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Etroplus maculatus fish fed with incorporated pigeon meal and its amino acid profile analysis

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ABSTRACT

The aim of the work is to analyse amino acid composition of pigeon meal from the pigeon species *Columba livia* incorporated feed fed to the fish *Etroplus maculatus*. Five pigeons from the local market Tirunelveli was selected for the study. The amino acid profile was deduced from HPLC analysis. Results obtained from the assessment indicate that the required amount of both essential and non - essential acids present in the Pigeon will eventually help in the growth of *Etroplus maculatus* taken for research.

Keyword: *Columba livia*, Cichlid, Amino acids, High Performance Liquid Chromatography

INTRODUCTION

Pigeons are selected for the study belongs to phylum: Chordata, class: Aves, super order Neognathae and order Columbiformes. These birds occupy all parts of India. They are fast and powerful in flight. Mostly they feed on paddy, corn, millet, groundnut, fruits and seed and their availability for study is more and hence the pigeon meat is selected as a nutritive food for ornamental fish.

The objective of the study is to select the nutritive food for the fish. In most of the other birds such as Turkey, chicken and in other animals such as poultry, beef and goat so many works have been carried out and the research work on Pigeon meal is meagre. Since the availability and reproducibility of the Pigeon is easy and it's a cheaper source as well as it is having a nutritive value so the meat of the pigeon has been selected as a source meal. The feed of fish and their nutritive value is one of the most important factors depends on production cost and health of fish. In case of ornamental fish, correct formulations of the diet improve the nutrient digestibility, supply the metabolic needs and reduce the maintenance cost and also the water pollution (Yohanna, 2011). The addition of mineral supplements to these diets improved growth and survival (Halver, 2002). Ornamental fish can absorb some water soluble minerals from water (Shim and Ho., 1989) of all the minerals required by fish. Phosphorous is one of the most important for growth and bone mineralisation. Also their contributions towards meat consumption of fish related to its nutritive values were evaluated.

Fish Tank maintenance: The ornamental fish, *Etroplus maculatus* was cultured in cement tanks such that ten fishes occupied a tank. The experimental tanks were supplied with ground water with continuous aeration maintained at a constant temperature (27±2˚C). Water exchange was done once fortnight. Measurements of dissolved oxygen and ammonia were carried out every 15 days in the water to give an indication of absence of stress exerted to the fishes. Walls and bottoms of the tanks were scrubbed on alternative days to minimize the growth of algae and fungi for the fish to feed on. The experiment was carried out in triplicates.

The feed was formulated with the inclusion of Pigeon meal (17%), Fish meal (17%), Prawn meal (17%), Groundnut oil cake (17%), Rice bran (15%), Tapioca (10%), Oil (1%) and Vitamin and mineral mix (2%), Carotenoids (2%), Binder (2%). The ingredients were cooked and made into dough which was pelleted using the hand extruder. The pellet was dried in hot air oven at 55˚C for 6 hours and crumbled to smaller size. The selected ornamental fish *Etroplus maculatus* was chosen with an approximate size of about 2gm from the nearby farm in Tuticorin and it was acclimatized in the tank for 2 days to which the experimental feed was given. The control tank was maintained separately where the fishes were fed with the commercial fish feed. (Table 1)

Orange chrome fingerlings obtained from Rajan farm, Sawyerpuram, Tamil Nadu were adapted to experimental conditions for 2 days before the start of the experiment. During the period their guts were cleared by making them starve and then they were cleansed in medicinal water to free them from bacterial and fungal attack. Sixty fish each weighing 2.5gm on an average were distributed into 18 cement tanks supplied with ground water. The water was continuously aerated and the temperature was controlled thermostatically 25˚C. Fish was held under the half shaded roof.

Each experimental diet was allocated to 3 tanks. During the 60 days of experimental period all fishes were hand - fed twice a day. Bulk weight of fish in each tank was recorded every fortnight. At the end of the experiment 5 fish from each tank were sampled. Fish were killed and then weighed and then taken for chemical analysis. They were then dried in a microwave oven at 55˚C for 6min and then ground into coarse powder which was sieved to separate the fine powder.

Table 2 indicates that the salinity, alkalinity, pH, temperature, hardness in the culturing tank of fish. The temperature was maintained at 25˚C throughout the experimental period. Water quality determines not only how fish will grow in an aquaculture operation, but how well they will survive. Fish influence water quality through processes like nitrogen metabolism and respiration. Some water quality factors are more likely to be involved with fish losses such as dissolved O_2, temperature and ammonia, others such as pH, alkalinity, hardness and clarity affect fish. The salinity ranges from 0.34±0.04 to 0.35±0.04ppm and in P3 showed the highest value of salinity in the present study.

