Journal of Fisheries and Aquatic Science, 2012
ISSN 1816-4927 / DOI: 10.3923/jfas.2012.

Lysine Requirement and its Effect on the Body Composition of *Oreochromis niloticus* Fingerlings

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ABSTRACT

In the use of plant based ingredients for production of cost effective feed, lysine being one of the deficient amino acid in such feed must be present in the required quantity for *Oreochromis niloticus* in order to impact on its production positively. This study was carried out to investigate the lysine requirement and its effect on the body composition of *Oreochromis niloticus* fingerlings. Two hundred and seventy fingerling *O. niloticus* (9.01-11.09 g) were reared in 35 L plastic troughs. Six diets were formulated, containing 35% crude protein. The diets were formulated with ingredients deficient of lysine (Groundnut cake -3.23 g lysine/100 g diet and guinea corn -1.9 g lysine/100 g diet) for *O. niloticus* having whole body content of 7.51 g lysine/100 g protein. The diets consist of a basal diet (Diet I, no added lysine), while the test diets (II, III, IV and V) had 4.56, 6.87, 7.30, 7.41 g lysine/100 g protein, respectively. The reference diet was made of Clupeids (mixture of *Pellonula afzeliusi* and *Physalia pellucida*), groundnut cake and guinea corn but no added lysine (5.37 g lysine/100 g protein). The fish were fed twice daily (09.00 and 18.00 h) at 3% body weight for 56 days. There was significant difference in the mean weight gain and food conversion efficiency ($p<0.05$). The four levels of lysine resulted in quadratic responses ($p<0.05$). Calculation using regression equations from the relationship between the specific growth rate, weight gain and lysine levels show that lysine requirement for fingerling was 7.12 g/100 g protein, respectively. The requirement of *O. niloticus* for lysine is 7.12 g lysine/100 g protein.

Key words: Lysine, *Oreochromis niloticus*, fingerlings, groundnut cake, protein

INTRODUCTION

Lysine has one major function of protein deposition in animal body. It is the second most limiting amino acid (Robinson and Li, 2007). Lysine helps the absorption of calcium, maintain healthy blood vessels, produce antibodies, enzymes, collagen and repairs of tissues. A typical commercial production diet formulated for tilapia or catfish contains approximately 32-40% protein (Miles and Chapman, 2008). Fish do not have a specific protein requirement but rather a definite requirement for essential amino acids that comprise proteins. Lysine is the most important amino acid, accounting for 7.2% of protein. Lysine is the first limiting essential amino acid in many protein sources used for feed and lysine rich-ingredients are often expensive. According to Miles and Chapman (2008), if fish feed contains the exact amount of EAA required by a fish species then the ideal protein for that species is met and so no amino acid deficiency or excess. The growing fish fed such diet would use very few amino acids for energy. The amino acids would be used efficiently for maintenance, health and synthesis of new structural proteins which would result in maximum feed efficiency and growth. National Research Council (1993) reported *O. niloticus* require 14 g lysine/kg diet while Corazon and Richard (1988) observed that *O. niloticus* requires 14.3 g lysine/kg diet. Other species of fish studied are *Oreochromis mossambicus* 16 g lysine/kg diet; *Ictalurus punctatus* 12 g lysine/kg diet; *Clarias gariepinus* 23 g lysine/kg diet and *Cyprinus carpio* 22 g lysine/kg diet. Encarnacao *et al.* (2004) reported increase in feed intake and growth with increased dietary lysine level. Further to this, they reported that efficiency of lysine utilization for whole body protein deposition was affected both by lysine and digestible energy levels in the diet.

