THE EFFECT OF PROCESSED *ALBIZZIA LEBBECK* SEED MEALS ON THE GROWTH AND FEED UTILIZATION OF *CLARIAS GARIEPINUS* (TEUGELS) JUVENILES

BY

ANWA, EKAETE PATRICK

B.Sc. Zoology (Hons), UniJos

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DECLARATION

I hereby declare that this work is wholly the author's and has not been part of any presentation for any other qualification.

ANWA Ekaete Patrick

DATE

CERTIFICATION

This thesis entitled "THE EFFECT OF PROCESSED *ALBIZZIA LEBBECK* SEED MEALS ON THE GROWTH AND FEED UTILIZATION OF *CLARIAS GARIEPINUS* (TEUGELS) JUVENILES" by Anwa, Ekaete Patrick meets the regulations governing the award of the degree of Master of Science in Fisheries of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

______________________________ _______________

Dr. J. Auta
(Chairman) Supervisory Committee
Department of Biological Sciences, A.B.U., Zaria.

Date

______________________________ _______________

Dr. S. A. Abdullahi
(Member) Supervisory Committee
Department of Biological Sciences, A.B.U., Zaria.

Date

______________________________ _______________

Dr. P. I. Bolorunduro
(Member) Supervisory Committee
NAERLS, A.B.U., Zaria.

Date

______________________________ _______________

Professor. A. U. Ezealor
Head of Department, Biological Sciences
A.B.U., Zaria.

Date

______________________________ _______________

Professor J. U. Umoh
Dean, Postgraduate School
A.B.U., Zaria.

Date

DEDICATION

Affectionately dedicated to my late dad
whose love for me while alive was not in doubt.

To my mentor, my role model and my mom.
I owe all that I am to your unique love and care.

To the best sister in the world.
You have been an inspiration.

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ABSTRACT

The seeds of *Albizzia lebbeck* were processed and chemically analysed for their proximate composition, anti-nutritional factors and amino acid profile. Results obtained from the first phase of the experiment determined the particular treatments that were used for the feeding trial. Four isoproteic (40% crude protein) diets were formulated with same protein ingredients and basal ingredients and fed at 5% body weight to groups of *Clarias gariepinus* juveniles of mean initial body weight of 15.8 ± 0.4g in eight out-door concrete tanks each measuring 1.3m x 0.9m x 1m capacity (1.17m^3) for 8 weeks. Proximate composition of the different Treatments showed that they had low moisture content (2.47 – 5.29%). The seeds were found to be rich in crude protein (29.73 – 38.04%) and carbohydrates (32.28 – 37.47%) but low in lipid content (5.08 – 6.87%). The phytochemical screening of the seeds showed the presence of anti-nutritional factors, however, tannin levels were negligible while cyanogenic glycoside and phytic acid levels were low. The amino acid levels of histidine and leucine were adequate when compared with the Essential Amino Acid requirements for *Clarias gariepinus*. Diets 1–4 contained *Albizzia* seeds processed as follows: raw (D1), boiled for 15 minutes (D2), boiled for 30 minutes (D3) and boiled for 1 hour (D4). Water quality parameters: dissolved oxygen, temperature, pH, electrical conductivity and total dissolved solids were monitored and no mortality was observed during the experimental period. Growth and nutrient utilization indices: Mean Weight Gain (MWG), Specific Growth Rate (SGR%), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were calculated. The best growth and nutrient utilization values were observed in D4 with significantly higher ($P<0.05$) MWG and SGR. Growth performance of the juveniles increased as heat exposure time of the seeds increased. Fish fed D1(Control) showed a significantly ($P < 0.05$) lower growth performance, higher carcass nitrogen free extract and lower levels of crude protein when compared with all other dietary groups. The present study indicates that the inclusion of *Albizzia* seed meal (replacement of 50% of the total dietary protein of feed) after boiling for one hour significantly improved the growth performance and feed utilization of *C. gariepinus* juveniles compared with that of the raw seed meal. This investigation provided useful information on the chemical constituents of differently treated seeds of *A. lebbeck* and their incorporation into fish feed meals for *Clarias gariepinus* juveniles was recommended.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Experiments to test lower feed costs for carnivorous fishes, while providing adequate nutrient bases was initiated with copra and certain high-protein legume leaf powders (Bardach, 1976). The demand for fish is expanding rapidly throughout the world because of increasing population and incomes (Shang, 1981). A couple of decades ago, fish was mainly the diet of low-income people. Presently, this is no longer the case as it is now popularly known that it possesses a finer protein than beef. Indeed, fish is an important component of total human food consumption and, to a lesser degree, of animal feed. It is nutritionally better than meat in protein, high in essential minerals, and low in saturated fats (Babbitt, 1990). Therefore, in overall performance, fish are a superior source of animal protein; 62-72% dry weight basis, depending on the species (Babbit, 1990). If the favourable amino acid pattern is considered, human beings can utilize at least 83 percent of the raw weight of fish, and its protein is especially valuable to humans because it is the richest natural source of two essential amino acids (lysine and methionine) which are the limiting amino acids in many vegetable diets (Barlow and Windsor, 1984).

Fish like other animals need protein in order to grow and perform optimally. This means they can become competitors for products which could otherwise be used for human consumption (Hilbrands and Yzerman, 1998). Since the inception of fish culture technology in Nigeria, imported commercial fish feeds have been promoted as the most favoured feed for fish rearing because they support satisfactory growth in fish, however the prohibitive cost of imported fish feeds has made catfish feeding economically unattractive for the small-scale fish farmer, therefore the need for a locally compounded feed that is nutritionally complete with ingredients not competed for by man nor animals but which would equally bring about optimal growth. The expenses incurred in feeding cultured fish may be lowered by utilizing locally available materials for feed instead of imported feeds. Decades ago, the demand for fish in Nigeria had previously been amply supplied by the wild and via importation of exotic species. Recently though, there has been an awareness among the nation's citizenry to save scant foreign resources which has led to a drive for self sufficiency. The resultant effect therefore, is fish production within the private and government sectors.

1.2 Statement of the Research Problem

In fish diets, protein is the most expensive portion and is usually the first nutrient that is computed in diet formulation (Hardy, 1989). The protein requirements of fish are more exacting than other cultured animal species (Chow, 1978). Therefore, fish not only require higher protein levels in their diets but the protein quality requirements are also higher. A major drawback with plant proteins is that the levels of the indispensable amino acids are not as high as those from animal proteins. Also, the presence of anti-nutritional factors presents a debilitating problem to their usage in the raw form. The high cost of feed inputs is a major problem to fish culturists in Nigeria (Madu *et al.*, 2003) and feed is an integral part and the most important cost item for intensive aquaculture. Shang (1981), Jauncey and Ross (1982), Hardy (1989), Lovell (1989) and Eyo (2003) all state that nutrition in aquaculture accounts for 40 – 65% of the total operating costs, depending on the level of aquaculture intensification. Research on a suitable low-cost feed is therefore a high priority.

1.3 Justification

Since aquaculture is a fast growing sector in Nigeria, to sustain such a high rate of increase in production, a matching increase in fish feed sources is imperative. Faced with the food supply problem for cultured fish, nutritionists have done more work evaluating alternate protein sources in aquaculture diets during the last 7 years than during the previous 50 years (Spinelli, 2006). Therefore, in order to attain more economically sustainable, environmentally-friendly and viable production, recently the research interest has been directed to the evaluation and use of unconventional protein sources. Information is available on the utilization of most well known and cultivated legumes as fish feed, however, there appears to be no work done on the utilization of *Albizzia* seeds as a non-conventional protein source for fish nor is there information on the use of *Albizzia* seed meals as supplements to other animals, however the leaves of the plant are utilized as forages to ruminants. This study was designed to provide some analytical information on *Albizzia lebbeck* when differently processed with a view to expand its present utilization.

Clarias gariepinus is the fish species that was used for this study because it is already an established aquaculture species in Africa and the most commonly cultured catfish in Nigeria. Faturoti (2003) reported that catfishes have taken the centre stage of the aquaculture sub-sector in Nigeria. They are suited to low-technology farming systems and have a propensity to consume a variety of supplementary feeds (Fagbenro *et al*., 2003). The international interest in this species with its astonishing tolerance of poor environmental conditions, its disease resistance, good growth rate and wide market acceptability makes it very suitable for marginal economies.

1.4 Objectives

The general objective of the study was to provide some analytical information on *Albizzia lebbeck* seeds when differently processed and to evaluate the usefulness of *Albizzia* seeds as feed both in raw and processed forms in the diet of the African mudfish, *Clarias gariepinus.*

The specific objectives were;

a To determine the proximate composition of the raw and processed seeds of *Albizzia*.

b To determine the anti-nutritional factors of the seeds of *Albizzia*.

c To analyse the amino acid profile of the *Albizzia* seeds.

d To evaluate the growth and nutrient utilization of the fish fed *Albizzia* seed based diets.

1.5 Statement of Research Hypothesis

For this study, the Null (H_0) hypotheses are postulated thus on the raw and processed *Albizzia lebbeck* seeds:

a There is no significant difference between raw and processed *Albizzia lebbeck* seeds in proximate composition, anti-nutritional factors and amino acid profile.

b Processing methods have no significant effect on the growth performance and nutrient utilization of *Clarias gariepinus* juveniles.

CHAPTER TWO

LITERATURE REVIEW

2.1 Ecology and Culture of *Clarias gariepinus*

2.1.1 Geographical distribution

The African mudfish is widely distributed throughout Africa. It has an almost Pan-African distribution, ranging from the Nile to West Africa and from Algeria to Southern Africa. It also occurs in Asia Minor (Israel, Syria and South of Turkey). It inhabits tropical swamps, lakes and rivers, some of which are subject to seasonal drying (Viveen *et al*., 1986).

2.1.2 Biological description

The taxonomic status of *Clarias gariepinus* is as illustrated below:

Taxonomic status

KINGDOM:	Animalia
PHYLUM:	Chordata
SUB-PHYLUM:	Vertebrata
SUPER CLASS:	Pisces
CLASS:	Actinopterygii (ray-finned fishes)
ORDER:	Siluriformes (catfish)
FAMILY:	Clariidae (Airbreathing catfishes)
GENUS:	*Clarias*
SPECIES:	*gariepinus*
COMMON NAME:	African mudfish

Clarias gariepinus has a scaleless slimy skin which is generally dark, greyish-black above and creamy-white below. The fish turns lighter in colour when exposed to light. During stress it will show a mosaic-like pattern of dark and light spots (Plate I). It possesses a fairly distinct black longitudinal band on each side of the ventral surface of the head. The young (less than 9 cm long) has a similar color pattern except that the black bands on the head are absent. The head is large, depressed, and heavily boned. The mouth is quite large and subterminal (Skelton, 1993). The upper surface of the head is coarsely granulate in adult fishes, and smooth in the young (van Oijen, 1995). It possesses a wide mouth with eight barbels (nasal, maxillary, outer mandibular and inner mandibular). With the wide mouth it has the ability to feed on a variety of food items, ranging from minute zooplankton to fish. Close to the nasal barbels, two olfactory organs are located.

