Effect Of Supplementing Methionine In *Clarias Gariepinus* Fry Diet

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ABSTRACT: Hatchery bred fry of *C. gariepinus* (0.440±.04) were stocked in eighteen 54L glass aquaria. Six diets (45% crude protein) consisting of a basal diet (1.81g methionine/100g protein),a reference (3.12g methionine/100g protein) and others having graded levels of crystalline methionine (2.87g, 2.97g, 3.00g 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.
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INTRODUCTION

Methionine is a nutritionally essential amino acid, meaning that it cannot be produced by the fish, and must be provided by the diet. It supplies sulphur and other compounds required by the fish for normal metabolism and growth. Methionine also belong to a group of compounds called lipotropics, or chemicals that help the liver process fats (Healthnotes, 2004). If an amino acid deficiency in a feed formulation did occur, it is generally not a problem because it can be overcome by supplementing the diet with the deficient amino acid or by using a feedstuff high in that particular amino acid (Robinson and Li, 2007). All essential amino acids have to be present in the diet for growth to occur. The primary plant protein sources used in catfish feed are oilseed meals, such as soyabean meal, cottonseed meal and groundnut cake. Generally most plant proteins are deficient in lysine and methionine. The advantages of using all – plant diets include, lower cost, milder flavour of fish flesh and less body fat because of a reduction in dietary energy (Robinson and Li, 2007). Catfish, traditionally have been fed relatively high protein diets (32 – 35%) but they grow just as fast and convert feed as efficiently on diets containing as low as 24% protein. According to Robinson and Li (2007) plant protein are recommended for growing catfish to table size but animal proteins are needed for growing fry and fingerlings. They further observed that no single feedstuff can provide all the nutrient needs of catfish, a mixture of feedstuffs is used.

Several studies on the methionine requirement of fish has been reported, juvenile Large mouth croaker (Kangsen Mai *et al*., 2006); Yellow tail (Ruchinat *et al*., 1997); Juvenile Cobia (Zhou *et al*., 2006); *C. mrigala* (Ahmad *et al*., 2006); *Epinephelus coioides* (Luo *et al*., 2005), Channel catfish (Cai and Burtle, 1996) and Fagbenro *et al*., (1998b).

Cai and Burtle (1996) observed that there is an increasing need for least cost formulated diets with minimal fish meal and maximal plant proteins. NRC (1993) showed that diets including large amounts of plant proteins contain antinutritional compounds, which may limit bioavailability of amino acids. Cai and burtle (1996) utilizing soyabean, corn diets observed rapid growth with catfish with a daily SGR of 2%. They concluded that such diet should contain 9.4g of methionine/kg diet (or 34.1g/kg of dietary protein) in the presence of cystine at 4g/kg of dry diet. Their results showed that an all – plant – protein diet formulated with an amino acid pattern similar to the carcass protein has much promise in channel catfish diet formulation. This study was carried out to investigate the optimal level of methionine required for growth of *C. gariepinus* fry.

MATERIALS AND METHODS

Clarias gariepinus fry (initial mean weight 0.44 ± 0.04g) were stocked 15 fish per glass aquaria for six treatments. Each treatment was replicated thrice in a factorial experimental design (3 x 6 x 15). The fish were fed five diets which had ingredients that are methionine deficient (Guinea corn 0.85 + soyabean 0.96 = 1.81g methionine/100g protein). The methionine content of *C. gariepinus* is 2.77g/100g protein as obtained from analysis using Technicon Sequential Multisample Analyzer. A basal diet

containing 1.81g methionine/100g protein was formulated. The proximate composition of the ingredients was analyzed as in Table 1. The experimental diets were supplied with 2.87g, 2.97g, 3.00g and 3.07g methionine/100g protein respectively (Table 2). 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 1: 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

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 amino acid composition of the diets was analyzed using Technicon Sequential Multisample Amino Acid Analyzer (TSM – 1 model DNA 0209) (Table 4).

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. One – Way Analysis of Variance (ANOVA), Student Newman Keul's (SNK) and Duncan's multiple range test was employed to determine the significance of the parameters.

The growth parameters were calculated as follows:

Mean Weight Gain (MWG) = (Wt – Wo)/Wo

Specific Growth Rate (SGR) (%/day) = 100 x (ln Wt – ln Wo)/t

Feed Conversion Efficiency (FCE) = Weight gain (g)/ dry food intake

Protein Efficiency Ratio (PER) = (weight gain per fish x 100)/ N x 6.25 given per fish

Table 2: PERCENTAGE COMPOSITION OF DIETS

	Soyabean	Fish meal	Groundnut cake	Guinea corn	Premix	Starch	Oil	Methionine
DIET I	760.30	-	-	89.70	30.00	20.00	100.00	-
DIET II	751.39	-	-	88.60	30.00	20.00	100.00	10.00
DIET III	733.5	-	-	86.50	30.00	20.00	100.00	30.00
DIET IV	715.6	-	-	82.90	30.00	20.00	100.00	50.00
DIET V	697.7	-	-	82.30	30.00	20.00	100.00	70.00
DIET VI	237.40	118.7	356.1	137.80	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	mg	50,000	Choline	mg	600,000
Vitamin C	mg	500,000	acid			chloride		
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

Table 3: 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 4: Amino acid composition of diets

