

Original Research Article

Dietary Crystalline DL-Methionine Supplementation of Oilseed Meal-Based Diets: Effects on Growth and Feed Utilization of Juvenile Nile Tilapia (*Oreochromis niloticus* L.)

ABSTRACT

Aim: This study evaluated the effect of dietary DL-methionine supplementation of diets containing mixtures of oilseed meals (i.e. soybean meal, SBM; cottonseed meal, CSM and groundnut cake, GNC) on the growth and feed utilization of Nile tilapia (*Oreochromis niloticus* L.) of mean initial weight 5.48 ± 0.20 g.

Study design and Methodology: Five isonitrogenous (320 g kg^{-1}), isolipidic (100 g kg^{-1}) and isoenergetic (18 KJ g^{-1}) test diets were formulated in which different mixture combinations of oilseed meal (i.e. EQ50, SBM50, CSM50 and GNC50) proteins enriched with crystalline DL-Methionine (0.5%) replaced fish meal (FM) protein at 50%. The control diet had FM as the major protein source. Fish were fed to satiation from the beginning and later fixed at 4% body weight per day and the study lasted for 56 days. The growth experiment was conducted in plastic tanks in a recirculation system with each dietary treatment in triplicate.

Results: Growth and feed utilization of fish fed the control diet were significantly higher ($P < 0.05$) level than for all the oilseed meal-based diets (supplemented with methionine). There were no significant differences in whole-body protein, moisture, lipid and energy contents among diets, with the exception of Diet 4 which had lower protein content. The cost analysis also showed that the control diet was the most cost effective.

Conclusion: Results in the present study demonstrated that utilization of crystalline DL-methionine (0.5%) by Nile tilapia was not effective in improving the growth, feed utilization and cost effectiveness of the diets containing oilseed meal compared with the fish meal based diet.

Keywords: Oilseed meal mixtures, growth, feed utilization, DL- methionine, supplementation, Nile tilapia.

1. INTRODUCTION

Good quality fish meal is becoming scarce and prices are escalating in recent times. This has generated a lot of interest in the use of alternative protein sources for fish. Plant proteins are the main alternative protein sources normally considered as a solution to the problem and these are

37 mainly from oilseed meals. However, oilseed meal proteins are inferior to fish meal proteins in
38 terms of essential amino acid (EEA) profile required in the diets of cultured fish [1]. The EAA
39 profiles of diets formulated with individual oilseed meals such as soybean meal (SBM),
40 cottonseed meal (CSM) and groundnut cake (GNC) as well as their mixtures in previous studies
41 [2,3,4] showed clear deficiency of one or more EAAs compared to the requirements of Nile
42 tilapia [5]. Crystalline amino acids supplementation in animal diets to enhance EAA profile has
43 been practised successfully in aquaculture but particularly in livestock production for many
44 years [6]. Supplementation with crystalline amino acids is actually an attempt to improve the
45 protein quality of fish feeds by addition of the EAAs that are in deficit in plant proteins [7, 8].
46 EAAs such as methionine and lysine are generally critical in the formulation of fish diets when
47 including plant protein sources [9]. For most EAAs, deficiency is manifest as a reduction in
48 weight gain. However, in some species of fish a deficiency of methionine or tryptophan may
49 lead to pathologies, since these EAAs are not only incorporated into proteins but also used for
50 the synthesis of other compounds [10].

51 Tilapias have a requirement for sulphur-containing amino acids (i.e. methionine, cystine and
52 cysteine) which can be met by either methionine alone or a proper mixture of methionine and
53 cysteine [11]. According to Jauncey and Ross [12] dietary cystine can replace up to 50% of the
54 total sulphur-containing amino acid requirement for *Oreochromis mossambicus*. Several studies
55 have indicated that fish utilize crystalline amino acids less efficiently than protein bound forms
56 [13,14]. Jauncey *et al.* [15] reported difficulty in using purified amino acid test diets with *O.*
57 *mossambicus*. Possible reasons for the poorer utilization of crystalline amino acids compared
58 with protein bound amino acids, may be attributed to different rates of absorption in the gut,
59 creating amino acid imbalances in the tissues [16] and also to leaching of dietary crystalline
60 amino acid during feeding [10] because these losses occur to a greater degree than those of
61 protein-bound amino acids [17]. However, other studies have reported successful use of
62 crystalline amino acids to supplement amino acid deficient diets in improving tilapia growth and
63 feed utilization efficiency [18, 19, 20, 21].

64 In view of contradictory reports on the efficacy of EAA supplementation of tilapia feeds, further
65 study and economic analysis is required to determine whether or not any improvements in
66 growth justify the use of EAAs and the additional feed cost that this would incur. The objective
67 of this study, therefore, was to investigate the effect of crystalline methionine supplementation in
68 oilseed meal based diets on growth, feed utilization and cost-effectiveness of Juvenile Nile
69 tilapia (*Oreochromis niloticus*).

