# THE NUTRITIONAL VALUE OF CASSAVA (*Manihot esculenta* Crantz) BASED DIET FOR AFRICAN CATFISH (*Clarias gariepinus*)

by

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**Thesis submitted in fulfillments of the**
**requirements for the degree**
**of Master Science**

**February 2007**

*In the name of Almighty Allah, the most gracious the most merciful*

## ACKNOWLEDGEMENTS

Alhamdulillah, after enduring all the challenges, this research has been accomplished. I feel pleasure to place my sincere appreciation and gratitude to my supervisor Prof. Roshada Hashim for her persistent effort, guidance, and encouragement during the course of my study at the School of Biological Science, University Science of Malaysia. I am also deeply grateful to her for her patiently reviewing and editing of this thesis. Special thanks to Dr. Khoo Khay Huat for his generous advice and assistance in some of my experiment.

I wish to express my sincere appreciation and thanks to En. Jamil and Auntie Anna for their friendly help and co-operation with all analyses in the laboratory and fish house. Thanks are also due to En. Abu Bakar and En. Jauhari for helping me in the histological analysis. To my fellow researcher of the Aquaculture Research Group, Khalidah, Finie, Che Pah, Karloon, Preeda, Mohammed and Ahmed, thanks for created a pleasant working environment, invaluable discussion and warm friendship. My thanks are also extended to my entire friend at the 'Achenese Student Club", the 'Unsyiah OECF Group" and my Malaysian friends. In Indonesia, I would like to thank my best friends and colleagues, Allahyarham Kak Susy, Kak Rini, Kak Nuna, Kak Eti, Pedy, Wahyu, Iyan and Arman for their excellent friendship and always helping me with the back home matters. Most specially I must thank my lovely friends and my well-wishers Sofia, Irin, Wina, Zuhra and Intan for their enormous support and always lend a helping hand when needed.

I am indebted to my employer, Veterinary Medicine Faculty of Syiah Kuala University, Aceh for providing me the opportunity to undertake this study. I do gratefully acknowledge Syiah Kuala University for granting the financial support and School of

Biological Science, University Science Malaysia that giving me the space and facilities to do research.

My heartfelt respect and special thanks must go to my beloved father Allahyarham Nurdin Aziz and mother Zainab Abbas, in-laws (Teuku Risyad and Nurbaya), and family back home for their never-ending support, blessings, love that encourages me to pursue higher studies. I also acknowledge my boundless gratitude to Kak Nana, Kak Mala, Kak Jeumpa, Kak Irma, Kak Lasmi and Inda for their never-stopped calling and message, sincere encouragement and constructive criticism.

From the bottom of my heart, I pledge my long-lasting gratitude and love to my husband Iskandar and my daughter Cut Najwa Azzahara for their love, patience, sacrifice and constant inspirations that undoubtedly helped me keep my wisdom all through these hard times. All these effort and success are meaningless without them by my side.

**Amalia Sutriana**

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| | |
|---|---|
| AABA | Alpha amino butyric acid |
| ADMD | Apparent dry matter digestibility |
| AIDAAA | Apparent indispensable amino acid availability |
| ALA | Alpha linolenic acid |
| APD | Apparent protein digestibility |
| CLM | Cassava leaf meal |
| CRM | Cassava root meal |
| DAA | Dispensable amino acid |
| FM | Fish meal |
| FCR | Food conversion ratio |
| GE | Gross energy |
| HCN | Hydrogen cyanide |
| HSI | Hepato somatic index |
| IDAA | Indispensable amino acid |
| LA | Linoleic acid |
| NFE | Nitrogen free extract |
| PER | Protein efficiency ratio |
| SBM | Soybean meal |
| SCN | Thiocyanate |
| SGR | Specific growth rate |
| WM | Wheat meal |
| HEPES | N-2 Hydroxyethylpiperazine-N-2 ethanesulphonic acid |

# NILAI NUTRISI DIET BERDASARKAN UBI KAYU (*Manihot esculenta* Crantz) UNTUK IKAN KELI AFRIKA (*Clarias gariepinus*)

## ABSTRAK

Empat kajian telah dilaksanakan untuk mengetahui nilai nutrisi serbuk daun ubi kayu (CLM) dan serbuk ubi kayu (CRM) ke arah membangunkan diet kos yang efektif bagi ikan keli Afrika *(Clarias gariepinus)*. Nisbah protein haiwan kepada protein tumbuhan dalam setiap eksperimen adalah sama iaitu 1 : 1. Eksperimen pertama dijalankan untuk menilai kesan penggantian serbuk kacang soya (SBM) dengan CLM secara berperingkat, terhadap penampilan anak ikan keli Afrika (1.45 g ± 0.14). Empat diet praktikal (30% protein) telah dirumuskan dengan setiap satunya mengandungi 0%, 10%, 20% dan 30% CLM. Selepas 12 minggu keputusan menunjukkan ikan keli yang diberi makan diet yang mengandungi CLM mengalami kemerosotan pertumbuhan dan penggunaan makanan serta keterhadaman protein berbanding yang menerima rawatan diet kawalan.

Eksperimen kedua telah dijalankan bagi menentukan kesan penambahan CLM dengan dan tanpa penambahan DL-metionina terhadap pertumbuhan anak ikan keli (2.84 ± 0.08 g) selama 8 minggu. Satu kumpulan diberi makan dengan diet kawalan yang sumber protein hanya terdiri daripada serbuk ikan (FM) dan serbuk kacang soya (SBM) sahaja (40% protein). Kumpulan 2, 3 dan 4 diberi makan dengan diet yang masing-masing mempunyai 10%, 20% dan 30% penggantian CLM daripada jumlah kandungan protein SBM. Bagi 3 kumpulan lagi (Diet 5, 6 dan 7) mempunyai komposisi yang sama dengan diet 2, 3 dan 4 kecuali terdapat penambahan DL-metionina bagi memadankan kandungan metionin yang sama dengan diet kawalan. Kepekatan kandungan tiosianat di dalam hati, insang dan otot di jalankan serta pemeriksaan histologi hati turut dilakukan. Diet kawalan telah menghasilkan

pertumbuhan, penggunaan makanan, keterhadaman protein dan keseimbangan kandungan asid amino perlu yang terbaik. Hanya Diet 2 (10 % CLM) boleh diterima jika dibandingkan diet kawalan dari aspek FCR dan PER. Peningkatan kandungan CLM mengakibatkan peningkatan tiosianat dalam tisu otot dan lebih banyak lagi dalam hati ikan. Siasatan histologi telah mendedahkan perubahan tisu hati ikan keli Afrika yang diberi makan CLM dengan adanya pembentukan vakuol periportal.

