EFFECT OF SUPPLEMENTATION OF SOYABEAN DIET WITH L-AND D,L-METHIONINE ON THE GROWTH OF MUDFISH *CLARIAS ANGUILLARIS* (L) FINGERLINGS

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ABSTRACT

Two experiments were carried out to study the effect of dietary supplements of methionine on the growth and food utilization of mudfish *Clarias anguillaris*. In both studies *C. anguillaris* fingerlings (17.20g and 1.02g. initial mean weight respectively) were fed full fat soybean diets supplemented with graded levels of L-and D, L-methionine and the growth was monitored for 12 and 14 weeks respectively. Growth rate and food utilization were significantly higher ($P<0.05$) when they were fed soybean diets supplemented with 0.2% L-and D, L-methionine than 0.4, 0.6 and 0.8%. An increase in the level of L-and D, L-methionine beyond 0.2% resulted in a significant reduction in the growth rate of the fish. Growth and food utilization were better in fish fed the casein and fish meal positive control diets than the 0.2% supplemented soybean diets. The unsupplemented soybean diets were poorly utilized by the mudfish fingerlings. The size disparity of fingerlings had no effect on the dietary requirement for the L-and D, L-methionine. Since the less expensive D, L-methionine can substitute L-methionine in the diet, the use of D, L-methionine in dietary supplements is more cost effective than the use of L-methionine.

INTRODUCTION

Soyabean meal is generally regarded as one of the best sources of plant protein available for animal feeding. Although the meal contains all the essential amino acids in its protein, the amount of methionine present is sub-optimal (McDonald *et al.,* 1973; Nose, 1978; Sadiku and Jauncy, 1995).

One solution to the use of plant protein in practical diets that are relatively deficient in one or more amino acids is to supplement the protein with appropriate amounts of the amino acid in question. Kaneko (1969) obtained positive results by feeding rainbow trout with feed containing 30% of soyabean meal enrich with methionine and lysine. Similarly, Koops *et al.,* (1976) found that one quarter of fish meal can be replaced by soyabean meal enriched with methione in the diets of rainbow trout. Soyabean meal has been supplemented with methionine in rainbow trout feeds with positive results (Steffens and Albrecht 1976). Murai *et al.,* (1982) demonstrated that channel catfish can utilize synthetic methionine added to soyabean meal protein.

The above results suggest that the quality of soyabean meal could be improved if the amino acid balance is adjusted by the use of crystalline amino acids.

This study was undertaken to determine the optimum supplemental level of L-and D, L-methionine in heat treated full fat soyabean diet that would guarantee the maximum utilization of soyabean protein by *Clarias anguillaris* fingerlings. Methionine alone was selected for this studies because it is the most limiting essential amino acid (EAA) in soyabean meal.

MATERIALS AND METHOD

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The experiments were conducted in the Institute's Hatchery Complex using indoor flow-through tanks for the soyabean diet containing L-methionine and hapas suspended in outdoor concrete tanks for the D. L-methionine-supplemented diet.

Indoor Experiment for L-Methionine

360 mudfish *Clarias anguillaris* fingerlings having mean weight of 17.20g and measuring from 13.0 to 15.4cm individual total length was used for the study. The fish ere distributed into 18 plastic tanks (20 fish per tank) each tank containing approximately 30 litres of water. The experiment was conducted for 12 weeks between June and September. Fish were acclimated to the indoor tanks for one week before the commencement of the experiment.

Outdoor Experiment for D, L-methionine

Hapa nets constructed into 18 hapa cages with dimension 1x1x1 metre were used for the outdoor experiment, and 360 catfish fingerlings having mean weight of 1.02g and individual total length of 5 to 7cm were acclimated for one week in the outdoor concrete tanks before they were stocked in the hapas. The hapas were suspended in concrete tanks measuring 10x10x2 metre with water one metre deep. The 14 weeks observation commenced from October to January.

Experimental Conditions

The pysico-chemical parameters such as temperature, pH and dissolved oxygen (DO) of water were analysed weekly using standard methods (APHA 1981).

The dissolved oxygen (DO) in the flow-through tanks was maintained at 6.9 + 0.6/mg/l, the pH was 7.5 + 0.2 and temperature ranged between 25.0 - 26.8°C. For the physico-chemical parameters of water in the outdoor hapas, the dissolved oxygen was 10.0 + 3.6mg/l, pH was 7.3 + 0.35 and temperature ranged between 18.4 - 30.0°C. The slightly low temperatures experienced during the last two weeks of the experiment in the hapas were due to the influence of harmattan. This was not expected to have profound effect on the growth rate of the fish.

