary inclusion of High Grade fish meal Protein (HGP) for *O. niloticus*. With regards to performance characteristics of the fish, this study further corroborates earlier reports[25-27] that good growth, feed consumption and feed utilization efficiency of cultured fish species depend to a large extent on good quality dietary inclusion of fish meal as shown by superlative ($p \leq 0.05$) growth performance of fish fed on fish meal based diet (Diet 1). The inferior growth performance of fish fed on diet 2 is obviously attributed to low level of lysine failing to meet the nutritional requirements of *O.niloticus*[28] as shown in Table 2. Sub-optimal amino acid balance in maize gluten meal in fish diets is one of the factors attributed to low fish growth performance and nutrient utilization efficiency of cultured fish[29,30,16]. This poor performance is indicative of high FRC and reduced SGR of the fish fed low level lysine in the diet.

There was a significant improvement in fish growth and nutrient utilization with corresponding increase in lysine supplementation in the diets up to 2.5%. However, further increase in lysine content of the diets did not bring about a corresponding increase in fish performances. This study agrees with the findings of[31,32] on rainbow trout that 2.25% dietary lysine level in fish meal-based diet resulted in the highest weight gain. Excess level of dietary lysine has been reported to increase the arginine requirement of animals causing reduction in fish growth performance[33]. Similar study,[34] showed that increase in dietary lysine of Atlantic Salmon (*Salmo salar*) fingerlings affects plasma arginine, urea levels and ammonia excretion of the fish. These changes were found to be due to a decrease in the relative rate of arginine degradation as the level of dietary lysine increased[35]. According to[16] Atlantic salmon had an overall reduction in lysine and threonine as dietary maize gluten content of the diets inceased and an increase in histidine and leucine. Feeding above optimum level of dietary lysine in Nile tilapia, has also been reported to cause reduction in weight gain[1]. It is supportive from this study that lysine supplementation up to 1.9% in maize gluten with low level of fish meal dietary inclusion appears feasible to support good growth and nutrient utilization in juvenile Nile tilapia. The favorable high rate of feed intake as shown in diets 6, 7 and 8 with higher lysine supplementation may be due to the palatability characteristics of maize gluten and fish meal proteins as major constituents of the diets. Maize gluten meal has been reported to be highly palatable protein source for fish and does not contain any anti-nutritional factors[36]. Other studies on channel catfish[37] and Nile tilapia[13] have also shown maize to be highly digestible (92-93% digestible protein) and similar to that of soybean meal. The inability of fish fed on maize gluten supplemented with lysine to perform favorably well compared to high grade fish meal based diet agrees with the findings of[38]. These workers reported that supplemented crystalline L-lysine in diets did not have positive effects on growth due to an amino acid imbalance resulting from disproportionate absorption rates and diverting amino acids into catabolic processes rather than anabolic processes. In a recent study,[32] showed that lysine supplementation in plant protein based diets allowed dietary crude protein to be reduced without reducing fish performance. Rodehutscord[39] obtained 1.7% dietary lysine at 55% crude protein for rainbow trout while similar study[5] obtained 2.5% dietary lysine level of 46% for rainbow trout.

The present investigation shows that maize gluten supplemented with L-lysine is better utilized and capable of enhancing growth performance of fish up to an optimum level of 1.9% in diet at 32% crude protein for Nile tilapia, *O. niloticus*. The findings would have practical implication in the production of balanced diets for tilapia using substantial levels of maize gluten meal. Further reduction of fish meal may be possible with the supplementation of lysine but the alternative strategy of blending complementary proteins for both animal sources with maize gluten meal should be explored. The cost implications of using crystalline amino acids must also be evaluated in least cost diet formulation strategies.

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