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71 **2. MATERIALS AND METHODS**72 **2.1 Experimental System and Animals**

73 This study was undertaken at the Institute of Aquaculture, University of Stirling, UK. Fingerlings
 74 of Nile tilapia (5.48 ± 0.20 g) were stocked in triplicates in 30-L tanks. Fish were distributed
 75 randomly in the 30-L tanks at a rate of 20 fingerlings per tank in a recirculation system which
 76 was supplied with aerated water at a flow rate of 1 L min^{-1} . Fish were subjected to a constant
 77 photoperiod of 12 hours Light/12 hours Darkness. Water quality parameters measured every
 78 week during the experiment included the following: temperature, 26.97 ± 0.26 °C; pH, $7.29 \pm$
 79 0.27 ; ammonia, $0.05 \pm 0.03 \text{ mg.L}^{-1}$; nitrite, $0.20 \pm 0.0 \text{ mg.L}^{-1}$; nitrate, $20 \pm 0.0 \text{ mg.L}^{-1}$ and
 80 dissolved oxygen, $7.54 \pm 0.52 \text{ mg.L}^{-1}$.

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82 **2.2 Diet Formulation and Preparation**

83 Five isonitrogenous and isoenergetic diets were formulated using a mixture of SBM (crude
 84 protein, 500.3 g kg^{-1}), CSM (crude protein, 441.4 g kg^{-1}), and GNC (crude protein, 430.5 g kg^{-1})
 85 as protein sources. Composition of the different oilseed meal mixtures used for diet formulation
 86 is presented in Table 1 and designation of diets shown correspondingly. FM (crude protein,
 87 716.3 g kg^{-1}) was substituted with different mixtures of SBM, CSM and GNC at 50% of total
 88 protein as presented in Table 2. Diets containing different mixtures of oilseed proteins were
 89 supplemented with 0.5% DL-methionine (M9500, 99.0% TLC; Sigma) to meet the minimum
 90 requirement for Nile tilapia, which is 2.7% of dietary protein [4]. In this study only methionine
 91 was supplemented because this was deemed more critical for Nile tilapia [11].

92

93 **Table 1. Specification of dietary protein levels (%) in the experimental diets fed to fish in**
 94 **this study**

Diet No.	Designation	Percentage of total protein contributed by various ingredients in the diets			
		Fish meal	Soybean meal	Cottonseed meal	Groundnut cake
1	Control	100.00	-	-	-
2	EQ50	50.00	16.67	16.67	16.67
3	SBM50	50.00	25.00	12.50	12.50
4	CSM50	50.00	12.50	25.00	12.50
5	GNC50	50.00	12.50	12.50	25.00

95 *EQ50 = equal contribution of protein from test ingredients to mixture, SBM50 = 50% contribution*
 96 *from soybean meal to mixture, CSM50 = 50% contribution from cottonseed meal to mixture,*
 97 *GNC50 = 50% contribution from groundnut cake to mixture*

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100 Preparation of experimental diets was similar to previous methods by Agbo *et al.* [2]. The diets
 101 were stored at -20°C until fed. Fish were hand-fed three times a day (09:30, 13:00 and 16:00)

up to satiation for the first three weeks and 4% of body weight for subsequent weeks with the experiment lasting for 8 weeks. Diets were dispensed in small portions to ensure prompt consumption and avoid amino acid leaching as recommended by Lovell [10].

Table 2. Composition of diets fed to juvenile *Oreochromis niloticus* using oilseed meal mixtures (g.kg⁻¹ of diet) supplemented with DL-Methionine

Ingredients	Control 1	EQ50 2	SBM50 3	CSM50 4	GNC50 5	Price of ingredients (\$kg ⁻¹)
Soybean Meal	-	100.2	150.4	75.2	75.2	0.432
Cottonseed Meal	-	113.5	85.2	170.4	85.2	0.162
Groundnut Cake	-	116.4	87.4	87.4	175.0	0.360
Fish Meal	420.0	210.0	210.0	210.0	210.0	1.053
Wheat Grain	208.0	211.0	210.5	211.0	210.2	0.090
Sunflower Oil	57.0	47.0	53.8	51.7	35.4	0.450
α- Cellulose	30.0	10.9	12.5	7.0	14.5	0.045
Corn Starch	205.0	106.0	105.2	102.3	109.5	0.090
Binder CMC ¹	20.00	20.00	20.00	20.00	20.00	0.225
Mineral Premix ²	40.0	40.0	40.0	40.0	40.0	1.350
Vitamin Premix ³	20.0	20.0	20.0	20.0	20.0	1.305
DL-Methionine ⁴	0.0	5.0	5.0	5.0	5.0	22.50
Diet cost, \$kg ⁻¹	0.504	0.486	0.495	0.477	0.486	