Feed and Feeding Levels

The raw soyabeans was roasted on a heated pan to destroy the trypsin inhibitor (Kunitz, 1945), and then milled after dehulling. The ground full fat soyabean meal was mixed with L-and D, L-methionine and other ingredients. The diet was made isonitroginous by the addition of aspartic acid. The nutrient composition of the experimental diet and the proximate composition are shown in Table 1. Fish were feed twice daily 9.00a.m. and 4.00p.m. with a total daily ration equal to 3% of their body weight. Measurement of fish weight and total length were taken fortnightly the tanks and hapas were washed throughly to remove algae and uneaten feeds.

Chemical Analysis

The crude protein was determined according to the AOAC (1980).

Food Utilization Parameters

The food utilization parameters used for the experiment were specific growth rate, feed conversion efficiency and protein efficiency ratio.

Statistical Analysis

Growth data were analysed statistically using the analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The data in Table 2 and 3 indicate the % weight gain, specific growth rate (SGR), feed conversion efficiency (FCE), and Protein Efficiency ration (PER) of mudfish fingerlings fed L-and D, L-methionine supplemented soyabean diets respectively. In general fish fed diets designated DT 3, DT 4 and DT 5 in Tables 2 and 3 were poorly utilized by the fingerlings. The best growth was obtained in fish fed Casein diet (DT 6 in Table 2) and fish meal diet (DT 6 in Table 3) followed by fingerlings fed 0.2% L-and D, L-methionine diets (DT2). Poor growth was observed in fish fed the unsupplemented heat-treated full fat soyabean diets (DT1).

The results obtained show that supplementation of heat-treated full fat soyabean diet with 0.2% crystalline methionine improved the growth and food utilization of mudfish fingerlings. The growth performance was significantly ($P<0.05$) better than fingerlings fed with soyabean diets supplemented with 0.4, 0.6 and 0.8% methionine. This was the case irrespective of whether the soyabean diet was supplemented with L- or D,L-methionine. This shows that mudfish fingerlings can utilise L-methione as effectively as D,L-methionine for protein synthesis. This agrees with the observation of Robinson *et al.*, (1978) using channel catfish.

Improved growth rate in fish fed methionine-supplemented soyabean diet has been reported for other species. Murai *et al.*, (1982) demonstrated improved growth rate in carp fed diets containing soyabean meal supplemented with methionine alone. Similarly addition of methionine to soy flour diet was responsible for the improvement in weight gain and feed efficiency of carp fed soy flour diet (Murai *et al.*, 1986). Kaneko (1969) working with rainbow trout also obtained positive results by feeding them with 30% of soyabean meal enriched with methionine and lysine.

Supplement of methionine beyond 0.2% in the soyabean diet caused a significant reduction ($P<0.05$) in the growth rate of *Clarias anguillaris* fingerlings corresponding to the relative amount of free methionine in the diet (Fig 1 and 2). The high level of methionine might have stimulated methionine degradation to its derivative such as taurine and methionine sulfoxide as reported by Murai *et al.*, (1989) for young carp fed crystalline methionine. This would render methionine unavailable for protein synthesis.

The growth rate of mudfish fed soyabean diets supplemented with crystalline L-methionine was significantly higher ($P<0.05$) than those fed the unsupplemeted soyabean diets (control A) in the indoor flow-through tank (Fig. 1). This confirms the observation that soyabean meal contain disproportinate levels of amino acids causing a reduction in growth rate which is corrected by the addition of amino acid (Harper *et al.*, (1970).

The higher growth rate of *C. anguillaris* fed the unsupplemented soyabean diets compared with those fed high (0.4, 0.6 and 0.8%) D,L-methionine levels in hapas (Fig. 2) indicate that higher levels of methionine is not beneficial to mudfish as it could have stimulated methionine degradation to taurine and methionine sulfoxide as reported by Murai (1989). In addition, *C. anguillaris* fed the unsupplemented soyabean diets could have obtained some of the methionine from the natural food in the hapas.

There was positive correlation between percentage weight gain and the specific growth rate, feed conversion efficiency and protein efficiency ratio (Table 2 and 3). These growth and food utilization indicators were better in the positive control ration than in the other diets. This demonstrates that the natural casein and fish meal diets were better utilized by catfish than the soyabean meal supplemented crystalline amino acid rations. This has ben observed for carp (Murai *et al.*, 1986).