The median fins consist of a dorsal (61-80 soft rays), a caudal, and an anal fin (45-65 soft rays), while the paired fins consist of the pectoral and ventral fins. It has a strong pectoral fin with spines that are serrated on the outer side (Teugels, 1986). The sharp spines are not poisonous. The eyes are small. The urogenital opening is situated at a papilla just behind the anus, the male possessing an elongated form of this papilla. According to IGFA (2001), the maximum size that it can attain is 1.7m (Total length) while the maximum published weight is 60.0 kg (Robins *et al*., 1991). The maximum reported age in the wild is eight years. It is a freshwater fish that can live in a depth range of 0 – 80 m.

The African mudfish lives in a variety of freshwater environments, i.e. it occurs mainly in quiet waters, lakes and pools but is also very prominent in flowing rivers, rapids, and around dams (Teugels, 1986). It is widely tolerant of extreme environmental conditions and can live in a pH range of 6.5-8.0. It is able to live in very turbid waters and can tolerate temperatures of 8-35°C.

PLATE I *CLARIAS GARIEPINUS*

Their optimal temperature for growth is 28-30°C (Teugels, 1986). Verreth and Den Bieman (1987) specifically recommend 28^{o}C as the optimum temperature for *Clarias gariepinus* larval rearing. The presence of an accessory breathing organ enables this species to breath air when very active or under very dry conditions. It is also able to secrete mucous to prevent drying and is able to burrow in the muddy substrate of a drying body of water (Skelton, 1993). It remains in the muddy substrates of ponds (benthopelagic) and occasionally gulps air through the mouth (de Moor and Bruton, 1988). As a result of being capable of atmospheric respiration it may exist in mud during the dry season. It is potamodromous, which means it can migrate between streams and rivers (Teugels, 1986). It can leave the water at night using its strong pectoral fins and spines in search of land-based food or can move into breeding areas through very shallow pathways (Burgess, 1989).

It is a bottom feeder which is also capable of feeding at the surface (Teugels, 1986). It is an omnivore. It is not specific in its food requirements. In its natural state, it is known to feed on insects, plankton, snails, crabs, shrimp, and other invertebrates (Burgess, 1989). It is also capable of eating dead or rotting animals, birds, reptiles, amphibians, small mammals, other fishes, eggs, and plant matter such as fruit and seeds (de Moor and Bruton, 1988). Because it is mobile on land, it is able to prey on terrestrial organisms. This species may also hunt in packs on occasion by herding and trapping smaller fish. It is also referred to as sharptooth catfish because of its fine, pointed bands of teeth (Skelton, 1993). This species can live in very poorly oxygenated waters. Since the fish can tolerate low dissolved oxygen levels in the water, it is very suitable for fish culture. In nature, it matures after 2-3 years. Under pond conditions however, mudfish mature at 7-10 months (Viveen *et al.*, 1986).

2.2 Amino Acid Requirements of Fish

In aquaculture, the protein requirement of the fish species being reared has to be satisfied. Proteins are composed of long chains of amino acids. Although proteins may be metabolized for energy, one goal in aquaculture is to utilize as much of the dietary protein as possible for growth, allowing the carbohydrates and lipids to provide metabolic energy. Proteins are the flesh-forming principles present in most foods. They are also known as 'proteids', 'albuminoids', or 'nitrogenous principles', the latter name being used on account of the presence of nitrogen in them. Proteins have in addition to carbon, hydrogen and oxygen, nitrogen and usually sulphur and sometimes phosphorous (William and West, 1967).

The quality of protein in any diet is principally based on the amount of essential amino acids present. Amino acids are the substances derived from the ultimate products of digestion. There are about twenty naturally available amino acids; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, aspartic acid, asparagine, cystine, glutamic acid, glutamine, glycine, proline, serine, tyrosine (Hardy, 1990) but only the first ten are essential for fish growth because they are not synthesized in fish and so must be provided in the feed. As such, they are referred to as "essential" or "indispensable" (Norris, 2005).

Most species of fish require a dietary source of at least one sulfur-bearing amino acid, although when the required one is available in sufficient quantity, other sulfur-bearing amino acids can be synthesized from it (Stickney, 1979). Methionine, cysteine and cystine contain sulphur and supply sulphur as the raw material for all the sulphur – containing compounds used in the body, such as glutathione, taurine, the thiosulphates and the thiocyanates. A particularly important amino acid for growth is lysine. Proteins rich in lysine can be obtained from legumes, fish meal, blood meal, or meat and bone meal. Tryptophan is very important for growth of all cells and normal development. It is used in cell proliferation. Arginine and histidine play an important role in maintaining a normal and healthy bloodstream and has other complex functions (William and West, 1967). Animals, fish inclusive are able to synthesize some of their amino acids (non-essential amino acids) from carbohydrates and lipids, and other nitrogen compounds, but generally from other non-essential amino acids.

Any diet deficient in any of these essential amino acids will cause depressed appetite and growth rate of fish (Ayinla, 1991). Due to a shortage of an economic source of animal protein, plant protein is increasingly being resorted to in spite of the fact that the best quality proteins come from fishmeal (Lovell, 1981). Fishmeal is not deficient in any of the essential amino acids. In fact, it contains an excess of those which are deficient in most plant proteins. This makes fish meal a valuable complement to fish diets containing large amounts of plant ingredients.

2.3 Supplementary Feeding in Aquaculture

Once an aquaculture species reaches the juvenile stage, the major expense involved in culture is often associated with the need to provide a suitable diet to the growing organisms. This may also be true of larvae and fry, but in species that will accept prepared feeds early in the life cycle, little expense may be incurred until the organisms attain larger size (Stickney, 1979). Mudfish juveniles require more protein than mature ones and this has been found to be 40 percent crude protein (Faturoti *et al*., 1986). Fish, like land animals, have higher protein requirements during early life than during the later phases of growth. Cultured fish are usually given supplemental feeding using nutrient-dense, dry feed pellets. Different feeds which are mainly distinguished by variations in pellet size, fat and protein content are fed according to the life stage of the fish; fry, fingerling, juvenile and adult.

The pivotal role of nutrition in aquaculture has been demonstrated in several studies (Adikwu, 1987, 1992a, b, 1993, 1994; NRC, 1993; Adikwu and Yusuf 1997; Haruna and Adikwu, 1998). High protein feed ingredients are essential for fish feed formulation since protein is the main structural component of the body tissues of fish. According to Falaye and Arogunjo (1989), protein is the building block nutrient which accounts for 60 – 80 % of the dry matter of fish. *Clarias gariepinus* has a high protein requirement, thus fishmeal as a protein source in their diets satisfies this requirement. Fishmeal made from whole fish contains from 60 – 80% protein in dry weight basis and is 82 – 95% digestible to most fishes (Lovell, 1981), however, expensive feeds marginalizes the profitability of mudfish rearing especially for the small-scale farmer.

2.4 Utilization of Unconventional Ingredients in Fish Feeds

Feedstuffs of vegetable origin are as a whole lower in protein content when compared with fish meal. In addition, the presence of high amounts of carbohydrates, fibre, and other organic molecules such as glycosides, phytates, and cyclopropenes in these sources present the nutritionist with problems that are generally not encountered with the latter. Despite these problems, feedstuffs like soya bean, cotton seed meal, and sunflower meal are being used to some extent in commercial aquaculture diets. None, however, perform as well as fish meal on a protein equivalent basis. These feedstuffs contain protein in amounts ranging from about 15 to 50 percent and so fall within the range of the protein requirements for optimum growth of several species of fish. However, proteins derived from vegetable sources are somewhat deficient in several key amino acids such as lysine, methionine, and tryptophan (Hardy, 1990).

Soya bean is one of the most abundant plant protein available for fish feed today in most parts of the world, Nigeria inclusive. Therefore, a considerable amount of work has been done for its use in fish diets (Eyo, 1989). It contains all the essential amino acids which are required by fish (Lovell, 1985; Eyo, 1989). Soya bean meal is therefore the highest quality (best balanced in essential amino acids) plant protein available. However, its use in fish feeds competes with other uses to which it is put. For instance, it is incorporated in livestock and poultry feeds. Man also uses it as food and the effect of all these is an ever increasing cost due to its demand. This situation leads us in the developing world to seek other under utilized legumes that may hold potentials as fish feed sources, one of such being the widely available *Albizzia lebbeck.*

Many researchers have studied the potentials of many non-conventional high protein feed sources that could serve as substitutes for fishmeal and have arrived at recommendations such as the use of feather meal, krill, maggots and so on. Their research efforts though commendable may not be feasible in practical terms as the production of maggots for fish food may lack consumer appeal nor is the collection of krill from their natural habitat an easy feat.

2.5 Use of plant sources as partial replacement of fishmeal in fish feeds

Various researchers have experimented with plants as unconventional sources of protein for fish: Leng *et al.* in 1995 established that duckweed was a potential high-protein feed resource for domestic animals and fish. From their study, they found out that duckweed contains up to 43% crude protein, 5% lipids and a highly digestible dry matter thereby closely resembling animal protein. Compared with most plants, duckweed leaves were seen to have little fibre (5% in dry matter of cultivated plants) as they do not need to support upright structures. The roots, however, appeared to be more fibrous. As a result, the plant had little or no indigestible material even for monogastric animals. This contrasts with many crops such as soya beans, rice, or maize, where approximately 50% of the biomass is in the form of high fibre and low digestibility residues.

Xie and Jokumsen (1995) replaced fish meal with potato protein concentrate (PPC) in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum). The results showed that fish fed diets containing PPC showed decreased growth, feed utilization and condition factor, and increased mortality when PPC constituted the sole protein source. Incorporation of PPC in the diets resulted in decreased dry matter, protein and fat and increased ash contents of the fish body. In conclusion, the type of potato protein concentrate used, especially at high levels, was not suitable as an ingredient in trout feed.

Siddhuraju and Becker (2003) carried out a comparative analysis on the nutritional evaluation of differently processed mucuna seeds, *Mucuna pruriens* (L.) DC. var. *utilis* on the performance of Nile tilapia (*Oreochromis niloticus*) L.; Reigh (2003) experimented with peanut meal, sunflower meal and unconventional plant protein supplements as alternative protein sources in aquafeeds. Also, Cai *et al*. (2005) investigated the growth performance, body composition and phosphorus availability of juvenile grass carp (*Ctenopharyngodon idellus*) as affected by diet processing and replacement of fishmeal by detoxified castor bean meal.