Fish adjust their body temperature and metabolic rate by moving into cooler or warmer water. At temperatures above or below optimum, fish growth is retarded. Mortalities occur at extreme temperature. The survival rate of fish in the tank PM4 found to be 100%.

Many fish excrete ammonia as their main nitrogenous wastes. The proportion of TAN (Total Ammonia Nitrogen) existing in ionized and unionized form increases. Ammonia is removed by bacteria that initially convert it into nitrite and subsequently into nitrate. The ammonia ranges from 0.3±0ppm to 0.45±0.2 in the experimental tank. A low pH is acidic and a high pH is basic, Mortalities occur below 4.5 and above 10. Fish grow best in water with pH between 6 and 9. The pH was calculated and found to be between 7.5 to 8.5 in the Pigeon meal fed fish.

Alkalinity is a measurement of carbonate and bicarbonate ions dissolved in water. Alkalinity can be increased by adding agricultural limestone to ponds or sodium bicarbonate to recirculating systems. Alkalinity ranges from 200±0.89 to 200±0.45 respectively. While alkalinity measures negative ions (carbonate, bicarbonate) hardness measures positive ions (Calcium, magnesium). Hardness should be above 50ppm; low hardness can be adjusted by the addition of lime or calcium chloride. The hardness ranges from 51.7±1 and to 56.70±1.80ppm

It is the total concentration of all ions in water. It not only affects osmoregulation but also influences the concentration of un - ionized ammonia. Careful investigation should be carried out before the start of fish culture. Fish that prefer turbid water if cultured in relatively clear water they will experience stress, survival and growth will be adversely affected filtration can clear the water of solids and discolouration.

Table.1.Formulation and chemical composition of the experimental diets

Ingredients	Amount %
Pigeon meal	17
Fish meal	17
Prawn meal	17
Groundnut oil cake	17
Rice bran	15
Tapioca	10
Oil	1
Vitamin mineral mix	2
Carotenoids	2
Sodium Alginate	2

Likewise the feed composition for P1 (0% pigeon meal), P2 (25% pigeon meal), P3 (50% pigeon meal), P4 (75% pigeon meal), P5 (100% pigeon meal) inclusion.

Table.2.Water analysis in the experimental fish tank

Parameter	C1	P1	P2	P3	P4	P5
Temperature	25˚C	25˚C	25˚C	25˚C	25˚C	25˚C
Salinity %	0.34±.04	0.18±0.06	0.34±0.04	0.35±0.03	0.04±0.03	0.08±0.06
Hardness (ppm)	56.70±1.80	52.87±1.84	54.33±0.95	55.3±1.01	54.37±1.14	51.7±1
Ammonia (ppm)	0.4±0.2	0.34±0.1	0.33±0.02	0.38±0.1	0.3±0	0.45±0.2
pH	8.34±0.04	8.41±0.04	8.63±0.03	8.55±0.06	7.86±0.04	7.62±0.06
Alkakinity (ppm)	300±1.19	300±0.45	300±0.51	250±0.65	200±0.89	255±0.36
Survival	95%	97%	97%	98%	100%	98%

PM - Pigeon Meal incorporated feed

MATERIALS AND METHODS

Amino acid profile was analysed in the pigeon meals, pigeon incorporated formulated fish feed and in the ornamental fish tissue. The analysis was carried out by separating protein which was later hydrolysed and subjected to

amino acid quantification by High Performance Liquid Chromatography. The protein was hydrolysed to amino acids using 6N Hydrochloric acid and known volume of the hydrolysate was injected into the injector of High Performance Liquid Chromatography and the amino acid composition was estimated. The injected sample from the injector was separated in the column. Secondary amines such as proline and hydroxyl proline were reduced to primary amines in the Reaction Coil 1. With the sodium chloride solution, all the amino acids reacted with o - Pthaladehyde and converted into fluorescent compounds in the Reaction Coil 2 and the produced fluorescent compounds were detected by the fluorescence detector.