Peres and Oliva-Teles (2008) also reported increased weight gain and final body weight with increased dietary lysine levels for *Scophthalmus maximus* juvenile, although they observed that voluntary food intake was not affected by dietary lysine diet. Ruchimat *et al.* (1997) observed that weight gain, feed efficiency, protein efficiency ratio and nitrogen retention increased for *Seriola quinqueradiata* with increasing levels of dietary lysine up to 1.85 g/100 g diet and remained nearly the same thereafter. According to Bureau and Encarnacao, 2006, published estimates of lysine requirements for rainbow trout varied as widely (1.3-2.9 g/100 g of diet). They further observed that the experimental design and condition between the different laboratories/experiments may be contributory (Kim *et al.*, 1992; Wilson, 1993; Cowey, 1994; Hauler and Carter, 2001a, b). National Research Council (1993) observed that the lysine requirements of fish range from 5.0 to 6.8% of dietary protein, the highest value being the nutritional requirements of carnivorous fish. Bureau and Encarnacao (2006) also observed that growth rates achieved with the semi purified diets used were much lower than when practical diets were used. Adequate dietary lysine contents improve survival and growth rate and prevent erosion and deformities of fish dorsal, pectoral and ventral fin (Keembiyehetty and Gatlin, 1992). Lysine is beneficial to fish because it helps absorption of calcium, maintain healthy blood vessels, produce antibodies, enzymes, collagen and repairs of tissues, it also produces carnitine which helps to convert fatty acids into energy and maintain cholesterol in the blood. This study was carried out to investigate the lysine requirement and its effect on the body composition of *O. niloticus* fingerlings.

MATERIALS AND METHODS

Feed and feeding/experimental design and water exchange: This study was carried out from 16th August 2009 to 10th October 2009 (56 days). Six diets were formulated, containing 35% crude protein. The diets were formulated with ingredients deficient of lysine (groundnut cake -3.23 g lysine/100 g protein and guinea corn -1.9 g/100 g protein) for *O. niloticus* fingerling as the whole body content is 7.51 g lysine/100 g protein (Table 3). The diets were made up of a basal diet (Diet I, no added lysine), while the test diets (II, III, IV and V) had 4.56, 6.87, 7.30, 7.41 g lysine/100 g protein, respectively. The reference diet (VI) was made of Clupeids (mixture of *Pellonula afzeliusi* and *Physalia pellucida*), groundnut cake and guinea corn but no added lysine (5.37 g lysine/100 g protein). The fixed components of all the diets were oil, premix, vitamin C and starch. Table 1 shows the composition of the ingredients while Table 2 shows the composition of experimental diets g kg^{-1}. The amino acid profile of *O. niloticus* was used as reference dietary amino acids profile (Table 3). The diets were formulated, pelleted and sun dried for three days. The amino acid composition of the diets was analyzed using Technicon TSM-1 multiple analyzer (Table 4). The experiment was in factorial design of six treatments, replicated thrice (6×3).

O. niloticus fingerlings of mean weight 9.01-11.09 g were obtained from the National Institute for Freshwater Fisheries Research hatchery complex New Bussa Nigeria and randomly distributed

Table 1: Proximate composition of ingredients

	Composition (%)					
Ingredients	Moisture	Protein	Lipid	Crude fibre	Ash	NFE
Groundnut cake	4.85	41.02	41.30	0.70	5.65	6.48
Fish meal	4.95	63.44	21.95	0.80	11.49	Nil
Guinea corn	6.93	11.17	17.10	1.60	2.18	61.02

NFE: Nitrogen free extract

Table 2: Composition of experimental diets (g kg^{-1})

	Diet (g kg^{-1})					
Ingredients	I	II	III	IV	V	VI
Groundnut cake	646.62	638.64	622.67	606.71	590.74	235.00
Lysine	0	10.00	30.00	50.00	70.00	0
Fish meal	-	-	-	-	-	235.00
Guinea corn	163.38	161.36	157.33	153.29	149.26	340.00
Cod liver oil	100.00	100.00	100.00	100.00	100.00	100.00
Premix	60.00	60.00	60.00	60.00	60.00	60.00
Starch	20.00	20.00	20.00	20.00	20.00	20.00
Vitamin C	10.00	10.00	10.00	10.00	10.00	10.00