In Nigeria, Olukunle (1982) reported that the growth performance and diet utilization of the fry of *Sarotherodon mossambicus* fed a 45% protein diet where 11% of the fish meal diet was replaced with groundnut meal and 11% by sunflower seed meal was similar to fry fed a fishmeal diet. Absalom *et al*. (1999) experimented with kidney bean, *Phaseolus vulgaris*, to find out its effects on *Oreochromis niloticus*. They obtained satisfactory results with the 60% kidney bean diet. Also, Balogun *et al*. (2004) observed that *Oreochromis niloticus* fed *Delonix regia* seed meal performed best as regards growth when the seeds were boiled for 80 minutes.

Seeds of legumes generally have a higher crude protein level than those of cereals. Also, among plant parts utilized as protein sources, legume seeds have a better potential in being used as partial replacements in fish diets because after collection they may be stored for long periods without a reduction in nutritional quality as long as certain precautionary steps are taken unlike other plant parts like leaves, whose quality may be drastically impaired as a result of long storage. For example, the viability of the seeds of *Albizzia lebbeck* remains well for at least one year (Salim *et al*., 2002). He however advised that they should be stored in sealed tins with insecticide since the seeds suffer from borer attack.

2.5.1 Anti-nutritional factors in plant sources

On the other hand, most legumes contain anti-nutritional factors which are natural toxins that at high enough levels are growth inhibitory and sometimes fatal to the animal consuming them. However, much of the toxicity is eliminated during processing of the raw material. For instance, cottonseed meal is used cautiously for fish because of the poisonous gossypol which may be present in grades of meal that contain appreciable amounts of the cottonseed hulls. Also, soya beans contain trypsin inhibitors, haemagglutins, saponins, phytoestrogens, and compounds that complex minerals. Raw groundnuts also contain trypsin inhibitors which form complexes with other elements (Eyo, 1999).

Anti-nutritional factors in most legumes prevent the digestion of proteins or the assimilation of amino acids. The heat-moisture-time conditions of processing usually minimize concern for these use-limiting factors. Smith (1986) asserts that proper processing of feeds require precise control of moisture content, temperature and processing time. Adequate moisture during processing facilitates destruction of the anti-nutritional factors. Both over and under-toasting of fish feeds can result in a meal of lower nutritional quality. Under heating produces incomplete inactivation of the anti-nutritional factors and over-toasting can reduce amino acid availabilities.

In the above cited work, heat treatment improved the digestibility of the amino acids and the nutritional value of soya beans. Heat treatment improves the nutritional value of legumes by increasing the utilization of proteins and amino acids, fats, and carbohydrates present in the meal. Therefore, digestibility is enhanced by cooking and thus the metabolizable energy value is increased. In soya bean meal, heat destroys the trypsin inhibitor, and wet heat is more effective than dry heat. Therefore, in this experiment both were utilized, analyses carried out before conclusion was made as to the type of heat that is preferable for *Albizzia* seeds.

Virtually all available ingredients of plant origin possess growth-inhibiting factors. They in most cases form a “shied effect” on the protein molecule thereby preventing the digestive enzymes from getting to them. This results in the passing out of all the proteinous molecules with the faeces undigested and hence unavailable to the fish for growth purposes (Eyo, 2003). These factors must be eliminated by special processing techniques to make them of maximum nutritional value. Also, most plant protein sources contain phytic acid which can interfere with both mineral and protein availability. Particular attention must be paid to mineral supplementation when formulating fish diets containing fairly high amounts of plant protein. Even though the temperature which destroys heat labile growth inhibitors is high, minimum protein damage is involved at these high temperatures provided the optimum peak of temperature is reached for a very brief period of time. The brevity of the period at peak temperature is more important than any other factor in obtaining optimum protein quality.

2.7 Botanic Description and Economic Importance of *Albizzia lebbeck* (Linn.) Benth.

2.6.1 Botanic description

The botanic status of *Albizzia lebbeck* is as illustrated below:

- **Taxonomy**

Family: Fabaceae (formerly Leguminoseae)

Sub-family: Mimosoideae

Synonyms: Mimosa lebbek Blanco

Mimosa sirissa Roxb.

- **Common names** (English):
  - Albizzia
  - Acacia amarilla
  - East Indian walnut
  - English woman's tongue
  - Fry wood
  - Indian siris
  - Lebbeck
  - Siris tree
  - Woman's tongue tree

Albizzia is a common name for a group of small trees or shrubs which are members of the mimosa subfamily of the pea family. They are native to warm regions of Asia, Australia and Africa. There are about 25 species of *Albizzia*. They commonly have graceful, finely cut foliage and numerous feathery clusters of white, pink, red, or yellow flowers in summer. Various species are grown in mild climates as ornamentals. For instance, in the drier areas of Thailand and Malaysia, they are grown on the road sides (Salim *et al*., 2002).

Albizzia lebbeck is an introduced species in Nigeria (Keay *et al*., 1964). It is hermaphroditic and so can reproduce itself readily. It occurs wild in forests as well as avenues and gardens where it serves as a windbreak. The tree is easily grown from seeds and can be found on a variety of soils. It grows well on laterites and deep loamy soils with good moisture supply, while stiff clay and gravelly soils restrict its growth. It is a dominant species in areas with a very dry winter. It can withstand long, hot dry periods and cold winters. After the first year, it can tolerate droughts and some frost (Salim *et al*., 2002). It can attain a height of 30m and a diameter of 1m. It is identified by greenish white flower heads, straw-coloured pods and a grayish bark with rusty brown breathing pores; rough and fissured. The flowers are bisexual. The compound, bipinnate leaves are sensitive; they bend together and droop upon slight stimulation by mechanical, chemical or electrical means.

Flowering occurs in Nigeria from November to February. Pods ripen during September to May and remain on the trees for a long time, i.e. after the new flowers have appeared. The fruits are pods and measure 15 to 30 by 2.5 to 5 cm in size. The pods are thin, flat, papery, strap-shaped, round at both the ends, straw coloured and reticulately veined. Seeds are 1 to 12 in each pod and measure 8-12 mm in size. They are ovate or oblong, compressed, brown and smooth with a hard testa (Microsoft ® Encarta ® Encyclopedia, 2005). The seeds are spaced uniformly along the whole length of the pod and each seed is attached to the margin by a hair-like thread [pappus] (Keay *et al*., 1964).

The genus is named after Filippo del Albizzi, a Florentine nobleman who in 1749 introduced *A. julibrissin* into cultivation. The species name is from the Arabic name for this plant, 'laebach'. When agitated by the wind, the pods and enclosed seeds are said to produce an incessant rattle likened to women's chatter, hence the common name 'woman's tongue' (Salim *et al*., 2002).

2.6.2 Economic importance

Albizzia lebbeck is a widely available plant in the tropics and subtropics with economic importance for industrial and medicinal uses. It is grown in some areas, like India, primarily as fodder for camels, water buffalo and cattle. In Sudan, goats eat the fallen leaves and flowers. The leaves are reported to be good fodder, with 17-26 % crude protein; 100kg of leaves yield 11-12 kg of digestible protein, and 37 kg of digestible carbohydrates. Lowry *et al*. (1994) reported that rabbits and bats feed on the leaves also. He stated that the seedlings cannot establish under the continuous grazing by cattle; and that the fallen flowers and pods can be used as feed early in the season since they are highly digestible. He went on to state that the leaves of *Albizzia lebbeck* are remarkably free of toxins and tannins and are low in phenolic compounds. The pods however contain saponin and are not eaten in large quantities by sheep, although cattle eat them readily (Salim *et al*., 2002).

Other functional uses are in the areas of; crop use: it is used as a windbreak crop (Fletcher, 1997). Also, *Albizzia lebbeck* is a nitrogen fixing woody legume. The amount of nitrogen fixed increases with the age of the plant (Kadiata *et al*., 1996). In addition, the nitrogen-rich leaves are valuable as mulch and manure; In the area of apiculture: its flowers are fragrant and so attract bees. It is highly regarded by bee-keepers for the light coloured honey its nectar provides (Salim *et al*., 2002); fuel: it serves as an excellent fuelwood species with a calorific value of 5200 kcal/g (Salim *et al*., 2002); gum or resin: the trunk yields a reddish gum that is used as an adulterant of gum Arabic (Salim *et al*., 2002); tannin or dyestuff: the bark is used locally in India for tanning fishing nets (tannin content, 7-11 %), (Salim *et al*., 2002); medicine: *Albizzia lebbeck* leaves possess anticonvulsant activity, (Kasture, 1996). The leaves or seeds are used for eye problems, and the bark to treat boils. Saponin from the pods and roots has a spermicidal activity (Salim *et al*., 2002); soap: when dried and pounded, the bark can be used for soap (Salim *et al*., 2002).

2.8 Water Quality in Aquaculture

The productivity of a given body of water is determined by its physical, chemical and biological properties. The environmental properties of water need to be conducive for fish to grow well; therefore an ideal water condition is a necessity for the survival of fish since the entire life processes of the fish is wholly dependent on the quality of its environment. These water quality parameters become more critical in intensive culture systems where fish are raised in artificial ponds with reduced self-purification capabilities as compared with natural systems.

The physical properties of water which are of paramount importance in outdoor digestibility studies include temperature and total dissolved solids (TDS), this is because the outdoor tanks are directly exposed to sunlight, therefore wide temperature values may occur while TDS affect turbidity or water transparency. The chemical properties of water include pH, dissolved oxygen and electrical conductivity (Lind, 1979). A drastic change, i.e. either a rise or fall from the optimum will affect the existence of fish and so is usually properly monitored.

2.7.1 Dissolved oxygen

In the absence of deliberate poisoning, dissolved oxygen is the single most important and critical water quality parameter for fish in pond culture systems (Boyd and Lichtkoppler, 1979). Nearly all aquatic organisms, with the exception of some bacteria, must have oxygen to survive (Wheaton, 1977). Most of these organisms must extract their oxygen from liquid water. Thus, the extraction of oxygen from water and the addition of this gas to water are operations of critical importance to fish.

Oxygen is often a limiting factor in aquatic systems. Primary production sources (i.e., photosynthetic plants) produce oxygen in the presence of light but require it when it is dark. Thus oxygen can limit both primary and secondary production. The two major sources of oxygen for water are plants in which photosynthesis occurs and the atmosphere. The amount of oxygen in water is increased by primary productivity which takes place in outdoor ponds and by wind action which aerates the water surface. Wheaton (1977) states that the rate of oxygen transfer from air to water depends on; water temperature, degree of saturation of the water and turbulence of the air-water interface.

Photosynthesis stops at night, but plants and animals continue to respire and consume oxygen. As a result, dissolved oxygen levels fall to a low point just before dawn. Photosynthetic oxygen production lags slightly behind the daily radiant energy cycle. Because of this, oxygen content of natural and pond waters usually reaches its daily minimum just at or slightly after daybreak. From then on the dissolved oxygen levels rise from morning through the afternoon as a result of photosynthesis, reaching a peak in late afternoon. Maximum oxygen concentrations are usually observed in the mid to late afternoon.