	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

RESULTS

The water quality parameters showed that dissolved oxygen ranged from 4 – 6.8mg/l, pH 7.4, temperature 27 – 30 ^{0}C, while conductivity 2100 - 3000µcm^3

All diets resulted in quadratic growth. The quadratic relationship between growth parameters and level of methionine showed a maxima at 2.97g methionine/100g protein for mean weight gain (MWG), specific growth rate (SGR) and protein efficiency ratio (PER). On using the regression equation to calculate the point at which dy/dx is equal to zero 2.97g methionine/100g protein was derived and this represents the requirement of *C. gariepinus* fry for methionine. All growth parameters were positively correlated in linear regression. The r^2 for MWG is 0.618, SGR, 0.668 and PER, 0.623 respectively. All diets were accepted and growth was positive. There was significant difference ($P<0.05$) in the mean weight gain, specific growth rate and protein efficiency ratio of fish fed the varying levels of methionine.

The carcass showed moisture and crude protein for all fish increased above initial. The fat content increased above initial except for fish fed 3.07g methionine/kg protein. The ash content of all fish fed varying levels of methionine was lower than initial.

Table 5: GROWTH PERFORMANCE OF *C. GARIEPINUS* FRY FED VARYING LEVELS OF METHIONINE IN 56 DAYS

	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET VI
Mean Initial weight	0.44±0.04	0.44±0.04	0.44±0.04	0.44±0.04	0.44±0.04	0.44±0.04
Mean Final weight	1.27±0.15	0.83±0.20	0.62±0.11	0.71±0.17	1.01±0.32	5.24±0.74
Mean weight gain	0.83 ± 0.15^{b}	0.39 ± 0.20^{ab}	0.18 ± 0.11^{a}	0.27 ± 0.17^{a}	0.57 ± 0.32^{ab}	2.94 ± 0.84^{c}
Specific Growth rate	1.88 ± 0.2^{b}	1.09 ± 0.42^{ab}	0.63 ± 0.28^{a}	0.69 ± 0.25^{a}	1.42 ± 0.63^{ab}	4.44 ± 1.15^{c}
Protein Efficiency ratio	0.33 ± 0.06^{b}	0.16 ± 0.08^{ab}	0.07 ± 0.04^{a}	0.11 ± 0.07^{a}	0.23 ± 0.13^{ab}	1.19 ± 0.35^{c}

Figures in the same row with the same superscript are not significantly different

Table 6: CARCASS COMPOSITION OF *C. GARIEPINUS* FRY REARED FOR 56 DAYS

	% MOISTURE	% CRUDE PROTEIN	% LIPID	%ASH	%CRUDE FIBRE	NFE
DIET I	74.10	16.83	7.10	1.50	Neg.	0.47
DIET II	70.00	19.18	6.30	2.70	1.25	0.57
DIET III	67.56	23.56	5.80	2.10	0.80	0.18
DIET IV	78.01	17.50	2.60	1.50	0.30	0.09
DIET V	79.84	13.79	3.91	2.20	Neg.	0.26
DIET VI	76.72	14.47	6.40	1.30	1.00	0.11
INITIAL	65.00	12.25	3.43	2.80	0.10	0.32

DISCUSSION

The second order regression analysis curve and calculation from the regression equation showed a maxima at 2.97g methionine/ 100g protein, an indication that this is the requirement of *C. geriepinus* fry. for methionine . Fagbenro *et al.*, (1998) reported 3.2g methionine/100g protein for *C. gariepinus* fingerlings, Harding *et al.*, (1977) observed 29g methionine/kg protein for *Anguilla japonica* and 31g methionine/kg protein for *Cyprinus carpio*, however in this study the requirement was 2.97g methionine/100g protein and this compares well with their observation. The SGR of the fish fed basal diet shows that the addition of methionine to the diets depressed the growth of the fish as none of the levels supplied improved growth over that of the basal diet. Although the SGR for the four levels of methionine were lower than that of the basal diet, these feed significantly influenced the growth of the fish. This corroborates the observation for *Rachycentron canadum* (Zhou *et al*., 2006). The correlation coefficients of MWG, SGR and PER also show that the diets were well utilized for growth. Fagbenro *et al.*, (1998) made similar observation working with *C. gariepinus*. The utilization of methionine among the fish in this study did not result in any clinical signs as was observed with *C. gariepinus*, when the level of methionine was 8 – 9.6g/kg diet (Fagbenro *et al*., 1998). Baker (1987) reported that methionine level twice the requirement is well tolerated by animals but at threefold or higher, toxicity results. There was no toxicity effect in this study as growth was experienced at all levels. Cai and Burtle (1996) observed decrease in growth rate from a diet 80% higher than the requirement of channel catfish. Other studies with the same observation of reduction in food intake include Mambrini and Kaushik (1994), Davis and Morris (1997), Green *et al*., (2002a), Encarnacao *et al.*, (2006), however, *C. gariepinus* in this study accepted the diets at all levels of methionine.

The carcass protein showed that the fish responded positively to all levels of methionine supplied. This also shows that feed intake was not negatively affected as reported in several studies (Cai and Burtle 1996, Davis and Morris 1997, Green *et al*., 2002a and Encarnacao *et al*., 2006).

In this study the basal diet and the reference diet which had no crystalline amino acid had better growth which corroborates the observation for catfish that intact protein are better utilized by fish (Zarate and Lovell, 1997).

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