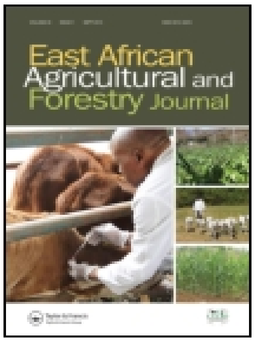

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Effect Of Replacing Fish Meal With Blood Meal On Chemical Composition Of Supplement For Nile Tilapia (*Oreochromis Niloticus*)

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ABSTRACT

The objective of the work was to evaluate the effect on the nutrient content of replacing fish meal (FM) with blood meal (BM) in fish supplement. Three isonitrogenous diets (35% crude protein) were formulated using FM as the main source of animal protein (BM0); 50% replacement of FM with blood meal (BM50); or 100% replacement of FM with BM (BM100). The chemical composition (ash, crude protein, crude fat and crude fibre) and amino acid composition were determined. Replacement of FM with BM did not affect the proximate composition of the diet apart from ash content which decreased with the level of substitution. Substituting fish meal with blood meal reduced the levels of methionine, lysine, isoleucine, leucine, proline, valine and increased the levels of arginine, phenylanine and alanine in the diet. Amino acid indices revealed that BM0 had more amino acids with the highest chemical scores followed by BM50 and BM100. In all the diets, methionine was the most limiting amino acid. The essential amino acid index of the diets reduced with the level of replacement of FM (0.94, 0.88 and 0.77). The study showed substitution of up to 50% FM with BM gave a useful protein diet and 100% gave almost a poor protein diet.

KEYWORDS
Amino acids; chemical score; essential amino acid index; nutritive value

Introduction

The nutrient quality of feed ingredients is one of the major prerequisites apart from their availability for the production of good quality feeds (Sogbesan & Ugwumba, 2008). In view of the high crude protein level in fish diet, protein is the most significant and expensive single nutrient in preparation of this diet. Formulating cost effective feeds that meet the essential amino acid (EAA) requirements of fish and shrimp can be a challenge (Kaushik & Seiliez, 2010) and depend on relevant data on both EAA requirements of the fish species and the EAA supplied with the feed. Fish meal has been the main protein source and the use of alternative feedstuffs requires a thorough understanding of amino acid requirements and their availability in feedstuffs.

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Proximate analysis is used in the initial evaluation of feeds and feedstuffs to provide information on their major nutrient and gross energy contents (Jobling, 2001; Bunda, *et al.*, 2015). Another evaluation criteria is the protein chemical score (CS) defined as the lowest ratio of the essential amino acid content in the test protein to the content of each amino acid in the muscle protein or to the EAA required level when the EAA requirement is already established. The assumption of chemical score was that whole egg protein is of the highest biological value (BV) and therefore the most suitable for growth and that growth is limited by that essential amino acid in the diet whose ratio to its content in the whole egg protein is the lowest (Hepher, 1988). Bunda *et al.* (2015), noted that although the first limiting amino acid has an important role in determining the relative value of the dietary protein, it was realized that other essential amino acids may also have some effect on it and this resulted in the development of the essential amino acid index (EAAI). According to Oser (1959), the EAAI is the geometrical mean of the ratio of all EAA in the evaluated protein relative to their content in a highly nutritive reference protein such as whole egg. The objective of this study therefore was to investigate the chemical composition (proximate and essential amino acid composition) of feed ingredients and the resulting ration when fish meal is replaced with blood meal from local slaughter houses.

Materials and methods

Study Site

The study was conducted at the National Aquaculture Research, Development & Training Centre, Sagana, altitude 1230 m above sea level, latitude 0°39′ S and longitude 37°12′ E, and 90 km north of Nairobi.

Preparation of Diets

The feed ingredients (fish meal, wheat bran, cotton seed cake, soya bean meal and blood meal) were obtained from the local markets. Bovine blood was collected from the local Sagana abattoir. Fresh blood drained from freshly slaughtered cattle was collected into a clean container, transported to Sagana National Aquaculture Centre and boiled immediately in a cooking container to 100 °C for 45 minutes in order to let the water evaporate and destroy pathogenic organisms (Khawaja *et al.*, 2007). As the blood boiled, it was continually stirred until it formed dough. The product was removed from the fire then drained and crushed manually to increase the drying surface area. The product was spread on a polythene liner and sun dried for three days to a moisture content of below 15%. The dried product was milled into a fine powder with a hammer mill.