Inadequate dissolved oxygen has many effects on fish; fish stop feeding, growth is impaired and fish become stressed thereby becoming more susceptible to diseases. Cold water fishes require large amounts of dissolved oxygen in water. This is only physically possible in cold water (5^o – 15^oC). Warm water fish, on the other hand, are able to survive in water with low oxygen content, which is the case in water between 20^o – 40^oC, where these fish are reared.

2.7.2 Temperature

Temperature is next to dissolved oxygen as the single most important factor affecting the welfare of cultured fish. Fish are poikilothermic and water temperature plays a tremendous role in their feeding. This parameter affects the metabolic activities, feeding potential, growth, survival, reproduction in all fishes (Dupree and Hunner, 1984) and efficiency of food conversion (Martinez-Palacios *et al*., 1993). It has been reported that a 5^oC sudden change in temperature will stress or even kill fish and this has formed the basis of the acclimatization of fish (Adeniji and Ovie, 1990).

Temperature has a pronounced effect on the rate of chemical and biological processes in water. For instance, fish require twice as much oxygen at 30^oC than at 20^oC (Adeniji and Ovie, 1990). Increment of the water level may therefore be necessary at higher temperatures. Temperature also affects the dominance and distribution of phytoplanktons in water. It is recommended that fish in the tropics be kept in water whose temperature range is between 25^oC and 30^oC (Auta, 1993).

2.7.3 Hydrogen ion concentration (pH)

The pH of water is a measure of how acidic or alkaline it is on a scale of 0 to 14 with 7 being neutral. In fish ponds, the time of day that a sample is taken often will influence the pH because of variations in the carbon dioxide concentration. As plants in the water remove carbon dioxide for photosynthesis, the pH will increase. At night, the pH will decrease as carbon dioxide accumulates. Increasing the total alkalinity concentration in water helps buffer against pH changes.

Chronic pH levels may reduce fish reproduction and are associated with fish die-offs (Stone and Thomforde, 2006). Adeniji and Ovie (1990) noted that acid and alkaline death points are approximately at pH 4 and 11 respectively and that waters with pH values ranging from 6.5 to 9 were observed to be the most suitable for fish production. Dupree and Hunner (1984) also observed that high pH increased the toxicity of sulphides. Also, Auta (1993) stated that the desirable pH range for fish is 6.7 to 9.0.

2.7.4 Total dissolved solids (TDS)

The term “Total Dissolved Solids” (TDS) refers mainly to the inorganic substances that are dissolved in water. These substances can include carbonate, bicarbonate, chloride, sulphate, phosphate, nitrate, calcium, magnesium, sodium, organic ions and other ions. A certain level of these ions in water is necessary for aquatic life (Stone and Thomforde, 2006). TDS (in mg/l or ppm) are solids in water that can pass through a filter (usually with a pore size of 0.45 micrometers).

Changes in TDS concentrations can be harmful because the density of the water determines the flow of water into and out of an organism's cells. Therefore, if TDS concentrations are too high or too low, the growth of fish can be limited, and death may occur. Similar to total suspended solids (TSS), high concentrations of TDS may also reduce water clarity, contribute to a decrease in photosynthesis, combine with toxic compounds and heavy metals, and lead to an increase in water temperature (Murphy, 2005).

2.7.5 Electrical conductivity (EC)

Electrical conductivity (EC) is a measure of how well a solution conducts electricity and is correlated with salt content. Conductivity is typically reported in units of µSiemens/cm (microsiemens per centimeter). Freshwater fish generally thrive over a wide range of electrical conductivity. Electrical conductivity (EC) also can be used to give a rough estimate of the total amount of dissolved solids (TDS) or the total amount of dissolved ions in water. Typically, the TDS value in mg/l is about half of the EC (µ Siemens/cm). High conductivity is an indication of the presence of large amounts of dissolved salts which may be detrimental to fish. The desirable range is 100-2,000 µSiemens/cm and the acceptable range is 30-5,000 µSiemens /cm (Stone and Thomforde, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Seed Collection

Dry pods of *Albizzia lebbeck* (Plate II) were collected from the trees in the main campus of Ahmadu Bello University, Zaria, where they are grown as aesthetic plants. They were thrashed in the bags with which they were collected and winnowed on a tray to get clear seeds (Plate III) by blowing air through in order to remove the chaff.

3.1.1 Seed processing

The *Albizzia* seeds were divided into eight portions and processed differently – raw (T1), boiled for 15 minutes (T2), boiled for 30 minutes (T3), boiled for 1 hour (T4), toasted for 1hour (T5), toasted for 2 hours (T6), toasted for 3 hours (T7) and roasted traditionally (T8).

The boiling was done with an aluminum pot using one part of the raw whole seeds to 10 parts of clean water on a gas burner for each processing that had to do with boiling. Traditional roasting was done by constantly stirring the seeds to prevent charring in a dry pot until browned. Toasting was done in an electric oven at 70^{o}C for each processing that had to do with toasting in the Amino acid Laboratory, University of Jos, Plateau State.

The boiled seeds were sun-dried while the roasted and toasted seeds were exposed to air in the laboratory to hasten cooling. The seeds were subsequently milled to obtain a homogeneous powder and stored in air-tight stoppered glassware before analysis was carried out. Proximate analysis and the levels of anti-nutritional factors were determined. The amino acid levels were also quantified. The results obtained served as a basis for determining the *Albizzia* meals that were used for the feeding trials.

PLATE II DRY PODS OF *ALBIZZIA LEBBECK*

PLATE III SEEDS OF *ALBIZZIA LEBBECK*

3.2 Proximate Analysis

The proximate composition (moisture, crude protein, crude lipid, crude fibre, ash and nitrogen free extract) of the raw, boiled, roasted and toasted seeds; experimental fish before and after the experiment were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 1980). All chemical analyses were replicated.

3.2.1 Moisture determination

Principle: This was done based on the difference between the net weight and the weight after drying to a constant (at 100^oC) for 24 hours.

Procedure: Crucibles were washed and dried to a constant weight in an oven at 100^oC. They were later removed and placed in a desiccator and weighed (W_1). 5g of the sample was placed in the weighed crucible (W_2). The crucible containing the sample was kept in an oven at 100^oC for 24 hours and then weighed. It was kept back in the oven and reweighed after about 3 hours to ensure a constant weight (W_3). Loss in weight was equal to water content of the original sample. The moisture content was calculated as:

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \quad X \quad 100$$

Where: W1 = Weight of an empty crucible

W2 = Weight of a known amount of sample (fresh) + crucible

W3 = Weight of oven dried sample

3.2.2 Crude protein determination

Principle: Nitrogen is used as an index of the protein and so is termed crude protein because it represents all of the nitrogen that is in the form of non-protein nitrogen (NPN) such as nitrates, ammonia, urea and single amino acids, as well as the nitrogen present as true protein. The multiplication by a conversion factor of 6.25 is based on the assumption that true protein contains 16% nitrogen.

Procedure: Protein determination was carried out in three stages, as follows:

Digestion: One gramme of sample was weighed and placed into a 50 ml digestion – flask and the Kjeldahl mixture which acts as a digestion catalyst was added with 5ml concentrated sulphuric acid (H_2SO_4). Some pumice stones (anti-bumping granules) were also added. The flask containing the sample mixture was heated gently at an inclined angle in a Kjeldahl digestion rack until frothing subsided. It was then boiled until the solution became colorless. Heating of the mixture released the nitrogen in the various samples which was then converted to ammonia with the concentrated sulphuric acid. It was later allowed to cool. The sample was transferred to a 100ml volumetric flask, diluted with distilled water to the mark and mixed thoroughly. The mixture was further allowed to cool before distillation. A blank containing only the sulphuric acid and catalyst was also heated.

Distillation: A known aliquot (10 ml) was transferred to the sample addition funnel of the distillation apparatus and then introduced to the sample chamber. 10 ml of 40% sodium hydroxide was added to the sample addition funnel and released to the sample chamber at a slow rate. The ammonia was entrapped in a receiving solution containing 10ml 2% boric acid solution into which 4 drops of bromocresol green/2 drops of methyl red indicator had been put. Distillation was continued until the pink colour turned greenish.

Titration: Titration with standard HCl acid (0.01N) was then carried out and the percentage of crude protein determined with the following calculation:

$$\% \text{ Nitrogen} = \frac{\text{Titre value (A-B) X Normality of acid X Vol of dilute X } N_2 \text{ X 100}}{\text{Sample wt (mg) X Vol of digest (aliquot)}}$$

$$= \frac{\text{Titre value X 0.01 X 100 X 14.007 X 100}}{\text{Sample wt (mg) X 10}}$$

Where: A = Titre value for the digestion sample

B = Titre value for the blank

% Crude protein = % N_2 X 6.25

3.2.3 Crude lipid determination

Principle: This is the continuous extraction of fat content from a sample using a suitable solvent in a soxhlet extractor.

Procedure: Two grammes of the dried sample (residue from moisture determination was used) was placed into a pre-weighed extraction thimble (W_1) and the weight recorded (W_2). A dry 250 ml round bottom quickfit flask was weighed (W_3) with a few anti-bumping granules inside. The thimble was fitted into the extraction unit of the Soxhlet extraction apparatus using a pair of forceps. 300 ml of petroleum ether (40-60°C boiling point) was poured into the quickfit flask. The apparatus was connected to a condenser. The heating rate was adjusted to give a condensation rate of 2 to 3 drops. After reflux extraction for 8 hours, the thimble was removed and the ether was reclaimed using the apparatus by distilling out some ether. The removal of ether from the flask containing a mixture of ether and extracted fat was completed on a boiling water bath and the flask dried in an oven at 105°C for 30 minutes. After this, the flask with the pure fat content was cooled in a desiccator and weighed (W_4). The percentage crude fat was calculated as follows:

$$\% \text{ Crude fat} = \frac{W_4 - W_3}{W_2 - W_1} \times 100$$

$$= \frac{\text{Fat}}{\text{Sample}} \times 100$$

3.2.4 Crude fibre determination

Principle: The bulk of roughage in foods is referred to as the fibre. This is the non-digestible portion of the carbohydrate contained in the sample.

Procedure: Two grammes of the defatted dried sample obtained from crude fat determination was transferred into a 600 ml beaker. The digestion mixture was prepared by mixing 500ml glacial acetic acid, 450ml distilled water and 50ml concentrated nitric acid. 20g of trichloro-acetic acid was dissolved in this mixture and mixed thoroughly. The quickfit flask was attached to a condenser. It was brought to a boil and refluxed for 40 minutes. Timing commenced from the time the mixture started to boil. Thereafter, the content of the flask was filtered through a weighed Whatman No. 4 ashless filter paper which was placed in a Buchner funnel over a conical flask. During filtration, residue from the digestion was washed six times with hot water and once with industrial methylated spirit to wash down particles adhering to the sides. The filtrate was dried at 105°C for 12 hours, cooled and weighed to get the residue. The residue now made up of crude fibre and ash was incinerated in weighed crucibles at 600°C for 12 hours. This was then cooled and weighed to obtain the weight of the ash.

