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Influence of Different Non-Conventional Feeds on Fatty Acid Profile of Mozambique Tilapia (*Oreochromis mossambicus*)

[1]Mukti Pada Bag, [2]Subhash Chandra Mahapatra, [2]Pavuluri Srinivasa Rao and [3]Debajyoti Chakrabarty
[1]Rural Development Centre, [2]Department of Agricultural and Food Engineering,
Indian Institute of Technology, Kharagpur, West Bengal, India
[3]Department of Zoology, Barasat Government College,
Barasat North 24 Parganas, West Bengal, India

Abstract: The present study was performed to investigate the influence of different non-conventional feeds on the Fatty Acid (FA) composition of Mozambique tilapia *Oreochromis mossambicus*. Three isonitrogenous and isoenergetic diets were used viz., Earthworm Meal (ETM), Poultry Offal Meal (POM) and Market Available Feed (MAF). Each diet was fed to triplicate groups of 20 fish. The feeds influenced ($p<0.05$) fatty acid profile of fish flesh. Fillet FA profile of fish fed MAF diets had significantly higher ($p<0.05$) concentrations of SFA and MUFA but lower PUFA compared to fish fed ETM and POM diets. Fillet of fish fed the ETM diet had higher ($p<0.05$) concentrations of PUFA as well as EPA and DHA compared with fish fed the other diets. The results of this study shows that feed formulated with non-conventional sources as key ingredient have better impact on FA profile of fish fillet than the feed available in market.

Key words: Non-conventional feeds, fatty acid profile, poly unsaturated fatty acid, *Oreochromis mossambicus*, MAF, POM, India

INTRODUCTION

Fish are a source of high quality protein, vitamins and essential minerals but above all, a virtually unique, rich source of omega-3 long-chain Poly Unsaturated Fatty Acids (PUFA). The PUFA are important for maintaining the integrity of membranes of all living cells for making prostaglandins which regulate many body processes such as inflammation and blood clotting. The fats are also needed in diets to absorb fat-soluble vitamins A, D, E and K from food and for regulating body cholesterol metabolism (Connor, 2000; Kris-Etherton *et al*., 2003). The fish fats contain essential PUFA like Eicosapentaenoic (EPA, C20:5 n-3), Docosahexaenoic (DHA, C22:6 n-3) and Arachidonic (C20:4 n-6) acids which are not synthesised in the human body but their inclusion in human diets is essential (Alasalvar *et al*., 2002; Glogowski and Ciereszko, 2001; Holub and Holub, 2004; Kolanowski and Laufenberg, 2006). Although, PUFA composition may vary among different fish species of both fresh water and marine origins (Rahnan *et al*., 1995), it is important for human health to increase the consumption of fish and its products (Burr, 1989; Sargent, 1997).

The knowledge of the fatty acid composition for important fish species; such as, the *carps* and *tilapia* is desirable due to the recent dietary and medical emphasis on the role of fatty acids in human health. Therefore, the aim of this study was to investigate the chemical and fatty acid profiles of OM fed with different diets.

Fish are unable to synthesize the EFA; such as, linoleic (18:2 n-6) and linolenic acid (18:3 n-3) *de novo*. Therefore, fish must obtain these substances via food (Takeuchi *et al*., 1980). The requirements of EFA for high growth rates of some cultured fish species are well investigated and most of them have a species-dependent demand for n-3 and n-6 FA. Some freshwater fish can elongate and desaturate FAs with 18 carbons, specifically linolenic acid to PUFA with 20-22 carbons of the n-3 series. This ability to synthesize EPA and DHA from linolenic acid allows the formulation of diets containing non-conventional sources. The market value of cultured fish largely depends on their quality and feed composition is one of the factors that control quality (Morris, 2001; Shearer, 2001). It is currently well established that increased fish consumption in human is associated with decreased mortality as well as morbidity from Cardiovascular Disease (CVD) and Coronary Heart Disease (CHD) (Connor, 2000; Arts *et al*., 2001). Consequently, the links between fish as food and human health are strongly related to the fatty acid composition of

Corresponding Author: Mukti Pada Bag, Rural Development Centre, Indian Institute of Technology, Kharagpur, West Bengal, India

the food (Crawford *et al*., 1993). The fatty acid profile of fish can be modified with diets containing non-conventional sources (Steffens, 1997).

