

**Determination of the Order of Amino acid Limitation in  
Slaughterhouse Poultry by-product Meal in African catfish diet by  
Amino acid Addition Assay**

El- Husseiny O.M., Issoufou H Mahamadou, Ashraf Suloma\*  
Animal Production Dept, Faculty of Agriculture, Cairo University, Egypt.

\*Corresponding Author E-mail address: [suloma2001@yahoo.com](mailto:suloma2001@yahoo.com)

**ABSTRACT**

An 8-week feeding trial was carried-out to more clearly establish and compare the order of amino acids (AAs) limitation in Slaughterhouse Poultry by-product (SPB) using AA addition assays in African catfish (*Clarias gariepinus*) diets. Seven isonitrogenous (40% crude protein) diets were formulated. Control diet (fish meal; FM) without amino acid supplementation contained 40% of FM and 20% of SBM which contribute 28% and 9.40% of crude protein, respectively, and formulated to meet all known nutrient requirements of catfish based on nutrient requirement values proposed by the NRC (2011). Diet 2 (SPB) contained SPB as the sole animal protein without amino acid supplementation. The SPB diet was individually supplemented in the other five diets with 1.2% Lys (SPB<sup>+Lys</sup>), 0.4% Met (SPB<sup>+Met</sup>), 0.1% Trp (SPB<sup>+Trp</sup>), 1.2% Arg (SPB<sup>+Arg</sup>) and a combination of 1.2% Lys+0.4% Met+0.1% Trp+1.2% Arg (SPB<sup>+Mix</sup>). Final weight of the fish fed FM diet increased 5 fold, while the group fed SPB<sup>+Lys</sup> and SPB<sup>+Mix</sup> increased 3 fold, respectively. However, fish weight fed diets SPB, SPB<sup>+Met</sup>, SPB<sup>+Trp</sup>, and SPB<sup>+Arg</sup> were duplicated at the end of the experiment. The least FCR value of 1.20 was obtained for group fed FM which produced the best growth performance followed by group fed SPB<sup>+Lys</sup> and SPB<sup>+Mix</sup> (1.52 and 1.66, respectively). Significant difference was observed for protein efficiency ratio (PER) of all experimental diets. Among the SPB diets used in the present study, the fish fed SPB<sup>+Lys</sup> and SPB<sup>+Mix</sup> had significantly ( $P < 0.05$ ) higher final body weight, WG, FI, FCR, SGR, PER, PR and ER than those of the groups fed diets SPB, SPB<sup>+Met</sup>, SPB<sup>+Trp</sup> and SPB<sup>+Arg</sup>. In this study, Lysine was found to be the first-limiting AA in SPB and deficiencies in African catfish performance were ameliorated by supplementing a 100% replacement diet with sufficient Lysine to match levels in a hypothetical diet in which all protein (40%) was provided by African catfish.

**Keywords:** African catfish, Slaughterhouse Poultry by-product, Amino acids.

**INTRODUCTION**

The African catfish (*Clarias gariepinus*), an air breathing fish, is a major warm water aquaculture species in Africa and Asia (Khan and Abidi, 2011). It is highly appreciated as an excellent aquaculture species for intensive culture because of their resistance to diseases, ability to tolerate a wide range of environmental conditions, high stocking densities and relative fast growth rate under intensive culture (Nyina-wamwiza *et al.*, 2007).

Protein is one of the most expensive components of aquaculture diets (Khan and Abidi, 2011 and Liu *et al.*, 2011). Animal protein sources, especially fish meal (FM), have relatively high cost, limited supply, and variable quality. The development of commercial aquatic feeds has been traditionally based on FM as the main protein source because of its high protein content and balanced essential amino acid (EAA) profile (Nguyen *et al.*, 2009). However, the limited supply of FM, coupled with its

increased demand in feeds for livestock and poultry, is likely to reduce the dependence on FM in aquatic feeds (Thompson *et al.*, 2008). Finding alternative protein sources for FM in the diet, either partially or completely, included terrestrial plant meals and animal by-products, have become the focus of research from the view point of producing a stable supply of commercial diets at a reduced price (Hardy, 2010).

