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The Fatty and Amino Acids Profiles of Cichlidae and Claridae Finfish Species

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Abstract: The paper profile the fatty and amino acids contents of *Oreochromis niloticus, Tilapia zilli, Sarotherodon galileaus, Clarias anguillaris, Clarias gariepinus* and *Heterobranchus longifilis* of the *Cichlidae* and *Claridae* families' respectively. The Essential fatty and amino acids profiles of a particular species is used in the formulation of diets for the grow-out phase of that particular species since maximum growth is obtained when the dietary essential fatty and amino acids are available at levels equal to or higher than essential amino acid body levels. This therefore leaves little space to economise on feed protein.

Keywords: Amino acids, Claridae, Cichlidae, Aquaculture, Fatty acids

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Introduction

Aquaculture has become the fastest-growing food production sector of the world, with an average annual increase of about 10% since 1984 compared with a 3% increase for livestock meat and a 1.6% increase for capture fisheries (FAO, 1997).This provides the potential to stimulate research into developing new species for aquaculture. Numerous different species of finfish are being identified as candidates for aquaculture. However, aquaculture presents unique challenges. Most problems encountered include the lack of data on the fatty and amino acids requirements of the fish species among others. This means that to sustain such a high rate of increase in aquaculture production a similar increase in the level of production of fish feed is required. However expenses for feed are the main operating costs in intensive aquaculture, with protein being the most expensive macro-ingredient of commercial feeds. Therefore, an important breakthrough will come when specific cost-effective formulated diets that provide adequate protein-to-fat ratios which balance energy requirements of fish with caloric intake are developed for each species, and accepted by farmers.

Commercial feeds are developed based on two aspects: the chemical composition of the potential feed ingredients, which requires knowledge on the nutritional requirements of the animal and the availability of the nutrients within the ingredients (Wee, 1992). Essential fatty and amino acids profiles of a particular species are used in the formulations of feeds for the grow-out phase of that particular species. The profile of mineral elements of fish is also known to reflect nutritional requirements (Steffens, 1989). The fatty and amino acid profile of eggs has been

used as an estimate of the dietary amino acid requirements of some species (Rumsey & Ketola, 1975; Arai 1981; Ketola 1982; Ogata, 1983), and these approaches have been evaluated (Cowey & Tacon 1983; Wilson & Poe 1985). Van der Meer (1995) observed that protein administered at feeding levels was linearly related to the amount of protein gained. This therefore, leaves little space to economise on feed protein.

Information on the essential fatty and amino acids (EFA and EAA) composition of a fish should give an insight into the EFA and EAA requirement of the species. Fatty and Amino acids profiles could be used as complementary tool for balancing fatty and amino acids in formulated feed for fish species, and in the validation of fatty and amino acids requirements determined in performance studies. This study provides information on the fatty and amino acids compositions of some *Cichlidae* and *Claridae* species.

Materials and Methods

The species of *Cichlidae* use in the study include *Oreochromis niloticus* (CON), *Tilapia zilli* (CTZ) and *Sarotherodon galileaus* (CSG) while the *Claridae* species used are *Clarias gariepinus (CCG)* and *Clarias anguillaris* (CCA).

Sample Preparation
Fresh captured were obtained from Fishermen at the Kainji Lake Dam site. The fishes were weighed, beheaded, eviscerated and cleaned prior to freezing. In an attempt to obtain a homogeneous sample from each species, their flesh were removed from their backbones, minced, blended and immediately extracted. Lipid extractions were performed on minced fish samples (10g each) using the extraction

Lipid Extraction
Methods of Folch *et al* (1957): chloroform-methanol. Methylene chloride (100µL) and 1 mL 0.5M NaOH in methanol were added to oil extracts in a test-tube and heated in a water bath at 90^{O}C for 10 min. The test tubes were removed from the water bath and allowed to cool before addition of 1 mL 14% BF_3 in methanol. The test tubes are heated again in a water bath for 90^{O}C for 10 min, and cooled to room temperature. One mL distilled water and 200-500µL hexane was added to the test tubes and then FAME was extracted by vigorous shaking for about 1 min. Following centrifugation, the top layer was transferred into a sample bottle for GC analysis.

Fatty Acids Analyses
The fatty acids profiles were determined in an Agilent Gas Chromatograph, Model 6890N fitted with an Agilent Mass Selective Detector, 5973 series. Separation was carried out in a capillary column (30 x 0.25mm id x 0.25µm DB wax). The starting temperature was 150^{o}C maintained for 2minutes at a heating rate of 10 ^{O}C/minutes. The total running time was 22 minutes. Helium was the carrier gas while the injection volume was 1µL. The fatty acids peaks were identified using Agilent Technologies software 5988-5871EN.