Fish feed generally constitutes 60-70% of the operational cost in intensive and semi-intensive aquaculture system (Singh *et al*., 2006). Naturally, there is a need for the preparation of healthy, hygienic fish feed which influences the production as well as determines the quality of cultured fish.

Considering the importance of nutritionally balanced and cost-effective alternative diets for fish, there is a need for research effort to evaluate the nutritive value of different non-conventional feed resources including earthworm (*Eisenia foetida*) and poultry offal. Earthworm is a bye product vermicompost farm contains substantial amount of protein and minerals (Tacon *et al*., 1983; Kostecka and Paczka, 2006; Sogbesan and Ugwumba, 2008). However, these materials are not evaluated for essential fatty acid content earlier. The beneficial fatty acid in fish body is synthesis from the feed materials they consumed (Horrobin and Manku, 1990). The beneficial effects of fish lipids on human health have already been well established (Mukhopadhyay, 2009). It is, therefore, of utmost importance to determine the influences of feed on growth as well as fat deposition in fish (Cengiz *et al*., 2003).

Poultry offal consists of ground rendered clean parts of the carcass of slaughtered birds including necks, feet, undeveloped eggs, intestines, etc. excluding feathers. Variation in the composition of this Poultry Offal Meal (POM) could be largely due to variability in raw material composition and quality. Numbers of researchers have reported the extensive use of POM as fish meal replacer for several species of fishes (El-Sayed, 1998; Webster *et al*., 2000) due to presence of higher quantity of protein (Crude protein 30-35%). Gradual increase in consumption of chicken in our country and West Bengal as well ensures the availability of such poultry offal for use as fish feed.

This study was undertaken to determine the effects of the non-conventional feeds on fatty acid composition of *O. mossambicus*.

MATERIALS AND METHODS

Experimental set up: A total of 20 fingerlings in triplicate groups used in 3 different treatments. Altogether 180 Nile tilapia (Male and female ratio 1:1) fingerlings were used in this experiment. The fish fingerlings were treated with potassium permanganate solution (1 mg L^{-1}) to remove any external parasites and were acclimatized in a big tank for 5 days. Experiments were carried out at the tanks of aquacultural engineering section of IIT Kharagpur, West Bengal and India. Each group of fingerlings also were initially weighed to record the initial biomass. They were stocked in nine rectangular cemented cement tanks (1000 L). The water system was static in nature and the bottom of the tank was filled with local inert soil (pH 6.4±0.05). The experiment was conducted for 90 days from June to August in the year 2011. Dechlorinated well water (pH 7.0±0.05 and dissolved oxygen (6±0.5 mg L^{-1}) was used in the experiment.

Feed formulation and preparation: The principal feed ingredient (Earthworms) was collected from local vermicom post farm at very low cost. These substances were economically cheap but contained significant amount (36-40%) of crude protein (Sogbesan and Ugwumba, 2008). Diets used for growth trial were prepared that feed formulations remain almost isoproteinaceous (30 g 100 g^{-1}) and isoenergetic (4 Kcal g^{-1}) in nature. The choice of these nutrient levels, particularly, protein was intended to reflect the practical diets used in India. Diet formulations are presented in Table 1. Mustard oil cake, wheat flour, rice bran, egg shell dust and vitamin premix were common ingredient in every feed tested. These ingredients were used to compensate lipid, protein and ash deficiency in formulated feed. Wheat flour was selected as binder. Each feed was fortified with eggshell dust which is available free of cost for calcium supplement. This was added keeping in mind that the developing fish needs huge quantity of calcium for its bone development. The different ingredients were thoroughly mixed using a food mixer (A200 Hobart Ltd.). The proportion of different feed ingredients was

Table 1: Formulation and composition of the experimental diets (%)

Name of feed	Ingredients	CP in ingredients (%)	Ingredient in formulated feed (%)	Crude protein in feed (%)	Lipid in feed (%)	Carbohydrate in feed (%)	Calorific value of feed (Kcalg^{-1})
ETM	Earthworm	40.43	32.2	25.80	9.2	9.2	4.1
	MOC	34.65	27.8	-	-	-	-
	Wheat flour	9.08	38.2	-	-	-	-
	Egg shell dust	1.80	1.8	-	-	-	-
POM	FO	29.80	39.0	25.25	8.9	9.5	4.0
	MOC	34.65	29.5	-	-	-	-
	Wheat flour	9.08	30.2	-	-	-	-
	Egg shell dust	1.80	1.3	-	-	-	-

determined by using Pearson's Square Method. The mixture was given the shape of pellets using a Pellet Mill (Model CL2) with a 12 mm die. The resulting pellets were dried in a hot air oven for 48 h at 50°C, packed in polythene bags and kept in dry and cool place.

