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# Protein Quality of Rations for Nile Tilapia (*Oreochromis niloticus*) Containing Oilseed Meals

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**Abstract**

Considering price as the main limiting factor in the use of animal proteins, this study evaluated the protein quality of diets for Nile tilapia containing oilseed meals as replacements of fishmeal. A control diet (FMBD) (30% crude protein and 2900Kcal DE/kg) was formulated using fishmeal (FM), soybean meal (SBM), canola meal (CM) and sunflower meal (SFM). The test diets SBBD, CMBD and SFBD were formulated by replacing 10% CP of FM by SBM, CM and SFM, respectively. The amino acid profile was determined by MPA FT-NIR spectrometer (Bruker, Germany). Fishmeal recorded higher levels ($p < 0.05$) of lysine (7.81 mg/100 g), methionine (2.89 mg/100 g), arginine (5.87 mg/100 g), threonine (4.28 mg/100 g) and isoleucine (4.55 mg/100 g). The chemical score for all essential amino acid in fishmeal were higher than 100. Sunflower meal was a superior ($p < 0.05$) protein source with an essential amino acid index (EAAI) of 1.14 compared to CM (0.80) and SBM (0.70). Substituting FM with SBM, CM or SFM, reduced ($p < 0.05$) the levels of all amino acids apart from methionine which was increased ($p < 0.05$) in the diet with SBM substitute. In all diets, methionine and isoleucine were the first and second limiting amino acids, respectively. Though the diet containing FM exhibited higher ($p < 0.05$) EAAI (0.97), it was not satisfactory because it was limiting in methionine. The EAAI reduced ($p < 0.05$) with replacement of fishmeal by SBM (0.78), CM (0.77) and SFM (0.76). The study showed that the control diet had good quality protein and substitution with SBM, CM and SFM gave useful protein diets.

**Keywords:** amino acids, canola meal, chemical score, essential amino acid index, fishmeal, Nile tilapia, protein quality, soybean meal, sunflower meal

## 1. Introduction

Fish do not have a true protein requirement but require a balanced combination of essential amino acids (EAA) and nonessential amino acids (NRC, 2011; Wilson, 2002). Supplying the essential amino acids requirements of cultured fish is extremely important because of significant effects of these nutrients on muscle deposition and feed cost (Small & Soares, 1999). However, formulating cost effective feeds that meet the essential amino acid requirement can be a challenge (Kaushik & Seiliez, 2010). This is because the use of high quality proteins to meet these requirements tend to be limited by their price (El-Sayed, 2006; Webster & Chhorn, 2006).

Fishmeal is considered the most desirable animal protein ingredient in aqua feeds because of its high protein content, balanced amino acid profile, high digestibility and palatability, and as a source of essential n-3 polyenoic fatty acids (Hardy & Tacon, 2002). However, plant protein feedstuffs have been used to replace fishmeal due to their more constant availability, and lower costs, despite presenting lower protein content, amino acid imbalances, antinutritional factors, and, digestibility and palatability than fishmeal (Hardy, 2010). A deficiency in certain essential amino acids is one of the major issues with plant protein sources, as it requires supplementation with other feedstuffs (Ogunji, Rahat-Ul-Ain, Summen, & Schulz, 2008).

When considering the value of different protein sources, the quality depends on the quantity of essential amino acids, the balance among the respective amino acids, on which the utilization of the protein depends. As a result, different methods of protein evaluation give different results for the nutritive value (Malomo & Alamu, 2015). Ramarao, Norton, and Johnson (1964) argued that essential amino acid requirements should serve as a standard for evaluating the quality of protein in foods, rather than the total essential amino acids content of the food. There are several reports now to confirm that amino acid profiles of whole body tissue of a given species of fish resemble those of the dietary requirements (Mambrini & Kaushik, 1995). Protein chemical score (CS) has been used to compare the essential amino acid content in the test protein when the requirement is already established (Hepher, 1988). It is based on the concept that utilisation of a dietary protein depends upon the level of the EAA in greatest deficit which is termed the 'first limiting EAA' (Jauncey, 1998). However, since other essential amino acids may also have effects on protein utilization, this resulted in the development of the essential amino acid index (EAAI) (Bunda, Tumbokon, & Serrano, 2015). The objective of this study therefore was to evaluate the protein quality, using the CS and EAAI, of oilseed meals (soybean meal, canola meal and sunflower meal) and their effect on protein quality when used as substitutes for fish meal in Nile tilapia rations.