Pengunaan kombinasi CLM dengan CRM sebagai sumber protein dan tenaga bagi ikan keli Afrika telah dijalankan pada eksperimen yang ketiga. Sepuluh diet telah dirumuskan bertujuan mempunyai tiga paras CLM yang berbeza dan digabungkan dengan tiga paras CRM. Diet 1 tidak mengandungi CLM mahupun CRM dan dianggap sebagai Diet kawalan. Diet 2, 3, 4 mengandungi 10% CLM di gabungkan bersama-sama dengan 10%, 20% dan 30% CRM masing-masing. Manakala Diet 5, 6 dan 7 mengandungi 20% CLM digabungkan bersama-sama dengan 10%, 20% dan 30% CRM masing-masing. Diet 8, 9 dan 10 mengandungi 30% CLM digabungkan dengan 10%, 20% dan 30% CRM. Keputusan menunjukkan peningkatan kandungan CLM dan CRM dalam diet menghasilkan pertambahan berat dan keefisienana penggunaan makanan yang lebih rendah secara signifikan (P<0.05) berbanding dengan Diet kawalan. Secara amnya, pada paras 10 % CLM, penambahan CRM menghasilkan pertumbuhan dan keefisienan makan yang lebih tinggi berbanding penambahan 20% dan 30 % CLM.

Penilaian kesan saiz ikan terhadap tindak balas toleransi diet berasaskan ubi kayu dijalankan dengan anak ikan keli Afrika (5.7 ± 0.09 g) dalam eksperimen 4. Lima Diet praktikal dirumuskan dengan diet berasaskan FM, SBM dan serbuk gandum (WM) ditambah dan bertindak sebagai diet kawalan. Diet 2 dan 3 mengandungi 20% CRM dan 40% CRM manakala Diet 4 dan 5 mengandungi 10% dan 20% CLM. Keputusan menunjukkan bahawa ikan keli Afrika (*Clarias gariepinus*) yang makan diet 10% CLM

mempunyai pertambahan berat, nisbah penukaran makanan, kadar pertumbuhan spesifik, nisbah keefisienan protein, dan keterhadaman protein, yang hampir sama dengan diet kawalan. Pertumbuhan dan keterhadaman nutrient oleh ikan yang makan diet 10% CLM adalah lebih tinggi secara signifikan berbanding ikan yang diberi makan diet 20% CLM. Dengan merujuk kepada diet CRM sahaja, tiada perbezaan signifikan dalam pertumbuhan ikan yang diberi makan diet 20% CRM dengan 40% CRM. Namun, perbezaan yang signifikan dapat dikenal pasti dalam aspek keterhadaman bahan mentah (ADMD) bagi ikan yang makan diet 20% CRM mempunyai nilai yang lebih tinggi berbanding ikan yang diberi makan diet 40% CRM.

Kajian ini telah menunjukkan bahawa tahap CLM yang rendah (10% dripada jumlah diet) boleh digunapakai dalam diet anak ikan kelu Afrika tanpa menjejaskan pertumbuhan, penggunaan makanan dan keterhadaman protein. Oleh kerana nilai APD dan PER hampir sama antara Diet kawalan dan Diet mengandungi 10 % dan 40 % CRM, untuk alasan ekonomi, 40 % CRM boleh diguna pakai dalam diet anak ikan keli afrika.

# THE NUTRITIONAL VALUE OF CASSAVA (*Manihot esculenta* Crantz) BASED DIET FOR AFRICAN CATFISH (*Clarias gariepinus*)

## ABSTRACT

Four studies to evaluate nutritional value of cassava leaf meal (CLM) and cassava root meal (CRM) (*Manihot esculenta* Crantz) in order to develop a suitable cost effective diet for African catfish (*Clarias gariepinus*) was conducted. All experimental diets were prepared with a 1 : 1 animal and plant protein ratio. The first experiment was carried out to evaluate the effect of graded levels CLM at the expenses of soybean meal (SBM) on African catfish fry (1.45 ± 0.14) performance. Four practical diets (30 % crude protein) were formulated to contain CLM at 0 %, 10 %, 20 % and 30 % levels. After 12 weeks, fish fed increasing levels of CLM showed growth depression, poor feed utilization, apparent protein digestibility (APD) as well as imbalance indispensable amino acid (IDAA) compared to the control diet.

The second experiment was conducted to determine the effect of graded levels of CLM with and without DL-methionine supplementation on performance of small African catfish fingerlings (2.84 ± 0.08) for 8 weeks. One group was fed on a control diet with fish meal (FM) and SBM as the protein source (40 % CP). Groups 2, 3 and 4 were fed graded levels of CLM to replace 10 %, 20 %, 30 % of the total diet at the expenses of SBM. Groups 5, 6, 7 were fed on CLM with levels similar to the groups 2, 3, 4 respectively, but supplemented with crystalline DL-methionine to match the methionine concentrations in the control diet. Thiocyanate concentration was determined in liver, gill and muscle and the liver histology determination were also carried out. The control diet produced the best growth performance, feed utilization, APD and IDAA composition. Only 10 % CLM diet compared favorably in terms of food conversion ratio (FCR) and protein efficiency ratio (PER) values with control diet.

Dietary inclusion of graded levels CLM resulted in increasing levels of thiocyanate in fish tissues and was more pronounced in the liver. Histology studies revealed alterations in African catfish liver tissue fed on CLM diet characterized by periportal vacuolation.

The use of combination of CLM and CRM as a protein and energy source respectively for African catfish fry was carried out in Experiment 3. Ten diets were formulated to contain 3 different levels of CLM and combine with 3 levels of CRM. Diet 1 was contained no CLM and CRM and considered as a control diet. Diets 2, 3, 4 contain 10 % CLM in combination with 10 %, 20 %, 30 % CRM respectively. Diets 6, 7, 8 contain 20 % CLM in combination with 10 %, 20 %, 30 % CRM respectively. Diets 8, 9, 10 contain 30 % CLM in combination with 10 %, 20 %, 30 % CRM respectively. The result showed that increasing the amounts of CLM and CRM in the diet resulted in significantly lower ($P<0.05$) weight gain and feed efficiency compared to control diet. Increasing levels of CRM at each level of CLM resulted in decrease growth performance numerically. Generally, at levels of 10 % CLM, inclusion of CRM resulted in higher value for growth and feed efficiency compared to 20 % and 30 % CLM inclusion.