Three isonitrogenous diets (35% CP) were formulated, in triplicate, using wheat bran, soybean meal, cotton seedcake and either fish meal as the main source of animal protein (BM0); 50% replacement of fish meal with blood meal (BM50); and 100% replacement of fish meal with blood meal (BM100) (Table 1).

The ingredients were ground using a hammer mill to be uniform and mixed thoroughly by hand in the desired proportion. Water was added to form dough and pelleted using a pelleter machine to particle size 4.5 mm diameter. The pellets were then dried in the shade.

Table 1. Ingredient composition and calculated chemical composition (%) of fish supplement containing blood meal as a replacement for fish meal (as fed basis).

	BM0†	BM50†	BM100†
Ingredient			
Fish meal	36.6	18.4	0
Blood meal	0	12.5	25.5
Wheat bran	48.4	53.8	59.5
Soybean meal	10	10.3	10
Cotton seed cake	5	5	5
Total	100	100	100
Calculated chemical composition (%)			
Crude protein (%)	35	35	35

†BM0, BM50 and BM100 represent replacement of fish meal at 0, 50 and 100%, respectively.

Analysis of Feeds

The proximate analysis of ingredients and diets were carried out as described by the AOAC (1995) for crude protein (CP), ether extracts (EE) and ash and crude fibre (CF). Nitrogen free extracts (NFEs) were estimated by subtracting the total moisture, crude protein, ether extracts, ash and crude fibre from 100. The method for protein extraction was adopted from Hamilton *et al.* (2012) for amino acid analysis using LC-Qtof-MS.

The amino acid score was calculated as:

$$\text{Chemical score (\%)} = [\text{essential amino acid of the sample} \\ /\textit{essential amino acid of the whole hen egg}] \times 100$$

While essential the EAAI was calculated as:

$$\text{EAAI} \ = n/\{(\text{aa}_1/\text{AA}_1)(\text{aa}_2/\text{AA}_2).(\text{aa}_n/\text{AA}_n)\}$$

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 *et al.*, 1993). Measurements were done in triplicate. The data were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences version 17.0 (SPSS Statistics) and where there were differences, mean separation was done by least significant difference (LSD).

Table 2. Proximate composition of feed ingredients (%) used to formulate fish supplements containing blood meal as a replacement for fish meal.

	Fish meal	Blood meal	Soybean meal	Cotton seed cake	Wheat bran
Proximate composition (%)					
Dry matter	$90.85^{c}\pm0.24$	$89.47^{e}\pm0.06$	$92.31^{a}\pm0.07$	$92.06^{ab}\pm0.17$	$89.55^{de}\pm0.07$
Crude protein	$64.20^{b}\pm0.27$	$80.41^{a}\pm0.13$	$11.47^{e}\pm0.04$	$27.07^{c}\pm0.40$	$14.49^{d}\pm0.02$
Ether extracts	$5.07^{c}\pm2.14$	$0.62^{e}\pm0.21$	$5.85^{bc}\pm0.44$	$8.52^{ab}\pm0.40$	$2.13^{abcde}\pm0.33$
Ash	$16.13^{a}\pm0.19$	$4.52^{b}\pm0.31$	$3.07^{c}\pm0.23$	$3.92^{bcd}\pm0.89$	$5.78^{be}\pm0.73$
Crude fibre	$0.52^{e}\pm0.12$	$1.34^{de}\pm0.28$	$9.75^{c}\pm0.87$	$23.43^{a}\pm0.77$	$12.58^{b}\pm0.85$
Nitrogen free extracts	$4.94^{d}\pm1.63$	$2.62^{de}\pm0.28$	$62.17^{a}\pm0.47$	$29.14^{c}\pm0.66$	$54.6^{b}\pm1.14$

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

Results

Chemical Composition of Diets

The proximate composition of the feed ingredients is shown in (Table 2). Blood meal recorded the highest crude protein content of 80.41% with soy bean meal recording the lowest crude protein content of 11.47%. Cotton seedcake had the highest crude fibre content (23.43%) with fish meal recording the lowest crude fibre content (0.52%). The ash content was high in fish meal (16.13%) compared with soybean meal (3.07%). Blood meal had a low residual oil compared to the cotton seedcake (0.62% vs 8.5%).

