PELAGIC FISH *Rastrineobola argentea* AS A PROTEIN SOURCE FOR BROILER CHICKEN

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Abstract

In recent years the small pelagic fish Rastriobola argentea found in Lake Victoria has become of interest as a protein source for both humans, non-ruminant animals and poultry and pigs in Kenya, Uganda and Tanzania. A study was conducted to evaluate the nutritional value and the potential of R. argentea fishmeal (RFM) as a protein and a lysine source for broiler chicks. When samples from different landing beaches were subjected to proximate analysis the mean composition was: dry matter 90.06%; crude protein 57.10%, ash 10.56 %; ether extract 12.39 % and crude fibre 1.11 %. The amino acid composition showed no differences except in proline and leucine. The lysine content was 6.08 %. A feeding trial was conducted where RFM replaced soybean at 0 %, 4 %, 8 % and 12 % in broiler diets. The diets with RFM elicited significantly better weight gains, feed intake and feed conversion, which increased with increases in the RFM levels. Broiler chicks fed on a diet of 89.3 % RFM grew normally after taking 3 days to adapt to the diet and had normal intestinal organs of the digestive system when they were killed and their organs compared to those of other chicks fed on diets with low levels of, or without RFM. This indicated that RFM had no gizzerozines that produce adverse effects in the digestive organs of broiler chickens. The bioavailability of lysine from RFM was estimated using a chick bioassay and found to be 97.3 %, an indication of little or no damage to lysine during processing. The high protein content (58.50 %), the essential amino acid profile, the performance of broiler chicks on RFM and the high bioavailability of lysine in RFM, all indicate the high potential this fishmeal has as a source of protein and essential amino acids in poultry diets.

Key words: *R. argentea* fish, protein, chicken.

INTRODUCTION

Rastrineobola argentea which are native zooplanktivores of lakes in East Africa (Manyala et. al., 1992) have been incorporated in poultry and pig diets (Tuitoek, 1992, Okot 1995) and in human diets (Steiner-Asiendu et al., 1993) but with little knowledge of their nutritive value, possible adverse affects or bioavailable amino acid content. There is a high production potential of this fish in L. Victoria (Othina and Odera, 1995). Fishmeals vary in their nutrient composition depending on their source, species, method of processing and storage (Wessels and Moodie, 1975;). This presents problems to feed manufacturers and users. Such diets may have low protein and/or imbalanced amino acids, or have excess protein and amino acids leading to wastage of nitrogen through inefficient utilization. Very little research has been done to evaluate *R. argentea* as a source of protein and essential amino acids for chickens although it is already being used in human, poultry and pig diets. The processing of *R. argentea* by sun drying is simple and inexpensive. The objectives of this study were therefore to:- 1) determine the nutrient composition of *R.. argentea* whole fish, 2) establish whether there are differences in the nutrient composition of *R. argentea* from different localities (landing beaches) along the Kenyan shore of Lake Victoria. 3) determine any adverse effects, of *R. argentea* diet in broiler chicken. 4) estimate the bioavailable lysine in *R. argentea* using broiler chicken, 5) determine the performance of broiler chicken fed on diets containing different levels of *R. argentea*.

MATERIALS AND METHODS

Proximate and amino acid analysis

Three *R. argentea* fish samples were collected as sun-dried whole fish from Sori, Sirongo, Port Victoria, Ragengni and Muhuru bay. In Muhuru bay two samples were taken; these were made up of small fish (2 to 4cm) long and large fish (5-7cm) long. The samples were ground into fishmeal using a hammer mill and a 3.5mm sieve for proximate analysis. The samples were analyzed for dry matter, crude protein, ether extract, ash and crude fibre according to the procedures of the Association of Official Analytical Chemists (AOAC, 1995). The amino acid composition was determined using High Pressure Liquid chromatography (HPLC). Samples were hydrolyzed in excess 6N hydrochloric acid for 12 hours at 110 º Celsius. The resultant hydrolysates were diluted using excess boric acid/sodium bicarbonate buffer of PH 8.4 and filtered. One µl of this was put in a vial and further diluted with one ml of buffer. To the vial was added one ml derivatiser (9-flourenylmethyl chloroformate). After 10 minutes the amino acids were extracted using excess pentane and put into the auto sampler. These were pumped through the Column (Micro Pak Amino Tag ™) and amino acids detected by fluorescent detector

(Varian Model 9070). The auto sampler, pump, column and detector were controlled by a computer using the Star Chromatography Software (version 4.0). Novaline, a derivative of valine was used as the internal standard. The resultant chromatograph was used to calculate the amino acids levels in the sample. The data generated from the proximate and amino acid analysis were subjected to analysis of variance and several statistical tests.