Evaluation of the effect of fish size on the tolerance response to cassava based diets was conducted with African catfish fingerlings (5.7 g ± 0.09) in Experiment 4. Five practical diets were formulated with FM, SBM and wheat meal (WM) based diets was included as a control diets. Diet 2 and 3 contained 20 % CRM and 40 % CRM, while Diet 4 and 5 containing 10 % and 20 % CLM. The results shows that African catfish fed 10 % CLM diet had similar growth response and digestibility to those of control diet. Growth performance and nutrient digestibility of fish fed 10 % CLM diet were significantly higher than those fed 20 % CLM diet. With respect to dietary of CRM alone, no significant different in growth response was obtained for fish fed 20 % CRM and 40 % CRM. However, significant different values were noted in term of apparent dry matter digestibility (ADMD) in which fish fed the 20 % CRM diet had higher value than those fed 40 % CRM.

These studies indicate that low level of CLM (10 % of total dietary) can be incorporated in the diet for African catfish fingerlings without adverse effects on growth performance, feed utilization and protein digestibility. Since Similar values were observed in terms of APD and PER between Diet control and Diet containing 20 % and 40 %, it may possible for economic reason, to use up to 40 % CRM in African catfish fingerlings diet, but over a longer feeding period.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

African catfish (*Clarias gariepinus)* is an importance species that is cultivated commercially world wide. The growing interest in culture of African catfish has been due to its high fecundity, fast growth, ability to utilize a wide range of food item and resistance to extreme environmental conditions (Hogendoorn, 1983, Hetch *et al.*, 1997, Fagbenro *et al.*, 1998, Maithya, 1998,). This fish has also been proven to be a very suitable species for high-density culture (Huisman and Richter, 1987).

As interest in the commercial culture of African catfish increase, the need to develop a cost effective feed becomes more critical. Currently, low cost feed used by catfish farmers are formulated using mixture of animal and plant based protein ingredient. Since catfish feeds tend to require high protein levels ranging from 25 % to 50 %, carbohydrate can be used to spare the protein, since carbohydrate is cheaper and more readily available than protein sources.

Nutrition represent over 50 % of operating cost in intensive aquaculture with protein from fishmeal (FM) being the most expensive dietary sources (El Sayed, 1999). Thus studies over decades have been focused on possibilities of total and partial replacement of fishmeal protein with several plant protein sources, which are less expensive as aquaculture feeds. Soybean meal (SBM) is currently a favorable choice due to its abundance and a relatively favorable essential amino acid profile of capable of meeting the essential amino acid requirements of fish except sulfur containing amino acids (NRC, 1993).

Considerable research has been done to asses the potential of using soybean meal as a substitute for fish meal in diets for African catfish (Degani *et al.,* 1989, Balogun and Ologhobu, 1989). Most studies conducted shown that total replacement of FM by SBM results in reduced growth and feed efficiency. This is most probably due to the presence of antinutritional factors and indigestible oligosaccharides in SBM (Lim *et al.*, 1998). Currently, soybean meal is still the major protein source used in African catfish feeds, and often this protein source constitutes up to 50 % and more of the diet by weight (Fagbenro and Jauncey, 1995). Since soybean meal still an imported ingredient in many tropical countries (Ng and Chen, 2002), alternative protein sources that are abundant and locally available are of considerable in these regional.

Cassava, *Manihot esculenta* Crantz, is a major agricultural crop which is plentiful available and inexpensive products in tropical countries. This plant is often considered as two distinct crops: roots rich in energy and leaves rich in protein (Okezie and Kosikowski, 1983). Old Cassava leaves generally go to waste after harvesting of cassava roots while young cassava shoots are used for human consumptions. Cassava leaves are rich in protein, mineral and vitamin (Muller *et al.*, 1974 as quoted by Eruvbetine *et al.*, 2003). Proteins of cassava leaves are rich in lysine, marginal in tryptophan and isoleucine but deficient in sulfur containing amino acids (methionine and cysteine) (Hutagalung *et al.*, 1974). However, Rogers and Milner, 1963 as cited by Jalaluddin (1977) have considered cassava leaf protein quality to be as superior as soybean protein quality.

Cassava leaf meal (CLM) has been used primarily as a feed for livestock and ruminant animals, but it utilization in fish feeds has been limited. Ng and Wee (1989) observed the depression of growth and decreasing feed utilization in tilapia (*Oreochromis niloticus)* when sun dried cassava leaves substituted the 20 % protein of FM. The depression of growth was attributed to low protein digestibility of CLM and to sub optimal concentration of sulfur amino acids. The situation in African catfish (*Clarias gariepinus)* is presently unclear. Twenty percent of cassava leaves can be incorporated in the diet of African catfish fingerling (initial weight of 6.2 g) of total dietary at the expense of SBM without resulting in significant difference of biomass yield and final weight as compared to the control diet containing FM and SBM as protein sources, (Bureau *et al.*, 1995). This study took place in cages located in ponds that enabled the fish to obtain indirect nutrition from natural food. However, Padua *et al.*, (1998) also found progressive reduction in growth performance of Pacu (*Piaractus mesopotamicus*) juvenile that were fed with increasing level of cassava foliage meal, even the fish were also reared in pond.

The use of CRM in aquaculture has also been conducted by several workers. Early research showed that CRM individually can comprise at least 40 % of the diet for common carp (*Cyprinus carpio*) (Ufodike and Matty, 1983) and 60 % of the diet for tilapia (*Oreochromis niloticus*) (Wee and Ng, 1986) without adversely affecting fish performance. However, Bureau *et al.*, (1995) conclude that the use of CRM based diets had adverse effects on the growth and feed conversion efficiency of African catfish as compared with corn based diets.

It seems no attempts has been made previously to evaluate the direct nutritive value of CLM and CRM as well as the percentage of CRM that can be included in African catfish diet when CLM used as a partial protein sources.

Cassava is also known to contain cyanoglucosides which hydrolyzed into cyanohydrin by enzyme linamarase is hydrolyzed into cyanohydrin and subsequently into cyanic acid (HCN). The hydrolysis takes place either during the preparation of cassava as a food or in digestive tracts of animals and human (Fukuba and Mendoza, 1984). The effectiveness of some of the traditional cassava processing such as drying, boiling, soaking and fermentation in reducing cyanide has been studied (Cooke and Maduagu, 1978, Best, 1979, Gomez and Valdivieso, 1985, Ravindran *et al.*, 1987, Fish and Trim, 1993, Saka, 1995, Essers *et al.*, 1991). It was found that these processes rapidly remove the HCN but was less effective in removing bound cyanide. Substantial amounts of bound cyanide may therefore remain in processed foods (Fukuba and Mendoza, 1984).

