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RESEARCH ARTICLE

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Suitability of Soybean-Blood Meal Mixture as Replacement of Fish Meal in Nile Tilapia (*Oreochromis niloticus*) Rations

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Abstract

Fish require high quality protein in their rations which is sourced from fish meal. However, its scarcity and competition for use in livestock feeds has caused escalation of prices thus limiting its use. A study was carried out to evaluate protein quality of soybean-blood meal mixture as fish meal substitute in diets for Nile tilapia. Control diet, Fish meal based diet (R1) with 30% crude protein was formulated using fish meal, maize flour, wheat bran, sunflower oil and mineral-vitamin premix. Test diets (R2, R3, R4 and R5) were formulated replacing 25%, 50%, 75% and 100% of fish meal protein with soybean-blood meal mixture. Amino acids were determined using HPLC (AOAC, 2005) procedure. Fish meal had higher ($p<0.05$) levels of lysine (78.1 mg/g), arginine (58.7 mg/g) threonine (42.8 mg/g), Valine (54 mg/g) isoluecine (45.5 mg/g) phenylalanine and tyrosine (51.6 mg/g), methionine and cysteine (38.4 mg/g) and tryptophan (11.5 mg/g) than soybean-blood mixture. Soybean-blood meal was superior ($p<0.05$) to fish meal in amino acid histidine (40.5 mg/g) and leucine (79.4 mg/g). Replacement demonstrated reduction ($p<0.05$) in all Essential amino acids, except Histidine and Leucine which increased ($p<0.05$) in amounts with fish meal replacement. Replacement of fish meal with soybean blood meal mixture indicated reduction ($p<0.05$) in chemical scores except Histidine and Leucine which increased. Tryptophan was the limiting amino acid in all replacement diets. Essential amino acid indices (EAAI) showed slight reduction ($p<0.05$) with replacement R1 (117%), R2 (113%), R3 (109%), R4 (104%) and R5 (100%). Though fish meal-based diet (R1) had highest EAAI, all replacement diets had EAAI above 90% hence Soybean blood meal mixture was identified to be of high nutritive value. The study revealed that fish meal substitution with soybean-blood meal mixture gave good quality feed for Nile tilapia.

Keywords: Soybean-blood meal, Essential Amino Acids

INTRODUCTION

Fish meal is reported to be desirable protein ingredient in fish feed rations because of its high palatability, high protein and amino acids (Hardy & Tacon, 2002). The prices of fish meal have doubled in recent years occasioned by its scarcity coupled with high competition for fish as human food and for use in diets for dairy, poultry and pigs hence limiting its use as a major protein ingredient in fish feeds (Hixson, 2014; Kirimi *et al*., 2016). Therefore, search for cost effective alternative sources of protein to fish meal have intensified to sustain rapid growth in aquaculture (Abou *et al.*, 2013, Ogello *et al*., 2014). Fish require essential amino acids for growth and development thus supplying these nutrients is important (NRC, 2011). Sadiku & Jauncey (1995) recommended mixing of plant protein and animal origin protein feed ingredients to enhance quality of compounded fish feeds. In Kenya, soybean meal which is a by-product from oil seed extraction at various factories is available in considerable quantities at lower prices compared to fish meal. Blood which is regarded as a waste in slaughter houses can be processed locally into blood meal and used in fish feed formulation as animal protein ingredient (Magondu *et al*., 2016). Beside availability of feed ingredients, their nutrient composition is the most critical part for formulation of cost-effective quality feeds that meet nutritional requirement for fish (Kaushik & Seiliez, 2010).

Bunda *et al.* (2015) reported proximate nutrient analysis as the first method in determining the main nutrients (crude protein, crude fiber, Ether extracts and nitrogen free extracts) and gross energy levels in feed. Since protein is the most critical nutrient for fish growth, its quality in feed ingredients and formulated diets must be ensured. Protein quality is evaluated by determining the number and amounts of at least 10 essential amino acids available in the feeds (Malomo & Alamu, 2015). Chemical score method is used to determine the lowest ratio of Essential Amino Acid (EAA) level in test feed relative to the same essential amino acid requirement (Bunda *et al*., 2015). Essential amino acid index (EAAI) method was developed after it was established that other EAA also affect protein nutritive value in feeds (Oser, 1959; Hepher, 1988). The study focused on proximate nutrient and amino acid composition, chemical scores and Essential Amino Acid Indices (EAAI) of feed ingredients and in diets formulated where fish meal was substituted with soybean-blood meal mixture.