Feeding: The feed was given *ad libitum* in a feeding bag hung from an iron rod in four locations in each tank. Unconsumed feed was removed after 1 h from the beginning of feed administration and dried in a hot air oven at 50°C. Feed consumption was estimated by subtracting the weight of the unconsumed feed from the weight of the feed offered. Fish, feed samples and unconsumed feeds were weighed on pan electric balance to an accuracy of 0.1 mg.

Extraction of Lipids: The total lipids were extracted from all the samples, (fish flesh-3, feed-3) following the method of Bligh and Dyer (1959) using methanol-chloroform (2:1, v/v), methanol-chloroform-water (2:1:0.8, v/v/v) and then again with the first solvent system viz., methanol-chloroform (2:1, v/v). Sample was ground with the solvent methanol-chloroform (2:1,v/v), filtered through Whatman No. 1 filter paper and residue was extracted with the next solvent system, consisting of methanol-chloroform-water (2:1:0.8, v/v/v). The process was repeated once again with methanol-chloroform (2:1, v/v). Finally, the 3 extracts were pooled, diluted with 3 volumes of water (100-200 mL, depending on the volume of pooled extracts) and layer was allowed to separate in a separatory funnel made by Pyrex glass Co. The chloroform layer at the bottom of the separatory funnel was withdrawn and dried over anhydrous sodium sulphate in glass stoppered conical flasks by Pyrex. The chloroform solution of lipid was evaporated in a rotary vacuum evaporator by Rotavap under a pressure of 40-50 mm of mercury, weighed on a micro-balance by Sartorius and redissolved in distilled n-hexane (10-20 mL) and kept at -20°C for future use. BHT (Butylated Hydroxy Toluene) was added at a level of 100 mg L^{-1} to the solvent as antioxidant.

Preparation of methyl ester of fatty acids: Total lipid of various (fish flesh-2, feed-2) samples was dissolved in anhydrous methanol containing concentrated sulfuric acid (1.0%, v/v) and the mixture was refluxed for 2 h. Methanol was evaporated to a small volume (1-3 mL) and cooled to 4°C in a freezer. Distilled water 10-15 mL was added to the cooled mixture (1-3 mL) in hard glass test tubes by Pyrex and the methyl esters of fatty acids were extracted 3 times with aliquots (5-10 mL) of diethyl ether, vortexed in a vortex mixer. The ethereal extracts were taken out by Pasteur pipettes, pooled and dried over anhydrous sodium sulphate, (1-2 gm) in conical flasks (25-50 mL capacity) with glass stopper, filtered through Whatman No. 1 filter study vacuum dried, redissolved in n-hexane (1-2 mL volume) and kept in a freezer at 4°C for future use.

Purification of Fatty Acid Methyl Ester (FAME) by Thin Layer Chromatography (TLC): Fatty acid methyl esters were purified by TLC using a solvent system of nhexane-diethyl ether (90:10, v/v). A standard methyl ester was also run on the same plate in a separate lane for identification of the methyl ester bands in the samples. The location of methyl ester bands were indicated by placing the TLC plate in an iodine vapour chamber by Pyrex glass Co. The methyl ester bands corresponding to the standard were marked and then scrapped off the plate with a sharp rajor blade. Methyl esters were recovered by extracting the silica gel bands containing the methyl ester samples in a mini glass column (10 cm length x 0.8 cm internal diameter by Pyrex) with chloroform (30-50 mL), the later was evaporated and the methyl esters were kept in n-hexane (1-2 mL) in a freezer at 4°C till analyzed by Gas Liquid Chromatography (GLC).

Gas Liquid Chromatography (GLC): GLC of fatty acid methyl esters were done on a Chemito 1000 instrument, equipped with Flame Ionization Detector (FID). Quantifications were done by computer using specific Clarity Lite software.