Kamalu (1993) found that the liver of the dog fed cassava based diets of 10.8 mg cyanide/ kg diet showed pathological changes which includes congestion, periportal vacuolation and pycnosis of some nuclei. However, long term feeding of giant rat (16 weeks) with cassava based diets showed no pathological lesions in liver (Tewe, 1984). To date there is no information available on the effect of cassava based diet with varying in cyanide content on fish liver histology, particularly African catfish (*Clarias gariepinus)*. Histological examination of fish tissue can yield useful information on chronic tissue damage before the effects become apparent at the organismal level (Ruby *et al.*, 1979).

Although cyanide is generally considered to be a potent poison, in low level it is probably less toxic. Cyanide is rapidly absorbed from the upper gastrointestinal tract followed by rapid convertion to thiocyanate in a reaction between cyanide and thiosulphate catalyzed by the enzyme rhodanese (thiosulphate sulfurtransferase) in the liver. This would be an effective mechanism to detoxify cyanide. Thiocyanate is less toxic than cyanide and major excretion product of cyanide metabolism (Solomonson, 1981).

In addition, Westley, (1981) reported that cyanide detoxification mechanism increase requirement for sulfur containing essential amino acids. Consumption of cassava is known to reduce the plasma levels of sulfur amino acids in human and several studies with farm animals have indicated that dietary supplementation of sulfur amino acids above the levels normally required, may be needed to enable high levels of performance (Davis, 1981). No previous work has been done to understand the effect of sulfur amino acids concentration in the dietary cassava for African catfish feeds and to investigate its effect on performance of African catfish.

Cassava based diets may have particular relevance in tropical developing countries where commercially produced pelleted fish feed are scarce and expensive. Small scale farmers can produce farm-made pelleted feeds using ingredients that are abundant, locally available and inexpensive products such as cassava by products, to reduce the cost of fish production. Therefore, the information about the nutritive value and antinutritive value of cassava based diets is important in the assessment for the development of economical use of this protein and energy source in aquaculture particularly in African catfish diets.

## 1.2 Objectives of the Study

The main objective of the study was to evaluate the nutritive value and antinutritive value of dietary cassava in African catfish (*Clarias gariepinus*) diets.

Measurable objectives:

1. To determine the growth performance, feed utilization and digestibility of African catfish (*Clarias gariepinus)* fry and fingerlings fed dietary cassava (leaf and root) based diets.
2. To investigate the effects of the use of CLM with dietary DL-methionine supplementation in African catfish (*Clarias gariepinus*) diets.
3. To examine the histological effect of dietary cassava which contain cyanogenic glucoside on the liver of African catfish

4. To evaluate the dietary effect of cyanogenic glucoside of cassava leaf on thiocyanate concentration in the liver, gill and muscle African catfish following the cassava meal.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Aquaculture

Aquaculture plays a vital role in many countries by offering better nutrition, higher income, earning foreign exchange and employment opportunities. Aquaculture is currently regarded as the fastest growing food production sector in the world, growing more rapidly than all others animal food production (Ali, 2001). Aquaculture production is expected to reach 45.7 millions tones in 2000 (Brugere and Ridler, 2004). During the 1990s, global aquaculture production increased 10 % / year (Pike and Barlow, 2002), rising from level of 13 % of world fisheries production (excluding aquatic plants) in 1990 to level of 26 % in 1999. Under favorable condition, aquaculture could supply up to 39 millions tones of fish by 2010, 70 % more than 1999 (Brugere and Ridler, 2004).

Global aquaculture production continues to be dominated by Asia whose role in aquaculture has risen through the centuries. At the beginning of 1990s, Asia provided 83 % of the world's total of aquaculture and by the year of 1999, it had risen to 89 %. Most the increase was attributable to China who share 50 % of the world's total aquaculture production in 1990s to 68 % in 1999 (Brugere and Ridler, 2004).In many Asian countries, fresh water aquaculture production has contributed to high total aquatic production and it is dominated by finfish. In Southeast Asia (Indonesia, Malaysia, Thailand, Philippines, Singapore, Viet Nam, Cambodia, Laos, Brunei Darussalam), fresh water fish dominate aquaculture production and account for 29 % of the total production (Subasinghe *et al.,* 1997 as cited by Ali, 2001). In 1995, world production of Clariidae was more than 0.2 million metric tones which was the second most important group of farmed catfish in the world (Hecht *et al.*, 1997).

Since fish considered as the source of high quality animal protein and relatively cheap for human consumption, the demand for this protein source will be increased. The growth of human population has lead to an intensified search for methods of producing fish protein other than capture fisheries as it face limit in production. Therefore, the potential of aquaculture as a source of protein will expand in terms of species cultured and technologies used.

## 2. 2 African Catfish (*Clarias gariepinus*)

### 2.2.1 Introduction

African catfish (*Clarias gariepinus)* is the second most important group of farmed fish in the world (FAO, 1983 as cited by Fasakin *et al*., 2003). This fish is increasingly becoming an important species in Africa, Europe and was introduced into Asian countries such as Republic of Korea, China, Taiwan, Philippine, Cambodia, Laos, India, Thailand, Malaysia and Indonesia in the late 1980s (FAO, 2002).

Myers *et al*., (2006) systematize the taxonomy of African catfish as follows:

Kindom : Animalia (animals)
Subkingdom : Bilateria (bilaterally symetrical animals)
Branch : Deuterostomia (deuterostomes)
Phylum : Chordata (chordates)
Subphylum : Vertebrata (vertebrates)
Superclass : Gnathostomata (jawed vertebrates)
Class : Actionopterygii (ray-finned fishes)
Subclass : Neopterygii
Infraclass : Telestoi
Superorder : Ostariophysi
Order : Siluriformes (catfishes)
Suborder : Siluroidae
Family : Clariidae (air breathing catfishes)
Genus : *Clarias*
Species : *Clarias gariepinus* (African catfish)

A recent revision in the systematic of clarias species has put several species being synonymyzed with *Clarias gariepinus*, these include *Clarias carpensis* of southern Africa, *Clarias mosambicus* of central Africa, *Clarias lazera* of west and north part of Africa and parts of Asia (Teugels , 1984).