¹Carboxymethyl cellulose (Sigma, C5013), ²Contained (as g kg⁻¹ of diet): MgSO₄·7H₂O, 20.40; NaCl, 8.00; KCl, 6.04; Fe SO₄·7H₂O, 4.00; ZnSO₄·4H₂O, 0.88; MnSO₄·4H₂O, 0.41; CuSO₄·5H₂O, 0.13; CoSO₄·7H₂O, 0.08; CaO₃·6H₂O, 0.05; CrCl₃·6H₂O, 0.02 (according to Jauncey and Ross [12]). ³Contained (as mg kg⁻¹ of diet): Thiamine (B₁), 85.00; Riboflavin (B₂), 60.00; Pyridoxine (B₆), 25.00; Pantothenic acid, 105.00; Inositol, 500.00; Biotin, 1.80; Folic acid, 20.00; Ethoxyquin, 4.00; Choline, 1481.00; Nicotinic acid (Niacin), 250.00; Cyanocobalamin (B₁₂), 0.03; Retinol palmitate(A), 20.00; Tocopherol acetate (E), 140.00; Ascorbic acid (C), 750.00; Menadione (K), 30.00; Cholecalciferol (D₃), 0.08 (according to Jauncey and Ross [12]). ⁴(Sigma, M9500) Sigma-Aldrich Chemie, Germany. (Price of ingredients are presented in US Dollars)

2.3 Analytical Techniques

Ingredients, diets and carcass were analysed in triplicates for proximate composition according to standard methods [22]. Energy was determined using an Adiabatic Autobomb Calorimeter (Parr 6100, USA) with benzoic acid as a standard. Amino acid content was analysed using LKB 4151 Alpha-Plus Amino Acid Analyser (LKB Biochrom Ltd, UK). Phosphorus was measured following the method by Stirling [23] using a spectrophotometer (Cecil Elegant Technology–Aquarius-P). Some antinutritional factors (ANFs) such as phytic acid, trypsin inhibitors and saponin were analysed following earlier methods used by Agbo *et al.* [24].

2.4 Chemical Composition of Diets

Chemical composition of the diets used in this experiment is presented in Table 3. Crude protein, CL and energy were similar for all diets as formulated. Ash and phosphorus contents of the control diet were higher than for the plant-based diets. Diet 4 (CSM50) had the highest fibre content reflecting higher inclusion level of cottonseed meal (50% of plant mixture). The ANFs

are much lower for the control (Diet 1) compare to Diets 2, 3, 4 and 5 (Table 3). The diets met the EAA requirements of Nile tilapia [5] and exceeded this in most cases with the exception of threonine which was slightly deficient for all the diets (Table 3).

2.5 Analysis of biological parameters

Weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU) and energy retention (ER) were used to evaluate growth and feed utilization as follows:

$$WG (\%) = (final \text{ body weight} - initial \text{ body weight}) / initial \text{ body weight} \times 100$$

$$SGR (\% \text{ day}^{-1}) = 100 \times [\ln(final \text{ body weight}) - \ln(initial \text{ body weight})] / \text{no. of days}$$

$$FI (g) = \text{Total feed intake per fish} / \text{no. of days},$$

$$FCR = \text{feed intake} / \text{live weight gain}$$

$$PER = \text{live weight gain} / \text{crude protein intake}$$

$$ANPU (\%) = 100 \times (\text{final fish body protein} - \text{initial fish body protein}) / \text{crude protein intake}$$

$$ER (\%) = 100 \times (\text{final fish body energy} - \text{initial fish body energy}) / \text{gross energy intake}$$

Whole body composition was determined where whole body samples were analyzed for moisture, crude protein, crude lipid and ash. Results were expressed as percentage of live weight.

Table 3. Biochemical composition of diets fed to Nile tilapia in this study

Proximate composition (g kg ⁻¹ as-fed)	Diets				
	Control	EQ50	SBM50	CSM50	GNC50
	1	2	3	4	5
Dry matter	976.7	971.8	973.0	958.9	973.7
Crude protein	333.4	342.7	342.1	337.2	343.6
Crude lipid	110.3	111.4	107.8	109.4	112.6
Crude fibre	22.8	28.2	26.6	31.9	25.5
Ash	97.8	89.1	88.3	89.7	89.1
Nitrogen free extract	412.4	400.5	408.1	390.7	402.9
Gross energy, (kJ g ⁻¹)	18.36	18.77	18.75	18.74	18.81
Essential amino acid composition (% of dietary protein)					
Arginine	4.20 ¹	5.01	6.11	5.97	6.23
Isoleucine	3.10	3.96	3.71	3.84	3.66
Leucine	3.40	6.41	6.08	6.24	6.03
Lysine	5.10	6.23	5.43	5.55	5.41
Methionine+Cystine ²	2.70	2.72	3.63	3.62	3.66
Phenylalanine+Tyrosine ²	3.80	5.26	5.90	5.96	5.89
Threonine	3.80	3.23	2.93	2.97	2.97
Valine	2.80	4.94	4.59	4.69	4.57
Phosphorous and antinutritional factors (g kg ⁻¹)					
Phosphorus	8.30	7.01	7.33	7.53	7.77
Phytic acid	0.5	7.6	7.1	8.5	7.1
Trypsin inhibitors	0.0	1.8	2.4	1.5	1.6
Gossypol	0.0	0.6	0.5	1.0	0.5
Saponin	1.1	3.4	3.3	3.4	3.5