Amino Acids Analysis
The amino acid profile in a known sample was determined using methods described by Sparkman *et. al.*, (1958). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and then located into the technician Sequential Multisampling Amino Acid Analyser (TSM) Model DNA 0209. The TSM analyzer is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysed. The period of an analysis lasted for 76 minutes.

Correlation coefficient was used to compare the total amino acid components of the each genus. Comparison of the amino acid composition of all the species was carried out using the Analysis of variance (ANOVA).

Results and Discussion
Amino acids profiles of *Cichlidae* and *Claridae* species

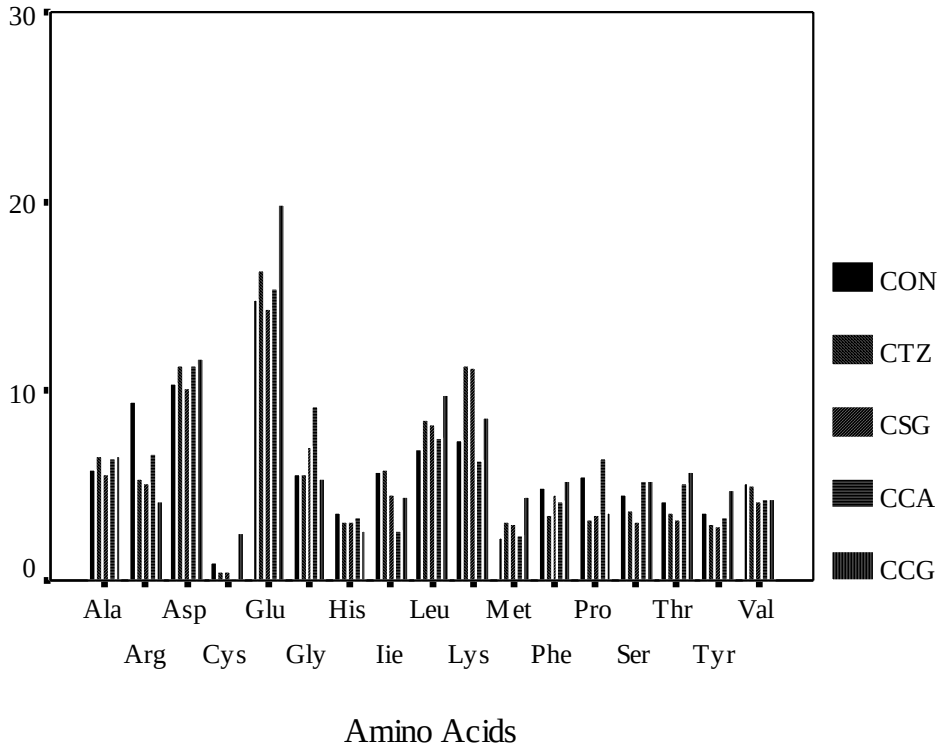

Figure 1: The amino acids of Claridae & Cichlidae species

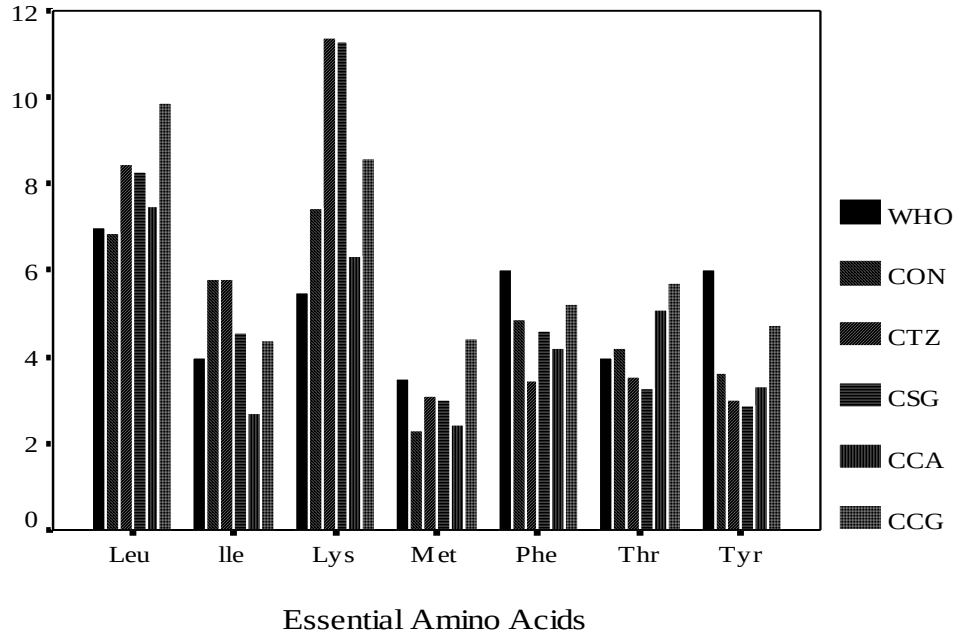

Figure 2: Essential Amino Acids composition of Claridae & Cichlidae

The glutamic acid and the aspartic acid contents were the highest amino acids in both genera (Fig 1). However, the glutamic acid content in all a species was higher than the aspartic acid. *Clarias gariepinus* contained more glutamic acid in the Claridae species while *Tilapia zilli* has the highest in the Cichlidae species. Figure 1 also shows that Cystine recorded the lowest values in all species. However, the Claridae had higher values than the Cichlidae. Tilapia zilli had the highest lysine value followed by *Sarotherodon galilaeus*. When the total amino acid for the Cichlidae species were statistically compared, there exists a highly significant variation while in the Claridae species, the variation was significant ($P<0.05$). Using the correlation coefficient, $r = 0.8787$ for the Claridae while $r = 0.9211$ for Cichlidae

Essential Amino Acids and Diet

Adults require lysine, methionine, phenylalanine, threonine, tryptophan, and valine for good health while infants need these six plus arginine and histidine. Cystine and tyrosine are substitutes in part for methionine and phenylalanine, so they are considered quasi-essential. Figure 2 suggests that the fish species under study may not be good sources of Cystine, methionine, tyrosine and phenylalanine due the low values of these amino acids in them. Tacon & Cowey (1985) observed that Cystine and tyrosine could only be synthesised by fish from methionine and phenylalanine respectively. Therefore, the methionine and phenylalanine requirement of fish will partially depend on the Cystine and tyrosine content of the diet. However, trypotophan, histidine, tyrosine, methionine, and cystein react with singlet oxygen to form peroxides, denaturing the protein in the process (Michaelia & Feitelson 1995, 1997). Tryptophan, histidine, and tyrosine contain double bonds while methionine and Cystein contain a sulphur atom with four nonbonding electrons. These attributes make these amino acids susceptible to attack by electrophilic singlet oxygen.