Replacement of FM in practical diets without reducing the performance would result in more profitable production of catfish. Therefore, the evaluation of other commercially available and potential alternative ingredients is necessary to insure future availability of sustainable and cost-effective aquatic feeds for the aquaculture industry (Waldemar, 2011).

Slaughterhouse poultry by-product meal (SPB) is rendered from the waste generated from small scale poultry slaughterhouse as Egyptian consumers prefer live slaughter. SPB differ with PBM as it included feather (Sevgili and Ertürk, 2004). This by-product can consider potential alternative feedstuffs to fishmeal because of its high protein contents (60-65% CP) and low price compared with the FM (Thompson *et al.*, 2007). SPB is high in ether extract (EE), ash and indigestible components (feathers, etc.), reduced digestibility (Sevgili and Ertürk, 2004). Thus, SPB varies in nutritional value and composition depending on the processing and the materials included in the meal (Nengas *et al.*, 1999).

Formulation of the practical diet at optimal protein level and an ideal essential amino acid (EAA) profile is a pre-requisite for fish growth (Peres and Oliva-Teles, 2009). Contents of the EAA, especially lysine (Lys), methionine (Met), arginine (Arg) and tryptophan (Try), are generally limiting amino acids in economical alternative protein sources (Xue *et al.*, 2012). The deficiency of an EAA will lead to poor utilization of the dietary protein (Jia *et al.*, 2012). A study by Wang and Persons (1998)

indicated that cystine, tryptophan, threonine and lysine were the first four limiting amino acids in PBM, while methionine may be slightly limiting in PBM protein in poultry diet. However, Main and Doghir (1982) reported that methionine and lysine were the first and second limiting amino acids in PBM, respectively, in poultry diet.

The present study was design to develop nutritionally balanced practical diets for the *Clarias gariepinus* based on the nutrient requirement of this species using SPB as mean protein source. No data about the limiting amino acids in SPB are available, therefore, the specific objective of this study was to more clearly establish and compare the order of AA limitation in SPB using AA addition assays.

## MATERIALS AND METHODS

### *Fish and experimental conditions*

An 8-week feeding trial was carried out at the laboratory of fish nutrition, Faculty of Agriculture, Cairo University, Egypt. African catfish (*Clarias gariepinus*) were purchased from a local commercial hatchery located in El-Morshedy, Abbassa area, Abou-Hammad, Sharkia Governorate, Egypt. Fish were acclimatized to experimental conditions for 7 days prior to the feeding trial. Two hundred and fifty two catfish fingerlings ( $5.36 \pm 0.03$ g) were randomly divided and distributed into 7 different groups with 3 replicates containing 12 fish each. The experiment was set up as a substitution experimental design, with seven diets fed in triplicate. The experiment was conducted in plastic tanks (70-L capacity) provided with well water having an average temperature of 25-28 °C. Compressed air was used to maintain the oxygen supply above 7 mg L<sup>-1</sup>. The fish culture tanks were covered with black plastic to prevent the fish escape. Fishes were counted and weighed at every 2 weeks and not fed on morning period of weighing day.

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***Experimental diets and feeding***

SPB was obtained from Ismailia investment company (El-wafa company, 31G region, kilometer 21, Ismailia desert hay way, Egypt). The chemical composition of the ingredients used in this study is presented in Table 1. Seven isonitrogenous (40% crude protein) diets were formulated (Table 2). Control diet (FM) without amino acid supplementation contained 40% of FM and 20% of SBM which contribute 28% and 9.40% of crude protein, respectively, and formulated to meet all known nutrient requirements of catfish based on nutrient requirement values proposed by the NRC (2011). Diet 2 (SPB) contained SPB as the sole animal protein without amino acid supplementation. The SPB diet was individually supplemented in the other five diets with 1.2%Lys (SPB<sup>+Lys</sup>), 0.4%Met (SPB<sup>+Met</sup>), 0.1%Trp (SPB<sup>+Trp</sup>), 1.2%Arg (SPB<sup>+Arg</sup>) and a combination of 1.2% Lys + 0.4 % Met + 0.1% Trp + 1.2 % Arg (SPB<sup>+Mix</sup>). The analysed AA profiles of the experimental diets and the AA requirements according to NRC (2011) are tabulated in Table 3.