The loss on ignition was the crude fibre.

3.2.5 Ash content determination

Principle: By ashing the sample, all the volatile organic constituents in the sample were burnt off leaving behind the non-volatile mineral elements.

Procedure: An empty crucible was dried in the oven. After drying, it was cooled in the desiccator and weighed (W_1). 2g of the already dried sample obtained after moisture extraction was put into the crucible and the weight recorded (W_2). This was then put into a muffle furnace and incinerated at 525°C for 24 hours. After this, it was placed in a desiccator for cooling and then re-weighed (W_3). Percentage ash content was computed by carrying out the following calculation:

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

$$= \frac{\text{Ash}}{\text{Sample}} \times 100$$

3.2.6 Nitrogen free extract

The nitrogen-free extract (NFE) which is the digestible portion of the carbohydrate contained in the sample was determined by subtracting the percentage sums of moisture, crude protein, crude fat, crude fibre, and ash from 100.

3.3 Anti-nutritional Factors

3.3.1 Cyanogenic glycoside

The procedure of A.O.A.C. (1984) was used in the determination of cyanogenic glycosides. To determine cyanogenic glycosides in the samples, the alkaline titration method was used. 5 g of sample was placed in a 200ml conical flask. 50ml of distilled water was added. The contents of the flask was stirred and allowed to stand for 4 hours. The filtrate was collected into a beaker using glass wool which was placed in a funnel. The filtrate was steam distilled into 20ml of 2.5% Sodium hydroxide. About 75ml of the distillate was collected. This was titrated with 0.02N Silver nitrate after the addition of 8ml of 6N Ammonium hydroxide and 2ml of 2% Potassium iodide. Permanent turbidity indicated end point.

1ml 0.02N $AgNO_3$ = 1.08 mg HCN

3.3.2 Phytic acid

Phytate phosphorus was determined by the method of Wheeler and Ferrel (1971). A known weight of each ground sample was soaked into 100ml of 2 % HCl for 5 hours and filtered. 25cm^3 of the filtrate was taken into a conical flask. 50cm^3 of 0.3% potassium thiocynate solution was added. The mixture was titrated in a standard solution of $FeCl_3$ until a brownish-yellow colour persisted for 5 minutes. The concentration of the $FeCl_3$ was 1.04% w/v.

Calculation: Mole ratio of Fe to phytate = 1:1

$$\text{Concentration of phytate phosphorous} = \frac{\text{Titre value X 0.064}}{1000} \text{ X weight of sample}$$

3.3.3 Oxalate

Oxalate was determined by using the method of Oke (1969). The total oxalic acid of the powdered samples was determined by weighing 2g into a 250ml flask. Then 190ml distilled water and 10ml of 6M hydrochloric acid were added. The mixture was warmed for 1 hour on boiling water bath, cooled, transferred into a 250 ml volumetric flask, diluted to volume and filtered. Four drops of methyl red indicator were added followed by concentrated ammonia till the solution turned faint yellow. It was then heated to 100^{o}C, allowed to cool and filtered to remove precipitate containing ferrous ions. The filtrate was boiled and 10ml of 5% calcium chloride was added with constant stirring. It was then allowed to stand overnight.

The mixture was filtered through Whatman No 40 filter paper. Then the precipitate was washed several times with distilled water and transferred to a beaker and 5ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80^{o}C and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute.

A blank was also run for the test sample. From the amount of potassium permanganate used, the oxalate content of the unknown sample was calculated using the equation below:

1ml of potassium permanganate = 2.24mg oxalate.

3.3.4 Tannin

Tannin was determined using the standard method described by A.O.A.C. (1980). Two grammes of the dried sample was boiled with 300ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non-absorbent cotton wool. A volume of 25ml of the infusion was measured into a 2 litre porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution changed green; then few drops of 0.1N potassium permanganate was added. The titre was multiplied by 0.0066235 to obtain the amount of tannin in the sample. The equation is below:

0.1N oxalic acid = 0.0066235g tannin

3.3.5 Saponin

The standard method of A.O.A.C. (1984) was used to determine saponin in the samples. A gravimetric method employing the use of a soxhlet extractor and two different organic solvents was used. The first solvent extracts lipids and interfering pigments while the second solvent extracts the saponins proper.

A known weight of the dried ground sample was weighed into a thimble and transferred into the soxhlet extraction chamber fitted with a condenser and a round - bottomed flask. Some quantity of acetone enough to cause a reflux was poured into the flask. The sample was exhaustively extracted of its lipids and interfering pigments for 3 hours by heating the flask on a hotplate and the solvent was distilled off.

For the second extraction, a pre-weighed round - bottom flask was fitted into the soxhlet apparatus which bore the thimble containing the sample. Then methanol was poured into the flask. The methanol was enough to cause reflux. The saponin was then exhaustively extracted for 3 hours by heating the flask on a hotplate after which the solvent was distilled off. The flask was reweighed. The difference between the final and initial weights of the flask represented the weight of the saponins extracted.

$$\% \text{ Saponins} = \frac{\text{Weight of saponins}}{\text{Weight of sample}} \times 100$$

3.4 Amino Acid Analyses

The amino acid profile of the seed samples were determined using methods described by Spackman *et al*. (1958).

Procedure: Amino acid analyses determination was carried out in three stages:

Defatting of samples: A known weight of the dried sample was weighed into an extraction thimble and the fat was extracted with chloroform/methanol mixture (2:1 ratio) using soxhlet extraction apparatus as described by A.O.A.C. (1980). The extraction lasted for 8 hours.

Hydrolysis of the samples: 0.5 g of the defatted sample was weighed into a glass ampoule. 7ml of 6N hydrochloric acid was added and oxygen was expelled by passing nitrogen into the ampoule. (This is to avoid possible oxidation of some amino acids during hydrolysis). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^0C \pm 5^0C$ for 22 hours. The ampoule was allowed to cool before being broken open at the tip to release the content which was filtered using a Whatman filter paper (No. 1).

The filtrate was transferred into a 250ml round-bottom flask, then placed in the rotary evaporator where it was evaporated to dryness at 40^0C under vacuum. The residue was subsequently washed twice with 10ml of de-ionized water and evaporated to dryness. The residue was picked up in 3ml of 0.01N HCl and preserved in a refrigerator at -6 ± 4^0C for loading into an amino acid analyzer when required.

Loading of the Hydrolysate: The hydrolysate was loaded into the Technicon Sequential Analyzer Multi-sample Amino acid analyzer (TSM): 1ml of the hydrolysed sample and a standard mixture were loaded with an equal volume of an internal standard solution (Norleucine) using a micro-pipette. These were dispensed into the cartridges of the analyzer. The cartridges were then placed on a sample tray of the TSM sampler. Two chromatographic columns were packed with 75% resin slurry and mounted directly below the TSM sampler. The front column was used for the separation of basic amino acids and the rear column was used for the separation of the acidic and neutral amino acids. Buffer solutions were connected to the systems via peristaltic valve.

The buffer solutions washed the column, thereby removing some of the amino acids bound to the resin by electrostatic forces. As the amino acids were eluted from the column, they were collected individually in an automatic fraction carefully partitioned by a bubble system into an analytical system. Here they were mixed with hydrin solution supplied through the proportioning pump. The reaction mixture now formed passed through a capillary tube immersed in a 100 ^{0}C water bath, where amino acids reacted with ninhydrin to produce a coloured product. The developed effluent (reaction steam) was led through the calorimeter where the absorbance was monitored continuously as it passed over a sensitive photoelectric unit. The signals from the calorimeter was finally fed into an automatic pen recorder which then plotted the intensity on a moving linear chart paper, the speed of which was carefully preset. The type of amino acid was established on the basis of its position on the chromatogram and it was quantitatively estimated on the basis of the area under the curve.

The chromatogram peaks (Appendices 1-9) obtained from the analysis was interpreted thus: The net height of each peak produced by the chart record (each representing an amino acid) was measured. The half–height of the peak on the chart was found and the width of the peak at half–height was accurately measured and recorded.

Approximate area of each peak was then obtained by multiplying the net height with the width at half-height.

The Norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{\text{Area of Norleucine peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture

$S_{std} = NE_{std}$ x Molecular weight x μMAA_{std}

Finally, the amount of each amino acid present in the sample was calculated in g/100g protein using the following formula:

Concentration (g/100g protein) = NH x Width at NH/2 x S_{std} x C

Where: NH = Net height

W = Width at half-height

Nleu= Norleucine

(Refer to Appendices 10-18).

3.5 Experimental Diets

3.5.1 Diet formulation and composition

The crude protein values of the *Albizzia* seeds derived from the proximate analysis were used to formulate feed at a crude protein level of 40% using Pearson Square method (Table 3.1). The experimental diets comprised of *Albizzia* meal, fish meal (clupeid), yellow corn, palm oil, bone meal, vitamin premix and salt. From the analyses carried out on the differently processed samples of *Albizzia*, the boiled seeds were chosen for feeding trial for the *Clarias* juveniles. Diet 1(raw) served as a control diet. Diets 2, 3 and 4 (boiled for 15 minutes, 30 minutes and 1 hour respectively) were those that had the best crude protein levels and amino acid array. Also, it was observed that the boiled seed samples had lowered anti-nutritional factors when compared with the toasted and traditionally roasted seed samples. All diets were isoproteic (40% protein).

3.5.2 Diet preparation

In preparing each of the diets, the proportions of the ingredients (Table 3.1) were weighed separately. Each diet was first mixed dry and later with just enough hot water to obtain a homogenous hard dough. Pelleting was done through compression to agglomerate ingredients into larger homogenous particles, thereby producing dense sinking pellets. Pelleting gelatinized the cassava flour, which acted as a binding ingredient. The locally fabricated pelleter produced pellets that were about 0.2cm in diameter and 2cm in length (Plate IV). The finished products were sun-dried and stored in labeled polyethylene bags. 100g sample of each diet was analysed in the laboratory for proximate composition of the fish feed pellets following standard methods (A.O.A.C., 1980). The chemical analysis was conducted to ensure that the final product contained all the nutritional and energy requirements needed for optimal fish growth and physiological functions.