The proximate composition of the formulated diets is shown in Table 3. Replacement of FM by BM increased (P<0.05) the crude protein values. Ether extracts were the same for BM50 and BM100 with a slight increase in BM0 (3.4%). Total replacement of fish meal had the lowest ash content with BM0 recording almost twice that of BM100. However, BM0 recorded the lowest crude fibre content (9.3%) with BM50 and BM100 recording almost the same figure.

Amino Acid Composition

Blood meal had the highest amount of isoleucine (118.07 mg/g protein) although phenylalanine combined with tyrosine had 137.01 mg/g protein (Table 4). Alanine was the lowest (13.97) followed by arginine and leucine. The chemical score was highest for phenylalanine and tyrosine (147.32%), methionine (71.91%) and lysine (64.06%).

Methionine, leucine, isoleucine, valine and proline contents of BM100 were lowest and highest in BM0. Arginine was high in BM100 with BM50 and BM0 being almost the same. BM50 recorded the highest content of tyrosine and lysine compared to the other diets (Table 5).

Data on chemical scores and EAAI (Table 6) shows that isoleucine had the highest percentage chemical score in the three supplements, i.e. 178.20, 142.31 and 136.81 for BM0, BM50 and BM100, respectively. However, the chemical score percentage for methionine was lowest in BM100 with BM0 recording the highest score. The chemical score for lysine decreased with the level of substitution of FM with BM (85.41, 68 and 64.83). BM0 had the highest chemical scores for methionine, leucine, isoleucine and valine while BM100 recorded the lowest chemicals scores for methionine, valine, isoleucine, leucine and phenylalanine+ tyrosine. The EAAI was highest in BM0 (0.94) followed by BM50 (0.88) and BM100 (0.77)

Table 3. Proximate composition of fish supplements containing blood meal as a replacement for fish meal.

	BM0†	BM50†	BM100†
Dry matter	90.59^{c}±0.10	91.15^{b}±0.13	91.37^{ab}±0.08
Crude protein	32.08^{c}±0.15	33.88^{a}±0.39	33.69^{ab}±0.03
Ether extracts	3.41^{a}±0.35	3.00^{a}±0.28	3.00^{a}±0.40
Ash	9.45^{a}±0.10	7.13^{b}±0.07	5.1^{c}±0.58
Crude fibre	9.3^{c}±0.23	11.37^{a}±0.20	11.28^{ab}±0.36
Nitrogen free extracts	36.36^{b}±0.25	35.78^{b}±0.49	38.3^{a}±0.66

Values are expressed as mean ± SE.
^{a,b,c}Values in the same row having different superscript letters are significantly different (P <0.05).
†BM0, BM50 and BM100 represent replacement of fish meal at 0, 50 and 100%, respectively.

Table 4. Amino acid composition (mg/g protein) and chemical score of blood meal.

Amino acid	Composition	Chemical score
Methionine	40.99	71.91
Lysine	44.84	64.06
Leucine	30.03	34.92
Isoleucine	118.07	218.65
Valine	61.43	93.08
Phenylalanine+tyrosine	137.01	147.32
Alanine†	13.97	
Arginine	23.45	
Proline†	39.01	

†Non-essential amino acids.

Discussion

Proximate Composition

The proximate composition of the test ingredients, i.e. fish meal, cotton seed cake, wheat bran and blood meal used in this study was within the range of values reported by other authors (Drew *et al.*, 2007; Um-E-Kalsoom *et al.*, 2009; Al Mahmud *et al.*, 2012). The crude protein (11%) was markedly low in soybean meal. Lovell (1988) noted that the nutrient composition of feedstuffs depends on the origin, state and processing methods used. However, according to the National Research Council (1993) solvent extraction of the oil results in soybean meal (SBM) containing 44% crude protein if the soybean hulls are included or 48% crude protein without the hulls. The low CP content was due to adulteration of the soybean meal by marketing agents using cheap low quality ingredients like sawdust. This was reflected in the high CF content of SBM (9.75%) which was more than double that recorded by Agbo (2008) and Noreen and Salim (2008), i.e. 3.82% and 1.09%, respectively. It is important to note that although the crude protein level of soybean was below the expected level, this had the same effect across the three diets formulated because inclusion levels of soybean meal were the same at 10%. This reveals that farmers can purchase adulterated ingredients leading to the formulation of substandard feeds which in turn is reflected by the poor performance of the animal.