The experimental diets were prepared by blending the ingredients into a homogenous mixture, and then the mixture was passed through laboratory pellet mill. The pellets were then dried overnight under forced air at room temperature and stored at 4 °C until used. Fish were fed to apparent satiation three times daily (at 09 am, 1 and 5 pm) for 56 days. Daily feed consumption was recorded for each tank by weighing the feed at the start and end of each day. To avoid excess feeding, food was offered with great care by giving small amounts of food at a time to ensure that fish ate all the food particles offered.

***Chemical analysis***

At the beginning of the trial, a pooled sample of 40 fish was collected to serve as an initial fish sample. At the end of the feeding trial, all fish were anaesthetized with t-amyl alcohol

**Table 1** *Composition of the dietary protein ingredients (g kg<sup>-1</sup>)*

Item	FM	SBM	SPB
Dry matter	918.2	902.2	948.4
Crude protein	698.2	415.6	615.0
Lipid	89.5	45.0	123.6
Ala	50.7	19.5	29.4
Asp	43.4	31.4	41.3
Glu	60.1	41.7	65.6
Gly	65.8	23.0	40.4
Pro	42.5	30.5	53.1
Ser	28.0	21.2	45.6
Tyr	23.6	16.6	19.0
Arg	48.5	31.8	39.3
His	15.1	11.3	6.4
Iso	32.6	20.3	26.7
Leu	52.4	34.4	43.0
Lys	52.9	28.0	20.9
Met	20.2	6.0	7.9
Cys	6.7	7.0	31.9
Phe	27.8	22.1	28.2
Thr	41.1	17.6	26.6
Val	38.0	20.9	35.1
Try	7.0	6.3	5.0

and killed by a cephalic blow. The fish were pooled, autoclaved, ground into homogeneous slurry, oven-dried, reground and stored at -20 °C in a freezer until analysed. Diet, ingredients, and fish samples were analyzed for dry matter (DM) and ash content according to AOAC (1995) methods, for crude protein (N x 6.25) by the Kjeldahl method using a Kjeltach auto-analyzer (Model 1030, Tecator, Hoganas, Sweden), and for crude fat (Bligh and Dyer 1959). Amino acids were analyzed at the Regional Center for Food and Feed (Giza, Egypt) using method 994.12 (AOAC 2005). Dietary gross energy was calculated using the conversion factors of 23.7, 39.5 and 17.2 kJ g<sup>-1</sup> for protein, lipid, and carbohydrate, respectively (Brett and Groves 1979).

**Table 2. Formulation and proximate composition (g kg<sup>-1</sup> dry weight basis) of experimental diets.**

Ingredients	FM	SPB	SPB+Lys	SPB+Met	SPB+Try	SPB+Arg	SPB+Mix <sup>1</sup>
Corn	200.0	193.6	180.0	190.0	192.4	180.0	170.0
Soybean meal	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Fish meal	400.0	0.0	0.0	0.0	0.0	0.0	0.0
SPB	0.0	455.9	457.5	455.9	456.1	457.7	461.5
Wheat	89.5	90.0	90.0	90.0	90.0	89.8	79.0
Starch	50.0	0.0	0.0	0.0	0.0	0.0	0.0
Salt	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vit C	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Carboxy methyl cellulose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Soybean oil	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin & Mineral Premix <sup>2</sup>	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Lysine	0.0	0.0	12.0	0.0	0.0	0.0	12.0
Methionine	0.0	0.0	0.0	4.0	0.0	0.0	4.0
Tryptophan	0.0	0.0	0.0	0.0	1.0	0.0	1.0
Arginine	0.0	0.0	0.0	0.0	0.0	12.0	12.0
<b>Chemical composition (g kg<sup>-1</sup>)</b>							
Dry matter	935.43	932.52	932.05	935.50	937.68	931.51	941.03
Crude protein	378.50	409.00	424.00	405.50	412.00	424.00	438.50
Crude lipid	85.78	109.18	111.14	128.01	113.83	142.86	132.24
Crude Ash	111.74	73.73	75.19	69.00	77.90	82.19	71.79
Gross energy ( KJ g <sup>-1</sup> )	19.59	21.03	21.14	21.50	21.08	21.73	21.76