An important indicator of protein quality is the indispensable/essential amino acid profile. To obtain maximum growth, the dietary EAA should be available at levels equal to or higher than EAA body levels in fish. Steffens (1989) stated that a close correlation exists between the pattern of EAA found in the body tissue of an animal and the dietary requirement pattern.

Statistically, the variations of the total EAA of CCA and CCG from the WHO model are insignificant, but highly significant in the other species. It is significant in the CON species. Fish have natural feeding rhythms and specific dietary preferences that relate to their environment and these should be taken into consideration in designing feeding regimes for aquaculture (Huntingford & Thorpe, 1992). Cichlids are known to exhibit considerable plasticity in feeding biology (Bowen, 1982).

Fatty Acids Profile of Some Freshwater Fish

Freshwater fish oils are unique in the variety of fatty acids of which they are composed and their degree of unsaturation. There are high levels of ω-6 polyethylenic than ω-3 polyethylenic fatty acids. The most common fatty acids presented below have an even number of carbon atoms per molecule and seldom contain functionalities other than cis and trans olefinic saturation, which usually occurs in a methylene-interrupted pattern in polyenes.

Long chain fatty acids (Table 1) are ubiquitous constituents noticed. In a given species, saturated and unsaturated fatty acids occur generally side by side, their structures varying widely in chain length and in degree of unsaturation.

The fatty acids profiles include minor amounts of odd-number, branched-chain, and even-number fatty acids. These varieties as well as the quantity and quality of fatty acids noticed are due to differences in sub-species, diet, spawning cycle, season and environment

Table 1.1: Fatty acids compositions of some freshwater fish species

FATTY ACIDS	CCA	CTZ	CCG	CSG	CON
4:0					
6:0	*				
10:0	*				
11:0	*				
12:0	*				
13:0	*				
14:0	*	*	*	*	*
14:1	*		*		
15:0	*	*	*	*	*
15:1	*		*		
16:0		*		*	*
16:1		*		*	*
17:0		*		*	*
17:1					
18:0	*	*	*	*	*
18:1cis			*	*	*
18:1 trans	*	*	*		
18:2cis		*			
18:2 trans			*	*	*
18:3n-3		*	*	*	*
18:3n-6			*		
18:4		*		*	*
20:0					
20:1n-9					
20:2					
20:3n-6			*		
20:3n-3					
20:4n-6		*	*	*	*
20:5n-3		*		*	*
21:0					
21:1					
22:0	*				
22:1					
22:2					
22:5n-3		*		*	*
22:6n-6		*		*	*
23:0	*				