¹Essential amino acid requirements for tilapia [5], ²Values of methionine and phenylalanine are the requirements in the presence of cystine and tyrosine of the diet respectively.

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2.6 Cost effectiveness of diets and statistical analysis

Cost effectiveness of diets was assessed using Profit Index (PI) as follows; *PI = value of fish/cost of feeding*. The value of fish and cost of diets were calculated using market prices in Ghana but presented in US Dollars.

Each experimental diet was fed to three groups of fish in a completely randomized design. Statistical analyses in this study were conducted using SPSS Statistical Package (Version 16.0, SPSS Inc., Chicago, IL). Data were subject to one-way ANOVA and the Tukey's Multiple Comparison Test applied to evaluate differences between means at $P=.05$. All percentages were arcsine transformed before analysis.

3. RESULTS

3.1 Growth performance and feed utilization

Data on growth performance and feed utilization of Nile tilapia fed the different experimental diets is presented in Table 4 and growth responses are also shown in Figure 1. All experimental diets fed to fish were well accepted and no pathological signs were observed during the trial. FW, WG, SGR, FCR, PER and ANPU in Nile tilapia were significantly higher for the control diet when compared to all the oilseed meal-based diets (supplemented with methionine). Feed intake of fish fed the control (Diet 1), Diets 4 and 5 was significantly higher than that of fish fed Diets 2 and 3 (Table 4). Fish fed Diet 3 (SBM50) had significantly lower energy retention than the control diet.

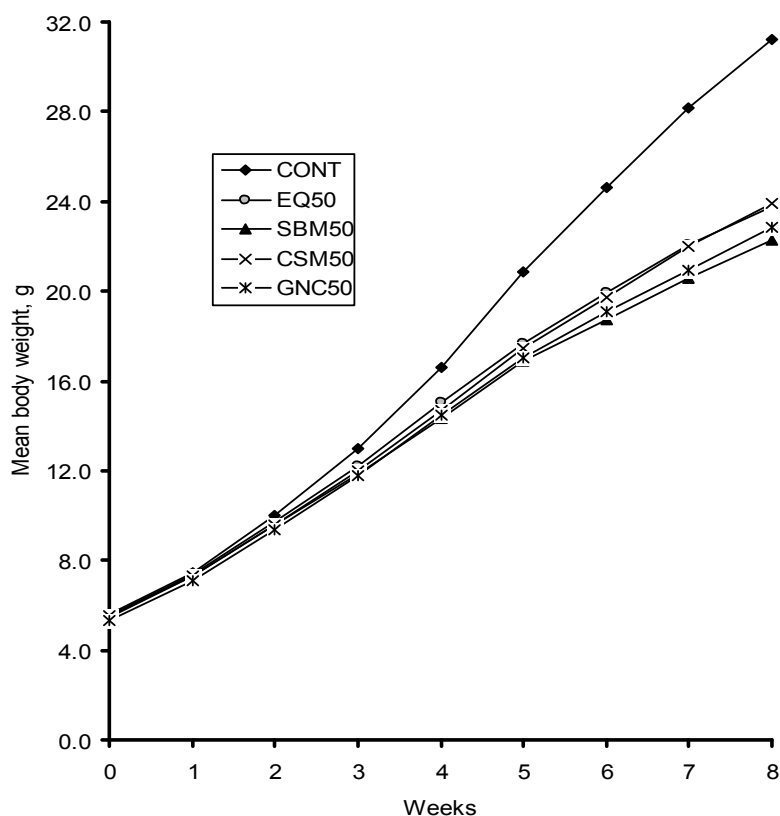


Fig 1. Growth response of fish fed oilseed based diets with DL-methionine supplementation for eight weeks

Table 4. Growth performance and feed utilization of Nile tilapia fingerlings fed diets containing oilseed meal mixtures with DL-methionine supplementation for eight weeks