African catfish species is characterized by an elongated body, a large head, depressed and bony with small eyes, narrow and angular occipital process. The gills open wide, and have a deep indentation with a strong operculum. The jaw extremity terminates in a special skin fold. They have four pairs of barbells (nasal, maxilar, outer mandibular and inner mandibular), long dorsal and anal fins, without adipose fin (FAO, 2004 and Fasakin *et al*., 2003). African catfish have a tight and smooth scaled mildly slimy skin which is darkly pigmented in the dorsal and lateral parts of the body. The fish turn lighter in color when exposed to light (Viveen *et al.*, 1986 as cited by Pantazis and Neofitou, 2003).

This species can be raised at high stocking densities and is well adapted to the wet and dry extremes of their habitat. The presence of an accessory breathing organ enables this species to breathe air during very active or under very dry conditions. Its ability to breathe air may be of importance particularly in reducing metabolic expenses for oxygen uptake as air contains about 30 times more $O_2$ than water and is probably easier to carry than water. A relatively high amount of the metabolized $CO_2$ is released into the water allowing the $O_2$ in the air to be taken into the labyrinth (Hogendoorn *et al.*, 1983). This fish is known as a bottom feeder but sometimes feeds at the surface and can be described as an omnivorous species with the ability to utilize a wide variety of both animal and plant protein efficiently (Hecht *et al.*, 1997). In natural conditions, the fish will feed on insects, crabs, plankton, snails and fish but will also consume rotting flesh, plants and fruits when prey animals become scarce (FAO, 2004).

Since this species is equipped with air breathing abilities and a tolerance of poor water quality, their culture does not require pond aeration or high water exchange rates (Hecht *et al.,* 1997).The rapid growth of this species under natural and controlled aquaculture conditions has been noted (Hogendoorn, 1983). Furthermore, it does not easily succumb to disease and this is an important cost-saving trait. The qualities described above make this species especially suitable for culture.

### 2.2.2 Nutritional Requirements

Feed is one of the main factors in aquaculture. The feed must contain the correct proportions of nutrients which are then to be digested and absorbed to provide energy and substrates for fish growth, reproduction and health (De Silva and

Anderson, 1995). This nutrient may come from natural food sources in the water and from all artificially prepared feeds. As aquaculture technology has advanced, there has been a trend toward higher yield and faster growth through intensive fish farming, which demand for improvement in the replacement of natural food with prepared feeds (Lovell, 1989).

Fish is fed adequately to generate the required amount of energy in the most economical manner. The majority of gross energy in food is contained in three types of molecules, namely the protein, lipid and carbohydrate molecules. No single feedstuff can supply all the nutrients and energy required for the optimum growth of catfish, therefore the nutritional requirements of cultured catfish are met by using a complete feed that are comprised of a mixture of feedstuffs and vitamins and mineral premixes that provide adequate amounts of essential nutrients as well as the energy necessary for normal growth and other physiological functions. As nutrient contribution from natural food organisms is considered to be minimal in intensive catfish farming, nutrients and energy are provided primarily by prepared feeds. The main objective in processing feedstuffs into feed is to maximize the nutritional value of various feed components to meet nutrient requirements (Hecht *et al.,* 1997).

Generally, nutritional requirement do not differ significantly among fish species and is often identified as requirement of warm water or cold water fish, finfish or crustacean, carnivorous or omnivorous and marine or fresh water fish. Therefore, when nutritional requirement are not available for one species, data obtained from other fish species could also be applied.

The following table (Table 2.1) presents the nutritional requirement of African catfish as summarized by Haylor, 1989.

Table 2.1 Nutritional data of African catfish (fry, fingerling and adult)

| Nutritional requirement (%) | African catfish | | |
|---|---|---|---|
| | Fry | Fingerling | Adult |
| Crude protein | 50 | 40 | 40 |
| Crude lipid | 9.5 | 10 | 10 |
| Carbohydrate | 20 | 30 | 30 |
| Fiber | 1.6 | <20 | <20 |

#### 2.2.2.1 Protein

Protein is a very important constituent in fish diet and the protein requirements of fish are generally higher than those of land animals (Lovell, 1989). The optimum levels and quality of protein are influenced by several factors including optimal dietary protein to energy balance, amino acids profile and digestibility of the protein, and the amount of non-protein source present in the diet (Wilson, 2002).

Catfish diets should be balanced to ensure that adequate levels of protein and less-expensive energy sources are supplied in proper proportions to minimize the use of protein for energy use and to maximize protein deposition (Robinson and Li, 1999). In general, dietary protein requirement of African catfish up to 40 % with energy levels range from 310 to 406 kcal/ 100 g diet (Van Weerd, 1995). However, the protein levels in the range of 22 % to 36 % were commonly used by several researchers with a good result (Bureau *et al.*, 1995, Akegbejo-Samsons, 1999, Fagbenro, 1998).

Fish consume protein to obtain amino acids. The protein is digested and absorbed from the gut in the form of amino acids which are then absorbed and distributed to the organs and tissues. They are then further used by various tissues to syntheses new proteins (during growth and reproduction) and to replace the existing proteins (Wilson, 2002).

The quality of protein sources used in catfish feeds must be taken into account to ensure that amino acid requirements are met. Ten indispensable amino acids (IDAA) are required by fish (NRC, 1993). Indispensable amino acids are nutrients that are cannot be synthesized in the body and thus must be supplied in the diet. Proteins of animal origin, particularly fish meal prepared from whole fish, are considered nutritionally superior to proteins of plant origin. This is because animal proteins generally contain a higher level of indispensable amino acids and are therefore more digestible (Robinson and Li, 1999).

Protein requirement also varies with water temperature, water quality, fish size, species and feeding rate (Steffens, 1989). Dietary protein levels can be reduced in feeds for bigger sized catfish. Feeding rate may also affect the optimum dietary protein level. For instance, fish fed at a restricted rate may require more or higher-quality protein, particularly if feeding is severely restricted (Robinson and Li, 1999).

Since the indispensable amino acids dietary requirement is closely related to the amino acid profile of muscle protein, the amino acids profile from the muscles provide the first approximation of the amino acids needs of a fish (Benitez, 1989).

#### 2.2.2.2 Lipids

Lipids are the second major group of dietary components after protein. Lipids contain more energy per unit weight than other dietary compounds. It increases feed palatability and assists in reducing dust. It also improves the stability of the pellet during the pellets manufacture, transportation and storage (Steffens, 1989).