## 2. Materials and Methods

### *2.1 Study Site*

The experiment was conducted at Chuka University laboratory and Fletcher Scientific Solutions, Nairobi, Kenya.

### *2.2 Preparation of Diets*

A control diet (FMBD) of 30% CP and 2900 Kcal/kg was formulated in triplicate using fishmeal (*Rastrionaebola argentea*), soybean meal, canola meal, sunflower cake, maize grain, wheatbran (Table 1), sourced from local feed dealers. The ingredients were ground using hammer mill to be uniform before mixing. The diets were pelleted using a pelletizer machine particle size 4.5 mm diameter, dried under shade and packed in polythene bags. The test rations were similarly prepared by replacing 10% of the CP supplied by FM by either SBM (SBBD), CM (CMBD) or SFM (SFBD) (Table 1).

Table 1. Ingredient composition (%), calculated crude protein (%) and digestible energy of diets for *Oreochromis niloticus* containing either soybean meal (SBBD), canola meal (CMBD) or sunflower meal (SFBD) as replacements of fishmeal (FMBD)

| Ingredients | FMBD | SBBD | CMBD | SFBD |
|---|---|---|---|---|
| Fish meal | 16.5 | 9 | 9 | 9 |
| Soybean meal | 13 | 24 | 15 | 16 |
| Canola meal | 16.5 | 16 | 31 | 15 |
| Sunflower cake | 18 | 19 | 18 | 43 |
| Maize grain | 18 | 16 | 13 | 10 |
| Wheat bran | 18 | 16 | 14 | 7 |
| Total | 100 | 100 | 100 | 100 |
| Calculated crude protein (%) | 30.17 | 30.06 | 30.15 | 30.06 |
| Calculated digestible energy(Kcal/kg) | 2997.08 | 2965.08 | 2949.63 | 2878.11 |

### *2.3 Analysis of Samples*

Amino acid analysis of feed ingredients and formulated rations. was performed by MPA FT-NIR spectrometer (Bruker, Germany) which is a non-destructive method of analysis. The crude protein was determined using the kjeldahl method as described in AOAC (1995).

The amino acid chemical score (CS) was calculated by the following formular (Jauncey, 1998):

$$\text{CS (\%)} = (\text{\% EAA in ingredient or diet/\% EAA requirement for fish}) \times 100 \quad (1)$$

Essential amino acid index (EAAI) was calculated by the equation:

$$\text{EAAI} = n/\{(aa_1/AA_1)\cdot(aa_2/AA_2)\ \ldots\ (aa_n/AA_n)\} \quad (2)$$

Where, EAAI is the *n* th root of the essential amino acids in the test diet (aa) to the content of each of those amino acids in the reference tissue (AA) and *n* is the total number of amino acids evaluated (Tidwell, Webster, Yancey, & Abramo, 1993).

### *2.4 Data Analysis*

The crude protein, amino acid content, chemical scores and essential amino acid index data were subjected to analysis of variance (ANOVA) using SPSS statistical package version 17.0 at P = 0.05 confidence level to determine whether there were significance differences and where the differences occurred, mean separation was done by least significance difference (LSD).

