PROTEIN AND AMINO ACID REQUIREMENTS OF WARM-WATER FISHES: A TOOL TO EFFICIENT AND LOW-COST FISH FEED PRODUCTION IN NIGERIA.

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ABSTRACT

Numerous investigations have utilized various semi-purified and purified diets to estimate the protein and amino acid requirements of several temperate fishes. The vast literature on the protein and amino acid requirements of fishes has continued to omit that of the tropical warm water species. The net effect is that fish feed formulation in Nigeria have relied on the requirement for temperate species.

This paper attempts to review the state of knowledge on the protein and amino acid requirements of fishes with emphasis on the warm water species, the methods of protein and amino acid requirement determinations and the influence of various factors on nutritional requirement studies. Finally evidence are presented with specific examples on how requirements of warm water fishes are different from the temperate species and used this to justify why fish feed formulation in Nigeria are far from being efficient.

1.0 INTRODUCTION

Since the early works by Halver (1957) the quantitative requirements for essential amino acid have evaluated in different species (Channel catfish, Robbinson et al 1980, Wilson *et al.* 1978, 1980, NRC 1983, 1991; Carp Nose 1979, Ogiao 1980; rainbow and lake NRC 1983; and many other temperate species including the cichlid fisheries, Ketola 1982, Santiago and Loveall 1988). Apart from the Cichlid fisheries the vast literature on the protein and amino acid requirement of fish has continued to omit the tropical fish species. The effect of using supplements of crystalline or synthetic amino acid to meet the requirement have not been thoroughly investigated in relation to Africa's peculiar environmental and economic circumstances. The net effect is that fish feed formulations in Nigeria have relied on the requirement data provided by the National Research Council for temperate fishes. This is not good enough as the feed ingredient situation in the developed countries is different from that in Nigeria.

The African sharp tooth catfish and the cichlid fishes for example are important component of Nigeria's aquaculture; most research efforts have concentrated on the feeding and breeding biology of these species with little effort on their nutritional requirement studies. Even in the search for alternative feed ingredient, it is imperative that the requirement for specific nutrients for these must be determined to facilitate the production efficiency and cost effective feeds. Most nutritional studies in Nigeria have relied on the protein and energy data provided by NRC data and meal on the classical work of Machiels and Henkels (1986) on protein and energy requirements of *Clarias gariepinus*. However protein requirement is determined by the component essential amino acids.

This paper therefore attempts to review the state of knowledge on the protein (amino acid) requirement of fishes, the methods of amino acid requirements determinations and the influence of various factors on requirement.

Examples are further presented based on recent studies on the amino acid requirement of some warm-water species in Nigeria.

3.0 STATE OF KNOWLEDGE ON PROTEIN AND AMINO ACID REQUIREMENT AND INTERPLAY OF ENERGY:

Protein is the basic component of animal tissues and is therefore an essential nutrient for both maintenance and growth. At maintenance level, the fish requires for replacement of worn-out tissues and proteinous products such as internal epithelial cells, Enzymes and hormones, which are vital for the proper function of the body, and are recycled quite rapidly. The requirement of protein is therefore obvious since protein constitutes 45-47% of the tissues dry matter.

The capacity of fish to synthesis protein de-novo from carbon skeleton is limited and most of the protein must, therefore be supplied through the diet. Thus the content of protein in the diet and its ratio to the metabolisable energy becomes of prime importance. Rate of protein synthesis varies among fishes but is generally lower in and animals. With intensive feeding, a broiler chicken will grow from 40g to 1.8kg or increase its weight 45 times in 7 to 8 weeks whereas a young channel catfish (20g) with intensive feeding will increase its weight only to 4 to 8 times during this time interval (Lovell, 1989). Information on protein synthesis rate in fish is scarce but in rats about 700mg of muscle protein per 100g weight/day is synthesized. Only about 150mg of new tissue is gained indicating a high rate of protein turnover.