Animals

The broiler chicks were kept in a brooder house heated and maintained at a temperature range of 25-30 degrees Celsius. The house had 24 hours of lighting. Commercial broiler chicks (Abor Acres) from Kenchick (K) Limited were used in experiments 1, 2 and 3. The chicks were purchased at one-day old and kept under brooding for 8 days, during which they were fed on a commercial broiler starter diet. Chicks were vaccinated against Marek's disease at day old and against New Castle Disease on day 4. On day 9, following an overnight period of feed withdrawal, the chicks were weighed, wing banded and randomly allocated to the treatment groups. Water and feed were given ad libitum. Experiments were terminated on day 23 by weighing the chicks and the remaining feed. The weight gain, feed intake and feed conversion efficiency (gain/feed) were then calculated.

Experiment 1: To test the response of broiler chicks to a maize-soybean diet supplemented with the *R. argentea* fishmeal (RFM). Three dietary (A,B,C) treatments (table 3) by four replicates (cages) with five chicks per replicate were used. A purely RFM diet (C) with maize bran added as source of fibre was included that had no mineral or vitamin supplementation. This diet C was to test for any adverse affects of feeding the RFM at the highest level.

Experiment 2: To assess the performance of broiler chicks fed on a maize soybean diet supplemented with 0, 4, 8 and 12 %. RFM. (Table 3 diets 1, 2, 3, 4, and 5). A Fishmeal diet (89.3% RFM diet 5) fortified with minerals and vitamins was included. This was to test for the efficiency of the utilization of the fishmeal when vitamins and minerals were adequate. The experiment was five treatment diets by four replicates and eight chicks per replicate giving thirty-two chicks per treatment diet.

Experiment 3 : To estimate the amount of bioavailable lysine in the RFM to broiler chicken**.** A basal diet (table 3) was formulated based on maize, maize gluten meal and crystalline amino acids. This basal diet was deficient in lysine, supplying a maximum of 30 % of the lysine requirements for broilers. Two other diets were formulated by the addition of lysine at 0.25 % and 0.50 % to establish a standard curve for lysine (Smith and Scott 1965a, Smith 1968). The chick assay consisted of four treatment diets by four replicates totaling thirty-two chicks per treatment diet. In treatments 1, 2 and 3, glutamic acid was used to equalize for nitrogen. The 5% RFM was added at the expense of maize gluten meal and was calculated to supply lysine in the range of 0.25 to 0.40 %. The data were subjected to analysis of variance and regression analysis. The parameters used to estimate the amount of bioavailable lysine were weight gains and lysine intake. Then weight gains and supplemental lysine intake were taken to be the major estimators of the bioavailable lysine by the linear regression equation Y = a x bx.

Statistical Analysis

The designs for experiments two, three and four were completely randomized designs of the basic model equation; Yij = U + Ti + Eij. In the simple regression analysis used in Experiment 3, the residual and error were combined and used to test treatment effect as well as the regression model. Data were subjected to analysis of variance using the general linear models procedures of Statistical Analysis Systems (SAS 1997). Means were tested by Fischer's LSD (Steel and Torrie 1984)

RESULTS

Proximate and amino acid analysis

A statistical test of the proximate composition data of the fish samples from different locations or landing beaches is presented in Table 1. There were significant differences in composition by location for crude fibre, ether extract and crude protein. However, there was no significant difference in proximate composition by size. Likewise there was no significant difference in the 14 amino acids present in fish from the different locations except proline and leucine (Table 2).

Experiment 1- Results presented in table 4. Chicks on the 8 and 12 % RFM diets were not significantly different in weight gains, feed intake and feed conversion. However, the two treatments were significantly (P<0.05) greater than those on the 93 % RFM diet in weight gain and feed intake but not in feed conversion.

Experiment 2. Results of table 5 indicate there was no significant difference (P<0.05) in weight gain and feed intake between the 8 and 12 % RFM supplemented diets. The 89.3 % diet when fortified with vitamins and minerals resulted in significantly high weight gain, feed conversion and the lowest feed intake.

Experiment 3. Results given in table 6.The simple regression curves showed good fits of R = 0.98, 0.91 and 0.97 for weight gain, feed intake and feed conversion respectively. The regression equation of supplemental lysine on weight gain, calculated as Y = 99.75 + 54.14x was used to estimate for the supplemental lysine from the 5 % RFM. From this equation the supplemental lysine intake from 5% RFM diet was 1.55 grams. After adjustment for the lysine in the basal, the lysine available in the 5% was calculated to be 97.3 %.