TABLE 3.1 GROSS COMPOSITIONS OF THE EXPERIMENTAL DIETS

	DIETS			
INGREDIENTS	D1	D2	D3	D4
Albizzia meal	32.72	33.46	33.13	33.49
Fish meal	32.72	33.46	33.13	33.49
Yellow corn	17.38	16.39	16.83	16.35
Cassava	8.69	8.19	8.42	8.18
Palm oil	4.0	4.0	4.0	4.0
Bone meal	2.0	2.0	2.0	2.0
Vitamin premix	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5
Total (%)	100.01	100.0	100.01	100.01

Albizzia meal/Fish meal — 1: 1

Yellow corn meal/Cassava meal — 2 : 1

Crude protein levels utilized in the Pearson Square method:

Fishmeal (small-sized clupeids) – 68.5

Yellow corn – 10.8

Cassava (peeled) – 2.6

SOURCE: Bolorunduro (2002)

D_1 D_2 D_3 D_4

PLATE IV SAMPLES OF PELLETED FEEDS

key: D1 – Raw

D2 – Boiled 15 minutes

D3 – Boiled 30 minutes

D4 – Boiled 1 hour

3.6 Experimental Fish

Two hundred juveniles of *C. gariepinus* were obtained from the Bagauda Fish Seed Multiplication Centre, Kano. They were transported in cool aerated water in partially-cut 50 - litres jerry can to the Biological Sciences Department, Ahmadu Bello University, Zaria. On arrival, the fish were transferred to a holding tank containing dechlorinated municipal tap water of temperature 26.5^oC and were allowed to acclimatize for one week. During this period, they were fed once a day on a commercial fish feed pellet (Coppen feed) of 2mm. This was necessary to allow the fish to adapt physiologically to a new set of ambient conditions which differed from its immediate preceding environment. There was no mortality during the acclimatization period.

Chemical analysis of samples of the fish was carried out at the beginning of the experiment, as well as samples of fish from each treatment separately at the end of the experiment, following standard methods (A.O.A.C., 1980).

3.7 Experimental Set-up

The experimental set-up consisted of eight out-door concrete tanks of 1.3m x 0.9m x 1m capacity (1.17m^3) situated in the out-door hatchery behind the Fish Laboratory at the Biological Sciences Department, Faculty of Science, Ahmadu Bello University, Zaria (Plate V). The concrete tanks were cleaned and cleared of debris. The sides and bottom of each tank was dampened with water and treated with agricultural lime ($CaCO_3$) which is judged to be the best liming material for fish ponds (Bolorunduro, 1999) at an application of 100g/m^2 as recommended by Ibiwoye (1996). This was achieved by scrubbing with a broom and was left to stand for 24 hours. The Liming was done as a prophylactic measure to eradicate any existing pathogens, parasites, cysts or spores of parasites and so served as disinfectant. The tanks were drained after a day and washed clear of lime.

PLATE V SIDE VIEW OF EXPERIMENTAL SET-UP SHOWING WIRE MESHES WITH WOOD TRIMMINGS

PLATE VI TOP VIEW OF EXPERIMENTAL SET-UP WITHOUT THE WIRE MESHES

Agricultural lime is cheap and readily available as it is a by-product of cement factories (Bolorunduro, 1999). The tanks were later refilled with water to a depth of 0.6m and maintained at that level as recommended by Viveen *et al*. (1986). The collected water was exposed to air to permit dechlorination. Wire mesh with wood trimmings were constructed and placed over each tank to prevent exogenous objects from dropping into the tanks during the duration of the experiment.

3.7.1 Stocking density

From the dimensions of the tanks and the water depth that was to be utilized, fish were stocked at a rate of 20 juveniles a tank for experimental purpose (Sogbesan, 2004).

3.8 Experimental Design

The statistical design of the experiment was Completely Randomised Block Design (CRBD). There were four treatments and two replications.

3.9 Feeding Rate and Practices

The fish were starved for 48 hours to empty their gastro-intestinal tract before the feeding trial (Eyo *et al*., 2004). An eight-week feeding trial was carried out with duplicate groups of twenty fingerlings each. Daily fish ration was calculated based on the body weight of the juveniles at 5% (2.5% in the morning; 8-9am and 2.5% in the evening; 5-6 pm) throughout the duration of the experiment. The reason for this feeding regime was because unlike tilapia that are frequent feeders due to their small rudimentary digestive systems, catfishes can consume a large meal at one feeding and therefore may not feed again for 8-12 hours after that (Castaldo, 1995). Feed rations were increased fortnightly after the weight determinations.

3.9.1 Fish weight measurement

The initial body weight of each set of fish was measured using a Mettler Top Loading balance before they were stocked. Thereafter, the experimental fish in each tank were weighed bi-weekly during the course of the experiment.

3.9.2 Fish length measurement

The initial body length of each set of fish was measured using a graduated meter rule before they were introduced into the experimental tanks. Thereafter, the lengths of the fish were measured fortnightly.

3.9.3 Monitoring of water quality

Careful monitoring was necessary in order that the water quality parameters be known and managed to maintain conditions within acceptable limits.

The dissolved oxygen in the experimental tanks was routinely monitored in the mornings between 9-10 a.m. weekly with the aid of a portable calibrated oxygen meter (HANNA HI 9142 model). Between readings, the meter tip was rinsed by dipping the probe into distilled water before dipping into the water in the tanks. The taking of readings was done once the value had stabilized. The meter provided sensitive and precise readings. Duplicate readings were taken at each experimental tank.

In addition, routine monitoring of other water parameters; temperature, pH, total dissolved solids and electrical conductivity was conducted using a HANNA model (HI 991300). The probe of the portable meter was let down into the sub-surface area of the water contained in the tanks. The readings were taken after they had stabilized. Rinsing of the probe in distilled water was done between readings.

3.10 Analysis of Fish Growth and Nutrient Utilization

Data Collection: Fish were measured at experiment initiation, week 2, week 4, week 6 and at the end of the study i.e. week 8 by transferring the fish from the test tanks into basins containing enough water to cover the fish to the laboratory. At the end of the experiment, the following growth indices of feed utilization were calculated:

3.10.1 Mean Weight Gain (MWG)

The fish fresh mean weight gain (MWG) was calculated as the difference between the final mean weight of the fish at the end of the experiment and the initial mean weight in grams (Castell and Tiews, 1980):

MWG = Final mean weight - Initial mean weight

3.10.2 Percentage Live Weight Gain (LWG%)

The percentage live weight gain was computed as the difference between the final mean weight and initial mean weight (i.e. MWG) divided by the initial mean weight expressed as a percentage (Wannigamma, Weerakoon and Muthukumarana, 1985):

$$\%LWG = \frac{W_f - W_i}{W_i} \times 100$$

Where: W_i = Initial mean weight

W_f = Final mean weight

3.10.3 Specific Growth Rate (SGR%)

This is the mean percentage increase in body weight per day over a given time interval (Brown, 1957):

$$SGR = \frac{\text{Ln Weight at time of observation} - \text{Ln Initial Weight}}{\text{Duration of experiment in days}} \times 100$$

Where Ln =Natural log

3.10.4 Feed Conversion Ratio (FCR)

The efficiency of a feed is normally measured by the amount necessary to produce a unit weight of fish. This is called the feed conversion ratio or FCR. The FCR is the unit weight of feed given, divided by the live weight or wet weight of the animal produced (Hepher, 1988):

$$FCR = \frac{\text{Total dry feed fed (g)}}{\text{Total wet weight gain (g)}}$$

3.10.5 Gross Food Conversion Efficiency (GFCE%)

The GFCE is the reciprocal of the FCR expressed as a percentage (Stickney, 1979):

$$GFCE = \frac{1}{FCR} \times 100$$

3.10.6 Protein Efficiency Ratio (PER)

This is the efficiency with which the fish utilizes dietary protein and is defined by the equation (Osborne *et al*., 1919):

$$PER = \frac{\text{Wet weight gained by fish (g)}}{\text{Weight of crude protein fed (g)}}$$

3.10.7 Apparent Net Protein Utilization (App. NPU)

The Apparent NPU is a measure of the efficiency of growth and is expressed thus (Miller and Bender, 1955):

$$\text{Apparent NPU} = \frac{\text{Protein retained in grams}}{\text{Protein consumed in grams}} \times 100$$

3.10.8 Condition Factor (K)

To ascertain the general ‘robustness’ or ‘plumpness’ of the fish, the Condition Factor is calculated thus (Brown, 1957):

$100W/L^3$

Where: W = Body weight (g)

L = Standard length (cm)

3.10.9 Carcass analysis

At the end of the study, four fish were selected from each tank and oven dried. The tissues from these representative samples of fish were ground using an electrical mill and subjected to proximate analysis. Percent moisture, crude protein, crude fat, crude fibre and ash were determined according to AOAC methods (AOAC, 1980). This was done to compare the concentrations of specific nutrients in whole body and tissues of the fish from the different treatment groups with the initial.

3.11 Statistical Analysis

The data obtained were subjected to Analysis of Variance (ANOVA) and where there was significant difference at 5% level or more, the differences in the means were separated by the use of Duncan Multiple Range Test (DMRT) (Duncan, 1955). Correlation Coefficient was used to determine the length-weight relationship of the fish studied.

3.12 Limitations to Study

In as much as efforts were made to minimize experimental errors, the following limitations were encountered in the course of this study.

a. Fortnight draining of the tanks to facilitate the removal of experimental fish for weight measurements might have acted as a stress factor to the fish.

b. Interruptions of electricity during weight measurements elongated the hours used in weighing fish.

c. Experimental diet was administered at a constant level of 5% body weight of fish. However, in the last two weeks, this percentage exceeded the fish appetite and resulted in low water quality due to uneaten feed.

d. Lack of certain reagents necessary for some analytical procedures influenced the number of anti-nutritional factors determined.

CHAPTER FOUR

RESULTS

4.1 Proximate Composition of Processed *Albizzia lebbeck*

Table 4.1 shows the percentage proximate composition of the seeds of *Albizzia lebbeck*. The results obtained determined the treatments that were used for the feeding trial. The treatments that had higher crude protein levels and low fibre content were used for fish feed (T1 - raw, T2 - boiled 15 minutes, T3 - boiled 30 minutes and T4 – boiled 1 hour). After incorporation with other feed ingredients, the finished product which was in a pelleted form was analysed for their proximate composition in the laboratory. Refer to Table 4.2.

TABLE 4.1 PROXIMATE COMPOSITION OF THE RAW AND PROCESSED SEEDS OF *A. LEBBECK*

COMPOSITION	DIETS TREATMENTS								SEM
COMPOSITION	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**SEM**
Moisture	4.55b	4.04c	3.74e	3.80de	3.73e	5.29a	3.91cd	2.47f	0.0025
Crude Protein	38.04a	35.95b	36.88ab	35.90b	30.55de	29.73e	31.52d	33.19c	0.2577
Crude Lipid	5.66c	6.53b	6.87a	5.70c	5.44cd	5.08d	5.43cd	5.39cd	0.0113
Crude Fibre	11.63g	12.65f	13.19e	14.51d	19.03a	18.05b	17.47c	17.38c	0.0272
Ash Content	7.84a	5.08b	5.04b	4.42de	4.66cd	4.85bc	4.20e	4.35e	0.0055
Nitrogen Free Extract	32.28e	35.75c	34.28d	35.67c	36.59b	37.00ab	37.47a	37.22ab	0.0351

Note: Means with the same letter(s) in rows are not significantly different (P>0.05).