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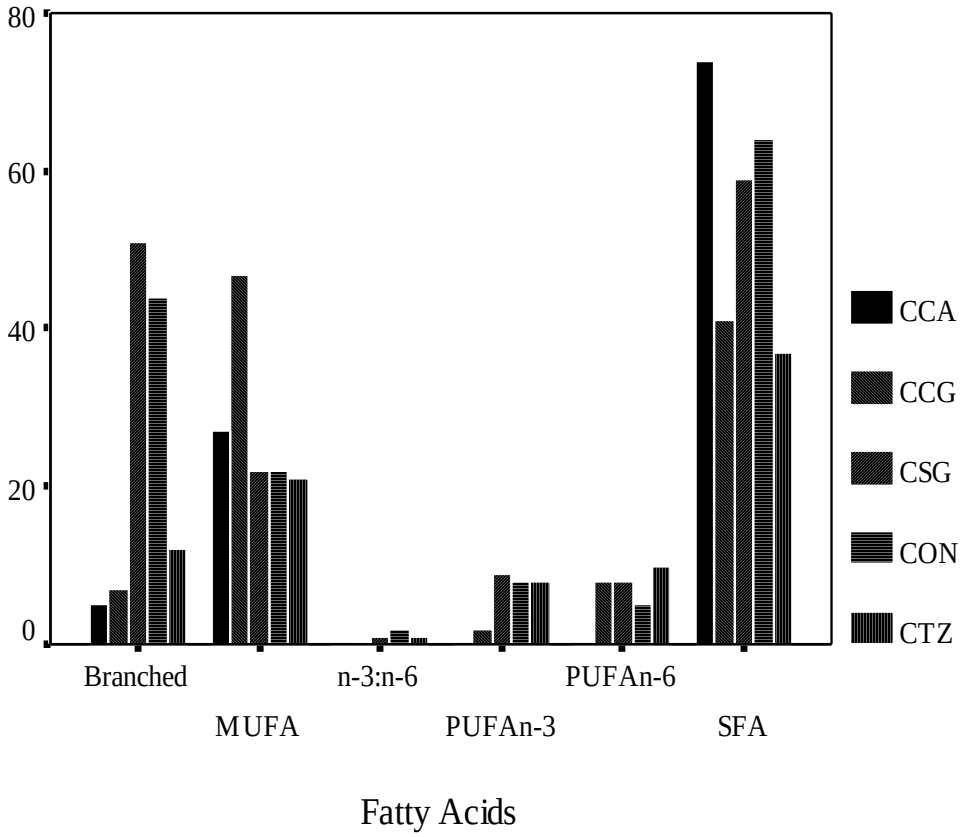

Figure 3: Major Fatty Acids components of Claridae & Cichlidae

The saturated components ranged from 37% to74%. Within these components the major fatty acids were C14:0 and C16:0. The mole percent of each fatty acid seems to vary. The monoene contents ranged from 21% to 47% with C18:1 the prominent MUFA. PUFA attained the lowest value (18%). The branched chain fatty acids identified are C15:0, C16:0, C17:0, C18:2 and C20:0 and is more in the CSG and CON. This high level of branched chain fatty acids in these species has an important advantage. Branched chain fatty acids influence lower melting point, lower cholesterol levels, provide energy, and form an integral part of biomembranes. Branched fatty acids because of their high temperature stability play an important role in the finished product of hydrogenated fish oils. Esterification of branched chain fatty acids to cholesterol causes the fatty acids to stimulate protein synthesis. The branched chain esters influence some ribosomal functions which are necessary for peptide elongation (Hradec *et al*, 1974).

In all the fish species analysed, the dominant PUFA are of the ω-6 series and are found chiefly in C18:2 fatty acids. The essential fatty acids compositions showed prominence in C18:3n-3 and C18:2n-6. The fatty acid, 18:2n-6 is highest in the CTZ but with high DHA content. C22:6n-3 was noted in the tilapia species.

CCA has more of saturated fatty acids than CCG. CCG has more MUFA (Figure 4). All the fish species contain almost same amount of PUFAn-3 and PUFAn-6 and significant saturated lipids but very low value of n-3: n-6. Wild fish has low ω-3: ω-6 ratios. This is needed to reduce high levels of ω-6: ω-3 in most human diets. The essential fatty acids (figure 4) were lacking in CCA while CCG has undetectable level of EPA and DHA. The tilapia species contain all the essential fatty acids although they vary in composition. The degree of unsaturation of fish oils vary with seasons. It rises as the water temperature falls and vice versa (IFFO BULLETIN No.18).

Conclusion

Since varying dietary energy protein availability, age of the fish, feeding habit, gender, genetics, EAA as well as the high cost of purified amino acids could affect studies on the amino acid requirements of these species, the use of information available in this work in diet formulation for these species, and the use of these fish species as food supplement, will help in producing nutritionally acceptable fish feed for the species. It will also give an insight into the quality and quantity of protein in the fish food. Diets are usually developed base on the knowledge of the chemical composition of the potential feed ingredients, which requires knowledge on the nutritional requirements of the animal and the availability of the nutrients within the ingredients.

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