<sup>1</sup>1.2%Lys+0.4%Met+0.1%Trp+1.2%Arg

<sup>2</sup>Provides per kg of diet: retinyl acetate, 3000 IU; cholecalciferol, 2400 IU; all-rac- $\alpha$ -tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49% ascorbic acid), 120 mg; cyanocobalamin, 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate (FeSO<sub>4</sub>.7H<sub>2</sub>O, 20% Fe), 65 mg; manganese sulfate (MnSO<sub>4</sub>, 36% Mn), 89 mg; zinc sulfate (ZnSO<sub>4</sub>.7H<sub>2</sub>O, 40% Zn), 150 mg; copper sulfate (CuSO<sub>4</sub>.5H<sub>2</sub>O, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1000 mg Agri-Vet Company, Cairo, Egypt

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**Table 3.** *Amino acid composition of experimental diets (g kg<sup>-1</sup> dry diet).*

Amino acid	FM	SPB	SPB <sup>+Lys</sup>	SPB <sup>+Met</sup>	SPB <sup>+Try</sup>	SPB <sup>+Arg</sup>	SPB <sup>+Mix*</sup>	Amino acid requirement of catfish <sup>1</sup>
	D1	D2	D3	D4	D5	D6	D7	
<b>Non-essential amino acid</b>								
<b>Ala</b>	25.6	18.7	18.6	18.7	18.7	18.6	18.7	-
<b>Asp</b>	24.5	25.9	25.9	25.9	25.9	26.0	26.1	-
<b>Glu</b>	33.7	39.5	39.5	39.5	39.5	39.5	39.7	-
<b>Gly</b>	31.7	23.8	23.8	23.8	23.8	23.8	23.9	-
<b>Pro</b>	25.2	32.4	32.3	32.4	32.4	32.3	32.4	-
<b>Ser</b>	16.3	25.9	25.9	25.9	25.9	25.9	26.0	-
<b>Tyr</b>	13.6	12.8	12.8	12.8	12.8	12.8	12.8	-
<b>Essential amino acid</b>								
<b>Arg</b>	26.5	25.0	25.0	25.0	25.0	<b>37.1</b>	<b>37.1</b>	10.3
<b>His</b>	8.8	5.6	5.6	5.6	5.6	5.6	5.6	3.7
<b>Iso</b>	17.8	16.9	16.9	16.9	16.9	16.9	17.0	6.2
<b>Leu</b>	30.1	28.7	28.6	28.7	28.7	28.6	28.6	8.4
<b>Lys</b>	27.3	15.6	<b>27.6</b>	15.6	15.6	15.6	<b>27.6</b>	12.3
<b>Met</b>	14.2	21.5	21.6	<b>25.6</b>	21.5	21.6	<b>25.6</b>	15.0
<b>Phe</b>	16.5	18.2	18.1	18.2	18.2	18.2	18.2	4.6
<b>Thr</b>	20.6	16.2	16.2	16.2	16.2	16.3	16.3	12.0
<b>Val</b>	20.3	21.0	21.0	21.0	21.0	21.0	21.1	7.1
<b>Try</b>	4.18	3.66	3.66	3.66	<b>4.7</b>	3.66	<b>4.7</b>	5.3

<sup>1</sup>NRC (2011)

***Water quality measurement***

Temperature was measured every day and pH was monitored in the tanks. The temperature was measured directly in the water column one time every day after feeding using a mercury thermometer suspended at 20-cm depth. The pH was measured directly in the water column of plastic tanks every week during experiment by pH meter (Orion pH meter, Abilene, TX, USA).

***Statistical analysis***

Data of fish growth performance, feed utilization and carcass traits were statistically analyzed by one-way analysis of variance (ANOVA), using Duncan's post hoc ANOVA test for individual comparisons (P≤0.05) level of significance. All statistics were performed using SPSS 17.0 (SPSS, Chicago, IL, USA).

**RESULTS**

***Nutrient composition of the experimental diets***

The proximate and amino acid composition of the imported FM and SPB are listed in Table 1. Both protein sources contained high protein levels in the range of 698.2-615 g kg<sup>-1</sup> and moderate levels of crude lipid at 89.5–123.6 g kg<sup>-1</sup>. The amino acid composition in SPB particularly, Arg (39.3 g kg<sup>-1</sup>), Met (7.9 g kg<sup>-1</sup>), Lys (20.9 g kg<sup>-1</sup>) and Try (5 g kg<sup>-1</sup>) are limited essential amino acid compared to FM (48.5, 20.2, 52.9 and 7 g kg<sup>-1</sup>, respectively).

