Dietary Methionine Requirement of *C. Gariepinus* Fingerlings and Its Effect On The Growth And Body Composition

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Abstract: The experiment was conducted to determine the methionine requirement for *Clarias gariepinus* and its effect on growth and body composition. Hatchery bred fingerlings of *C. gariepinus* (2.97±.036g) were stocked in eighteen 54L glass aquaria. Six diets (40% crude protein) consisting of a basal diet containing 1.81g methionine/100g protein from the ingredients soyabean and guinea corn was formulated, a reference diet (3.12g methionine/ 100g protein) and others having graded levels of crystalline methionine (2.87, 2.97, 3.00 and 3.07g/100g protein). Each diet was fed to three aquaria twice daily (8.00 – 18.00hrs) for 56 days. The mean weight gain, specific growth rate, food conversion efficiency and protein efficiency ratio were significantly influenced by the level of methionine ($P<0.05$). Second order polynomial regression analysis showed that the requirement of *C. gariepinus* for methionine is 2.97g/100g protein. Carcass protein showed an increase above that of the initial fish stocked before feeding commenced. The percentage lipid also increased except for the fish fed 3.07g methionine/ 100g protein. [Report and Opinion. 2010; 2(1):22-27]. (ISSN: 1553-9873).

Keyword: Clarias fingerlings, methionine, body composition, requirement

Introduction

In feed formulation the present trend and most beneficial is to consider least-cost, with low fish meal and high level of plant proteins. All plant proteins contain anti-nutritional compounds (NRC 1993) which limit bioavailability of some amino acids (Cai and Burtle 1996). Methionine and lysine are the limiting essential amino acids in plant based fish diets. Methionine also serves as a precursor to carnitine (Tacon 1990). It is also required for building muscles and detoxifying the liver (Health Vitamin Guide.com). Its availability in the appropriate quality is important. The methionine requirements of several species have also been reported (Table 1).

Table 1: Quantitative methionine requirement of various fish species

Fish Species	Methionine requirement	Reference
Channel catfish	9.4g/kg	Cai and Burtle (1996)
Catla catla	3.55g/100g protein	Ravi and Devaraj (1991)
Seriola quinqueradiata	2.56g/100g protein	Ruchinat et al., (1997)
Pseudosciaena crocea	3.34g/100g protein	Mai et al., (2005)
Cirrhinus mrigala	3g/100g protein	Ahmed et al., (2006)
Epinephelus coioides	2.73g/100 protein	Luo et al., (2005)
Rachycentron canadum	2.64g/100g protein	Zhou et al.,(2005)
Dicentrachus labrax	2.0g/100g protein	Thebault et al., (1985)
Oncorhynchus mykiss	2.2g/100g protein	Walton et al., (1982)
Channel catfish	2.3g/100g protein	Nose (1989)
Oreochromis niloticus	2.7g/100g protein	Santiago and Lovell (1988)
Anguilla japonicus	2.9g/100g protein	Harding et al., (1977)
Cyprinus carpio	3.1g/100g protein	Harding et al., (1977)
Clarias gariepinus	3.2g/100g protein	Fagbenro et al., (1998)
Sparus aurata	4.0g/100g protein	Walton et al., (1982)
Seriola quinqueradiata	3.1-3.4g/100g protein	Twibell et al., (1999)

According to Lim and Dominy (1989) a protein with an essential amino acid composition which closely matches the essential amino acid requirements of the fish is described as being of high nutritive value. A protein that is deficient in one or more essential amino acids is of low biological value. Previous

analytical work on the whole body protein of *C. gariepinus* shows that methionine makes up 2.77g/100g protein of the fish (Fagbenro *et al*., 2001). This study was conducted to determine the methionine requirement and its effect on the body composition of *Clarias gariepinus* fingerlings

2. Experimental Design, Feed Preparation and Sampling

Clarias gariepinus fingerlings (initial mean weight 2.97 ± 0.36g) were stocked 6 fish per glass aquaria (54L) for six treatments. Each treatment was replicated thrice in a 2 x 3 x 6 factorial experimental design. The fish were fed five diets which were having ingredients that are methionine deficient (Guinea corn 0.85 + soyabean 0.96 = 1.81g/100g protein). The methionine content of *C. gariepinus* is 2.77g/100g protein. A basal diet containing 1.81g methionine/100g protein was formulated. The proximate composition of the ingredients was analyzed as in Table 2. The experimental diets were supplied with 2.87g, 2.97g, 3.00g and 3.07g methionine/100g protein respectively (Table 3). A reference diet was formulated with fish meal and groundnut cake in addition to the ingredients in the basal diets. They were pelleted and sun dried for three days. The diets were fed to three replicates of fish stocked in 54L glass aquaria twice daily for 56 days.

Sampling was carried out biweekly by bulk weighing the fish. On sampling days complete replacement of water was carried out, while on other days remnant of feed and faecal wastes were siphoned out. Partial replacement of water was done on those days.