Generally, inclusion of 10-20 % of lipid in fish diet results in optimal growth rate without producing an excessively fatty carcass (Obey and Sargent, 1979 as cited by De Silva and Anderson, 1995). Many information available on the lipid uses for African catfish. Uys (1989) as cited by Van Weerd (1995) found that at 42 % protein level inclusion, the optimum dietary lipid for African catfish is in between 10-12 %. Ng *et al.,* (2001) reported that the inclusion of at least 8 % lipid of refined, bleached, deodorized palm oil and crude palm oil in African catfish diet under their experimental conditions improved the growth performance, protein ratio and vitamin E concentration of this fish. In most studies, lipid was incorporated up to 10 % of dietary lipid when formulating diet for African catfish (Sadiku and Jauncey, 1995, Fagbenro *et al.*, 1998, Fagbenro, 1998, Ng *et al.*, 2003 , Fagbenro, 2004).

Besides acting as energy sources, dietary lipids are also important sources of essential fatty acids and serve as a vehicle for the absorption of fat-soluble vitamins A, D, and K (Watanabe, 1981). The essential fatty acids affect the fluidity, flexibility and permeability of the membrane. They also function as precursors of the eicosanoids, which are necessary in the formation of an impermeability skin barrier and are involved in cholesterol transport and metabolism (Steffens, 1989).

There are two series of essential fatty acids, namely, the n-6 series that is derived from linoleic acid (LA) and the n-3 series from alpha linolenic acid (ALA). Both cannot be synthesized by fish and therefore must be supplied in the diet (Steffens, 1989 and Troutwein, 2001).

Studies on essential fatty acid requirement have demonstrated that its requirement in fish differs from species to species. Rainbow trout (*Salmo gairdneri)* requires fatty acid of the linoleic acid (n-6) series for maximum growth, better feed utilization and freedom from pathology. In contrast, African catfish require both n-3 and n-6 fatty acids for maximum growth, with optimum ratios of 0.88 and 1.09 respectively generating optimum growth (Ng *et al.*, 2003).

### 2.2.2.3 Carbohydrates

Carbohydrates are consists of carbon, hydrogen, and oxygen, which include sugars, starch, cellulose, gum, and other closely related compounds. Plants are the major sources of carbohydrates. Carbohydrate comprises about 50% to 80% of the dry weight of various plants. The primary form of energy from plant is stored in seeds, roots, and tubers. Plants synthesize carbohydrate from solar energy, carbon dioxide, and water through the process of photosynthesis (Robinson and Li, 1996).

Carbohydrates can be classified according to their degree of natural sugar complexicity namely monosacharides (such as glucose and fructose), disaccharides (such as sucrose and maltose) and polysacharides (such as starch and cellulose) (De Silva and Anderson, 1995).

Carbohydrates are limited use as an energy source and also for sparing protein in the diet for finfish, due to their relatively low digestibility of high molecular carbohydrate compounds (Steffens, 1989). Generally, fish possess varying degrees of carbohydrate utilization. Warm water fishes are able to utilize much higher levels of dietary carbohydrate compared to cold water or marine fishes. Cold water fishes require $\leq$ 20 % of digestible carbohydrate, whereas higher levels are used by fresh and warm water fishes (Wilson, 1994) (Table 2.2).

No information available on the dietary carbohydrate requirements of in clarias species, however Jantrarotai *et al.*, (1994) observed that hybrid catfish *Cl. Macrocephalus x Cl. gariepinus* are capable of utilizing carbohydrate and can tolerate up to 50 % carbohydrate in their diets which is higher than values reported for channel catfish (28 %) (Anderson *et al.*, 1984) and *Tilapia zilii* (40 %) (El Sayed and Garling, 1988). For African catfish, the use of 15 % to 35 % carbohydrate has been reported (Van Weerd, 1995).

Table 2.2. Optimum or recommended dietary level of digestible carbohydrate for various fishes (reviewed by Wilson, 1994)

| Fish species | % digestible carbohydrate |
|---|---|
| **Marine or cold water** | |
| Asian seabass | ≤ 20 |
| Atlantic salmon | ≤ 20 |
| Plaice | ≤ 20 |
| Pacific salmon | ≤ 20 |
| Rainbow trout | ≤ 20 |
| Yellow tail | ≤ 10 |
| **Fresh or warm water** | |
| Channel catfish | 25-30 |
| Common carp | 30-40 |
| Eel | 20-30 |
| Grass carp | 37-56 |
| Milkfish | 35-45 |
| Red drum | -25 |
| Striped bass and hybrid | 25-30 |
| Tilapia | -40 |

The relative utilization of dietary carbohydrate varies and appears to be related to the complexity of the carbohydrate. In general, starch and dextrin (partially hydrolyzed starch) are better utilized by catfish than simple sugars such as glucose and sucrose (De Silva and Anderson, 1995). Glucose is highly digestible by catfish, but apparently a large portion of the absorbed glucose is excreted. The use of hydrolyzed or cooked starch is to be preferred as it is more digestible than native or raw starch although the dietary level must not be allowed to change the maximum levels of native starch (Bergot and Breque, 1983). Besides this, the present of crude fiber must always be given due consideration. Jantrarotai *et al.*, (1994) found that a crude fiber content of around 18 % as cellulose caused poor performance in hybrid clarias catfish.

However, carbohydrates are still considered as important dietary components since they have been used as an inexpensive source of energy as precursors for various metabolic intermediates, as an aid in pelleting practical catfish feeds, and as an inexpensive source of energy.

### 2.2.2.4 Vitamins

Vitamins are low molecular weight compounds that act as cofactors or substrates in some reactions. Their deficiency causes serious metabolism disorders known as avitaminosis and other disorders signs of vitamin deficiency in salmonids include impaired carbohydrate metabolism, nervous disorders, poor appetite, poor growth and increased sensitivity to shock from physical blows or from flashes of light (Halver, 2002).

Vitamins are synthesized in small quantities and are insufficient for animals and fish, and therefore must be given as dietary supplements (Steffens, 1989). The vitamin requirements of most fish species in fish culture have not been determined. The data obtained from salmonid, carp or catfish are usually applied to other species and in the case of African catfish, the vitamin requirements of channel catfish are used based on the assumption that they have similar dietary needs (Table 2.3). Recent work on vitamin C requirements for African catfish has shown that larvae and juveniles require at least 1500 mg ascorbic acid/g dry weight of food (FAO, 2004).