The proximate and amino acid composition of the experimental diets are tabulated in Table 2. The analysed values of crude protein, crude lipid and gross energy of the experimental diets were laid in the ranges of 368.50-424 g kg<sup>-1</sup> diet, 85.78-142.86 g kg<sup>-1</sup> diet and 19.59-21.76 KJ g<sup>-1</sup> diet, respectively. The amino acid composition in diets reflected the composition of the protein sources. Arginine, methionine, lysine and tryptophan contents of the experimental diets were reduced when FM was totally replaced by slaughterhouse poultry by-product meal. However, the levels of these AAs increased in diets supplemented with these crystalline amino acids (Table 3).

#### **Growth performance and feed utilization**

The water quality parameters were within the acceptable range for African catfish. Fish appeared healthy at the end of the experiment and no mortality during the experimental period was observed. The initial weights of the experimental fish for all treatments did not vary significantly (Table 4).

The performance of catfish fingerlings are significantly differed ( $P < 0.05$ ) in terms of final weight, WG, FI, FCR, SGR, PER, PR and ER. Growth response and feed utilization were less in groups fed diets with SPB compared to those fed the control diet that sustained FM. Final weight of the fish fed FM increased 5 fold, while the group fed SPB<sup>+Lys</sup> and SPB<sup>+Mix</sup> increased 3 fold, respectively. However, fish fed diets SPB, SPB<sup>+Met</sup> SPB<sup>+Try</sup>, and SPB<sup>+Arg</sup> were doubled at the end of the experiment (Table 4).

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The lowest FCR value of 1.20 was obtained for group Fed FM which showed the best growth performance followed by group fed SPB<sup>+Lys</sup> and SPB<sup>+Mix</sup> (1.66 and 1.52, respectively). Fish fed SPB<sup>+Lys</sup> and SPB<sup>+Mix</sup> had significantly ( $P < 0.05$ ) higher final weight, WG, FI, FCR, SGR, PR and ER compared to the groups fed diets SPB, SPB<sup>+Met</sup> SPB<sup>+Try</sup>, and SPB<sup>+Arg</sup>. Significant ( $P > 0.5$ ) differences in the PER figures of all the experimental diets were noticed (Table 4).

#### **Whole-body proximate composition**

Body composition of *Clarias gariepinus* fed different experimental diets are figured in Table 5. The whole-body moisture and crude protein content did not differ significantly ( $P > 0.05$ ). Fish fed SPB<sup>+Mix</sup> had significantly ( $P < 0.05$ ) higher levels of fat and less ash than fish fed the other diets. The group fed SPB<sup>+Mix</sup> recorded the least ( $P < 0.05$ ) crude lipid content.

#### **DISCUSSION**

Experimental diets were formulated to meet all nutrient requirements of African catfish fingerlings. Fish fed with SPB produced the lowest performance and feed utilization of all the experimental diets compare with fish fed FM diet. The diets containing SPB gave significantly limited final weight, WG, FCR, SGR, PR and ER., being worsened when total replacement of FM by SPB. Their poor performance may be due to limiting amino acid (Arg, Met, Lys and Try) content (Nengas *et al.*, 1999), feather, connective tissue and skin contents which are considered to be difficult for fish to digest (Hardy, 2000), subjection of the product to high temperature (150-200°C) for a long time (10 hours) during the processing (Nengas *et al.*, 1999), or combination of all. High temperature during the raw material processing leads to amino acid losses and thus, digestibility of protein and amino acids is reduced (Li and Robinson, 2013). According to the National Research Council (1993), crude protein digestibility of poultry by-products are lower than those reported for FM.