Cottonseed cake recorded a higher crude fibre of all the ingredients (Table 2) which is considered a limiting factor in its use as feed. Nagalakshmi *et al.* (2007) observed that a

Table 5. Amino acid composition (mg/g protein) of fish supplements containing blood meal as a replacement for fish meal.

Amino acid	BM0†	BM50†	BM100†
Methionine	38.31^{a}±0.08	35.11^{a}±2.39	30.09^{a}±3.00
Lysine	45.38^{a}±14.35	59.79^{a}±3.01	47.60^{a}±9.50
Leucine	75.62^{a}±1.40	61.43^{b}±2.24	47.18^{c}±1.35
Isoleucine	96.23^{a}±0.20	76.85^{a}±10.43	73.88^{a}±11.07
Alanine	33.54^{a}±4.82	30.89^{a}±7.44	46.62^{a}±8.99
Valine	62.06^{a}±2.54	50.44^{b}±0.52	46.34^{c}±0.78b
Arginine	20.87^{a}±0.85	20.03^{a}±0.88	24.05^{a}±1.58
Proline	57.09^{a}±2.24	47.15^{b}±0.51	45.19^{bc}±2.12
Phenylalanine	71.36^{a}±7.42	70.48^{a}±1.00	74.04^{a}±3.30
Tyrosine	27.31^{a}±3.73	34.09^{a}±1.76	27.93^{a}±1.69

Values are expressed as mean ± SE.
[a,b,c]Values in the same row having different superscript letters are significantly different (*P* <0.05).
†BM0, BM50 and BM100 represent replacement of fish meal at 0, 50 and 100%, respectively.

Table 6. Chemical scores (%) and essential amino acid index (EAAI) of fish supplements containing blood meal as a replacement for fish meal.

	BM0†	BM50†	BM100†
Amino acid	Chemical score		
Methionine	67.21^{a}±0.14	61.59^{a}±4.19	52.79^{a}±4.64
Lysine	64.83^{a}±20.50	85.41^{a}±4.30	68.01^{a}±13.57
Leucine	87.93^{a}±1.60	71.43^{b}±2.62	54.86^{c}±1.56
Isoleucine	178.20^{a}±0.37	142.31^{a}±19.31	136.81^{a}±20.49
Valine	94.03^{a}±3.84	76.42^{b}±0.79	70.21^{bc}±1.17
Phenylalanine + tyrosine	106.08^{a}±11.99	112.44^{a}±2.97	109.61^{a}±1.73
Essential amino acid index	0.94^{a}±0.60	0.88^{a}±0.35	0.77^{a}±0.20

Values are expressed as mean ± SE.
[a,b,c]Values in the same row having different superscript letters are significantly different (P <0.05).
†BM0, BM50 and BM100 represent replacement of fish meal at 0, 50 and 100%, respectively.

high level of crude fibre in cotton seed cake is inversely proportional to the concentration of protein and further revealed that the crude protein of undecorticated cotton seed meal ranged from 22.2 to 30.31%.

The crude protein content of fish meal (64.20%) was below that obtained by Otubusin (2009) who recorded 70% CP. This figure together with the ash content (16.3%) were within the normal range which according to Drew *et al.* (2007), may vary from 50 to 70% and 10 to 21%, respectively, depending on fish species, the source and processing method. The crude protein of wheat bran was the same as that obtained by Um-E-Kalsoom (2009) and Al Mahmud *et al.* (2012) but ash and ether extracts were relatively low. The crude protein of blood meal (80%) was close to the results by Drew *et al.* (2007) while the ash content was double (4.5%). However, Otubusin *et al.* (2009) recorded similar results for crude protein but ash content was zero.

The proximate analysis of the three supplements (Table 3) shows that the crude protein had slight variation from the formulated diet on as fed bases (Table 1). This was due to fluctuation in the crude protein content of the ingredients (Table 2), in particular soybean meal and cottonseed cake. Substitution of FM with BM gave a higher crude fibre content of 11.37% for diet BM50 and 11.28% for BM100. This was due to increased amounts of wheat bran incorporated in the diet to adjust for the CP content in the formulation of the diets (Table 1). A high fibre content reduces the total dry matter and nutrient digestibility of the diet, resulting in poor performance (De Silva & Anderson, 1995). In addition it adds to the faecal waste which affects the water quality and hence fish performance (Lovell, 1998). According to De Silva and Anderson (1995), crude fibre was within the normal range of 8–12% for diets of fish.