TABLE 4.2 PROXIMATE COMPOSITION OF THE EXPERIMENTAL DIETS

	D1	D2	D3	D4	
Moisture	3.01a	3.22a	3.66a	3.22a	0.0347
Crude Protein	54.98b	55.19a	55.73a	55.57a	0.0612
Crude Lipid	7.13a	6.52a	6.92a	6.86a	0.1057
Crude Fibre	8.49a	8.27a	8.52a	8.87a	0.124
Ash Content	5.01a	5.43a	5.21a	5.48a	0.0249
Nitrogen Free Extract	21.40a	21.38a	19.97b	20.02a	0.035

Note: Means with the same letter(s) in rows are not significantly different (P>0.05).

4.2 Anti-nutritional Factors

The raw and heat-processed *Albizzia* seed samples contained some anti-nutritional factors as can be seen in Table 4.3. Cyanogenic glycoside, tannins and phytic acid were very low in quantity. Heat was also found to reduce certain anti-nutritional factors like oxalate and saponin but the levels of cyanogenic glycoside and tannins were not significantly different in raw and heat-processed samples. For instance, tannins in T1 (raw) were 0.002mg/g, in T2-T4 (boiled) were also 0.002mg/g, but in all the toasted samples were 0.003mg/g while in the roasted sample they were 0.002mg/g.

ANTI-NUTRITIONAL FACTORS	TREATMENTS								
	T1	T2	T3	T4	T5	T6	T7	T8	SEM
Cyanogenic glycoside (mg/g)	0.11a	0.13a	0.13a	0.13a	0.09a	0.09a	0.09a	0.09a	0.0034
Phytic acid (mg/g)	0.256a	0.192b	0.192b	0.16c	0.16c	0.16c	0.128d	0.096e	0.1203
Oxalate (mg/g)	2.80a	2.41b	1.85c	1.29d	2.19b	2.07c	1.46d	1.51d	0.0031
Saponin (%)	18a	9c	7c	4d	16b	15b	14b	14b	0.0132
Tannin (mg/g)	0.002a	0.002a	0.002a	0.002a	0.003a	0.003a	0.003a	0.002a	0.1320

TABLE 4.3 LEVELS OF ANTI-NUTRITIONAL FACTORS IN RAW AND PROCESSED SEEDS OF *ALBIZZIA LEBBECK*

Note: Means with the same letter(s) in rows are not significantly different (P>0.05).

4.3 Amino Acid Analyses

The levels of the Essential Amino Acids (EAA) required by C*larias gariepinus* for growth in raw and processed seeds of A*lbizzia lebbeck* are shown in Table 4.4. A Chemical Score of the individual amino acids in the *Albizzia* seed meals was conducted using a reference protein; in this case, fishmeal. The result obtained showed the essential amino acids that were limiting to the growth of fish in each of the treatments. The general trend was that lysine, methionine, phenylalanine and valine were the limiting amino acids in all the treatments as none of them met the Essential Amino Acid requirements for *Clarias gariepinus*, which are; 5.0, 2.3, 5.0 and 3.0 respectively in g/100g.

The mean Chemical Score (CS) of the different treatments showed that T3 had the best essential amino acid array while the second best was T4. The results of the amino acid analysis contributed to the choice of the boiled treatments rather than the toasted and roasted.

There were seventeen amino acids that were quantified using the Technicon Sequential Analyzer. The chromatographs obtained are placed in Appendices 1 to 9. The interpretations of the chromatographs are placed in Appendices 10 to 18.

TABLE 4.4 LEVELS OF THE ESSENTIAL AMINO ACIDS (EAA) REQUIRED BY *CLARIAS GARIEPINUS* FOR GROWTH IN RAW AND PROCESSED SEEDS OF *ALBIZZIA LEBBECK*, EAA REQUIREMENTS (EAA, g/100g PROTEIN) OF *CLARIAS GARIEPINUS* AND CHEMICAL SCORE FOR RAW AND PROCESSED SEEDS OF *ALBIZZIA LEBBECK* USING FISHMEAL AS A REFERENCE PROTEIN

AMINO ACIDS	TREATMENTS								*EAA REQ. (g/100g)	**REFERENCE PROTEIN: FISHMEAL
	T1	T2	T3	T4	T5	T6	T7	T8		
Arginine	3.06	3.06	4.42	3.23	3.32	3.57	4.00	3.91	**4.3**	**5.82**
CS (%)	53	53	76	55	57	61	69	67	**74**	
Histidine	1.88	1.69	2.01	1.57	1.57	1.35	1.47	1.50	**1.5**	**2.22**
CS (%)	85	76	91	71	71	61	66	68	**68**	
Isoleucine	2.07	1.88	1.98	2.95	2.04	2.26	2.01	1.82	**2.6**	**4.85**
CS (%)	43	39	41	61	42	47	41	38	**54**	
Leucine	4.01	4.31	4.89	5.05	3.02	4.20	4.78	3.10	**3.5**	**7.35**
CS (%)	55	59	67	69	41	57	65	42	**48**	
Lysine	3.22	3.01	3.25	2.95	3.89	3.19	3.54	3.60	**5.0**	**7.85**
CS (%)	41	38	41	38	50	41	45	46	**64**	
Methionine	1.02	1.30	1.49	0.89	1.20	1.15	0.89	1.28	**2.3**	**2.84**
CS (%)	36	46	52	31	42	40	31	45	**81**	
Phenylalanine	2.70	3.38	3.97	3.80	2.87	2.96	2.54	2.45	**5.0**	**4.35**
CS (%)	62	78	91	87	66	68	58	56	**115**	
Threonine	1.78	1.44	2.11	1.94	2.33	2.89	2.16	2.25	**2.0**	**4.55**
CS (%)	39	32	46	43	51	64	47	49	**44**	

Valine	2.03	2.27	2.93	2.79	2.09	1.89	2.53	1.80	**3.0**	**5.65**
CS (%)	36	40	52	49	37	33	45	32	**53**	
MEAN CS (%)	50	51	62	56	51	52	52	49	**67**	

SOURCE: **Uys (1989)*

********Okoye (2003)*

4.4 Growth and Feed Performance of *Clarias* Juveniles

There were no mortality or external clinical symptoms recorded in any Treatment during the period of this study. There was no significant difference (P>0.05) in the initial mean body weights of the juveniles. However, differences were observed in the final mean body weights with diet 4 recording the highest final mean body weight which was significantly different (P<0.05) from the other diets. Growth response was significantly (P<0.05) affected by the length of boiling of the *Albizzia* seeds. Weight gain increased in response to longer heat exposure of the seeds. Growth response of fish fed diet 4 (*Albizzia* boiled 1 hour) increased the most, followed by D3, D2 and then D1 in that order. There was a trend of increasing growth performance on the basis of mean body weight gain (MWG), percentage life weight gain (%LWG) and specific growth rate (SGR%).

Food conversion ratio (FCR) decreased as heat exposure time of the *Albizzia* seeds increased. The FCR was lowest in D4 (P<0.05) and highest in D1, with values 0.37 and

0.60 respectively. The gross food conversion efficiency (GFCE %) was highest in D4 (270.3), followed by D3 (200.0) and D2 (185.2), then lastly by D1 (166.7). The condition factor (K) in all the dietary treatments were not significantly different (P>0.05) from one another. The growth responses and nutrient utilization indices measured are summarized in Table 4.5. Also, graphical representations of the mean body weight gain and the mean standard length gain in 8 weeks are shown in Figures 4.1 and 4.2 respectively. Figure 4.3 shows the mean length-weight relationship of the experimental fish throughout the duration of the experiment. The trend shows that length was positively correlated (Y = -30.006 + 3.7821X; r= 0.97; P < 0.05) with weight.

TABLE 4.5 GROWTH INDICES, FEED UTILIZATION AND SURVIVAL RATE OF *CLARIAS GARIEPINUS* JUVENILES FED EXPERIMENTAL DIETS FOR EIGHT WEEKS

GROWTH INDICES	**DIETS**				
	D1	**D2**	**D3**	**D4**	**SEM**
Initial Mean Weight (g)	16.2a	15.8a	15.8a	15.4a	0.3309
Final Mean Weight (g)	22.2b	22.7b	23.3b	26.3a	0.5714
Mean Weight Gain (g)	6.0b	6.9b	7.5b	10.9a	1.5751
Initial Mean Standard Length (CM)	12.4a	12.4a	12.1a	12.2a	0.1635
Final Mean Standard Length (CM)	13.8b	13.9b	14.2b	14.8a	0.1389
Mean Standard Length Gain (CM)	1.4b	1.5b	2.1b	2.6a	0.498
Percentage Live Weight Gain (%)	37.04b	43.67b	47.47b	70.78a	81.347
Specific Growth Rate (%)	0.56c	0.65b	0.69b	0.96a	0.0137
Feed Conversion Ratio	0.60a	0.54b	0.50b	0.37c	0.0255
Gross Food Conversion Efficiency (%)	166.7c	185.2b	200.0b	270.3a	14.797
Protein Efficiency Ratio	1.86a	1.85a	1.80b	1.77b	0.0234
Apparent net protein utilization	4.14c	3.69c	7.31a	6.00b	0.9871
Condition factor	0.86a	0.85a	0.78a	0.81a	0.9082
Survival Rate (%)	100a	100a	100a	100a	0.0002

Note: Means with the same letter(s) in rows are not significantly different (P>0.05).