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**Table 4. The performance of experimental treatments.**

Parameters	FM	SPB	SPB <sup>+Lys</sup>	SPB <sup>+Met</sup>	SPB <sup>+Try</sup>	SPB <sup>+Arg</sup>	SPB <sup>+Mix*</sup>	SEM <sup>1</sup>
Mean initial weight (g/fish)	5.43	5.43	5.43	5.28	5.41	5.34	5.36	0.03
Mean final weight (g/fish)	28.80 <sup>a</sup>	10.56 <sup>d</sup>	15.29 <sup>c</sup>	10.52 <sup>d</sup>	11.19 <sup>d</sup>	10.75 <sup>d</sup>	16.96 <sup>b</sup>	1.72
Weight gain (g/fish) <sup>2</sup>	23.43 <sup>a</sup>	5.13 <sup>d</sup>	9.86 <sup>c</sup>	5.24 <sup>d</sup>	5.79 <sup>d</sup>	5.42 <sup>d</sup>	11.60 <sup>b</sup>	1.71
Feed intake (g)	28.18 <sup>a</sup>	14.46 <sup>de</sup>	16.36 <sup>bc</sup>	13.48 <sup>e</sup>	16.16 <sup>bc</sup>	15.31 <sup>cd</sup>	17.65 <sup>b</sup>	1.29
FCR (feed: gain) <sup>3</sup>	1.20 <sup>b</sup>	2.82 <sup>a</sup>	1.66 <sup>b</sup>	2.60 <sup>a</sup>	2.84 <sup>a</sup>	2.83 <sup>a</sup>	1.52 <sup>b</sup>	0.19
SGR <sup>4</sup>	3.00 <sup>a</sup>	1.19 <sup>d</sup>	1.85 <sup>c</sup>	1.23 <sup>d</sup>	1.30 <sup>d</sup>	1.26 <sup>d</sup>	2.06 <sup>b</sup>	0.17
PER <sup>5</sup>	2.26 <sup>a</sup>	0.96 <sup>c</sup>	1.42 <sup>b</sup>	0.96 <sup>c</sup>	0.94 <sup>c</sup>	0.84 <sup>c</sup>	1.52 <sup>b</sup>	0.13
PR <sup>6</sup>	26.07 <sup>a</sup>	7.47 <sup>b</sup>	10.10 <sup>b</sup>	7.07 <sup>b</sup>	8.68 <sup>b</sup>	7.36 <sup>b</sup>	13.60 <sup>b</sup>	1.79
ER <sup>7</sup>	21.73 <sup>a</sup>	8.48 <sup>b</sup>	9.81 <sup>b</sup>	7.19 <sup>b</sup>	7.98 <sup>b</sup>	7.84 <sup>b</sup>	13.42 <sup>b</sup>	1.37
Survival rate	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00

\*Lys+Met+Try+Arg

<sup>1</sup>Pooled standard error of a means.

<sup>2</sup>Weight gain (WG) = Final body weight (g) – Initial body weight (g).

<sup>3</sup>Feed conversion ratio (FCR) = feed intake (g) / body weight gain (g).

<sup>4</sup>Specific growth rate (SGR) = (In final body wt - In initial body wt) / feeding days X 100.

<sup>5</sup>PER = body weight gain (g) / protein intake (g).

<sup>6</sup>Protein retention = 100 x (the total protein gain in fish body (g) / the total protein consumed (g)).

<sup>7</sup>Energy retention = 100 x (the gross energy gain of the fish / gross energy in the feed consumed (g)).

Mean in the same raw with different superscription are significantly different (P≤0.05) by Duncan's test.

**Table 5 Chemical composition of the whole body (g kg<sup>-1</sup>) of African catfish fed the experimental diets for 8 weeks**

Composition	Initial	FM	SPB	SPB <sup>+Lys</sup>	SPB <sup>+Met</sup>	SPB <sup>+Try</sup>	SPB <sup>+Arg</sup>	SPB <sup>+Mix</sup>	SEM <sup>1</sup>
Moisture	713.83	778.11	746.14	775.81	773.53	759.99	755.87	747.72	4.87
Crude protein	143.09	130.64	139.27	134.97	135.58	142.41	142.69	148.08	2.25
Crude lipid	43.39	56.26	58.97	51.67	50.30	54.34	55.26	62.78	1.40
Ash	97.36	33.39	48.44	34.42	35.16	42.96	39.72	34.05	1.71

<sup>1</sup>Pooled standard error of a means.