On the economic aspect, the current marked prices for L-and D,L-methionine are ₦450.00 and ₦80.00 per kg respectively. Thus L-methionine is more than five times as costly as D, L-methionine. The reason is that D. L-methionine is less expensive to synthesize than the pure L-form (Freedland and Briggs 1977). Since D, L-methionine can substitute L-methionine, the use of D, L methionine in synthetic diets or in dietary supplements is more economical than L-methionine.

Table 1: Composition of experimental diets (% by weight

COMPONENT	DIET COMPOSITION						
	DT1	DT2	DT3	DT4	DT5	DT6(1*)	DT(ii**)
soyabean meal	77	77	77	77	77	-	-
Casein	-	-	-	-	-	40	-
Fish meal	-	-	-	-	-	-	50
+Methionine	-	0.2	0.4	0.6	0.8	-	-
Aspartic acid	1	0.8	0.6	0.4	0.2	3.5	-
++Cod liver oil	2	2	2	2	2	2	2
Corn meal	18	18	18	18	18	52.5	45
+++ Vitamin and Mineral	2	2	2	2	2	2	2
Premix	36.68	36.68	36.68	36.68	36.68	36.68	37.00
Calculated crude Protein %	4.3	4.3	4.3	4.3	4.3	4.3	4.3
Gross energy KCal/kg							
Proximate Composition							
Moisture	9.25	9.45	9.18	8.98	9.38	7.75	6.96
Crude Protein	35.82	35	35.03	34.85	35.40	35.75	35.55
Lipid	14.66	14.35	13.85	13.65	14.00	10.57	15.44
Crude fibre	5.25	5.18	4.95	4.85	5.05	1.13	1.25
Ash	5.43	5.40	5.17	5.35	5.15	3.25	18.20
Nitrogen free Extract (NFEE)	29.59	30.67	31.82	32.32	31.02	43.55	22.60

\+ L-Methionine was substituted for D.L-Methionine in the second experiment.

++ Cod liver oil supplied per 10 ml, Vitamin A 7000 I.U, Vitamin D 800 I.U, Vitamin E 1 I.U.

+++ Vitamin and mineral supplied as follows (mg/kg) of premix). Riboflavin 6,000, Pyridoxine 24,000, Folic acid 12,000, Manganese 60,00, Iron 40,000, Copper 5,000, Choline 30,000, Selenium 100, Iodine 1,100, Ascorbic acid 25,000, antioxidant 125,000, Vitamins A, D and E were added at 350,000, 130,000, and 7,500 I.U/kg respectively.

* Casein diet (control B) for the experimental fish in indoor flow through tank fed L-methionine.

** Fish meal diet (control B) for the experimental fish in outdoor fed in outdoor hapas fed D, L-methionine.

Table 2: Growth and food utilization of *Clarias anguillaris* fed soyabean diets containing different levels of L-Methionine for 12 weeks in indoor flow-through tanks

	DIET DESIGNATION						
	DT 1	DT 2	DT 3	DT 4	DT 5	DT 6	SEM
Dietary Methionine (g/mg)	-	0.2	0.4	0.6	0.8	-	
Mean initial Weight (g)	17.10b±4.20	17.00b±4.86	17.00b±2.73	17.10b±2.52	17.70e±4.62	17.30b±1.00	0.10
Mean final Weight (g)	19.90b±3.15	29.59b±2.50	21.00d±4.10	21.26d±3.21	22.67e±1.68	35.70c±2.17	2.56
% Weight gain	16.37b	74.06d	23.53d	24.33e	28.08e	106.36c	2.45
Specific Growth Rate (SGR)	0.18b	0.66a	0.25d	0.26d	0.29d	0.86c	0.11
Feed Conversion Efficiency (FCE)	6.07b	21.85d	7.44d	8.20e	7.58d	25.32c	3.47
Protein Efficiency Ration (PER)	0.17b	0.63a	0.21d	0.24d	0.21d	0.71c	0.10

Note: Figures followed by the same letters in each row are not significantly different (P>0.05).

Table 3: Growth and food utilization of *Clarias anguillaris* fingerlings fed soyabean diets containing different levels of D, L-Methionine for 14 weeks in outdoor hapas

	DIET DESIGNATION						
	DT 1	DT 2	DT 3	DT 4	DT 5	DT 6	SEM
Dietary Methionine (g/mg)	-	0.2	0.4	0.6	0.8	-	-
Mean initial Weight (g)	1.05a±0.01	1.02a±0.01	1.03a±0.02	1.01a ±0.0	10.0a±0	1.01a±0.01	n.s.
Mean final Weight (g)	3.83b±0.44	4.25a±0.65	3.70b±0.33	3.05b±0.55	3.00b±0.55	5.04c±0.54	0.31*
% Weight gain	271.84b	316.76a	260.19b	201.98b	200.98b	339.00a	30.72*
Specific Growth Rate (SGR)	1.34b	1.46a	1.31b	1.13b	1.12b	1.64c	0.08*
Feed Conversion Efficiency (FCE)	28.51b	30.13b	24.63b	26.29b	25.22b	41.59c	2.58*
Protein Efficiency Ration (PER)	0.80b	0.86b	0.70b	0.70b	0.71b	1.20c	0.08*