## 3. Results

### *3.1 Amino Acid Composition of Ingredients*

The CP content and amino acid composition of ingredients are shown in Table 2. Sunflower had the lowest ($p < 0.05$) CP content (24.81%) among the protein supplements. Amino acid analysis revealed that Fish meal (*Rastrionaebola argentea*), recorded highest level for essential amino acids. Fish meal had highest level of lysine (7.81 mg/100g) followed by canola meal (4.01 mg/100 g) with maize meal recording the least (1.42 mg/100 g). Methionine content was higher in fishmeal (2.89 mg/100 g) compared to the oilseed meals.

Table 2. Amino acid composition (mg/100 g protein) of feed ingredients used to formulate diets for *Oreochromis niloticus*

| | Fish meal | Soybean meal | Canola meal | Sunflower meal | Maize meal | Wheat bran |
|---|---|---|---|---|---|---|
| Crude protein (%) | 62.60±038[a] | 47.38±0.32[b] | 34.39±0.18[c] | 24.81±0.03[d] | 10.65±0.27[f] | 16.04±0.43[e] |
| *Essential amino acids* | | | | | | |
| Lysine | 7.81±0.07[a] | 3.01±0.01[d] | 4.01±0.01[b] | 3.14±0.01[c] | 1.42±0.00[f] | 1.75±0.01[e] |
| Methionine | 2.89±0.01[a] | 0.61±0.01[de] | 0.61±0.01[ed] | 0.51±0.01[f] | 2.16±0.01[b] | 1.44±0.01[c] |
| Cysteine | 0.95±0.01[e] | 0.66±0.00[f] | 1.16±0.00[c] | 1.54±0.02[b] | 1.04±0.00[d] | 1.82±0.02[a] |
| Histidine | 2.43±0.01[b] | 1.26±0.01[f] | 1.57±0.00[e] | 5.44±0.01[a] | 2.12±0.01[c] | 1.81±0.01[d] |
| Arginine | 5.87±0.04[a] | 3.39±0.01[b] | 3.05±0.02[c] | 2.96±0.00[d] | 2.42±0.01[f] | 2.81±0.01[e] |
| Threonine | 4.28±0.01[a] | 1.96±0.04[f] | 2.1±0.06[e] | 3.87±0.00[b] | 2.6±0.01[d] | 3.16±0.01[c] |
| Valine | 5.4±0.01[b] | 2.24±0.01[f] | 2.34±0.01[e] | 6.27±0.02[a] | 4.09±0.01[d] | 4.93±0.01[c] |
| Isoleucine | 4.55±0.07[a] | 2.36±0.01[c] | 2.55±0.01[e] | 0.97±0.02[f] | 3.26±0.01[d] | 3.83±0.01[b] |
| Leucine | 7.55±0.04[b] | 3.69±0.01[f] | 3.78±0.01[e] | 10.06±0.01[a] | 7.15±0.01[c] | 6.85±0.02[d] |
| Phenylalanine | 4.2±0.01[c] | 2.71±0.02[f] | 3.84±0.01[d] | 5.83±0.01[a] | 4.24±0.01[b] | 3.79±0.01[e] |
| Tryptophan | 1.15 | 0.68 | 0.62 | ND | ND | ND |
| *Non essential amino acids* | | | | | | |
| Tyrosine | 3.32 | ND | ND | ND | 3.25 | 2.32 |
| Aspartic acid | 9.29 | ND | ND | ND | 3.46 | 2.83 |
| Serine | 3.83 | ND | ND | ND | 6.47 | 6.61 |
| Glutamic acid | 12.88 | ND | ND | ND | 13.21 | 17.11 |
| Proline | 4.28 | ND | ND | ND | 8.06 | 12.61 |
| Glycine | 5.74 | ND | ND | ND | 4.17 | 6.01 |
| Alanine | 6.24 | ND | ND | ND | 10.14 | 4.32 |

*Note.* Values are expressed as mean±SE; [a], [b], [c], [d], [e], [f] Values in the same row having different superscript letters are significantly different ($p < 0.05$); ND: Not detected.