Mean in the same raw with different superscription are significantly different (P≤0.05) by Tukey's test.

Imbalanced amino acid concentration is associated with lower protein synthesis or increased degradation, or simultaneous change in both components of protein turnover (Hansen *et al.*, 2007). Therefore, it is necessary to satisfy the dietary essential amino acid needs fish by formulating amino acid-balanced fish feeds.

In the present study, supplementation of the SPB diet with each of Met, Try or Arg had no positive effect, except for SPB<sup>+Lys</sup> and SPB<sup>+Mix</sup> which enhanced fish growth performance and feed utilization. The latter finding was probably due to the fact that Lys

was much more limiting than the others in SPB. Wang and Parsons (1998) indicated that cystine, tryptophan, threonine and lysine were the first four limiting amino acids in PBM, while methionine may be slightly limiting in PBM protein in poultry diet. However, Main and Doghir (1982) reported that methionine and lysine were the first and second limiting amino acids in PBM, respectively, in poultry diet.

Up to 40% of protein in practical diets for African catfish could be provided by PBM before significant reduction in performance (Abdel-Warith *et al.*, 2001). Total replacing protein provided by fishmeal (24.5% total crude protein) with PBM produced a little bit reduction in performance indices of tilapia and a large increase in the profit index for the replacement diets (El-Sayed, 1998). Hybrid tilapia fed a replacement diet in which PBM provided 17% crude protein, or 60% of dietary protein, exhibited equivalent weight gains, feed conversions and protein efficiencies as fish fed a fishmeal-based control diet (Fasakin *et al.*, 2005). Juvenile red drum were able to use up to 13% crude protein or 30% of the total dietary protein provided by either PBM or enzyme-digested PBM with no differences in performance with respect to fish fed a fishmeal-based control diet (Kureshy *et al.*, 2000). Nengas *et al.* (1999) were able to replace 50% of the protein provided by fishmeal, or 18% crude protein, with PBM in diets for gilthead sea bream; however, reduction in growth performance was seen at the 75% level of fishmeal replacement. Similarly, Quartararo *et al.* (1998) were able to replace 27% of the protein provided by fishmeal, or 12% crude protein, with protein from PBM and supplemental Met (0.34%) and Lys (0.24%) in diets for Australian snapper, but reduction in weight gain and specific growth rate were evident at higher levels of replacement. Nevertheless, an economic analysis revealed that up to 50% replacement of fishmeal protein with PBM was cost-effective in Australian snapper diets in spite of the reduced

performance at that level of substitution. Yang *et al.* (2004) also found PBM was able to replace 50% of the protein provided by fishmeal in diets for gibel carp. On the other hand, Emre *et al.* (2003) observed drastic reduction in growth and nutrient retention of mirror carp fingerlings fed diets containing as little as 33%PBM without amino acid supplementation.

Some studies have shown that PBM could replace even 100% of FM without significant decrease in fish growth (Saadiah *et al.*, 2011). The differences between the present results and those is probably because of the better amino acids profile of the PBM diet used there versus those of the SPB diet evaluated in study reported here in Similarly, the use of PBM in fish diets has been reported to reduce growth in some carnivorous fish, especially when totally replacing FM in the diet (Rawles *et al.*, 2011).

The worst FCR value was observed in the treatment with 100% SPB, this may be due to the high ash content of SPB, which produced a faster gut transit rate, and force fish to feed more associated with poor feed efficiency and growth performance (Goda *et al.*, 2007).

In the present study, the African catfish fed diets with different protein sources had the PER values in the range of 0.84-2.26. The PER depend on the weight gain in relation to protein. Protein intake depends on the source of protein in the diet (Mohanta *et al.*, 2013). Therefore, the source of protein may be responsible for the wide variation on PER values.

The proximate compositions of the initial and final carcass are recorded in Table 5. Whole body compositions of African catfish fed diets containing 100%PBM were not significantly differed. These findings are in agreement with the values reported by Emre *et al.* (2003). The final body crude lipid levels are higher (Emre *et al.*, 2003) and ash is lower (Nengas *et al.*, 1999) than initial levels. However, final body protein level obtained in this trial was slightly lower compared to initial value except for fish

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fed SPB<sup>+Mix</sup>. This is contradictory findings reported by Hasan and Das (1993).