A solution of dissolved mixed amino acids (1mg / ml) was prepared in a simple diluent (0.2N sodium citrate pH 2.2). Twenty µl of this solution was injected into the sample injector which contained 1N mole (1×10^{-9}) of each amino acid. Twenty mg of the sample was hydrolysed in a hydrolysis tube using 2ml of 6N Hydrolchloric acid under vacuum for 12 hours at 110˚C. After hydrolysis the tube was left open to evaporate hydrochloric acid in a rotary evaporator. The hydrolysed sample was dissolved in the sample diluents (0.2N Sodium citrate pH 2.2) into a known volume, filtered and 20 µl was injected into the column.

The mobile phases is the solution containing 15.2g triethylamine R in 800ml of water R which was adjusted to pH 3.0 with phosphoric acid R and diluted to 1000ml with water R. About 850ml of this solution was added to 150ml to a mixture of 2 volumes of propanol R and 3 volumes of acetonitrile R. The stationary phase used was octadecylsilyl silica gel for chromatography R (3µm). The flow rate was maintained at 1.0 - 1.5ml / min with the column oven temperature maintained at 60˚C. The run time of the sample was 90min. The separated amino acids were detected by post column derivation using reaction reagents, 0.04% sodium hypo chlorite - carbonate - borate buffer and 0.04% ophthaldehyde, 0.1% acetyl cystine in carbonate - borate buffer with the flow rate of 0.25ml / min. The fluorescence was measured at Ex 348 m microns and EM 460m microns using FLD6A fluorescence detector. The amino acid composition of the sample was estimated based on the retention time values and peak areas of the standards and samples.

RESULTS AND DISCUSSION

Table 3 represented the estimated value of amino acids sometimes appears to be quite highly variable across the different species of pigeon and even between the same species of pigeon. The estimated value of amino acid may also vary while using different drying techniques. The present study represented the availability of amino acids in fish tissue fed with different levels of pigeon meal incorporation in different percentage such as 0%, 25%, 50%, 75% and 100% replacement of fish meal with pigeon meal. And the graph in the following (Fig 1) represented the estimated amino acid values.

In p4 the essential amino acid aspartic acid is high (0.45±0.04), glutamic acid (0.45±0.04), Arginine (0.48±0.05), Proline (0.26±0.02), glutamine (0.25±0.03), glycine (0.34±0.03) and the non – essential amino acid Tryptophan has the high value of (0.45±0.04) , Lysine (1.08±0.08). In P3 the essential amino acid such as Threonine has the highest value of (0.35±0.03), Alanine (0.45±0.04), Serine (0.26±0.03) and the non – essential amino acids such as Tyrosine is high (0.46±0.04), Methionine (0.35±0.03), Isoleucine (0.44±0.04).

In P5 the essential amino acid such as Threonine has the highest value (0.38±0.04), Cysteine (0.94±0.08), Asparagine (0.32±0.03) and the non-essential amino acid has high value in amino acid valine (0.35±0.03), Phenyl alanine (0.87±0.07). In P2 the essential amino acid i.e Histidine has high value (0.54±0.05). In P1 the essential amino acid asparagine has the highest value of (0.34±0.02) and the non-essential amino acid in leucine (0.86±0.06) and the valine value is high in 0.40±0.04.

In C1 both essential and non-essential amino acid are only in the average amount. The total amount of amino acid is high in P4 (75% of fish meal replaced with the pigeon meal). The lysine requirement is needed for maximizing weight gain and protein deposition, in rainbow trout was estimated between 2.3 to 2.7% (DM basis) Rodehutscord et al., 1997, 2000; Encarnacao et al., 2004.So it is deduced from the experiment on amino acids that P4 excels P3 in value as it has several peak values compared to P3.

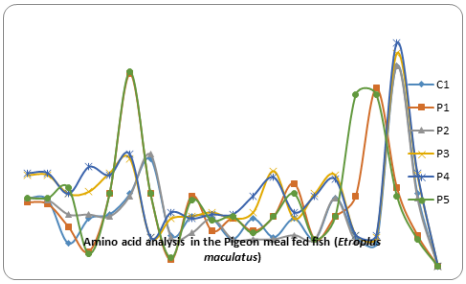

Fig.1.Represents the amino acid analysis in the Pigeon meal fed fish (*Etroplus maculatus*)

Table.3.Amino acid profile for the fish fed with feed incorporating pigeon meal (%)