Fish use protein efficiently as a source of energy. A high percentage of the digested energy in proteins is metabolisable in fish than in land animals. The heat increament for protein consumed is lower in fish than in mammals or birds, which gives a higher productive energy value for fish. This is attributed to the efficient manner of nitrogen excretion in fish; Studies with channel catfish (Lovell, 1989) in which dietary protein was increased above 45% without proportionate increases in non-protein energy suppresses the growth rate of fish. Thus the content of protein in the diet and its relationship to the metabolisable energy is very important in protein requirement (amino acid requirement) of fishes. The most efficient level of feeding is attained only when the correct supply of energy and essential nutrients are available in the proportions required by the fish for maintenance and growth. Any deviation from this will change the quantitative food requirement.

In the transformation of food energy to net energy available for metabolism, and growth, a considerable portion is lost. The digestible energy is part of its food energy which is absorbed by the fish, while energy ejested in the faeces is lost. Some of the digestible energy is lost through the gills. This loss is largely the result of catabolism of protein and their deamination when amino acids are utilized for energy rather than for synthesis of new tissue proteins. The energy assimilated in the body is metabolisable energy, but part of it is again lost through a number of processes. The loss of energy through these processes grouped together is known as the "SPECIFIC DYNAMIC ACTION"

The metabolisable energy value of dietary protein varies with the amino acid composition of the protein its digestability, and whether the amino acids are retained by the animal for protein synthesis or are deaminated and their carbon skeleton utilized for energy.

For mammals, the metabolisable energy value of dietary protein has been firmly established. The value of 4.1kcal/g has also been used for fish (Phillips et al. 1966, Hastings and Dupree, 1969).

However, with fish, ammonia being the excretory product, a different ME value for protein has been established.

Smith (1971) and Gropp (1979) have quoted a range of 4.2 kcal/g and 4.9/kcal/g for fish taking into account (i) the calorific value of NH_3 and substracting this value from the calorific value of protein (ii) the average true digestibility of protein by fish is 94-95% (Ogino and Chem 1973). These values are higher than those for mammals and fowls and should be taken into consideration in nutritional experiments with fish. A more precise value should of course, be calculated according to the digestibility of the various feedstuffs by the different fish species.

3.0 METHODS OF QUANTITATIVE PROTEIN AND AMINO ACID REQUIREMENT DETERMINATION:

Quantitative methods through growth and labelling studies have been used to establish the essential amino acid requirement of fish. Halver (1957) developed the amino acid test diets and determined that ten amino acids were essential for the growth of fish. Aoe et al (1970) Dupree and Halver (1970) Nose et al. (1974) have used the amino acid test diet of Halver (1957) in a neutralized form to determine the qualitative amino acid requirement of the channel catfish and the common carp. Convey et al (1971) have also used isotopic labelling technique to determine the essentiality of some amino acids in plaice and sole. These studies and others showed that all fin fish studied require the same ten amino acids which are considered essential for most animals.

Quantitatively, amino acid requirements have been studied through the use of growth studies (Nose 1979) Wilson et al. 1977; amino acid test diets, Kaushik, 1979, Dabrowski 1981, Halver *et al.*1958, semi purified diets; Walton et al 1958, semi purified diets, Walton et al 1984; Jackson and Capper 1982, practical - type diets.) Amino acid requirement values have thus been estimated based on the growth response or Almquist plots in which a break point with increasing level of test amino acid corresponds to the requirement of the specific amino acid at which growth rate levels - off or plateaus.

METHODS USED TO CALCULATE THE BREAKPOINT LEVEL ARE

(1) Normal almquist plot (Delong et al 1962)

(2) Use of regression analysis to generate the Almquist plot (Abiyamn *et al.* 1985).

(3) Broken like model (Problems *et al.* 1979).

(4) Quadratic regression and the broken - like model (Santiago and Lovell 1989). The model which gave the lower Mean square Error (MSE) was used to determine the requirement values. Convey and Luquett (1983) have critisised the first 3 methods enumerated above that they do not represent an accurate determination of the amino acid requirements of fish because (a) they represent a limited number of studies for fish. (b) factors such as fish size temporation genetics, feeding rate, energy concentivasim and other diet factors rate, that influence the reported requirements for amino acids.