The data revealed that L-lysine was found to be first-limiting AA in the SPB. Deficiencies in African catfish performance were ameliorated by supplementing the 100% replacement diet with sufficient L-lysine to match levels in a hypothetical 40% protein diet formulated for the African catfish.

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## تحديد ترتيب الأحماض الأمينية المحددة لاستخدام مسحوق مخلفات مجازر الدواجن في علائق القراميط الأفريقي باستخدام طريقة الإضافة

أسامة محمد الحسيني ، محمد يوسف حسن، اشرف سلومه  
قسم الانتاج الحيواني – كلية الزراعة – جامعة القاهرة

أجريت هذه الدراسة بهدف تحديد ترتيب الأحماض الأمينية المحددة لاستخدام مسحوق مخلفات مجازر الدواجن في علائق القراميط الأفريقي باستخدام طريقة الإضافة. واستخدمت ٧ علائق متماثلة في محتوى البروتين الخام ( ٤٠ %). عليقة الكنترول تحتوي على ٤٠% مسحوق السمك و ٢٠% مسحوق فول الصويا و بدون إضافة أحماض أمينية، و تم تكوين العليقة لتغطي بالاحتياجات الغذائية لأسماك القراميط الأفريقي. أما في العليقة الثانية تم إحلال مسحوق السمك بنسبة ١٠٠% باستخدام مسحوق مخلفات مجازر الدواجن و بدون إضافة أحماض أمينية. ثم تم إضافة الليسين و الميثيونين و التربتوفان و الأرجينين منفردا في العلائق  $SPB^{+Lys}$  ،  $SPB^{+Met}$  ،  $SPB^{+Try}$  و  $SPB^{+Arg}$  على التوالي ثم مجموعة في العليقة رقم ٧. و غذيت بها أسماك القراميط ثلاث مرات يوميا حتي الشبع. وكانت هناك فروق معنوية في النمو و كفاءة الإستفادة من الغذاء. أسماك القراميط التي غذيت على عليقة  $SPB$  سجلت أقل معدل نمو و كفاءة الإستفادة من الغذاء مقارنة بالمجموعة التي غذيت على عليقة الكنترول. المجموعة التي غذيت على عليقة الكنترول سجلت أعلى وزن نهائي حيث زادت بمعدل ٥ إضعاف في نهاية التجربة بينما المجموعات التي غذيت على علائق  $SPB^{+Lys}$  و  $SPB^{+Mix}$  تضاعفت ٣ مرات، أما المجموعة التي غذيت على علائق  $SPB$  و  $SPB^{+Try}$  و  $SPB^{+Met}$  و  $SPB^{+Arg}$  تضاعفت مرة واحدة فقط. المجموعة التي غذيت على  $SPB^{+Met}$  سجلت أقل وزن نهائي. أفضل معامل تحويل غذائي (١,٢٠) سجلت للمجموعة التي غذيت على عليقة الكنترول مع عدم وجود فروق معنوية بينها و بين المجموعات التي غذيت على  $SPB^{+Lys}$  و  $SPB^{+Mix}$  (١,٥٢ و ١,٦٦ على التوالي). المجموعات التي غذيت على  $SPB^{+Lys}$  و  $SPB^{+Mix}$  سجلت أفضل معدل نمو و كفاءة الإستفادة من البروتين مقارنة بالمجموعات التي غذيت على  $SPB$  ،  $SPB^{+Met}$  ،  $SPB^{+Try}$  و  $SPB^{+Arg}$ . المجموعة التي غذيت على عليقة الكنترول سجلت أعلى كفاءة الإستفادة من البروتين بينما المجموعة التي غذيت على  $SPB^{+Arg}$  سجلت أقل قيمة. من النتائج المتحصل عليها يمكن إستنتاج ان الليسين يعتبر الحامض الأميني المحدد الأول في مسحوق مخلفات مجازر الدواجن و إضافة الليسين في علائق القراميط المحتوية على نسب عالية من مسحوق مخلفات مجازر الدواجن تحسن النمو.

**الكلمات الدالة:** القراميط الأفريقي، مسحوق مخلفات مجازر الدواجن، أحماض أمينية