	Control	EQ50	SBM50	CSM50	GNC50
	1	2	3	4	5
IW	5.59 ± 0.27	5.50 ± 0.28	5.47 ± 0.02	5.50 ± 0.19	5.32 ± 0.17
FW	31.20 ± 0.99 ^a	23.80 ± 0.92 ^b	22.29 ± 1.16 ^b	23.88 ± 1.97 ^b	22.90 ± 1.07 ^b
WG	458.63 ± 28.60 ^a	333.12 ± 23.95 ^b	307.65 ± 20.14 ^b	333.78 ± 21.46 ^b	330.34 ± 6.84 ^b
SGR	3.07 ± 0.09 ^a	2.62 ± 0.10 ^b	2.51 ± 0.09 ^b	2.62 ± 0.09 ^b	2.61 ± 0.03 ^b
FCR	1.34 ± 0.05 ^a	1.82 ± 0.09 ^b	1.99 ± 0.13 ^b	1.84 ± 0.16 ^b	1.94 ± 0.10 ^b
FI	34.31 ± 0.07 ^a	33.22 ± 0.11 ^b	33.42 ± 0.03 ^b	34.09 ± 0.28 ^a	34.12 ± 0.04 ^a
PER	2.24 ± 0.08 ^a	1.61 ± 0.08 ^b	1.47 ± 0.10 ^b	1.60 ± 0.16 ^b	1.50 ± 0.08 ^b
ANPU	32.68 ± 1.16 ^a	24.00 ± 1.13 ^b	21.75 ± 1.4 ^b	23.10 ± 2.28 ^b	23.26 ± 1.20 ^b
ER	26.70 ± 1.03 ^a	20.37 ± 2.73 ^{ab}	19.91 ± 0.90 ^b	22.46 ± 4.36 ^{ab}	20.48 ± 1.16 ^{ab}
S	100.00	100.00	100.00	100.00	100.00
PI	2.66	2.04	1.82	2.04	1.90

IW (g) = Initial weight, FW (g) = Final weight, WG (%) = Weight gain, SGR (%.day⁻¹) = Specific growth rate, S = Survival (%), FCR = Feed conversion ratio, FI (g) = Feed intake, PER = Protein efficiency ratio, ANPU (%) = Apparent net protein utilization, ER (%) = Energy retention, PI=Profit index. Values are means ± SD of three replicates, and means within the same row with different letters are significantly different at P=.05. The prices of ingredients used in this study are presented in Table 2 (all prices are in US Dollars; Sale price of tilapia is 1.80 \$kg⁻¹).

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199 **3.2 Body Composition**

200 At the end of the growth study there were no significant differences in whole-body protein,
 201 moisture, lipid and energy contents among diets, with the exception of Diet 4 which resulted in
 202 lower protein content (Table 5). These values were, however, higher than for initial whole-body
 203 composition. Ash contents of fish fed the control diet were significantly higher than those of fish
 204 fed the plant-based diets.

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206 **Table 5. Whole body composition (% wet weight) of Nile tilapia fed oilseed meal-based**
 207 **diets with DL-methionine supplementation for eight weeks**

	Initial	Control	EQ50	SBM50	CSM50	GNC50
		1	2	3	4	5
MC	75.48	72.66 ± 0.61	72.44 ± 1.07	71.61 ± 0.20	70.85 ± 1.36	70.98 ± 0.27
CP	13.85	14.45 ± 0.17 ^{ab}	14.66 ± 0.06 ^{ab}	14.56 ± 0.24 ^{ab}	14.27 ± 0.45 ^b	15.13 ± 0.43 ^a
CL	5.73	7.78 ± 0.75	8.43 ± 1.19	9.13 ± 0.42	9.93 ± 1.58	8.89 ± 0.37
Ash	3.90	4.31 ± 0.05 ^a	3.57 ± 0.16 ^b	3.77 ± 0.11 ^b	3.83 ± 0.12 ^b	3.78 ± 0.16 ^b
GE	5.41	6.36 ± 0.27	6.57 ± 0.48	6.93 ± 0.10	7.22 ± 0.63	7.00 ± 0.08

208 *MC = moisture content, CP = crude protein, CL = crude lipid, CF = crude fibre, GE = gross*
 209 *energy (kJ.g⁻¹). Values are means ± SD of three replicates, and means within the same row with*
 210 *different letters are significantly different at P=.05.*

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212 **3.3 Cost-effectiveness Analysis of Diets**

213 The costs of ingredients used in this analysis are presented in Table 2. The cost per kilogram of
 214 experimental diets varied little with the control having the highest (\$0.504 kg⁻¹) and Diet 4
 215 (CSM50) the least (\$0.477 kg⁻¹) (Table 2). The cost analysis however, showed that the highest
 216 profit was obtained by the control diet and the lowest by Diet 3 (SBM50) (Table 4). Among the
 217 oilseed meal-based diets, Diets 2 and 4 were the most profitable.