### *3.2 Amino Acid Composition of Diets*

Amino acid composition of the diets (mg/100 g) is as shown in Table 3. The diet with sunflower meal had lowest content of essential amino acid methionine (0.86), lysine (6.83), phenylalanine (2.54), histidine (1.50) and valine (2.40) ($p < 0.05$). FMBD recorded highest values ($p < 0.05$) for essential amino acid lysine (8.12), phenylalanine (3.42), histidine (2.32), valine (2.79) and threonine (2.65). Methionine content of SBBD was highest (1.05) followed by FMBD (0.93), CMBD (0.92) with SFBD (0.86) recording least. Statistically, methionine content of FMBD (0.93) and CMBD (0.92) was similar ($p > 0.05$).

Table 3. Amino acid composition (mg/100g Protein) of diets for *Oreochromis niloticus* containing either soybean meal (SBBD), canola meal (CMBD) or sunflower meal (SFBD) as replacements of fishmeal (FMBD)

| | FMBD | SBBD | CMBD | SFBD |
|---|---|---|---|---|
| *Essential amino acids* | | | | |
| Isoleucine | 2.3±0.01[b] | 1.34±0.01[c] | 1.23±0.01[d] | 1.42±0.00[a] |
| Leucine | 4.43±0.01[a] | 3.12±0.00[d] | 3.89±0.01[b] | 3.57±0.01[c] |
| Arginine | 5.52±0.01[a] | 4.55±0.01[d] | 4.61±0.01[c] | 4.73±0.01[b] |
| Valine | 2.79±0.01[a] | 2.46±0.01[b] | 2.42±0.00[cd] | 2.40±0.01[dc] |
| Methionine | 0.93±0.01[bc] | 1.05±0.01[a] | 0.92±0.01[cb] | 0.86±0.01[d] |
| Lysine | 8.12±0.01[a] | 7.35±0.01[b] | 7.19±0.01[c] | 6.83±0.01[d] |
| Phenylalanine | 3.42±0.01[a] | 2.57±0.01[bc] | 2.56±0.00[cbd] | 2.54±0.01[dc] |
| Histidine | 2.32±0.01[a] | 1.65±0.00[c] | 1.70±0.01[b] | 1.50±0.01[d] |
| Threonine | 2.65±0.01[a] | 2.30±0.01[b] | 2.15±0.01[d] | 2.25±0.01[c] |
| *Non essential amino acids* | | | | |
| Aspartic acid | 5.13±0.01[a] | 4.68±0.01[b] | 3.58±0.01[c] | 3.04±0.01[d] |
| Glutamic acid | 8.76±0.01[a] | 7.53±0.01[b] | 7.08±0.01[d] | 7.35±0.01[c] |
| Proline | 0.18±0.00[a] | 0.11±0.00[cd] | 0.11±0.00[dc] | 0.13±0.00[b] |
| Serine | 3.31±0.01[a] | 2.64±0.01[c] | 2.52±0.00[d] | 2.67±0.01[b] |
| Glycine | 4.25±0.01[a] | 3.22±0.00[c] | 3.11±0.01[d] | 3.31±0.01[b] |
| Tyrosine | 1.00±0.00[a] | 0.78±0.01[b] | 0.66±0.01[d] | 0.73±0.01[c] |
| Alanine | 0.19±0.00[b] | 0.11±0.00[d] | 0.15±0.00[c] | 0.21±0.00[a] |

*Note*. Values are expressed as mean±SE; [a], [b], [c], [d] Values in the same row having different superscript letters are significantly different ($p < 0.05$).

### *3.3 Chemical Scores and Essential Amino Acid Index o f Feed Ingredients*

Fishmeal recorded highest chemical scores for all essential amino acids which were above 100 (Table 4). In all the ingredients, chemical scores for lysine were below 100 except for fishmeal (152.47). The chemical score for oilseed meals of EAAs were below 100 apart from SFM histidine (316.47), threonine (103.11), valine (224.04), leucine (296.75) and phenylalanine + tyrosine (155.46), SBM (leucine (108.75) and CM (leucine (111.50) and phenylalanine + tyrosine (102.47). Maize meal and wheatbran had scores above 100 except lysine (27.79 and 34.25) and arginine (57.54 and 66.98), respectively.