**Table 2.3** Tentative vitamin requirements of African catfish (*Clarias gariepinus*) diets, based on levels used in commercial diets for channel catfish (FAO, 2004)

| Vitamins (premix requirements in grammes or IU/kg | |
|---|---|
| Thiamine | 11 g |
| Roboflavine | 13 g |
| Pyridoxine | 11 g |
| Panthothenic acid | 35 g |
| Nicotinic acid | 88 g |
| Folic acid | 2.2 g |
| B 12 | 0.09 g |
| Choline | 550 g |
| Ascorbic acid | 350 g |
| A (IU) | 4400 (IU x 1000) |
| D (IU) | 2200 (IU x 1000) |
| E (IU) | 55 (IU x 1000) |
| K (IU) | 11 (IU x 1000) |

Vitamin requirements depend on number factors such as raw materials, composition of the diet, the age of fish, temperature, stocking density and disease (Steffens, 1989).

#### 2.2.2.5 Minerals

Minerals are essential chemical elements which are involved in the formation of skeletal structure, maintenance of colloidal system and regulation of acid-base equilibrium (Lall, 2002). They also act as component of enzymes, hormones and proteins (Steffens, 1989). Generally minerals can be divided into 2 groups. First group is macro elements (calcium, phosphorous, kalium, natrium, and sulfur) which are required in large quantities above 100 mg/ kg diet. Second group is micro elements (ferum, cuprum, mangan, zink, cobalt, molibdum, chromium, selenium, flour, iodine, nikel) which are required in small amount below 100 mg/kg diet (Steffens, 1989).

The concentration of minerals in the body of the organism depends on food sources, environment, species, and stage of development and physiological status of the animal. Most fish accumulate and retain minerals from the environment and from their diets (De Silva and Anderson, 1995). Purified diets without mineral supplementation cause loss of appetite, growth depression, hypochrome anemia, high mortality and cranial deformity (Ogino and Kamizono, 1972 as cited by Lall, 2002). Although the dietary mineral requirements for African catfish (*Clarias gariepinus*) are not well documented, it is not uncommon to use the data from channel catfish as a reference (Table 2.4).

**Table 2.4 Minimum requirement of minerals to prevent deficiency symptoms for African catfish (*Clarias gariepinus*) diets, based on levels used in commercial diets for channel catfish (Lall, 2002)**

| Minerals | |
|---|---|
| Calcium | Below level of detection |
| Phosphorus | 0.45 % |
| Magnesium | 0.04 % |
| Sodium | Below level of detection |
| Potassium | Below level of detection |
| Chloride | Below level of detection |
| Zinc | 20 mg/ kg |
| Selenium | 0.25 mg/kg |
| Manganese | 2.40 mg/kg |
| Iron | 30 mg/kg |
| Copper | 5 mg/kg |
| Iodine | 1.1 ug/kg |

## 2.3 Protein and Amino Acids

### 2.3.1 Introduction

Protein is a high molecular weight nutrient that contains about 50 % carbon, 22 % oxygen, 7 % hydrogen and 16 % nitrogen. It also contains a small amount of sulfur and phosphorus (Steffens, 1989).

The buildings blocks from which proteins are formed are the amino acids. The amino acid composition in a protein determines the quality of the protein and its value as a feed constituent (Steffens, 1989). Amino acids are linked by peptide bonds that connect the carbonyl group of one amino acid to the amino group of the next amino acid (De Silva and Anderson, 1995) (Figure 2.1).

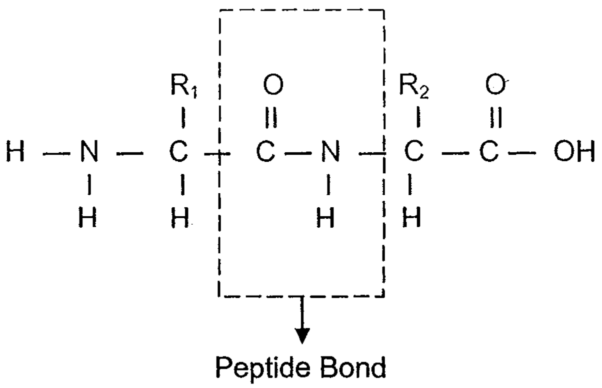

**Figure 2.1. A peptide bond outline in broken lines which links two amino acids**

Amino acids are composed of the amino group (-NH2) and the carbonyl group (-COOH). Amino acids have a particular general structure NH2 - CH - RCOOH where R is any one of a number of organic side chains containing carbon, hydrogen, nitrogen and sulfur atoms (De Silva and Anderson, 1995).

Amino acid analysis can be used to quantify protein and peptides, to determine the identity of proteins and peptides based on their amino acid composition, to support protein and peptide structure analysis, to evaluate fragmentation strategies for peptide mapping, and to detect atypical amino acids that might be present in protein or peptide (FAO, 2004). It is necessary to hydrolyze a protein or peptide to its individual amino acid constituents before amino acid analysis

### 2.3.2 Significance of Amino Acid in Fish Nutrition

Sources of dietary protein are not similar in their nutritional and biological values. The nutritive value of a dietary protein is primarily dependent on the digestibility and amino acid composition particularly to the extent the composition of amino acid fulfills the requirements of the organism. The closer the profile from this requirement, the greater is the nutritional value of the dietary protein (Benitez, 1989). Moreover, the lack of sufficient information on amino acids requirement, availability and interaction has hindered the successful replacement of fish meal in feed with less expensive sources of protein (Borlongan, 1992).

There are 20 amino acids commonly found in animals. Ten of them cannot be synthesized within the organism and therefore must be supplied in a diet and are referred as indispensable amino acids (Table 2.5). In contrast, the other 10 amino acids can be synthesized in the body and are called dispensable amino acids (De Silva and Anderson, 1995). Wilson (1989) noted that the same 10 Indispensable amino acids are required in diets of some aquaculture species such as common carp, rainbow trout, chinook salmon, Japanese and European eels, channel catfish, sea bream, sole, plaice, tilapia and also the African catfish.