Other methods which have been excellently reviewed by Halver (1989) include the use of serum or tissue amino acid levels (kaushik, 1979, Wilson *et al.* 1980) and the use of oxidation studies (Walton et al. 1984). D'Abramo and Lovell (1991) reported that an investigation into a possible relationship between levels of free essential amino acids might assist in the estimation of some amino acid requirements. A strong relationship between the requirement pattern and whole body pattern of essential amino acids has been demonstrated for carp (Convey and Tacon, 1983) and channel catfish (Wilson and Poe(1985). With this relationship established, an account determination of the requirement of the most limiting dietary essential amino acid, could lead to estimate of the dietary requirements of all other essentials amino acids. Such as interpolative approach would circumvent the logistic difficulties and inordinate time demands currently encountered in attempts at empirical determinations.

Amino Acid requirements commonly presented generally represent maximum performance levels and not necessarily the most economic levels. In view of the economic importance of protein and amino acid requirements in the formulation of cost-effective feeds, their requirement should be further evaluated. One way is to subject growth response data to quadratic Regression analysis which can yield amino acid requirements for maximum and less than maximum growth rates. For example, Santiago (1985), determined with Nile Tilapia that the dietary requirement for arginine for maximum growth in an amino acid balanced diet has 4.4% of the protein, while 3.5% (25% less) could produce a growth rate that was within the 95% confidence limit of maximum growth. These results indicate that 20% less arginine can be fed to Nile tilapia in an otherwise amino acid balanced diet with probably a significant reduction in feed cost but with only 5% likelihood of a reduction in growth rate. In the use of alternative ingredients for the production of least-cost ration for fish, linear programming is the commonly used method. This method selects the least - cost contribution of ingredients to meet certain fixed nutrients specifications, such as the requirement levels reported for optimum growths presented by the National Research Council. Linear programming, does not take into account "diminshing Marginal Productivity which is defined as a dimination in growth response as nutrient level in the diet approaches the requirement for maximum growth. When the cost of dietary protein (amino acid) source is low in relation to the price of fish feeding for near maximum weight gain may be most profitable but as protein becomes more expensive, a lower amount in the diet may be more economical. Thus, in realisation of the high cost of protein ingredient in Nigeria it is advocated that the use of quadratic programming in place of linear programming should be used in least - cost fish feed formulation. This is in accordance with the views of D'Abrano and Loveall (1991). Quadratic Programming to formulate fish feeds uses diminshing marginal productivity in selecting the most-effective combination of feed ingredients. The model contains a "biological response function" which describes the change in growth that will result as a given level of the nutrient (protein) increases in a feed under a selected set of conditions.

4.0 RELATED STUDIES ON PROTEIN AND AMINO ACID REQUIREMENTS OF FISH.

Many experiments have been conducted to determine the optimal level of protein in the diet for various fish species. However the interpretation of the results of these experiments is not always easy. Since protein and amino acid requirements are affected by and interacts with many factors such as environmental conditions specific physiology and feeding habits as well as age and developmental stage of the fish, the results of the experiment, under a set of conditions are necessarily true for a different set of conditions without understanding more about the relationship between protein and amino acid requirement and factors affecting it the results of the experiments cannot be generally applied.

The dietary protein, energy and amino acid requirements in various fish species are presented in Tables 1 (Hepher, 1988) and 2 (NRC, 1991). One important observation in these tables is the fact that most of the species quoted are those for the temperate countries. In few cases for Oreochromis and tilapia species (tropical) the experimental conditions and procedure for obtaining these values are different from conditions operating in the tropical environment. Values quoted in the NRC (1993) publications on the nutrient requirement for warm water fish species did not also reflect the requirements of the indigenous tropical warm-water fishes. The result is that feed formulation for fish in this part of the world relies heavily on figures based on the requirement that were established on environments completely allien to the tropical environments.

This approach is wrong, because, in accordance with the views of Robbinson (1989), it is difficult to set a level of requirement that is optimum for all situations because requirement of fishes are affected by the factors already enumerated earlier on in section of along with other factors such as feed allowance, and the management practices. The feeding habits and the costs of ingredients to meet these requirement levels are additional important considerations for a cost-effective fish production in a tropical environment such as Nigeria.

The minimum amount of protein needed to supply the necessary nitrogen and amino acids has been studied in fishes reared in ponds or in the laboratory under controlled conditions using either practical or purified diets (see Tables 1 & 2).