Table 4. Chemical scores (%) and essential amino acid index (EAAI) of ingredients (%) used to formulate diets for *Oreochromis niloticus*

| Amino acid | Fish meal | Soybean meal | Canola meal | Sunflower meal | Maize meal | Wheat bran |
|---|---|---|---|---|---|---|
| Lysine | 152.47±1.30[a] | 58.79±0.22[d] | 78.32±0.22[b] | 61.32±0.19[c] | 27.79±0.06[f] | 34.25±0.28[e] |
| Methionine + Cystine | 143.28±0.21[a] | 47.63±0.33[f] | 66.29±0.33[e] | 76.49±0.98[d] | 119.52±0.33[c] | 121.39±0.89[b] |
| Histidine | 140.39±0.46[a] | 73.06±0.77[e] | 91.08±0.19[f] | 316.47±0.70[a] | 123.25±0.67[c] | 105.43±0.70[d] |
| Arginine | 139.76±0.96[a] | 80.64±0.28[b] | 72.70±0.51[c] | 70.39±0.15[d] | 57.54±0.21[f] | 66.98±0.20[e] |
| Threonine | 114.22±0.38[a] | 52.18±1.10[f] | 56.00±1.46[e] | 103.11±0.09[b] | 69.24±0.23[d] | 84.17±0.17[c] |
| Valine | 192.97±0.24[b] | 79.88±0.31[f] | 83.45±0.31[e] | 224.04±0.66[a] | 145.95±0.31[d] | 176.07±0.41[c] |
| Isoleucine | 146.30±2.09[a] | 75.77±0.39[e] | 81.99±0.19[d] | 31.19±0.67[f] | 104.82±0.32[c] | 123.15±0.32[b] |
| Leucine | 222.81±1.04[b] | 108.75±0.43[f] | 111.50±0.45[e] | 296.75±0.17[a] | 211.01±0.43[c] | 202.16±0.49[c] |
| Phenylalanine + Tyrosine | 200.53±1.46[a] | 72.26±0.55[f] | 102.47±0.40[e] | 155.46±0.15[d] | 199.91±0.23[b] | 162.84±0.44[c] |
| EAAI | 1.48±0.01[a] | 0.70±0.01[f] | 0.80±0.01[e] | 1.14±0.00[b] | 0.93±0.00[d] | 1.02±0.06[c] |

*Note.* Values are expressed as mean±SE; [a], [b], [c], [d], [e], [f] Values in the same row having different superscript letters are significantly different ($p < 0.05$).

### *3.4 Chemical Scores and Essential Amino Acid Index of Diets*

Chemical scores and essential amino acid indices for the diets are as shown in Table 5. Diet containing FM recorded chemical scores above 100 for all essential amino acids except Isoleucine (73.95), methionine + cysteine (34.58) and threonine (70.58). Replacement of FM with oil seed meals recorded scores below 100 except Arginine, Lysine and leucine (CM and SFC). The values for methionine were all below 100. Fish meal diet recorded highest essential amino acid index (0.97) while its replacement by SFM had the lowest (0.76).