DISCUSSION

The mean composition of *R. argentea* samples from all the locations by proximate analysis shows that it is a good source of protein (58.50%) and lysine (6.03%). The significant differences in dry matter, crude protein, ash, ether extract and crude fibre between samples from different landing beaches may be due to several factors. The difference in crude protein and ether extract is possibly due to age or seasonal variation. Okot (1995) reported that pelagic fishes like *R. argentea* have two oil cycles, the first beginning December-January (low oil content) which ris es to a peak in May-June. Contamination with sand and organic matter from the drying surfaces may cause the differences in ash and crude fiber contents.

However, the proximate composition results are comparable to data reported in the studies of similar fish by Tuitoek (1992), Steiner-Asiendu et. al. (1993) and Okot (1995). The amino acids results are comparable to those reported for RFM (Okot 1995). However, the results for RFM show a high lysine content (6.08 %) compared to 5.70 % for Herring fishmeal (NRC, 1984). In Experiment 1, chicks on the fishmeal diet of 93 % RFM performed below those on the other diets (Table 4).

This poor performance can be attributed to non-supplementation of the fishmeal diet (Table 3 diet c) with vitamins and minerals. However, chicks on this fishmeal diet did not die. The excess protein in the fishmeal depressed intake, stressed their metabolism and resulted to diarrhoea for 3 - 5 days, but then the chicks adapted to it. This indicates that the RFM as processed and used, had no adverse effects on the chicks. It did not contain any toxic factors such as gizzerosines or histamines (Harry et. al. 1974), but it was deficient in vitamins and or/minerals; hence the low weight gains. When the fishmeal diet (89.3 % RFM) was supplemented with vitamins and minerals it produced significantly high weight gain.

RFM is an excellent source of lysine as shown by results of Experiment 3. The bioavailable lysine estimated by regression was 97 % and appears to be as good as that of Menhaden at 89 % (Sibbald and Wolynetz 1984). Our calculations were based on the assumption that the supplemental crystalline lysine used to establish the regression line was 100 % bioavailable to the broiler chicken (Smith 1968). Studies indicate that bioavailability figures obtained using weight gains regressed on supplemental intake are the most reliable, because it measures the direct result of lysine supplementation in the diets (Parsons, 1991). The 97 % bioavailable lysine in RFM is an indication that its simple processing by sun drying preserves most of the lysine. Processing of conventional fishmeal such as Menhaden and Herring by heating has a detrimental effect on the availability of lysine (Smith and Scott, 1965a; Carpenter and Booth, 1973). The biological assays tend to give values, which are correlated with values of chemical assays (Barlow et. al. 1984). This seems to be the case in our study, in that the lysine value (6.03 %) found by amino acids analysis is closely reflected in the 5.87 % calculated using the bioavalaiblity figure of 97 %. Supplementing the 89.3 % with vitamins and minerals greatly increased weights gain. Results of experiment 2 Table 5 demonstrated that RFM is a good source of protein and that the optimal inclusion level in broiler diets would be between 8 and 12 %. The maximum level of fishmeal in chicken diets established by studies is 10 %. Beyond this level fishy taint occurs in broiler meat and eggs (Pearson et. al. 1983,). Fishy taint was not tested for in this study. However the use of RFM at 8 or 10 % will depend on the prevailing costs of the fishmeal.

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Tab. 1: Proximate composition of *R. argentea* from different sites.

	Proximate Composition in (% as fed)				
Landing Beach	DM	CP	Ash	EE	CF
Muhuru	89.56b	56.47cd	10.22b	11.51c	1.337ab
P.Victoria	90.45b	54.90d	10.61	12.10c	1.247ab
Sori	89.79b	58.85a	10.21b	13.26b	0.357bc
Sirongo	89.25b	58.25ab	10.66ab	10.11d	0.910bc
Ragengni	91.24	57.01bc	11.11a	14.95a	1.710a
Mean	90.10	58.50	10.40	12.60	0.08
S.E.M.	0.369	0.518	0.185	0.240	0.176

DM= Dry matter; CP= Crude protein; EE= Ether extract; CF= Crude fibre.
a,b,c,d, - values having different superscripts in the same column are significantly different.(P<0.05).

Tab. 2 : Some amino acid composition of *R. argentea* from different landing beaches.

Amino Acids g/100g of RFM	Muhuru	Port Victoria	Sori	Sirongo	Overall mean	S.E.M
Arginine	3.10	3.24	4.66	3.57	3.64	0.578
Threonine	1.70	1.79	2.47	1.34	1.83	0.324
Proline	1.98b	2.49b	3.40a	1.80b	2.42	0.266
Valine	2.47	2.39	2.78	1.49	2.28	0.267
Phenylalanine	1.96	3.41	2.88	2.36	2.65	0.313
Isoleuncine	1.34	1.54	1.59	1.16	1.41	0.312
Leucine	3.23b	4.96a	2.06c	2.68c	3.23	0.236
Lysine	6.73	6.26	6.03	5.30	6.08	1.052
Histidine	2.87	3.05	3.82	3.41	3.29	0.263

a,b,c, - values with different letters in the same row are significantly different (P<0.05).