Machiels and Henken (1986) worked with purified feed ingredients and concluded that *Clarias gariepinus* requires a dietary protein content in excess of 40% for maximal growth irrespective of dietary energy level. In cases where practical diets were used, either a single animal protein sources that are defficient in one essential amino acid or the other used. For example, Ufodike and Ekokutu (1986) using blood meal as the sole protein source found that diet mixtures containing 50.2% protein resulted in better growth responses in *Clarias gariepinus* than diets containing 38.0% or 62.9% protein.

Preliminary studies on the gross protein requirement of *Clarias gariepinus* were carried out by Faturoti, Balogun, Ugwu (1986) and Ayinla (1988). The summary of their findings revealed the following protein requirements at the different growth phases.

(a)	Fingerling to Juvenile	37.5%
(b)	Juvenile to Adult	32.5%
(c)	Fingerling to Adult	35.5%

In a recent study, Balogun (1990) determined the Lysine and methiomine requirements of Clarias gariepinus at the fingerling stage using 37.5% protein diets. The results (table 3) showed that for maximum weight gain PER, protein gain, a dietary lysine level of 2.25% corresponding to a level of 6.00% of dietary protein was obtained. Best feed, efficiency was obtained at 1.50% dietary lysine level which corresponds to 4.00% of dietary protein.

However, when summed across the parameters used an average lysine requirement of 2.04% corresponding to 5.44% of dietary protein was obtained.

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Table 1. Dietary protein and energy levels r[illegible] in highest growth rates in vari[illegible] [illegible]cies [illegible]

Fish species Source	Crude dietary protein (%)	Gross dietary energy (kcal/kg)	Protein energy ratio (mg/kcal)	
Anguilla japonica	44.5	--	---	
Chanos chanos	40.0	3650	110	
Chrysophrys auratus	40.0	5370	74	
Clarias gariepinus	40.0	4441	90	Machiels & Henke 1986
Ctenopharyngodon idella	45.6	--	---	
Cyprinus carpio	31.0	3100	100	
	33.0	3060	108	
	35.0	5430	64	
	38.4	3860	99	
	38.5	3700	104	
	40.6	3265	124	
	42.0	--	---	
	42.0	4883	86	
	45.0	4920	91	
	45.9	--		
	45.9	5330	86	
Dicentrarchus labrax	40.0	3000	133	
Epinephelus salmoides	50.0	3410	147	
Fugu rubripes	50.0	--	---	
Ictalurus punctatus	25.0	2670	9[illegible]	
	25.3	--	---	
	35.0	--	---	
	35.0	--	---	
	35-40	4040	93	
	36-40	2750-3410	85	

Source: Hepher, 1988 (Nutrition of Pond Fishes)

Table 2. Nutrient Requirements for Channel Catfish, Salmonids, Carps, Tilapias, and Eels as Percentages or Milligrams per Kilogram of Diet (as fed basis)[a]

		Channel catfish	Salmonid	Carp	Tilapia	Eel
Energy base[b]						
(Kcal DE/ kg diet)		3,000	3,600	3,200	3,000	4,200
Dig. Protein	%	32	36	35	32	44
Argine	%	1.38	1.51	1.50	1.34	1.98
Histidine	%	0.48	0.65	0.74	0.54	0.92
Isoleucine	%	0.83	0.86	0.88	0.99	1.76
Leucine	%	1.12	1.41	1.16	1.09	2.33
Lysine	%	1.63	1.80	2.00	1.63	2.33
Methionine + cystine	%	0.74	1.04	1.09	1.02	1.41
Phenylalanine + tyrosine	%	1.60	1.84	2.28	1.82	2.55
Threonine	%	0.64	0.79	1.37	1.15	1.76
Tryptophan	%	0.16	0.18	0.28	0.32	0.48
Valine	%	0.96	1.15	1.26	0.90	1.76

Source: NRC (1991) UNPUBLISHED)

Table 3. Summary of Lysine Requirement of Clarias Gariepinus Fingerlings

Parameter	Lysine Requirement (% of Diet)
Weight gain (g)	2.25 (6.00)*
Specific growth rate (%)	1.75
Protein efficiency ratio	2.25 (6.00)
Protein gain	2.25 (6.00)
Feed/gain ratio	1.50 (4.00)
Mean	2.04 (5.44)

* Figures in parenthesis are requirement expressed as dietary protein.

Source: Balogun (1990) Unpublished data