Table 3: Amino acid composition of *Oreochromis niloticus*

	Composition (g/100 g protein)	Composition (g/100 g protein)
Amino acids	Fry	Fingerlings
Lysine	5.30	7.51
Histidine	2.24	2.39
Arginine	5.01	6.04
Aspartic acid	7.85	8.19
Threonine	3.23	4.03
Serine	3.66	4.01
Glutamic acid	10.23	13.63
Proline	3.87	4.45
Glycine	4.13	7.25
Alanine	4.48	6.46
Cystine	0.79	0.71
Valine	4.10	4.80
Methionine	2.19	2.40
Isoleucine	3.49	3.99
Tyrosine	3.18	7.15
Phenylalanine	3.51	2.86
Leucine	6.25	3.77

into eighteen 35 L plastic troughs. The fish were fed twice daily (09.00 and 18.00 h) at 3% b.wt. for 56 days. All troughs were well aerated using air pumps, cleared of feed remnants and faecal materials. Water was replaced daily to make up for that siphoned out, while complete replacement was done on every sampling day. Fish were sampled by bulk weighing biweekly. Samples of five fish was analyzed before and after the experiment for carcass composition and amino acid composition.

Table 4: Essential amino acid composition of diets

	Composition of diet (g/100 g protein)					
Amino acid	I	II	III	IV	V	VI
Lysine	4.56	6.87	7.30	7.41	7.51	5.37
Histidine	2.32	2.19	2.32	2.38	2.57	2.38
Threonine	2.39	2.55	2.72	2.83	2.77	2.66
Arginine	4.76	5.28	4.93	5.44	5.79	4.76
Valine	3.83	3.95	4.24	4.41	4.18	4.07
Methionine	1.67	1.72	2.11	1.77	1.88	2.14
Isoleucine	3.08	3.26	2.89	2.95	3.32	3.14
Leucine	7.58	7.80	8.24	8.34	7.80	8.13
Tyrosine	3.38	3.38	3.61	3.70	3.54	3.31
Phenylalanine	5.92	4.40	4.23	4.40	4.56	4.22

Measurement of growth parameters: The growth parameters were calculated as follows:

- Mean weight gain (MWG) = Wt-Wo
- Wo= Mean initial weight; Wt= Mean final weight
- Specific growth rate (SGR) (%/day) = 100×(ln Wt-ln Wo)/days
- Feed conversion efficiency (FCE) = Weight gain (g)/dry food intake
- Protein efficiency ratio (PER) = (weight gain per fish×100)/N×6.25 given per fish
- N= Nitrogen content of feed

Statistical analysis: Statistical analysis was done using SPSS version 10. Polynomial regression curves were graphically represented for the mean weight gain Food conversion efficiencies, protein efficiency ratio and the specific growth rate were utilized to determine the requirement. The second order polynomial equation was also calculated for the requirement of lysine. One-Way Analysis of Variance (ANOVA), Student Newman Keuls (SNK) and Duncan's multiple range test was employed to test for the significance of the parameters.

RESULTS AND DISCUSSION

The lysine content of *O. niloticus* fry and fingerlings was 5.3 and 7.51 g/100 g protein, respectively (Table 3). The lysine composition of the diets ranged from 4.56 to 7.51 g/100 g protein (Table 4). There was significant difference in the mean weight gain and food conversion efficiency ($p<0.05$) (Table 5). The four levels of lysine resulted in quadratic responses ($p<0.05$).

The mean weight gain, food conversion efficiency and protein efficiency ratio all resulted in minima at diet III (7.30 g lysine/100 g protein). When the specific growth rate was regressed on levels of lysine there was an exponential relationship from fish fed 7.30 to 7.51 g lysine/100 g protein. The regression coefficients (R^2) for mean weight gain and food conversion ratio are 0.854 and 0.54, respectively. Analysis of the growth data showed a minimum at 7.30 g lysine/100 g protein of diet and rose to the highest point of 7.51 g lysine/100 g protein. The quadratic equation of this relationship between weight gain, specific growth rate and lysine content shows that lysine requirement for *O. niloticus* fingerlings was 7.12 g/100 g protein.