MATERIALS AND METHODS

Feed Sourcing, Nutrient Determination and Processing

Feed ingredients and feed processing was carried out at Chwele fish farm located in Bungoma County-Kenya. Fresh blood from slaughtered cattle at the Chwele slaughter house was collected into clean stainless steel containers. The blood was transported to the farm where it was boiled at 100^0C, while being stirred for 15 minutes as described by MSOP (2015) to get rid of pathogens. The liquid part was drained and blood clots crushed manually by hand into small pieces. The blood pieces were dried in the sun on a polythene liner for two days and milled into powder called blood meal using a hammer mill machine. The other feed ingredients (soybean meal, maize flour, wheat bran, sunflower oil and vitamin-mineral premix) were purchased from animal feed shops in Bungoma town and transported to the farm. Sun dried *Rastrineobola argentae* locally known as "Omena" in Kenya was purchased from fish traders at Chwele Market Centre. All the feed ingredients were ground separately using a hammer mill. Samples of each ingredient were taken and sent to feed analysis laboratories at Kenya Industrial and Research Development Institute (KIRDI). Proximate determination of Crude Protein (CP), Ether Extracts (EE), Crude Fiber (CF), Nitrogen Free Extracts (NFE) and Ash was carried out in triplicates following the procedure of AOAC (1995).

Diet Formulation and Processing

Information from the laboratory nutrient determination was used in formulating rations for Nile tilapia. Ground soybean meal was first mixed with blood meal at a ratio of 1:2 in a motorized mixer to produce soybean-blood meal blend. Protein content of the mixture was calculated before it was used to substitute fish meal protein at four levels. Five diets were compounded containing 30% crude protein with control diet (R1) made up of fish meal, maize flour, wheat bran, sunflower oil and mineral-vitamin premix. Test diets R2, R3, R4 and R5 were formulated substituting fish meal protein with soybean-blood meal mixture at four levels (25, 50, 75 and 100%) as shown in Table 1.

Table 1: Treatment diets and ingredients used in formulation of five levels of fish meal substitution using soybean-blood meal mixture

Ingredients (Kg)	Dietary treatments (% fish meal substitution)				
	R1(0)	R2 (25)	R3 (50)	R4 (75)	R5 (100)
Fish meal	38.27	28.7	19.14	9.57	0
Soybean-blood meal	0	8.26	16.52	24.77	33.03
Maize flour	20.73	21.84	22.94	23.96	24.97
Wheat bran	39	39	39	39	39
Sunflower oil	1	1.2	1.4	1.7	2
Mineral-vit premix	1	1	1	1	1
Total	100	100	100	100	100
C.P	29.9	29.9	30	30	30

The amount of feed ingredients indicated in the formula were weighed and poured into a motorized mixer. They were mixed for three minutes, then 25% water mixed with vitamin - mineral premix and sunflower oil was added to moisten the feed and re-mixed for three minutes to get dough. The dough was transferred into the pellet making machine to produce floating pellets of 3.5 mm size. The pellets were dried in the sun for one day and packaged in airtight bags. Nutrient and amino acid composition were determined in triplicates using AOAC (1995) and High Performance Liquid Chromatography AOAC (2005) procedures respectively at the KIRDI Laboratory. Chemical scores and Essential Amino Acid Indices (EAAI) were calculated using the formulae bellow;

Chemical score (CS) = is the ratio of essential amino acids in the test diets relative to the same essential amino acid requirements (Bunda *et al*., 2015).

$$CS = \frac{\text{EAA in formulated diet}}{\text{Corresponding EAA required level in Nile tilapia}} x100$$

Where EAA is Essential Amino Acids

Essential amino acid index (EAAI) is defined as mean geometrical ratio of all essential amino acids in test diets to their required levels for Nile tilapia (Bunda *et al.*, 2015).

$$EAAI = \sqrt[n]{\frac{100a \text{ x } 100b \text{ x} ..100j}{a1 \text{ x } a2 \text{ x} \ldots .a10}}$$

Where a, b..j were amounts of essential amino acids in the formulated diets, while a1, a2…a10 were the corresponding essential amino acid requirement levels for Nile tilapia fish and n was amino acid number used in calculation, counting combined amino acids as one.