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219 **4. DISCUSSION**

220 Results from the present study indicated that there was significantly lower growth performance
 221 and feed utilization in Nile tilapia fed oilseed meal-based diets supplemented with methionine
 222 compared with the control diet (fish meal-based diet). However, ER of fish fed Diets 2, 4 and 5
 223 were not significantly different from the control diet. Amongst the test diets, Diets 2 and 4 gave
 224 better growth performance and feed utilization even though this was not significantly different
 225 from the other diets. Despite the fact that growth and feed utilization of fish fed oilseed meal-
 226 based diets were lower than that of the control in this study, they showed considerable
 227 improvements particularly in FCR, PER and ANPU compare to earlier studies. Values are
 228 significantly higher than in previous studies using the same oilseed meals [2,3,4] at similar plant
 229 protein levels and comparable to those of various researchers who reported improved growth of
 230 fish with amino acid incorporation at similar inclusion levels of plant protein [20,21,25,26].

231 Crystalline amino acids supplementation in fish diets has had variable success. According to
232 Shiau *et al.* [27], male tilapia (*O. niloticus* x *O. aureus*) fed diets in which 100% of the fishmeal
233 was replaced with SBM either with or without methionine supplementation had significantly
234 lower weight gain, feed utilization and protein digestibility than in groups fed diets containing
235 fishmeal as the sole source of protein. Andrews and Page [28] observed no improvement in
236 growth of channel catfish when L-methionine was supplemented to a soybean meal-based diet.
237 Teshima [29] also reported similar observation for Nile tilapia when he supplemented SBM with
238 methionine, and concluded that it was unnecessary. Davis and Stickney [30] and El-Saidy and
239 Gaber [20] reported that feeding blue tilapia and Nile tilapia respectively with 100% SBM, firstly
240 with methionine supplementation and secondly with methionine and lysine, had no significant
241 effect on growth parameters and feed utilization.

242 In contrast, Shiau *et al.* [31] reported that the growth of tilapia was improved with addition of
243 supplemental methionine and Murai *et al.* [32] also reported improvement in the nutritional
244 value of soy flour by addition of 0.4% crystalline L-methionine. In other studies partial
245 replacement of fish meal was achieved using a combination of plant proteins supplemented with
246 amino acids. A mixture of plant proteins (corn gluten, wheat gluten, extruded peas, rapeseed
247 meal and sweet white lupin) balanced with EAAs could provide between 50 - 75% of protein in
248 diets fed to gilthead sea bream, *Sparus aurata* without significantly affecting performance [33].
249 A sizeable proportion of the fish meal in diets for juvenile turbot, *Psetta maxima* could be
250 replaced by a mixture of plant proteins from lupin, corn gluten and wheat gluten meal
251 supplemented with amino acids without affecting growth and nutrition [34].

252 Although supplementation of diets with DL-methionine in the present study resulted in
253 improvements in feed utilization compared to previous studies [2,3,4], the oilseed meal-based
254 diets did not do well in terms of growth and feed utilization compared to fish fed the fish meal-
255 based diet. The superiority of fishmeal in this respect may be attributed to the absence of any
256 known antinutritional factors (Table 3), higher protein levels and to the generally higher
257 availability of amino acids in fish meal protein [35]. Reduced efficacy of crystalline-amino acid
258 compared to protein-bound-amino acid was also observed in different fish species
259 [14,17,36,37]. This was attributed to differences in amino acid absorption rates and the leaching
260 of dietary crystalline amino acid during feeding of fish. In the present study in order to avoid
261 leaching of amino acids, an attempt was made to feed fish more frequently with smaller
262 quantities of feed as recommended by Lovell [10].

263 Whole body composition of fish was little affected by dietary treatments at the end of the feed
264 trial. Total crude protein and energy contents of Nile tilapia were not significantly different
265 between dietary treatments and the control with the exception of CP of fish fed Diet 4, which
266 was significantly lower than the control. However, there was significant increase in protein and
267 lipid in comparison with the initial carcass values in all the dietary treatments. Similar results

268 were reported by Polat [26] in *Tilapia zillii*, El-Saidy and Gaber [20] in Nile tilapia and
269 Mukhopadhyay and Ray [25] in rohu.

270 Cost effectiveness analysis of diets in this study revealed that the oilseed meal based diets with
271 methionine supplementation had slightly lower cost per kilogram compared to the control diet.
272 Nonetheless, PI for the control diet was higher indicating that the control was more profitable
273 than the oilseed meal-based diets supplemented with DL-methionine. The result showed that
274 DL-methionine supplementation of tilapia diets in this study did not lead to improved cost-
275 effectiveness. This appears to concur with concerns raised by Jauncey [38] about EAA
276 supplementation in tilapia feeds and whether any improvements in growth justify the additional
277 costs. The cost of the methionine used in this study might be higher than other commercial ones
278 on the market since it was based on feed grade price in the USA (MP Biomedicals, Solon,
279 USA). It is therefore likely that if cheaper sources of methionine are found cost effectiveness
280 could improve substantially, especially for Diets 2 and 4.