A high ash content of BM0 (9.45%), which was almost double when 100% of FM was substituted with BM, was due to the high ash content in fish meal (Table 2). The FM is usually dried on sandy ground along the lake, which contributes to the high ash content. There was not much variation in the lipid content (3–3.4%) with the substitution of FM. However, this was below the recommended levels of 5–12% for tilapia (Suresh, 2003).

Amino Acid Composition

Low methionine content on substitution of FM was due to low methionine in blood meal compared to fish meal. Similarly, substituting fish meal wholly with blood meal, as in the case of BM100, reduced the level of some amino acids in the supplement, i.e. methionine, lysine, isoleucine, leucine, proline, valine and increased arginine, phenylanine and alanine in the supplements. Thus, BM0 displayed a better amino acid profile with higher levels of methionine, isoleucine, leucine, lysine, valine and proline.

It is worth noting that cystine was not detected in the supplements (Table 5) and according to the National Research Council (1993) there exists a relationship among amino acids such that cystine can be formed metabolically from dietary methionine at a rate sufficient to meet the requirements of fish but the reverse sequence of reactions does not occur. Methionine can thus meet the total sulphur amino acid requirement of fish, although some of this requirement may be met by cystine (National Research Council, 1993). Based on this, the cystine requirement for fish can be met by the methionine content in the diet. In addition, phenylalanine and tyrosine were present in the three supplements but a similar relationship exists between aromatic amino acids (phenylalanine and tyrosine). Fish readily convert phenylalanine to tyrosine so that phenylalanine alone can meet the requirements for aromatic amino acids but the presence of tyrosine in the diet reduces some of the requirement for phenylalanine (National Research Council, 1993).

Considering the amino acid profile in the supplements against the recommended levels for Nile tilapia (National Research Council, 1993), the amino acid profiles were low which can lead to poor utilization of the dietary protein and consequently reduce growth and decrease feed efficiency (Halver & Hardy, 2002). Dietary amino acid utilization requires that all amino acids are simultaneously present in adequate concentrations at sites of protein synthesis. Hence, deficiency of an essential amino acid limits protein synthesis to the level of that particular essential amino acid, the remainder being catabolized (Sveier *et al.*, 2001). For most essential amino acids, deficiency translates to a reduction in weight gain. In some species of fish, a deficiency of methionine or tryptophan leads to pathologies, because these amino acids are not only incorporated into proteins but also used for the synthesis of other compounds (Lovell, 1998). For example, cataracts occur in salmonids and rainbow trout as a consequence of methionine (sulphur amino acids) and tryptophan deficiency, respectively, in their diets (Lovell, 1998). The amino acids balance in the supplements for the present study were not met because they were not formulated based on an ideal protein concept and according to Yamamoto *et al.* (2004), the diets could depress feed intake and growth of fish.

Chemical Score and Essential Amino Acid Index

In the present study (Table 4), BM as an ingredient displayed a high chemical score for phenylalanine + tyrosine and isoleucine. The most limiting amino acid in BM100 and BM50 was methionine. This could be attributed to the use of blood meal. In the present study, high chemical scores were associated with low substitution of fish meal, which is better balanced in amino acids than blood meal.

Although the chemical score is important in determining the relative value of dietary protein, other essential amino acids could also have an effect on the nutritive value of the dietary protein, as reflected in the EAAI (Table 6). The chemical score is based on the assumption that whole egg protein is of the highest biological value and thus the most suitable for growth, which could be limited by the EAA in the diet whose ratio to its content in the whole egg protein is the lowest (Hepher, 1988). The EAAI of the three supplements, BM0, BM50 and BM100 was estimated to be 0.94, 0.88 and 0.77, respectively. Good quality protein sources have an EAAI greater than or equal to 0.90, useful protein sources have a value of 0.80 whereas sources with values below 0.70 are considered to be inadequate (Oser, 1959; Penaflorida, 1989). Thus, BM0 in the present study could be considered to be a good quality protein supplement, BM50 a useful protein supplement and BM100 was closer to a poor protein source.

Conclusion

The proximate contents of the three supplements were within the required level for growing fish. However, considering the amino acid composition, substitution of up to 50% FM with BM gave a useful protein diet and 100% gave almost a poor protein diet. Based on this, it can be concluded that blood meal can partially replace 50% fish meal in the diets of *Oreochromis niloticus*. Further studies on the use of blood meal as a replacement for fish meal on the nutritive value of diets for *Oreochromis niloticus* is recommended.

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