Data Analysis

Data were subjected to Analysis of Variance at $p \leq 0.05$. Where differences occurred, mean separation was carried out using LSD (Least Significant Difference) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Proximate Nutrient Composition

The amount of crude protein slightly increased ($p<0.05$) with fish meal substitution levels due to high protein level in soybean meal and blood meal. Formulated diets had all their nutrient values within the range recommended for *Oreochromis niloticus* fish (Table 2). Jauncey (1998) recommended crude protein range from 30% to 35% for fingerlings and 25% to 30% for growers and adult Nile tilapia respectively. Soybean meal has been used in formulating fish feeds because of its constant availability at lower cost than fish meal despite being less palatable, low protein content and deficient in some essential amino acids (Hardy, 2010; Caruso, 2015). Combining soybean meal with blood meal in this study improved amount of protein in the feed as blood meal is reported to have protein content of above 80% which is higher than fish meal.

Table 2: Proximate nutrient composition (%) of the treatment diets

Nutrient	Treatment diets (% fish meal substitution)						
	R1(0)	R2(25)	R3(50)	R4(75)	R5(100)	SEM	p-value
DM	89.85^{e}	90.1^{d}	90.91^{a}	90.63^{b}	90.35^{c}	0.01	<0.001
CP	30.23^{e}	30.3^{d}	30.38^{c}	30.43^{b}	30.5^{a}	0.03	<0.001
CF	3.95^{e}	4.13^{d}	4.32^{c}	4.51^{b}	4.7^{a}	0.07	<0.001
EE	6.89^{a}	6.39^{b}	5.9^{c}	5.49^{d}	5.1^{e}	0.17	<0.001
Ash	9.52^{a}	8.12^{b}	6.78^{c}	5.32^{d}	3.92^{e}	0.58	<0.001
NFE	39.25^{e}	41.16^{d}	43.54^{c}	44.87^{b}	46.14^{a}	0.67	<0.001

Values presented as means with different superscripts in the same row are statistically ($p<0.05$) different. Note: DM= Dry Matter, CP= Crude Protein, CF =Crude Fiber, EE =Ether Extracts and NFE= Nitrogen Free Extracts

Increase in crude fiber amounts with increase in fish meal substitution levels was because of increase in amounts of soybean meal which had high levels of crude fiber since wheat bran with highest crude fiber content was constant in all diets. Ether extracts and ash contents reduced with fish meal replacement due to increase in amount of soybean-blood meal mixture which was low in the same nutrients. Nitrogen free extracts (NFE) increased with fish meal replacement as a result of increase in amount of soybean meal which has high NFE values.

Essential Amino Acids in Feed Ingredients

Fish meal recorded higher levels of Essential Amino Acid (EAA) profile than soybean-blood meal mixture (Table 3). Amino acid lysine had highest concentration of 78.1 mg/g followed closely by leucine 75.5 mg/g despite soybean and blood meal having higher amount of lysine than fish meal as reported by NRC (1993), heat processing may have denatured amino acid reducing their concentration. The results were in agreement with those recorded by Maina *et al.* (2007) and Kirimi *et al.* (2016). Kirimi *et al.* (2020) reported higher value of arginine (75.5 mg/g) in fish meal contrary to this study which recorded 58.7 mg/g. Soybean-blood meal mixture had higher values of histidine (40.5 mg/g) and leucine (79.4 mg/g) compared to fish meal, this is because blood meal is reported by NRC (1993) to have high concentration of amino acids histidine and leucine and played part in increasing their concentration in soybean-blood meal mixture as it had highest ratio of two parts to one (2:1). Concentration of amino acid methionine and cystein combined in soybean-blood meal mixture was 17.9 mg/g which was higher than values of 7.66 mg/g reported by NRC (2012) in soybean alone. Increase in amount of methionine and cystein amino acid in soybean-blood meal mixture qualifies the statement by Sadiku & Jauncey (1995) that combining plant and animal protein feed ingredients improves the quality of feeds which translates to efficient utilization of dietary amino acid resulting to increased growth of fish.