281 4. CONCLUSIONS

282 To conclude, the present study demonstrated that utilization of crystalline DL-methionine (0.5%)
283 by Nile tilapia was not effective in improving the nutritive value and cost effectiveness of the
284 diets containing oilseed meal mixtures (SBM, CSM and GNC replacing 50% FM protein)
285 compared with the fish meal based diet. However, FCR, PER and ANPU improved compared to
286 previous studies where there was no methionine supplementation. The lower performance of
287 fish fed with the oilseed meal-based diets compared to the control diet may be attributed to poor
288 utilization of the crystalline methionine and the presence of various antinutritional factors,
289 among others in the selected oilseed meals. The present study was carried out under laboratory
290 conditions. It is recommended a similar research be conducted under field conditions,
291 particularly in semi-intensive system commonly used for tilapia production in earthen ponds.
292 There is the possibility of achieving better and practical results which would directly be
293 beneficial to farmers.

294

295 COMPETING INTERESTS

296 Authors have declared that no competing interests exist.

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299 REFERENCES

- 300 1. De Silva SS, Anderson TA. Fish nutrition in aquaculture. London: Chapman & Hall;
301 1995.
- 302 2. Agbo NW, Adjei-Boateng D, Jauncey K. The potential of groundnut (*Arachis hypogaea*
303 L.) by-products as alternative protein sources in the diet of Nile tilapia (*Oreochromis*
304 *niloticus*). Journal of Applied Aquaculture. 2011;23(4):367-378.

3. Agbo NW, Madalla N, Jauncey K. Effects of dietary cottonseed meal protein levels on growth and feed utilization of Nile tilapia, *Oreochromis niloticus* L. Journal of Applied Science and Environmental Management. 2011;15(2):235-239.
4. Agbo NW, PhD Thesis, University of Stirling (Stirling, UK); <https://dspace.stir.ac.uk/dspace/handle/1893/984>, 2008.
5. NRC. Nutrient Requirements of Fish. National Research Council. Washington D.C.: National Academy Press; 1993.
6. Lewis AJ, Bayley HS. Amino acid bioavailability. In: Ammerman CA, Baker DH, Lewis AJ, editors. Bioavailability of Nutrients for Animals: Amino Acids, Minerals and Vitamins, pp.35-65. San Diego, CA: Academic Press, Inc.; 1995.
7. Robinson EH. Improvement of cottonseed meal protein with supplemental lysine in feeds for channel catfish. Journal of Applied Aquaculture; 1991;1: 1-14.
8. Webster C.D., Goodgame-Tiu L.S. and Tidwell J.H. Total replacement of fish meal by soybean meal, with various percentages of supplemental L-methionine, in diets for blue catfish, *Ictalurus punctatus* (Lesueur). Aquaculture Research; 1995;26:299-306.
9. Tacon AGJ. Essential Nutrients, Proteins and Amino Acids. Standard Methods for the Nutrition of Farmed Fish and Shrimp. Redmond, Washington: Argent Laboratories Press; 1990.
10. Lovell T. Nutrition and feeding of Fish. 2nd ed. Boston, MA; London: Kluwer Academic; 1998.
11. Shiao SY. Tilapia. In: Webster CD, Lim C, editors. Nutrient requirements and feeding of finfish for aquaculture. CAB International; 2002.
12. Jauncey K, Ross B. A guide to Tilapia Feeds and Feeding. Stirling (UK): Institute of Aquaculture, University of Stirling; 1982.
13. Davies SJ, Morris PC. Influence of multiple amino acid supplementation on the performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed soya based diets. *Aquaculture Research*; 1997;28(1):65-74.
14. Sveier H, Nordas H, Berge GE, Lied E. Dietary inclusion of crystalline D- and L-methionine: effects on growth, feed and protein utilization, and digestibility in small and large Atlantic salmon (*Salmon salar* L.). *Aquaculture Nutrition*; 2001;7(3):169-181.
15. Jauncey K, Tacon AGJ, Jackson AJ. The quantitative essential amino acid requirements of *Oreochromis mossambicus*. In: Fishelson L, Yaron Z, editors. *Proceedings of the First International Symposium on Tilapia in Aquaculture*, pp. 328-334. Israel: Tel Aviv University; 1984.
16. Cowey, C.B. and Walton, M.J. Studies on the uptake of (14C) amino acids derived from both dietary (14C) protein and dietary (14C) amino acids by rainbow trout, *Salmo gairdneri* Rich. *Journal of Fish Biology*; 1988; 33:293-305.
17. Zarate, D.D. and Lovell, R.T. Free lysine (L-lysine. HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (*Ictalurus punctatus*). *Aquaculture*; 1997;159:87-100.
18. Jackson AJ, Capper BS. Investigations into the requirements of the tilapia, *Sarotherodon mossambicus* for dietary methionine, lysine and arginine in semisynthetic diets. *Aquaculture*; 1982;29:289-297.
19. Odum O, Ejike C. (Aspects of amino acid utilization in the cichlid *Oreochromis niloticus* L. *Acta Hydrobiology*; 1991;33:345-352.
20. El-Saidy DMSD, Gaber MMA. Complete Replacement of Fish Meal by Soybean Meal with Dietary L-Lysine Supplementation for Nile Tilapia *Oreochromis niloticus* (L.) Fingerlings. *Journal of the World Aquaculture Society*; 2002;33(3):297-306.
21. Furuya WM, Pezzato LE, Barros MM, Pezzato AC, Furuya VRB, Miranda EC. Use of ideal protein concept for precision formulation of amino acid levels in fish-meal-free diets for juvenile Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research*; 2004;35(12):1110-1116.