Table 5. Chemical scores (%) and essential amino acid index (EAAI) of diets for *Oreochromis niloticus*, containing either soybean meal (SBBD), canola meal (CMBD) or sunflower meal (SFBD) as replacements of fishmeal (FMBD)

| Chemical Scores | FMBD | SBBD | CMBD | SFBD |
|---|---|---|---|---|
| Isoleucine | 73.95±0.18$^{b}$ | 42.98±0.28$^{c}$ | 39.44±0.28$^{d}$ | 45.55±0.11$^{a}$ |
| Leucine | 130.58±0. 97$^{a}$ | 91.94±0.10$^{d}$ | 114.85±0.43$^{b}$ | 105.31±0.17$^{c}$ |
| Arginine | 131.51±0.08$^{a}$ | 108.41±0.16$^{d}$ | 109.84±0.16$^{c}$ | 112.54±0.08$^{b}$ |
| Valine | 99.76±0.31$^{a}$ | 87.74±0.31$^{b}$ | 86.55±0.12$^{cd}$ | 85.83±0.24$^{dc}$ |
| Methionine + Cystine | 34.58±0.25$^{bc}$ | 39.06±0.25$^{a}$ | 34.33±0.21$^{cb}$ | 32.21±0.25$^{d}$ |
| Lysine | 158.59±0.11$^{a}$ | 143.49±0.17$^{b}$ | 140.37±0.17$^{c}$ | 133.46±0.17$^{d}$ |
| Phenylalanine + Tyrosine | 118.04±0.23$^{a}$ | 89.24±0.44$^{b}$ | 86.05±0.32$^{d}$ | 87.38±0.09$^{c}$ |
| Histidine | 134.88±0.34$^{a}$ | 96.12±0.19$^{c}$ | 99.03±0.51$^{b}$ | 87.21±0.58$^{d}$ |
| Threonine | 70.58±0.18$^{a}$ | 61.24±0.23$^{b}$ | 57.24±0.23$^{d}$ | 60.09±0.18c |
| EAAI | 0.97±0.00$^{a}$ | 0.78±0.00$^{bc}$ | 0.77±0.08$^{cb}$ | 0.76±0.00$^{d}$ |

*Note.* Values are expressed as mean±SE; $^{a}$, $^{b}$, $^{c}$, $^{d}$ Values in the same row having different superscript letters are significantly different ($p < 0.05$).

## 4. Discussion

### *4.1 Amino Acid Profile of Feed Ingredients*

Fish meal (*Rastrionaebola argentea)* displayed better and higher amino acid profile than other ingredients and according to Drew, Borgeson, and Thiessen (2007) it is the "gold standard" to which plant proteins must be compared in terms of protein quality. These results were comparable to those reported by Maina et al. (2007), and Oduho, Baker, and Tuitoek (2005). Lysine had the highest concentration with values of (7.81 mg/100 g), closely followed by arginine with a value of (7.55 mg/100 g). High levels of essential amino acids in fish meal as in the present study make it more desirable in fish feed. However, the high costs and scarcity in Kenya is the major limitation to its use (Kirimi, Musalia, Magana, & Munguti, 2016b).

Soybean meal in the present work recorded low levels for methionine and cysteine which have been reported as the two most limiting amino acids (NRC, 1993). Whereas, Banaszkiewicz (2000) reported low levels of tryptophan, NRC (2012) reported slightly higher levels for methionine (0.66 mg/100 g) and cystine (0.70 mg/100 g) but lower levels of lysine (2.96 mg/100 g) and phenylalanine (2.40 mg/100 g). The present study agrees with Storebakken et al. (2000) that soybean is a widely used plant protein ingredient in many aquaculture species due to relatively well balanced amino acid.

The essential amino acid compositions of canola meal figures in this study were below those obtained by Adem, Tressel, Pudel, Slawski, and Schulz (2014). However, levels of methionine and cystine (0.61 and 1.16 mg/100 g, respectively) contradicts with the findings of Chen et al. (2015) who reported higher values (0.91 and 1.21 mg/100 g, respectively) but lower than that recorded by NRC (2012). More so, the levels recorded by NRC, (2012) for lysine (4.01 mg/100 g), leucine (3.78 mg/100 g) and phenylalanine (3.84 mg/100 g), were lower. According to Bell and Keith (1991), and Newkirk (2009), the concentration of amino acids in canola meal varies depending on varieties, environmental factors, seed composition, and amount of residual oil and carbohydrates in the meal. Level of heat duration in processing also contributes to degradation of heat sensitive amino acids such as lysine (Newkirk, 2009).