O. niloticus fingerlings responded positively to the highest level of lysine. This is an indication that at this level the relationship of lysine and other essential amino acids was not impaired. Jackson and Capper (1982) reported 4.1 g lysine/100 g protein for *O. mossambicus* while

Table 5: Growth parameters of *O. niloticus* fingerlings fed varying levels of lysine for 56 days

	Diet (g/100 g protein)					
Growth parameters	I	II	III	IV	V	VI
MIW	11.09±3.84	9.01±1.84	9.09±1.90	11.09±3.84	11.09±3.84	9.20±1.54
MFW	24.96±6.93	13.70±2.32	17.81±4.12	17.57±3.20	21.14±5.92	10.28±2.17
MWG	13.89±1.67^{d}	4.69±0.73^{b}	8.72±1.14^{c}	8.15±1.36^{c}	13.38±2.48^{d}	1.08±0.07^{a}
SGR	0.72±0.71^{c}	0.44±0.30^{b}	0.77±0.37^{b}	0.80±0.33^{b}	1.09±0.53^{c}	0.16±0.01^{a}
PER	4.8±3.32^{a}	1.42±1.08^{a}	2.91±1.90^{a}	3.07±1.49^{a}	4.60±2.70^{a}	0.54±0.09^{a}
FCE	14.37±4.24^{c}	2.97±0.29^{b}	3.51±2.33^{b}	4.39±1.06^{b}	14.18±8.21^{c}	0.79±0.09^{a}

Values in the same row with the same superscript are not significantly different at (p>0.05), MIW: Mean initial weight, MFW: Mean final weight, MWG: Mean weight gain, SGR: Specific growth rate, PER: Protein efficiency ratio, FCE: Food conversion efficiency

Table 6: Carcass composition of *O. niloticus* fingerlings fed varying levels of lysine for 56 days

	Composition (%)					
Diet	Moisture	Ash	Crude fibre	Crude protein	Lipid	NFE
1	71.30	2.40	0.90	18.17	7.06	0.17
2	74.08	3.60	0.30	18.51	3.30	0.21
3	70.98	3.40	0.10	17.84	7.60	0.08
4	76.00	2.30	0.20	18.17	3.30	0.03
5	71.20	3.60	0.10	15.82	8.60	0.68
6	73.00	4.10	0.25	18.85	3.80	Neg
Initial	78.60	3.69	2.28	12.30	3.00	0.03

NFE: Nitrogen free extract, Neg: Negligible

Corazon and Richard, 1988) and National Research Council (1993) reported 5.13 g lysine/100 g protein and 5.1 g lysine/100 g protein respectively for *O. niloticus* requirement which are slightly lower than the requirement obtained in this study. This may be attributed to the observation made by Bureau and Encarnacao (2006) that growth rates achieved with the semi purified diets in experiments were much lower than when practical diets were used.

Carcass composition shows that protein and fat increased while moisture, ash and crude fibre decreased except for ash in the reference diet (Table 6). The positive increase in carcass protein observed in all treatments shows that there was deposition of tissue. Fish fed diet I, which is the basal diet had crude protein level similar to others with additional lysine indicating that the levels of lysine supplied did not make any difference in the deposition of protein observed although in the somatic growth of the fish it contributed positively throughout the experiment. According to Bureau and Encarnacao (2006) the efficiency of utilization of dietary free amino acids differs among published reports. Williams *et al*. (2001) observed that the efficiency of the utilization of crystalline amino acids is dependent on dietary protein level. In this study, the reference diet had only intact protein as the basal diet but the response of the fish to the diets varied being better in the basal diet than the reference. The carcass protein of the fish fed the basal diet (18.17%) and that fed the reference diet (18.85%) were not significantly different (p>0.05). This shows that the utilization of animal protein in the diet of *O. niloticus* does not have an advantage (Robinson and Li, 2007). The diets supplied also positively affected the percentage fat deposited during the rearing period, this may have contributed to the weight gain observed at harvest. Nitrogen free Extract (NFE) of the fish did not follow the trends of other components in that Fish fed diet VI had lower levels than the fish stocked while fish fed diet IV had the same level.

Water quality parameters in this experiment showed that temperature ranged from 28-31°C, pH 7.4, Dissolved oxygen from 0.5-7.8 mg L^{-1} and conductivity from 44-3100 μcm^3.

No morphological deficiency signs were observed in fish fed the varying levels of lysine. This is similar to the observation of Fagbenro *et al.* (1998). According to Keembiyehetty and Gatlin (1992) lysine helps production of antibodies and prevents erosion and deformities in fins. In conclusion the lysine requirement of *Oreochromis niloticus* is about 7.12 g/100 g protein.

ACKNOWLEDGMENT

This study was funded by the Federal Republic of Nigeria, through National Institute for Freshwater fisheries Research, New Bussa.

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