Note: Figures followed by the same letters in each row are not significantly different (P>0.05).
n.s. = not significant
* = significant at 0.05

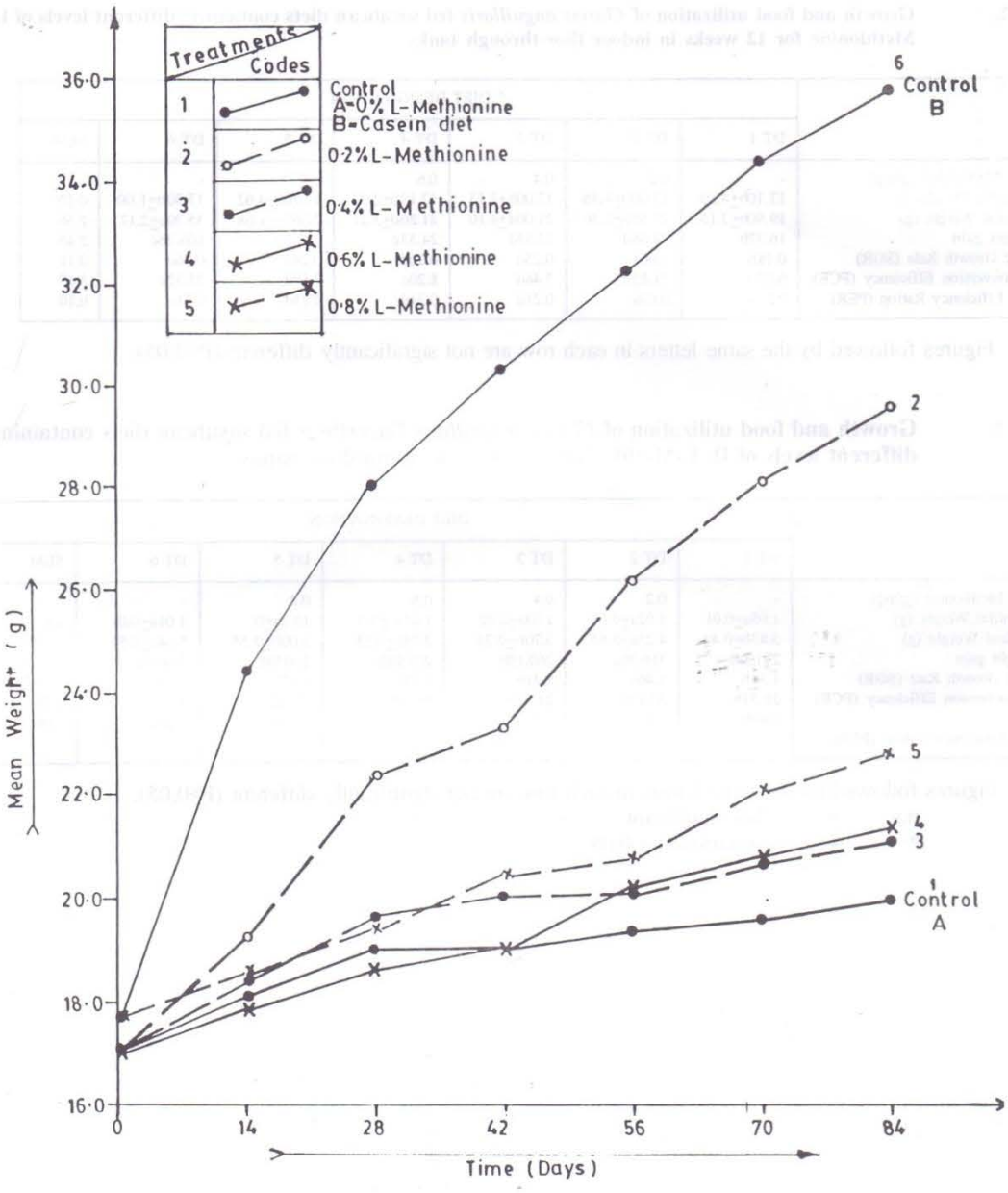

Fig 1: Growth of *Clarias anguillaris* fingerlings fed soyabean diets contain various level of L-methionine in indoor mini flow-through tanks.

