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LYSINE REQUIREMENT AND ITS EFFECT ON THE BODY COMPOSITION OF *OREOCHROMIS NILOTICUS* FINGERLINGS

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ABSTRACT

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 - 11.09g) were reared in 35L plastic troughs. Six diets were formulated, containing 35% crude protein. The diets were formulated with ingredients deficient of lysine (Groundnut cake -3.23g lysine/100g diet and guinea corn -1.9g lysine/100g diet) for O. niloticus having whole body content of 7.51g lysine/100g protein. The diets consist of a basal diet (Diet I, no added lysine), while the test diets (II, III, IV and V) had 4.56g, 6.87g, 7.30g, 7.41g lysine/100g 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 (5.37g lysine/100g protein). The fish were fed twice daily (0900 and 18.00hrs) 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 7.12g/100g protein respectively. The requirement of O. niloticus for lysine is 7.12g lysine/100g 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. NRC (1993) reported *O. niloticus* require 14g lysine/kg diet while Santiago and Lovell (1988) observed that *O. niloticus* requires 14.3g lysine/kg diet. Other species of fish studied are *Oreochromis mossambicus* 16g lysine/kg diet; *Ictalurus*

punctatus 12g lysine/kg diet; *Clarias gariepinus* 23g lysine/kg diet and *Cyprinus carpio* 22g 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.

Pere and Oliva – Teles, (2007) 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.*, (1998) observed that weight gain, feed efficiency, protein efficiency ratio and nitrogen retention increased for *Seriola quinqueradiata* with increasing levels of dietary lysine up to 1.85g/100g 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 – 2.9g/ 100g of diet). They further observed that the experimental design and condition between the different laboratories/experiments may be contributory (Kim *et al.*, 1992a; Wilson, 1993; Cowey, 1994; Hauler and Carter 2001a and b). NRC (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

Six diets were formulated, containing 35% crude protein. The diets were formulated with ingredients deficient of lysine (Groundnut cake -3.23g lysine/100g protein and guinea corn -1.9g/100g protein) for *O. niloticus* fingerling as the whole body content is 7.51g lysine/100g 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 4.56g, 6.87g, 7.30g, 7.41g lysine/100g 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 (5.37g lysine/100g 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. 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)

Table 1: Proximate composition of ingredients

Ingredients	Moisture	Protein	Lipid	Crude fibre	Ash	NFE
Groundnut cake	4.85	41.02	41.30	0.70	5.65	6.48
Fishmeal	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

The experiment was in factorial design of six treatments, replicated thrice (6x3).

O. niloticus fingerlings of mean weight 9.01-11.09g were obtained from the National Institute for Freshwater Fisheries Research hatchery complex New Bussa Nigeria and randomly distributed into eighteen 35L plastic troughs. The fish were fed twice daily (0900 and 18.00hrs) at 3% body weight 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 2: Composition of experimental diets (g/kg)

Ingredients	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet 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*

AMINO ACIDS	COMPOSITION g/100g protein for Fry	COMPOSITION g/100g protein for 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

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 Keul's (SNK) and Duncan's multiple range test was employed to test for the significance of the parameters. The

growth parameters were calculated as follows:

Mean Weight Gain (MWG) = $W_t - W_o$

W_o = Mean initial weight; W_t = Mean final weight

Specific Growth Rate (SGR) (%/day) = $100 \times (\ln W_t - \ln W_o) / \text{days}$

Feed Conversion Efficiency (FCE) = $\text{Weight gain (g)} / \text{dry food intake}$

Protein Efficiency Ratio (PER) = $(\text{weight gain per fish} \times 100) / N \times 6.25$ given per fish

N = Nitrogen content of feed

Table 4: Essential Amino Acid composition of diets

AMINO ACID	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET 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
Isoleusine	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
Phenylalnine	5.92	4.40	4.23	4.40	4.56	4.22

RESULTS AND DISCUSSION

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 (7.30g lysine/100g protein). When the specific growth rate was regressed on levels of lysine there was an exponential relationship from fish fed 7.30g to 7.51g lysine/100g 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 7.30g lysine/ 100g

protein of diet and rose to the highest point of 7.51g lysine/ 100g protein. The quadratic equation of this relationship between weight gain, specific growth rate and lysine shows that lysine requirement for *O. niloticus* fingerlings was 7.12g/100g 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 4.1g lysine/100g protein for *O. mossambicus* while Santiago and Lovell, (1988) and NRC, (1993) reported 5.13g lysine/100g protein and 5.1g lysine/100g 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 Encaarnacao (2006) that growth rates

achieved with the semi purified diets in experiments were much lower than when practical diets were used.

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

Growth parameters	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet 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±2.7 ^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

Figures in the same row with the same superscript are not significantly different ($P>0.05$)

MIW- Mean Initial Weight ; MFW- Mean Final Weight; MWG- Mean Weight Gain; SGR- Specific Growth Rate; PER- Protein Efficiency Ratio; FCE- Food Conversion Efficiency

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 Menghe, 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.