22. AOAC (Association of Official Analytical Chemists) Official Methods of Analysis of the Official Association of Analytical Chemists. 15th ed, 1990.
23. Stirling HP. Chemical and biological methods of water analysis for aquaculturists, Institute of Aquaculture, University of Stirling, Scotland; 1985.
24. Agbo NW, Madalla N, Jauncey K. The biochemical composition of some Ghanaian oilseed by-products and their digestibility by Nile tilapia, *Oreochromis niloticus* L. Journal of the Ghana Science Association; 2009;11:136-148.
25. Mukhopadhyay N, Ray AK. Effects of amino acid supplementation on the nutritive quality of fermented linseed meal protein in the diets for rohu, *Labeo rohita*, fingerlings. Journal of Applied Ichthyology; 2001;17:220-226.
26. Polat A. The effects of methionine supplementation to soybean meal (SBM)- based diets on the growth and whole body-carcass chemical composition of tilapia (*T. zillii*). Turkish Journal Zoology; 1999;23:173-178.
27. Shiau SY, Kwok CC, Hwang JY, Chen CM, Lee SL. Replacement of fish meal with soybean meal in male tilapia (*Oreochromis niloticus* x *O. aureus*) fingerling diets at a suboptimal level. *Journal of the World Aquaculture Society*; 1989;2(4):230-235.
28. Andrews WJ, Page WJ. Growth factors in the fish meal component of catfish diets. *Journal of Nutrition*; 1974;104(8):1091-1096.
29. Teshima SKA. Nutritive value of methionine-enriched soy plastein for *Oreochromis niloticus* fry. In: Pullin RSV, Bhukaswan T, Tonguthai K, Maclean JL, editors. Second International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings No. 15, pp. 393–399 Manila, Philippines: ICLARM; 1988.
30. Davis AT, Stickney RR. Growth responses of *Tilapia aurea* to dietary protein quality and quantity. *Transactions of American Fisheries Society*; 1978;107:479-483.
31. Shiau SY, Chuang JL, Sun CL. Inclusion of soybean meal in tilapia (*Oreochromis niloticus* x *O. aureus*) diets at two protein levels. *Aquaculture*; 1987;65(3-4):251-261.
32. Murai T, Ogata H, Kisutarak P, Arai S. Effects of amino acids supplementation and methanol treatment on utilization of soy flour by fingerling carp. *Aquaculture*; 1986;56:197-206.
33. Sitj-Bobadilla A, Peña-Llopis S, Gómez-Requeni P, Médale F, Kaushik S, Pérez-Sánchez J. Effect of fishmeal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). *Aquaculture*; 2005;249:387-400.
34. Fournier V, Huelvan C, Desbruyeres E. Incorporation of a mixture of plant feedstuffs as substitute for fish meal in diets of juvenile turbot (*Psetta maxima*). *Aquaculture*; 2004;236(1-4):451-465.
35. Hertrampf JW, Piedad-Pascual F. Handbook on Ingredients for Aquaculture Feeds. The Netherlands: Kluwer Academic Publishers; 2000.
36. Mambrini M, Kaushik SJ. Partial replacement of dietary protein nitrogen with dispensable amino acids in diets of Nile tilapia, *Oreochromis niloticus*. *Comparative Biochemistry and Physiology Part A: Physiology*; 1994;109(2):469-477.
37. Zarate DD, Lovell RT, Payne M. Effects of feeding frequency and rate of stomach evacuation on utilization of dietary free and protein bound lysine for growth by channel catfish, *Ictalurus punctatus*. *Aquaculture Nutrition*; 1999;5:17-22.
38. Jauncey K. Tilapia Feeds and Feeding. Stirling, Scotland: Pisces Press Ltd; 1998.