Table 2: Proximate Composition of Ingredients

Ingredients	% Moisture	% Protein	% Lipid	% Ash	% Crude Fibre	% NFE
Fishmeal	4.81	63.44	21.95	9.00	0.80	Neg.
Soyabean	1.73	43.49	34.60	6.26	6.80	7.12
Guinea corn	6.93	11.17	17.10	2.18	1.60	61.02
Groundnut cake	4.85	41.02	41.30	5.65	0.70	6.48

Table 3: Percentage Composition ingredients in Diets

	Soyabean	Fish meal	Groundnut cake	Guinea corn	Premix	Starch	Oil	Methionine
DIET I	758.30	-	-	91.70	30.00	20.00	100.00	-
DIET II	757.4	-	-	91.60	30.00	20.00	100.00	10.00
DIET III	755.6	-	-	91.40	30.00	20.00	100.00	30.00
DIET IV	753.8	-	-	91.20	30.00	20.00	100.00	50.00
DIET V	752.1	-	-	90.9	30.00	20.00	100.00	70.00
DIET VI	234.2	117.1	351.4	147.30	30.00	20.00	100.00	-

Aqua Biomix Fish Premix

Vitamin A	i.u	20,000,000	Vitamin B2	mg	30,000	Folic acid	mg	4,000
Vitamin D3	i. u	2,000,000	Niacin	mg	150,000	Biotin	mg	800
VitaminE	mg	200,000	Pantothenic acid	mg	50,000	Choline chloride	mg	600,000
Vitamin C	mg	500,000						
Vitamin K3	mg	8,000	Vitamin B6	mg	12,000	Cobalt	mg	2,000
Vitamin B1	mg	20,000	Vitamin B12	mg	50	Copper	mg	4,000
Iodine	mg	5,000	Selenium	mg	200	Antioxidant	mg	100,000
Inositol	mg	200,000	Zinc	mg	40,000	Lysine	mg	100,000
Iron	mg	40,000	Manganese	mg	30,000	Methionine	mg	100,000

2.1 Chemical Analysis

At the beginning of the experiment five pieces of fish was analyzed for their chemical composition. At the end of the experiment five pieces of fish from each treatment was also analyzed (AOAC 2000). Proximate analysis of feed was also carried out

according to AOAC (2000). The proximate composition (percentage) of the diets was also analyzed (Table 4) using the methods in AOAC (2000). The amino acid composition (g/100g protein) of the diets was analyzed using Technicon Sequential Multisample Amino Acid Analyzer (TSM – 1 model DNA 0209) (Table 5).

2.2 Statistical Analysis

Statistical analysis was carried out using computer package SPSS version 10, One – way Analysis of Variance (ANOVA) was utilized to test for significance of growth parameters. Regression graphs were drawn to show the relationship between level of methionine in diet and growth, food conversion efficiency and protein efficiency ratio.

Table 4: Proximate Composition Of Diets

	% Moisture	% Crude Protein	% Crude Lipid	% Ash	% Crude Fibre	% NFE
DIET I	4.02	40.01	24.18	7.45	1.04	23.3
DIET II	3.43	40.00	23.98	8.62	6.24	17.73
DIET III	3.38	40.83	24.10	6.82	2.79	22.08
DIET IV	3.56	40.12	23.94	8.26	4.08	20.04
DIET V	4.14	40.80	24.24	7.63	3.93	19.98
DIET VI	3.72	40.67	24.19	8.43	2.47	20.52

Table 5: Essential Amino acid composition of diets (g/100g protein)

Amino acid	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET VI
Lysine	3.92	5.21	6.01	5.21	6.01	5.69
Histidine	3.32	2.51	2.38	2.13	2.44	3.01
Arginine	7.16	6.98	6.63	6.47	6.80	6.98
Threonine	2.89	2.72	2.55	2.68	3.21	3.00
Methionine	1.81	2.87	2.97	3.00	3.07	3.12
Isoleusine	4.07	4.20	3.95	3.85	4.33	4.20
Leusine	6.09	6.09	5.93	5.71	6.37	6.42
Tyrosine	3.22	3.38	3.22	3.06	3.38	3.54
Phenylalanine	4.06	4.22	4.39	4.05	4.56	4.73
Valine	3.95	4.01	4.30	3.89	4.01	4.18

3. Results Analysis

3.1 Regression analysis, significance and percentage Growth

The quadratic regression graphs of all growth parameters with level of methionine showed maxima at 2.97g methionine/100 protein. The mean final weight, specific growth rate, protein efficiency ratio and food conversion efficiency varied significantly ($P<0.05$) (Table 6). Percentage growth of fish ranged from 96% to 155% for fish fed the varying levels of methionine. The growth of the fish fed the basal diet was not significantly different from the fish fed additional methionine ($P<0.05$).