Table 3: Amino acid composition (mg/g Protein) of the feed ingredients used to formulate diets

Amino acid	Maize meal	Wheat bran	Soybean-Blood meal	Fish meal	SEM	p-value
Lysine	14.23^{d}	17.53^{c}	64.19^{b}	78.06^{a}	0.79	<0.001
Arginine	24.17^{d}	28.13^{c}	31.43^{b}	58.7^{a}	0.57	<0.001
Histidine	21.2 ^{c}	18.13^{d}	40.54^{a}	24.33^{b}	0.39	<0.001
Threonine	25.97^{d}	31.57^{c}	32.33^{b}	42.83^{a}	0.22	<0.001
Valine	40.87^{d}	49.3^{c}	49.82^{b}	54.03^{a}	0.37	<0.001
Leucine	71.53^{c}	68.53^{d}	78.98^{a}	75.53^{b}	0.54	<0.001
Isoleucine	32.6^{c}	38.3^{b}	16.57^{d}	45.5^{a}	0.33	<0.001
Ph + Tyr	74.97^{a}	61.07^{b}	47.86^{d}	51.56^{c}	0.42	<0.001
Met + Cyst	32.03^{b}	32.53^{b}	17.95^{c}	38.4^{a}	0.25	<0.001
Tryptophan	2.37^{d}	2.53^{d}	8.68^{b}	11.53^{a}	0.10	<0.001

Values presented as mean with different superscripts in the same row are statistically (p<0.05) different

Essential Amino Acids in Formulated Feeds

Essential amino acids, lysine, arginine, threonine, valine, isoleucine, phenylalanine+tyrosine, methionine+cystein and tryptophan significantly decreased (p<0.05) except histidine and leucine which increased with increase in fish meal substitution level (Table 4). This indicated that soybean-blood meal mixture had low levels of all other amino acids which reduced with replacement compared to fish meal. The mixture was high in amino acids histidine and leucine than fish meal hence their increase with replacement levels.

Considering essential amino acids in the feed ration in this study against recommended requirement for *Oreochromis niloticus* fish (NRC, 1993), all essential amino acid were high except Tryptophan which can limit dietary protein utilization resulting to reduced fish growth. According Sveier *et al.* (2001), utilization of essential amino acids requires presentation of all essential amino acids in adequate amounts at protein synthesis site and a deficiency of any single essential amino acid reduces protein synthesis to that level of the essential amino acid. Tryptophan deficiency in fish diets leads to pathological developments such as cataracts in rainbow trout as reported by Lovel (1998).

Table 4: Amino acid composition (mg/g Protein) of formulated treatment diets

Amino acid	Amino acid content in dietary treatments (fish meal substitution, %)						
	R1(0)	R2(25)	R3(50)	R4(75)	R5(100)	SEM	p-value
Lysine	39.67^{a}	37.66^{b}	35.67^{c}	33.65^{d}	31.64^{e}	0.76	<0.001
Arginine	38.45 ^{a}	35.66^{b}	32.87^{c}	30.06^{d}	27.24^{e}	1.06	<0.001
Histidine	20.78 ^{e}	22.03^{d}	23.28^{c}	24.51^{b}	25.74^{a}	0.47	<0.001
Threonine	34.07^{a}	32.94^{b}	31.80^{c}	30.63^{d}	29.47^{e}	0.44	<0.001
Valine	48.38 ^{a}	47.73^{b}	47.08^{c}	46.39^{d}	45.71^{e}	0.25	<0.001
Leucine	70.46 ^{d}	70.89^{cd}	71.29^{bc}	71.29^{ab}	71.99^{a}	0.16	<0.001
Isoleucine	39.11^{a}	36.30^{b}	33.49^{c}	30.35^{d}	27.81^{d}	1.07	<0.001
Ph + Tyr	68.13 ^{a}	65.73^{b}	63.32^{c}	60.84^{d}	58.35^{e}	0.93	<0.001
Met + Cyst	34.02^{a}	32.19^{b}	30.35^{c}	28.48^{d}	26.57^{e}	0.71	<0.001
Tryptophan	5.89 ^{a}	5.52^{b}	5.15^{c}	4.78^{d}	4.41^{e}	5.33	<0.001