Analysis of Fatty Acid Methyl Esters (FAME): GLC of FAME was done on a BPX-70 megabore capillary column of 30 mm length and 0.53 mm internal diameter obtained from SGE, Australia. Oven temperature was programmed from 150- 240°C with a rate of 8°C min^{-1}. Initial and final temperatures were kept isothermal for 1 and 20 min, respectively. Injection port and detector temperatures were 250 and 300°C, respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.18 mL min^{-1}.

Statistical analysis: Data were analysed using one-way ANOVA (Snedecor and Cochran, 1967) and differences between the means of treatments were examined using Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Ackman (2000) stated that only 14 fatty acids are really needed to describe the fatty acids of fish. However, Ackman *et al.* (2002) listed 64 fatty acids from 5 fresh water fishes of West Bengal, India. The two fishes under discussion recorded 28 fatty acids of the Total Lipid (TL)

and the result is more or less similar to those reported from other tropical and certain temperate zone fresh water fishes.

Among different classes of FAs, MUFA was recorded maximum in amount followed by DUFA, SFA and PUFA in supplied feeds. The amount of SFA found to be maximum in ETM (11.5) and minimum in MAF (11.1). Palmitic (16:0) was the main SFA and it constituted >50% of total SFA (Table 2).

MUFA was found to be maximum in MAF (59.7) feed followed by POM (59.0) and ETM (58.5) feed. Docosaenoic acid (22:1ω11) and oleic acid (18:1ω9) are the two principal MUFA. Only one DUFA (Linoleic-18:2ω6) was detected which was found to be maximum in POM (18.3) feed and minimum in ETM (17.1) feed (Table 2). As far as total PUFA is concerned, it was considerably superior in ETM to others. ALA (18:3ω3) was the chief contributor of total PUFA. It was maximum in ETM (5.1) followed by MAF (5.0) and POM (4.9) feed. The n-3/n-6 ratio was highest in ETM (0.64). In contrast to feeds, in all fed fish SFA was highest in amount followed by MUFA and PUFA (Table 3). The reason behind that fish may convert MUFA of the feed into the SFA of its flesh. Amount of DUFA was also reduced nearly 3 times in all fed fish. The recorded values of PUFA of fed fish were always high in comparison to the feed supplied. According to Ackman *et al*. (2002) dominant fatty acids in lipids of all the fishes were myristic (14:0), palmitic (16:0), stearic (18:0), palmitoleic (16:1ω7), oleic (18:1ω9), linoleic (18:2ω6), linolenic (18:3ω3), arachidonic (20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids. The present results corroborate with the above findings. PUFA was maximum in ETM (18.7) fed fish. ALA exhibited lowering tendency in all fed fish. On the other hand AA, EPA and DHA expressed increasing trend in all fed fish. EPA and DHA recorded maximum in ETM fed fish but highest amount of AA was obtained

Table 2: Fatty acid profiles of ETM, POM and MAF feeds (% w/w of each component in total fatty acids)

Components	ETM	POM	MAF
Saturated			
14:0	0.60	0.70	0.70
15:0	0.20	0.50	0.20
16:0	6.00	5.40	5.30
17:0	0.10	0.30	0.40
18:0	2.40	2.10	2.00
20:0	1.00	0.70	0.60
22:0	0.60	0.60	0.80
24:0	0.60	0.90	1.10
Σ SFA	11.50	11.20	11.10
Monoene			
14:1	0.00	0.00	0.00
15:1	0.00	0.00	0.00
16:1	0.90	1.10	1.20
17:1	0.00	0.00	0.00
18:1ω9	20.90	22.00	22.60
20:1ω9	7.10	7.50	7.00
22:1ω11	28.50	26.90	27.20
24:1	0.80	1.50	1.20
Σ MUFA	58.50	59.00	59.20
Diene			
16:2	0.00	0.00	0.00
18:2ω6	17.10	18.30	17.90
20:2	0.00	0.00	0.00
Σ DUFA	17.10	18.30	17.90
Polyene			
18:3ω6	0.20	0.30	0.50
18:3ω3	5.10	4.90	5.00
20:3ω6	0.70	0.50	0.00
20:3ω3	0.50	0.03	0.60
20:4ω6	0.20	0.40	0.40
20:5ω3	1.50	1.00	1.10
21:5ω3	0.10	0.40	0.50
22:5ω6	0.20	0.20	0.20
22:5ω3	1.90	1.80	1.90
22:6ω3	2.80	2.00	1.50
Σ PUFA	13.20	11.53	11.80
Total -ω3	11.90	10.13	10.60
Total -ω6	18.40	19.30	20.10
n-3/n-6	0.64	0.52	0.52