3.2 Carcass content

The moisture, crude protein and fat content of all experimental fish were higher than initial. The ash and fibre content of the initial fish stocked before feeding commenced were higher than that of the fish harvested at the end of the experiment. The crude protein content of all fish fed methionine was higher than the those fed the basal and reference diets. (Table 7)

Table 6: Growth of *C. gariepinus* fingerlings fed varying levels of methionine in 56 days

	Mean Initial Weight (g)	Mean Final Weight (g)	Mean Weight Gain (g)	Specific Growth Rate (%)	Protein Efficiency Ratio	Food Conversion Efficiency
DIET I	2.97±0.36	6.55±0.3 [a]	3.58±0.3[a]	1.41±0.09[ab]	1.02±0.05[a]	0.033±0.004[a]
DIET II	2.97±0.36	5.84±0.29 [a]	2.87±0.29[a]	1.21±0.09[a]	0.91±0.05[a]	0.041±0.003[bc]
DIET III	2.97±0.36	7.59±1.0 [a]	4.62±1.0[a]	1.66±0.24[b]	1.19±0.16[a]	0.047±0.01[cd]
DIET IV	2.97±0.36	6.02±1.28 [a]	3.05±1.28[a]	1.35±0.31[ab]	0.94±0.2[a]	0.03±0.004[a]
DIET V	2.97±0.36	6.46±1.07 [a]	3.99±0.81[a]	1.37±0.3[ab]	1.01±0.17[a]	0.035±0.001[ab]
DIET VI	2.97±0.36	12.04±1.34 [b]	9.07±1.34 [b]	2.5±0.19[c]	1.88±0.21[b]	0.053±0.01[d]

Figures with the same superscript in a column are not significantly different (P>0.05)

Table 7: Carcass composition of *Clarias gariepinus* fingerlings fed varying levels of methionine in 56 days

	MOISTURE	% CRUDE PROTEIN	% CRUDE FAT	% ASH	% CRUDE FIBRE	NFE
DIET I	73.00	19.38	4.30	2.00	0.5	0.82
DIET I	74.05	20.29	3.05	1.85	0.6	0.16
DIET I	72.00	22.59	4.40	1.00	0.2	0.31
DIET I	72.60	21.67	4.00	1.65	0.6	Neg.
DIET I	73.30	21.11	3.30	2.15	0.7	Neg.
DIET I	72.30	19.88	4.05	1.10	0.54	Neg.
INITIAL	71.40	18.90	2.13	3.95	2.45	0.17

4. Conclusion

In using the second order quadratic regression equation derived from the graphs of growth of *C. gariepinus* and level of methionine the point at which dy/dx was equal to zero was 2.97g methionine /100g protein. This result corroborates the earlier experiment carried out with *C. gariepinus* fry (Ovie and Eze, in press). In both fry and fingerling experiments the regression graphs had maxima at 2.97g methionine/100 protein. This is similar to the observation for channel catfish (Wilson and Poe, 1985); Ahmed *et al*., (2006) for *Cirrhinus mrigala*, Zhi *et al*., (2005) for *Epinephelus coioides*; Ruchinat *et al*., (1997) and Twibell *et al*. (2000), for *Seriola quinqueradiata*; Harding *et al*., (1977) for *Anguilla japonicus* and *Cyprinus carpio*; Santaigo and Lovell (1988) for *O. niloticus*, Borlongan and Coloso (1993) for *Chanos chanos*; Jackson and Capper (1982) for *S. mossambicus*; Keembiyehetty and Gatlin, (1993) for hybrid striped bass; Moon and Gatlin, (1991) for *Sciaenops ocellatus*; Nose, (1979) for Carp and Rodehutscord *et al*., (1995) for *O. mykiss*. However, this does not compare well with the observation for red sea bream *Pagrus major*, whose amino acid requirement changed with growth and or age (Takagi *et al*., 2001). This study shows a slightly lower methionine level than that observed for the same species by Fagbenro *et al*. (1998) for *C. gariepinus*. The slight variation could be due to several factors such as fish size, culture protocols, model used, and basal diets composition (Bureau and Encarnacao, 2006). The fact that there was no significant difference in the growth of fish fed varying levels of methionine and that fed the basal diet shows that *C. gariepinus* grows appreciably with feed of low biological value. This result is similar to an earlier study on *C. gariepinus* fry (Ovie and Eze, in press). When the growth of fish of all diets containing methionine is compared with the fish fed the reference diet the latter is superior, an indication of the bioavailability of the essential amino acid than that of the experimental diets (Miles and Chapman, 2007). This result is similar to the observation made for *Ictalurus punctatus* that supplementation of methionine in low protein but

amino acid replete diets were not beneficial (Li and Robinson 1998). The percentage weight gain in this study exceeds that reported by Twibell *et al*., (2000) for *Seriola quinqueradiata* but lower than that reported by Fagbenro *et al*., (1998) for *C. gariepinus.*

The carcass composition of the fish fed varying levels of methionine varied significantly (P<0.05). This agrees with Ahmed *et al*., (2003) for *Cirrhinus mrigala*. Fish fed methionine above or below the requirement level did not show any nutritional defects. The increase in the methionine content in the diets is not reflected in the protein content of the body, however, all fish fed crystalline amino acid, i.e. methionine showed higher protein content than that fed basal and the reference diets

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