**Table 2.5 Indispensable and Dispensable Amino Acid in Fish (De Silva and Anderson, 1995)**

| Indispensable Amino Acid (IDAA) | Dispensable Amino Acid (DAA) |
|---|---|
| Arginine | Alanine |
| Histidine | Asparagine |
| Isoleucine | Aspartate |
| Leucine | Cysteine |
| Lysine | Glutamate |
| Methionine | Glutamine |
| Phenylalanine | Glycine |
| Threonine | Proline |
| Tryptophan | Serine |
| Valine | Tyrosine |

Dietary amino acids are required for 2 purposes, firstly for growth which mainly consists of protein deposition and secondary for maintenance (Cowey, 1994). Deficiency of IDAA results in the poor utilization of dietary protein and consequently reduced growth and decrease in feed efficiency. Lovell (1989) stated that if some amino acids are fed in excess of the requirement levels, they cause an increase in the requirement for other structurally similar amino acids and this phenomenon is called amino acid antagonism. If the dietary excess of certain amino acids in some cases are directly toxic and their negative effect cannot be improved by the addition of other amino acids, a phenomenon termed amino acid toxicity occurs.

The values of IDAA requirement for several species of fish when expressed as a proportion of their diet indicate that there is a large variation in the requirement of different species. Table 2.6 summarizes the range of indispensable amino acids requirement that have been determined for a variety of species of fish in general (De Silva and Anderson, 1995). Knowledge of the amino acids requirement of fish is useful in formulating optimal fish diets.

**Table 2.6 The range of Indispensable amino acid requirements of fish (De Silva and Anderson, 1995)**

| Amino acid | Requirement (g/100 g Protein) |
|---|---|
| Arginine | 3.3-5.9 |
| Histidine | 1.3-2.1 |
| Isoleucine | 2.0-4.0 |
| Leucine | 2.8-5.3 |
| Lysine | 4.1-6.1 |
| Methionine[a] | 2.2-6.5 |
| Phenylalanine[b] | 5.0-6.5 |
| Threonine | 2.0-4.0 |
| Tryptophan | 0.3-1.4 |
| valine | 2.3-4.0 |

[a] Requirement varies depend on the amount of cysteine in the diet

[b] Requirement varies depend on the amount of tyrosine in the diet.

The amino acid pools in the body are derived from 3 principal sources which are the diet, the catabolism of body protein and the synthesis of DAA (Cowey and Walton, 1989) (Figure 2.2). The catabolism of body proteins in fish supplies less than 50 % of the free amino acid in fish (Cowey and Luquet, 1983). Therefore, fish seems to require more amino acid from the diet than do omnivorous mammals (Wilson, 2002).

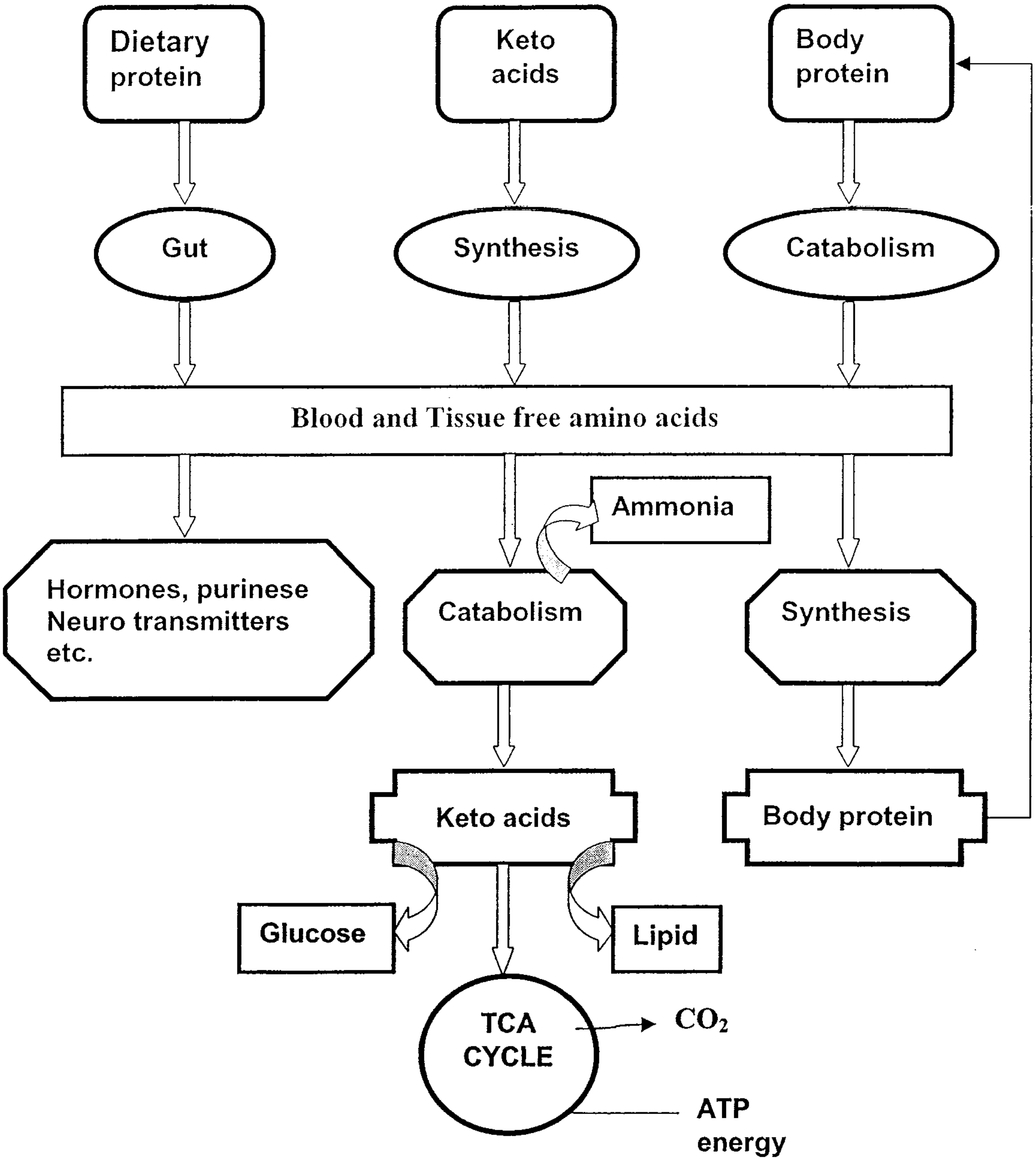

**Figure 2.2 Main pathway of amino acid metabolism (Cowey and Walton, 1989)**

The proteins in the diet are converted to amino acids in the body through the action of various digestive enzymes. Free amino acid is released and absorbed in the intestinal tract and used by various tissues in the synthesis of tissue protein (Benitez, 1989).