Essential a.a (%)	Cl	P1	P2	P3	P4	P5
Aspartic acid	0.33±0.03	0.31±0.03	0.33±0.03	0.44±0.04	0.45±0.04	0.33±0.03
Glutamic acid	0.32±0.03	0.30±0.02	0.32±0.03	0.44±0.04	0.45±0.04	0.33±0.03
Threonine	0.11±0.02	0.19±0.01	0.25±0.02	0.35±0.03	0.35±0.03	0.38±0.04
Arginine	0.23±0.02	0.07±0	0.25±0.02	0.36±0.03	0.48±0.05	0.06±0.00
Alanine	0.25±0.02	0.35±0.04	0.24±0.02	0.45±0.04	0.44±0.04	0.35±0.03
Cysteine	0.35±0.02	0.93±0.05	0.34±0.03	0.52±0.04	0.54±0.05	0.94±0.08
Histidine	0.52±0.03	0.35±0.02	0.54±0.05	0.14±0.01	0.14±0.01	0.35±0.03
Proline	0.15±0.02	0.03±0.05	0.14±0.01	0.23±0.02	0.26±0.02	0.04±0.00
Aparagine	0.24±0.04	0.34±0.02	0.16±0.01	0.24±0.02	0.23±0.02	0.32±0.03
Serine	0.23±0.03	0.17±0.01	0.23±0.02	0.26±0.03	0.25±0.03	0.22±0.02
Glutamine	0.13±0.01	0.23±0.02	0.13±0.01	0.23±0.02	0.25±0.03	0.24±0.02
Glycine	0.23±0.03	0.17±0.01	0.13±0.01	0.26±0.04	0.34±0.03	0.16±0.01
Non- Essential a.a						
Tyrosine	0.14±0.01	0.24±0.03	0.13±0.01	0.46±0.04	0.43±0.04	0.24±0.02
Valine	0.23±0.03	0.40±0.04	0.15±0.01	0.23±0.02	0.26±0.02	0.35±0.03
Methionine	0.13±0.01	0.13±0.01	0.13±0.01	0.35±0.03	0.34±0.03	0.13±0.01
Isoleucine	0.33±0.04	0.24±0.02	0.33±0.03	0.44±0.04	0.42±0.04	0.24±0.02
Phenylalanine	0.14±0.01	0.34±0.03	0.12±0.01	0.15±0.01	0.15±0.01	0.87±0.07
Leucine	0.11±0.01	0.86±0.06	0.14±0.01	0.14±0.01	0.15±0.02	0.83±0.06
Lysine	0.97±0.05	0.38±0.02	0.97±0.07	1.03±0.06	1.08±0.08	0.34±0.03
Tryptophan	0.35±0.03	0.15±0.01	0.33±0.02	0.44±0.04	0.45±0.04	0.13±0.01
Sum **F value** **P value**	**5.49** **0.8412** **0.52**	**6.18**	**5.36**	**7.16**	**7.46**	**6.85**

a.a - Amino acids. The group of amino acids was found to be highly significant since the P value is (> 1) when compared with the control feed

DISCUSSION

Using pigeon meal incorporated feed in P3 feed the iso leucine concentration was 0.44±0.04 and that of P4 feed0.42±0.04 when compared with that of rainbow trout 1.4% was required and in case of blood meal the isoleucine present was 1.2% (NRC, 1993) and Rodehutscord et al., 1997. Many studies reveal that the time of exposure to heating and actual temperature of processing and heat treated ingredients, are critical factors for the digestibility of protein and amino acids (Cook and Dumont, 1991).

Several studies were conducted with terrestrial (Rolls et al., 1972; Butts et al., 1993; Schumacher et al., 1997) and studies also conducted with aquatic animals (Yamada et al., 1981; Kaushik and Dabrowski, 1983; Murai et al., 1987; Cowey and Walton, 1988) have proved that free bound amino acids are easily digested and absorbed than protein bound amino acids.These amino acids gets deposited in the plasma membrane and thus there is increase in the higher tissue concentration and major amino acids being catabolised (Batterham, 1984; Schumacher et al., 1997), Batterham and Murison (1981), Batterham (1986), Tantikitti and March (1995), and Zarate et al., (1999). Bleaching of amino acid was prevented by coating and encapulaion.

CONCLUSION

The results of this study highlights the importance of processing and drying technique on the availability of amino acids in the formulated feeds. The Five feeds P1, P2, P3, P4, P5 were highly significant since the p value was less then (> 1 value) when compared to control feed and among the Five feeds P4 feed where there is 75% of the pigeon meat is included showed that total amino acid was found to be high. So the partial replacement of the 75% fish meal with the pigeon meal showed the significant result.

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