Table 3: FA profiles of *O. niloticus* fed with ETM, POM and MAF feeds (% w/w of each component in total fatty acids)

Components	ETM fed fish	POM fed fish	MAF fed fish
Saturated			
14:0	4.80	4.50	5.00
15:0	1.00	1.90	0.90
16:0	29.10	28.00	30.50
17:0	0.70	2.40	1.00
18:0	6.80	9.10	7.80
20:0	0.60	0.40	0.50
22:0	5.10	4.80	3.90
24:0	0.90	1.00	0.90
Σ SFA	49.00	52.10	50.50
Monoene			
14:1	0.90	0.40	1.00
15:1	0.40	0.20	0.30
16:1	7.60	7.20	7.50
17:1	0.40	0.90	0.40
18:1ω9	12.10	12.50	13.40
20:1ω9	1.40	1.80	1.50
22:1ω11	1.10	0.90	1.60
24:1	1.60	1.90	1.80
Σ MUFA	25.50	25.80	27.50
Diene			
16:2	0.30	0.50	0.20
18:2ω6	6.50	6.20	6.20
20:2	0.00	0.00	0.00
Σ DUFA	6.80	6.70	6.40
Polyene			
18:3ω6	0.40	0.50	0.30
18:3ω3	3.90	3.10	2.80
20:3ω6	1.10	0.90	1.40
20:3ω3	0.10	0.05	0.07
20:4ω6	0.90	0.60	1.00
20:5ω3	1.90	1.50	1.30
21:5ω3	0.70	0.50	0.50
22:5ω6	0.50	0.60	0.40
22:5ω3	3.40	2.60	2.50
22:6ω3	5.80	5.40	5.50
Σ PUFA	18.70	15.75	15.77
Total -ω3	15.80	13.15	12.67
Total -ω6	9.40	8.80	9.30
n-3/n-6	1.68	1.49	1.36

from MAF fed fish. In all feeds total amount of ω_6 fatty acid was higher than ω_3 fatty acid. But, in all fed fish the situation is just reverse. This is the most interesting phenomenon of the present study. Fish were able to covert n-6 fatty acids to n-3 fatty acids efficiently to a very low n-3:n-6 ratio (<1) to higher value of n-3:n-6 (>1). The mechanism of this conversion could not be explained at present. However, it proves that these fishes have the ability for such a conversion for the maintenance of physiological homeostasis. Ackman *et al*. (2002) stated that n-3/n-6 ratio should range 1-2 for fresh water fish. The recorded n-3/n-6 ratio of fed fish was within the same limit. The highest n-3/n-6 ratio was obtained from ETM fed fish indicates that the test fish convert the n6 fatty acid of the feed into n-3 fatty acid of its fillet more than the other feeds. This improves the flesh quality of the fish which makes the fish more acceptable to health conscious customers.

CONCLUSION

The feed prepared from Earthworm (ETM) influence the flesh quality through accumulating n-3 more n-3 PUFA in the flesh of the fish as well as increasing the n-3/n-6 ratio which is beneficial for human health. Moreover, the feed can be formulated at local level leading to employment generation in rural areas.

REFERENCES

Ackman, R.G., 2000. Fatty Acids in Fish and Shell Fish. In: Fatty Acids in Foods and their Healh Implication, Chow, C.K. (Ed.). M. Dekker, New York, Basel, pp: 153-172.

Ackman, R.G., C. Mcleod, K.K. Misra and S. Rakshit, 2002. Lipids and fatty acids of five freshwater food fishes of India. J. Food Lipids, 9: 127-145.

Alasalvar, C., K.D.A. Taylor, E. Zubcov, F. Shahidi and M. Alexis, 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): Total lipid content, fatty acid and trace mineral composition. Food Chem., 79: 145-150.

Arts, M.T., R.G. Ackman and B.J. Holub, 2001. Essential fatty acids in aquatic ecosystems: A crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci., 58: 122-137.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Burr, M.L., 1989. Fish and cardiovascular system. Prog. Food Nutr. Sci., 13: 291-316.