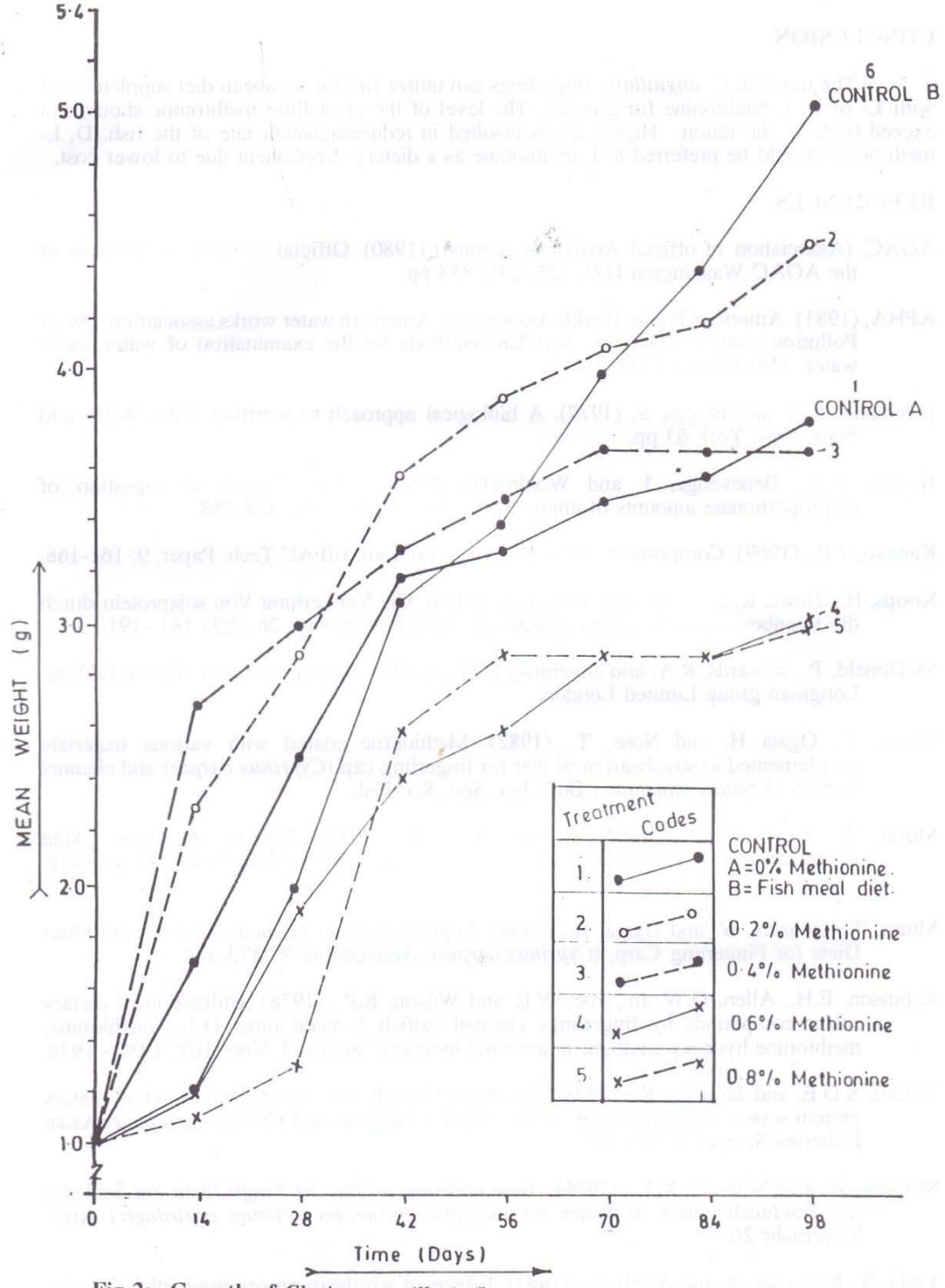

Fig 2: Growth of *Clarias anguillaris* fingerlings fed soyabean diets contain various level of D.L-methionine in outdoor hapas.

CONCLUSION

The mudfish *C. anguillaris* fingerlings can utilize full fat soyabean diet supplemented with L- or D, L-methionine for growth. The level of the crystalline methionine should not exceed 0.2% of the ration. Higher levels resulted in reduced growth rate of the fish. D, L-methionine should be preferred to L-methionine as a dietary supplement due to lower cost.

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