Tab. 3: The Composition of diets in Experiment 1, 2 and 3.

Ingredients	Experiment 1			Experiment 2					Experiment 3
g/kg	A	B	C	1	2	3	4	5	Basal
Maize (corn)	600	600	-	600	600	600	600	-	611
Soybean meal	195	155	-	305	255	195	155	-	-
Maize Gluten 56%CP	-	-	-	-	-	-	-	-	250
R. argentea	80	120	930	-	40	80	120	893	-
Maize bran	75	70	70	40	55	65	70	70	70
Corn oil	15	15	-	28	18	15	15	-	28
Mineral mix [1]	23.5	23.5	-	23.5	23.5	23.5	23.5	23.5	28
Vitamin mix [2]	2	2	-	3.5	3.5	3.5	3.5	3.5	3.2
Diluent	9.5	14.5	-	-	5.5	18.0	13	10	-
Amino acid Mix 3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	6.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Calculated Analysis									
Energy MJ,ME/Kg	11.71	11.72	10.53	12.29	11.87	11.70	11.79	10.53	12.32
Crude Protein g/100g	19.00	19.00	55.00	18.5	18.6	18.5	19.00	52.00	19.40
Lysine g/100g	1.20	1.2	5.70	1.15	1.20	1.20	1.20	5.50	0.40

Mineral mixture [1] provided per kg diet: Calcium, 15g; Iron, 18.4mg; Manganese, 50.4mg; Zinc, 50mg; Cobalt, 0.8mg; Iodine, 1.6mg; Copper, 11mg; Selenium, 0.08mg; Nacl, 8.5g.
Vitamin mixture [2] provided per kg diet: thiamine HCL, 20mg; riboflavin, 10mg; D-capantothenate, 30mg; Vitamin B12, 0.04mg; Pyridoxine HCL, 6.0mg; D-biotin, 0.9mg; folic acid, 4mg; ascorbic acid, 240mg; retinyl acetate, 180 ug; D3, 15 ug.
Amino acid mixture [3] provided per kg diet :- L-Isoleucine 1.0kg, L-Valine 1.0kg, L-Threonine 1.0kg, L-Tryptophan 1.0kg, L-lysine 1.0kg, L-Glutamine 1.0kg.

Tab. 4: Performance of chicks fed on diets with increasing levels of *R. argentea* Fishmeal over 13 days. Experiment 1

RFM levels %	Weight gains (g/chick)	Feed intake (g/chick)	Feed conversion (g/kg feed)
8 %	191.5 ab	593.8a	0.325a
12 %	233.0	629.4a	0.375a
93 %	134.5b	327.5b	0.410a
S.E.M.	15.41	38.85	0.23

SEM Standard Error of mean.
- a,b,- values with different superscripts in the same column are significantly different (P<0.05).

Tab. 5: Performance of chicks fed on diet with increasing levels of *R. argentea* fishmeal over 13 days. Experiment 2

RFM levels	Weight gains (g/chick)	Feed intake (g/chick)	Feed conversion (g/kg feed)
0 %	132.50c	479.50c	0.2668c
4 %	171.87c	567.25b	0.3028c
8 %	229.81b	688.75	0.3350c
12 %	238.12b	622.00ab	0.3342b
89.3 %	298.44a	457.75c	0.6560a
S.E.M.	14.51	22.68	0.022

SEM Standard Error of mean.
- a,b,c- values with different superscripts in the same column are significantly different (P<0.05).

Tab. 6: Performance of chicks fed on diets with graded levels of lysine over 14 days. Experiment 3

Diet	Supplemental Lysine g/chick	Weight Gains g/chick	Feed intake g/chick) (g/kg feed)	Gain/Feed
Basal + 0 % lysine	0.00	88.69a	479.40a	0.185a
Basal + 0.25% lysine	1.47	201.03b	586.10b	0.343b
Basal + 0.5 % lysine	3.00	251.87c	599.70c	0.420c
Basal + 5% RFM2	1.40	138.53b	528.90	0.347b
S.E.M. [1]		11.74	18.62	0.036

SEM Standard Error of mean. [2]RFM - lysine values calculated by regression equation.
- a,b,c- values with different superscripts in the same column are significantly different (P<0.05).