Water quality parameters in this experiment showed that temperature ranged from 28 – 31⁰C, pH 7.4, Dissolved oxygen from 0.5 – 7.8mg/L and conductivity from 44 – 3100 μ cm³.

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

	% Moisture	% Ash	% Crude fibre	% Crude protein	% Lipid	% NFE
Diet I	71.30	2.40	0.90	18.17	7.06	0.17
Diet II	74.08	3.60	0.30	18.51	3.30	0.21
Diet III	70.98	3.40	0.10	17.84	7.60	0.08
Diet IV	76.00	2.30	0.20	18.17	3.30	0.03
Diet V	71.20	3.60	0.10	15.82	8.60	0.68
Diet VI	73.00	4.10	0.25	18.85	3.80	Neg.
Initial	78.60	3.69	2.28	12.30	3.00	0.03

No morphological deficiency signs were observed in fish fed the varying levels of lysine. This is similar to the observation of Fagbenro *et al* (1998a). According to Keembiyehetty and Gatlin (1992) lysine helps production of antibodies and prevents erosion and deformities in fins.

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REFERENCES

- Bureau Dominique P, Encarnacao Pedro M (2006). Adequately defining the amino acid requirements of fish: The case example of lysine. UG/OMNR Fish nutrition Research Laboratory, Department of Animal and Poultry Science University of Guelph, Guelph, Ontario, N1G2W1 Canada
- Cowey CB (1994). Amino acid requirements of fish: a critical appraisal of present values. *Aquaculture*. 124, 1-11
- Encarnacao, P., Cornelis de Lange, Markus Rodehutschord, Dirk Hoehler, Wafa Bureau, Dominique, P. Bureau (2004). Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout (*Oncorhynchus mykiss*) for maximum growth. *Aquaculture* 2335: 569 – 586
- Encarnacao P, CFM de Lange, Bureau DP. (2006). Diet energy source affect lysine utilization for protein deposition in rainbow trout (*Onchorhynchus mykiss*). *Aquaculture* (in press).
- Fagbenro OA, Balogun AM, Fasakin EA, Bello-Olusoji (1998a) Dietary lysine requirement of the African catfish, *Clarias gariepinus*. *Journal of Applied Aquaculture* 8 (2) 71-77.
- Hauler R, Carter C (2001a). Re-evaluation of the quantitative dietary requirement of fish. *Rev. Fish. Sci.* 9, 133 – 163.
- Hauler R, Carter C (2001b). Lysine deposition responds linearly to marginal lysine intake in Atlantic salmon (*Salmo salar* L) parr. *Aquaculture Research*, 32 (suppl. 1), 147 – 156
- Jackson AJ, Capper BS (1982). Investigation into the requirements of the , *Sarotherodon mossambicus* for dietary methionine, lysine and arginine in semi – synthetic diets. *Aquaculture*, 29, 289 - 297
- Kim KI, Kayes TB, Amundson CH (1992a). Requirements for lysine and arginine by rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 106, 333 – 344
- Keembiyehetty CN, DM. Gatlin III (1992). Dietary requirement of juvenile hybrid striped bass (*Morone chrysops* x *M. saxatilis*). *Aquaculture* 104: 271 – 277. *Ictalurus punctatus*.

- Aquaculture
http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B...
- Miles Richard D, Chapman Frank A (2008). The concept of ideal protein in formulation of Aquaculture feeds. Department of Fisheries and Aquatic sciences, University of florida IFAS Extension. FA 144
- National Research Council (1993). Nutrient requirements of fish. Washington: National Academic Press. 114p
- Peres Helena, Oliva – Teles Aires (2007). Lysine requirement and efficiency of lysine utilization in turbot (*Sophthalmus maximus*) juveniles. Aquaculture
http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4D-4RDS3YC-2&_user=10&_o
- Robinson Edwin H, Li Menghe H (2007). Catfish protein nutrition. Delta Research and Extension Centre, Mississippi State University. MAFES Bulletin 1090.
<http://www.thefishsite.com/articles/293/catfish-protein-nutrition>
- Ruchimat Toni, Toshiro Masumoto, Hidetsuyo Hosokawa, Yoshiaki Itoh, Sadao Shimeno (1998). Quantitative lysine requirement of yellow tail (*Seriola quinqueradiata*).
[http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4D-3553VSF-&_user=10\\$or](http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4D-3553VSF-&_user=10$or)
- Santiago Corazon B, Lovell Richard T (1988). Amino acid requirements for growth of Nile Tilapia. J. Nutr 118,1540 – 1546
- Williams K, Barlow, Rodgers L (2001). Efficacy of crystalline and protein bound amino acid enrichment of diets for barramundi/asian seabass (*Lates calcarifer* Bloch). Aquaculture Research 32, 415 – 429
- Wilson RP (1993). Amino acid nutrition of fish. A new method of estimating requirement values. Proceeding of the 20th US- Japan Symposium on Aquaculture Nutrition. Newport, Oregon. 28 – 39 October 1991. Collie, M. R. & McVey, J. P. (Eds) pp. 49-54