In the present study, sunflower meal amino acid analysis showed that, methionine recorded the lowest figure (0.51 mg/100 g) but the most limiting amino acid was isoleucine followed by lysine, arginine and methionine+cystine (Table 4). The levels of leucine and valine in this study were high, similar to the report made by Akande (2011). Sunflower meal ranked second to FM in EAAI followed by CM and SBM had the least score among the three oilseed meals. In other studies SFM proteins have well balanced amino acid composition apart from lysine and contain more sulphur amino acids which are deficient in other plant proteins (Gassman, 1983; Ribarova et al., 1987; Canibe et al., 1999).

Fishmeal as an ingredient recorded highest chemical scores for all essential amino acids and no essential amino acid was limiting. This is in agreement with De Silva and Anderson (1995) that there is no single foodstuff that can serve as an alternative to fish meal. Generally, the oilseed meals were poor sources of EAA for fish apart from leucine, phenylalanine + tyrosine (CM and SFM), valine (SFM), threonine (SFM) and histidine (SFM) (Table 4). Apparently among the oilseed meals, sunflower meal had the highest EAAI (1.14%) and this was mainly due to high levels of leucine (10.06 mg/100 g), valine (6.27 mg/100 g) and histidine (5.44 mg/100 g) (Table 2). However, isoleucine was the 1$^{st}$ limiting amino acid in SFM.

Amino acid profile for maize meal was slightly lower than those reported by Lasek et al. (2012) except for methionine which was higher. Cystine (1.04 mg/100 g) was the first limiting amino acid in maize meal closely followed by lysine (1.42 mg/100 g) and tryptophan was not detected. This agrees with the observation of Olson and Frey (1987) that maize meal is deficient in the essential amino acids tryptophan and lysine and this limits the amount of protein available for monogastric animals. In contrast, Paes and Bicudo (1995) argued that special maize with increased levels of the essential amino acids lysine and tryptophan have been produced in developing countries as a strategy to improve protein quality of maize. However, the level of aromatic amino acid (phenylalanine, 4.24 and tyrosine, 3.25 mg/100 g) was high which corroborate with Makanjuola and Olakunle (2017) that maize meal is high in aromatic amino acids.

*4.2 Amino Acid Profile of Diets*

In the diets, all the essential amino acids except tryptophan were detected though there was slight variation among them. This variation in amino acid contents of the diets could have been attributed to the varying proportion of the ingredients inorder to balance for the crude protein. The control diet displayed a better amino acid profile with higher levels of leucine, arginine, valine, lysine, histidine, threonine and phenylalanine ($p < 0.05$). The high concentration of the essential amino acids could be attributed to the high levels of fish meal. According to De Silva and Anderson (1995), the EAA profiles of plant proteins used in feed formulation are usually poor, implying they are deficient of one or more EAAs, compared to the requirements of the animal. This contributed to the low amino acid levels observed in diet containing plant proteins as replacement of FM that formed the bulk of protein. According to Jubie et al. (2015), animal nutritionist recommend varied sources of protein for reasons that each source could be complimentary with another source which ultimately balance the essential amino acids.

In the present study, the concentration of sulfur containing amino acids (methionine and cystine) in the diets decreased with substitution of fishmeal though SBBD recorded slightly higher levels (1.08) ($p < 0.05$). However, methionine levels in the four diets were low (Table 3) against the recommended (2.68) for Nile tilapia (NRC, 1993). This was attributed by low levels of methionine recorded in the ingredients (Table 2). This agrees with Lovell (1989) and Furuya et al. (2004) that methionine is an essential amino acid for fish and it is usually the first limiting amino acid in many fish diets. Methionine acts as methyl donor, precursor of several substrates, including nucleic acids, proteins, phospholipids, biogenic amines, carnitine, cystine, choline, polyamines and other metabolic intermediates (Mato et al., 1997; Zhou et al., 2006).