Values presented as means with different superscripts in the same row are statistically (p<0.05) different

Chemical Scores and Essential Amino Acid Indices of Diets

Chemical scores for all amino acids reduced apart from histidine and leucine which increased with fish meal replacement indicating their concentration in soybean-blood meal mixture. Bunda *et al*. (2015) noted that essential amino acid requirement provides a good standard protein in calculation of chemical scores and EAAI than whole egg protein as this provides a good measure of protein quality in relation to fish nutrition. In this study, amino acids requirement from National Research Council fish requirement table NRC (1993) was used in chemical score and Essential Amino Acid Index (EAAI) calculation. Rao *et al.* (1964) derived another protein evaluation method from EAAI mathematical model called Protein Requirement Index which they defined as the geometrical mean ratio of essential amino acid in test feed to their requirement levels but values which exceeded 100% were reduced to 100%. Kapour & Heiner (1982) used the model and found a high correlation (r = 0.88) with biological value in wheat. Formulated diets had high EAAI exceeding 100% except 100% fish meal substitution which had a mean of 99.91% (Table 5). Essential Amino Acid Indices (EAAI) indicated that all feed rations were of good quality for Nile tilapia feeding. Bunda *et al*. (2015) stated that a protein feed is considered to be of good nutritive value when EAAI is above or equal to 90%, useful nutritive value when is about 80% and incomplete or poor protein quality when is less than 70%. All formulated diets had EAAI above 90% and therefore were of good nutritive value; therefore soybean-blood meal mixture can totally replace fish meal to produce good quality feeds for Nile tilapia.

Table 5: Chemical scores (%) and essential amino acid indices (EAAI) in (%) of formulated diets

Amino acid	Dietary treatments (fish meal substitution, %)						
	R1(0)	R2(25)	R3(50)	R4(75)	R5(100)	SEM	p-value
Lysine	77.47^{a}	73.56^{b}	69.66^{c}	64.72^{d}	61.79^{e}	1.54	<0.001
Arginine	91.54^{a}	84.90^{b}	78.26^{c}	71.57^{d}	64.87^{e}	2.52	<0.001
Histidine	120.8^{e}	128.1^{d}	135.3^{c}	142.5^{b}	149.6^{a}	2.73	<0.001
Threonine	90.86^{a}	87.85^{b}	84.80^{c}	81.69^{d}	78.58^{e}	1.16	<0.001
Valine	172.8^{a}	170.5^{b}	168.1^{c}	165.7^{d}	163.2 e	0.90	<0.001
Leucine	207.9^{d}	209.1^{cd}	210.3^{bc}	211.3^{ab}	212.4^{a}	0.46	<0.001
Isoleucine	125.7^{a}	116.7^{b}	107.7^{c}	98.6^{d}	89.4^{d}	3.44	<0.001
Ph + Tyr	181.7^{a}	175.3^{b}	168.8^{c}	162.2^{d}	155.6^{e}	2.47	<0.001
Met + Cyst	127^{a}	120.1^{b}	113.2^{c}	106.3^{d}	99.2^{e}	2.63	<0.001
Tryptophan	58.9^{a}	55.20^{b}	51.50^{c}	47.77^{d}	44.1^{e}	1.38	<0.001
EAAI	116.9^{a}	113.01^{b}	108.90^{c}	104.35^{d}	99.91^{e}	1.61	<0.001

Values presented as means with different superscripts in the same row are statistically (p<0.05) different

CONCLUSION

Based on proximate nutrient and amino acids analysis and calculated Essential Amino Acid indices, soybean-blood meal mixture can be used as alternative protein source to fish meal to produce good quality feeds for Nile tilapia fish. Chemical scores indicated that tryptophan was limiting amino acid which can limit protein utilization by the fish.

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