Cengiz, E.I., E. Unlu and M. Bashan, 2003. The effect of dietary fatty acids on the fatty acid composition in the phospholipids fraction of *Gambusia affinis*. Turk. J. Biol., 27: 145-148.

Connor, W.E., 2000. Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr., 71: 171S-175S.

Crawford, M.A., S.C. Cunnanes and L.S. Harbige, 1993. A New Theory of Evolution: Quantum Theory. In: Essential Fatty Acids and Eicosanoids, Sinclair, A.J. and R. Gibson (Eds.). Association of Official Analytical Chemists Press, Adelaide, Australia, pp: 87-95.

Duncan, D.B., 1955. Multiple range and multiple F-tests. Biometrics, 11: 1-42.

El-Sayed, A.F.M., 1998. Total replacement of fish meal with animal protein sources in Nile tilapia, *Oreochromis niloticus* (L.) feeds. Aquacult. Res., 29: 275-280.

Glogowski, J. and A. Ciereszko, 2001. Why we should increase food consumption, especially that of rainbow trout. Magazine Przemysl. Ryb., 2: 95-102.

Holub, D.J. and B.J. Holub, 2004. Omega-3 fatty acids from fish oils and cardiovascular disease. Mol. Cell. Biochem., 263: 217-217.

Horrobin, D.F. and M.S. Manku, 1990. Clinical Biochemistry of Essential Fatty Acids. In: Omega-6 Essential Fatty Acids: Pathophysiology and Roles in Clinical Medicine, Horrobin, D.F. (Ed.). Wiley-Liss, New York, pp: 21-53.

Kolanowski, W. and G. Laufenberg, 2006. Enrichment of food products with polyunsaturated fatty acids by fish oil addition. Eur. Food Res. Technol., 222: 472-477.

Kostecka, J. and G. Paczka, 2006. Possible use of earthworm *Eisenia fetida* biomass for breeding aquarium fish. Eur. J. Soil Biol., 42: S231-S233.

Kris-Etherton, P.M., W.S. Harris and L.J. Appel, 2003. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. Arterioscler. Thromb. Vasc. Biol., 23: e20-e30.

Morris, P.C., 2001. The Effect of Nutrition on the Composition of Farmed Fish. In: Farmed Fish Quality, Fishing News Books, Kestin, S.C. and P.D. Warriss (Eds.). Blackwell Science, London, pp: 161-179.

Mukhopadhyay, P.K., 2009. Fish as a nutritional source of long chain fatty acids. Sci. Culture, 75: 53-60.

Rahnan, S.A., T.S. Huah, O. Nassan and N.M. Daud, 1995. Fatty acid composition of some Malaysian freshwater fish. Food Chem., 54: 45-49.

Sargent, J.R., 1997. Fish oils and human diet. Br. J. Nutr., 78: S5-S13.

Shearer, K.D., 2001. The Effect of Diet Composition and Feeding Regime on the Proximate Composition of Farmed Fishes. In: Farmed Fish Quality, Kestin, S.C. and P.D. Warriss (Eds.). Blackwell Science, Oxford, UK., pp: 31-41.

Singh, P.K., S.R. Gaur and M.S. Chari, 2006. Growth performance of *Labeo rohita* (Ham.) fed on diet containing different levels of slaughter house waste. J. Fish. Aquatic Sci., 1: 10-16.

Snedecor, G.W. and W.G. Cochran, 1967. Statistical Methods. 6th Edn., Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, Pages: 593.

Sogbesan, A.O. and A.A.A. Ugwumba, 2008. Nutritive values of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture in Nigeria. Turk. J. Fish. Aquat. Sci., 8: 159-164.

Steffens, W., 1997. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquaculture, 151: 97-119.

Tacon, A.G.J., E.A. Stafford and C.A. Edwards, 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture, 35: 187-199.

Takeuchi, T., S. Arai, T. Watanabe and Y. Shimma, 1980. Requirement of eel anguilla japonica for essential fatty acids. Bull. Japanese Soc. Sci. Fish., 46: 345-353.

Webster, C.D., K.R. Thompson, A.M. Morgan, E.J. Grisby and A.L. Gannam, 2000. Use of hempseed meal, poultry by-product meal and canola meal in practical diets without fish meal for sunshine bass (*Morone chrysops* x *M. saxatilis*). Aquaculture, 188: 299-309.