The results of this study indicate that lysine in all the diets were above the levels recommended by Santiago and Lovell (1988) of 5.1 mg/100 g crude protein for Nile tilapia. This could have been attributed by the high levels of lysine in all the ingredients except in maize meal and wheatbran (Table 2). Thus, lysine requirements were met. However, excess lysine reported in this study could lead to amino acid toxicity. It has been reported that excessive levels of amino acids may become toxic and have an adverse effect on growth because the disproportionate amount of one amino acid affects the absorption and utilization of other amino acids (Murthy & Varghese, 1996).

Phenylalanine + tyrosine were present in the four diets (Table 3). However, only FMBD met the requirement of (3.75 mg/100 g) for Nile tilapia recommended by NRC (1993). Phenylalanine, an aromatic indispensable amino acid is required for normal growth and metabolic processes. It is the sole precursor of tyrosine. Phenylalanine can be converted to tyrosine by tetrahydrobiopterin-dependent phenylalanine hydroxylase in liver and kidneys but phenylalanine cannot be synthesized back from tyrosine (NRC, 1993). Inclusion of adequate levels of tyrosine reduces the phenylalanine requirement. Hence, to satisfy the total aromatic amino acid requirement of fish, feeds should be formulated with required amount of phenylalanine and adequate tyrosine.

In the present study, only FMBD met the requirements for histidine (2.32 mg/100 g) against the recommended (1.75 mg/100 g). This is attributed to the low levels of histidine in the ingredients.

Valine levels in the present study were slightly lower than the recommended levels (2.8 mg/100 g). Chung and Bakerv (1992) described that valine deficiency reduces the use of other limiting amino acids for protein deposition, and is thus essential. Isoleucine was significantly low in all the diets. According to Khan and Abidi (2007), the determination of essential amino acid requirements, besides methionine, lysine, and threonine is mainly important in low protein diets and those developed from plant foods, and isoleucine is highlighted because of its important role in protein synthesis and in energy metabolism of skeletal muscle. Antagonism between branched-chain amino acids arises in animals from an excess of leucine over isoleucine and valine; the first two steps of the catabolic breakdown of all three branched-chain amino acids are catalyzed by the same enzymes (NRC, 1993). Based on this, in the present study, there could be antagonism of the branched chain amino acids because leucine content in all the diets were in excess of isoleucine and valine. Diets with branched amino acid deficiencies result in decreased response from lymphocytes and stimulating agents, increased susceptibility to infections, and growth reduction (Calder, 2006).

In all the diets, methionine had the lowest chemical scores hence the first limiting amino acid and isoleucine second limiting amino acid. The Essential Amino Acid Index (EAAI) recorded in the present study varied significantly among the four diets ($p < 0.05$) with FMBD recording highest (0.97). This variation in EAAI is attributed to the corresponding fluctuations in respective amino acids in the diets. It's worth noting that in SFM based diet, EAAI was lowest (0.76) despite high EAAI in sunflower meal (1.14). This scenario could be attributed to the low CP in the SFM (24.81%) hence large quantity was required to balance for the CP in SFM based diet. The findings in the present study closely agree with the study done by Kirimi et al., (2016a) where diet with more fishmeal recorded highest EAAI. Based on Oser (1959) criterion for classifying protein quality, FMBD (0.97) could be considered good quality protein while SBBD (0.78), CMBD (0.77) and SFBD (0.76) useful protein sources.

In conclusion, all the diets were not satisfactory in the content of essential amino acids despite higher EAAI for FM based diet. They were all limiting in methionine, with chemical score lower than the standard. However, whereas the control diet was classified to be good quality protein source, substitution of FM with SBM, CM and SFM gave useful protein diets that can be supplemented with synthetic methionine and isoleucine.

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