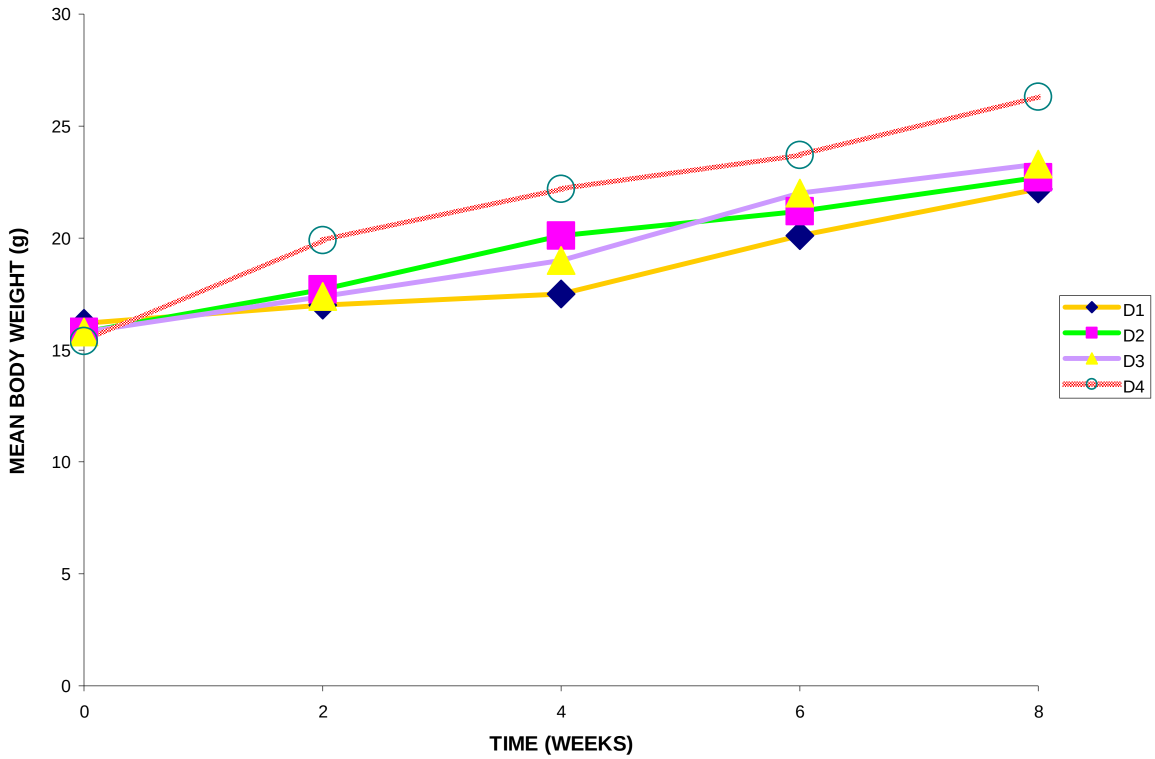

FIGURE 4.1 BI-WEEKLY MEAN BODY WEIGHT GAIN OF *CLARIAS GARIEPINUS* FED THE EXPERIMENTAL DIETS

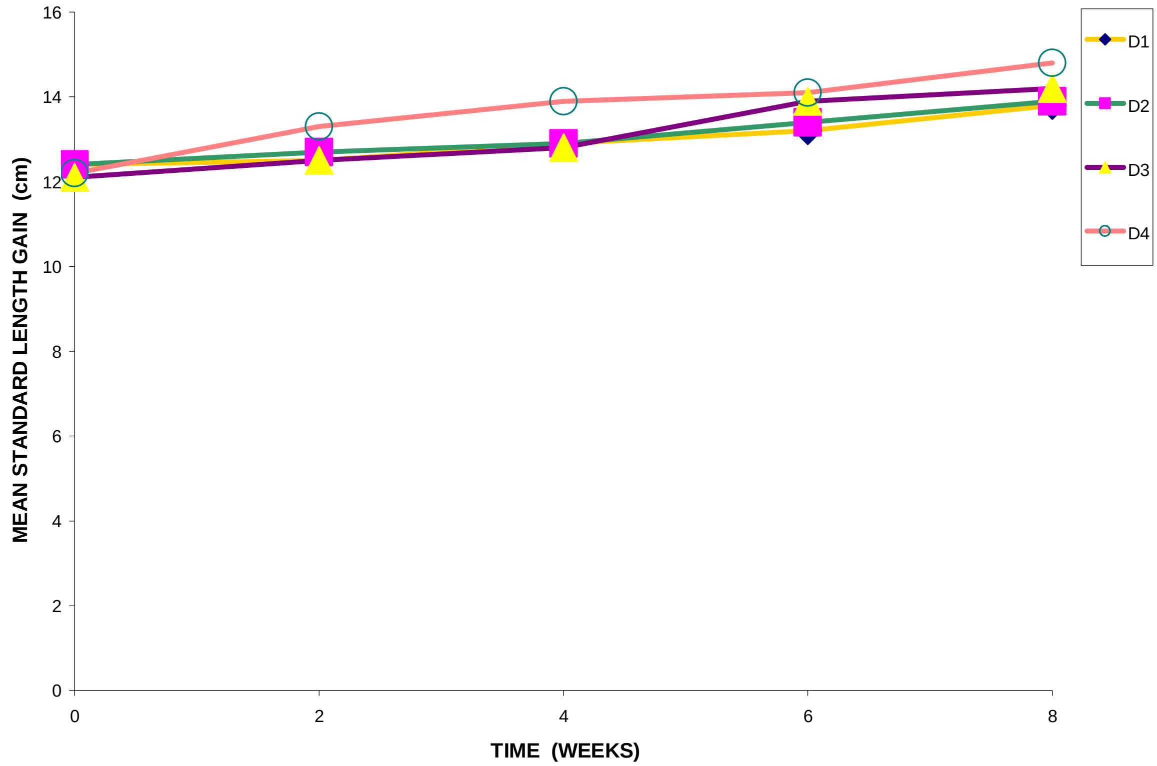

FIGURE 4.2 BI-WEEKLY MEAN STANDARD LENGTH OF *CLARIAS GARIEPINUS* FED THE EXPERIMENTAL DIETS

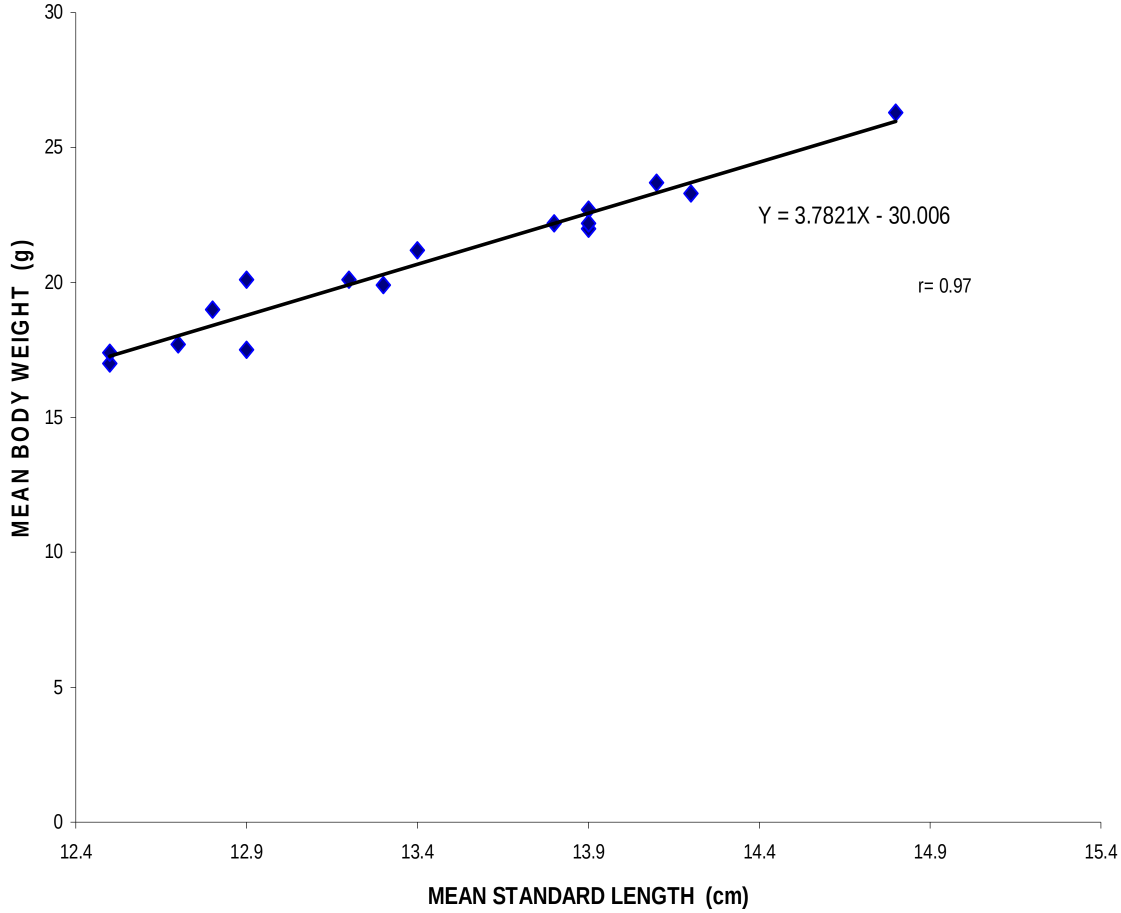

FIGURE 4.3 LENGTH-WEIGHT RELATIONSHIP OF EXPERIMENTAL FISH OVER AN EIGHT-WEEK PERIOD

4.5 Carcass Composition

The results of the body composition data for the whole fish carcasses are shown in Table 4.6. At the end of the experiment, compared to initial values, all experimental groups exhibited higher percentages of crude protein with D4 being significantly ($P < 0.05$) higher than D1 and D2, but not significantly different from D3. Lower percentages of crude lipid was observed in D1 and D3 when compared with the Initial, however D2 and D4 were significantly ($P < 0.05$) higher than the Initial in lipid contents. Body ash content ranged from 19.95 (D1) to 25.33 (D2), although D2 was not significantly different ($P>0.05$) from the Initial value of 24.08. The lowest percentage of nitrogen free extracts was recorded in D4 though it was not significantly different ($P>0.05$) from D2 at 4.54.

TABLE 4.6 PROXIMATE COMPOSITION OF THE FISH CARCASS BEFORE AND AFTER THE EXPERIMENT

		DIETS				
COMPOSITION	**INITIAL**	**D1**	**D1**	**D1**	**D1**	**SEM**
Crude Protein	47.41c	48.35c	50.25bc	53.77ab	54.81a	1.3368
Crude Lipid	16.28b	15.71b	19.89a	12.96c	20.28a	0.1050
Ash Content	24.08a	19.95c	25.33a	23.97a	22.24b	0.1638
Nitrogen Free Extract	12.24ab	16.00a	4.54c	9.31b	2.69c	1.5950

Note: Means with the same letter(s) in rows are not significantly different (P>0.05).

4.6 Physico-Chemical Parameters

Water quality conditions in the experimental system showed some variations especially during the last two weeks of the feeding trial period when the dissolved oxygen content reduced considerably to critically low levels. D1 and D2 had comparatively lower DO values. Other weekly mean water quality parameters were relatively stable. A summary of the mean water quality parameters is shown in Table 4.7.

TABLE 4.7: MEAN WATER QUALITY VALUES OBTAINED DURING THE EIGHT-WEEK EXPERIMENTAL PERIOD

WATER PARAMETERS	**DIETS**			
	D1	**D2**	**D3**	**D4**
Dissolved Oxygen (mg/l)	4.94 ± 0.24	3.76 ± 0.23	8. 78 ± 0.36	7.85 ± 0.24
Temperature (^{o}C)	26.7 ± 0.15	27.4 ± 0.17	26.9 ± 0.04	27.5 ± 0.32
pH	7.74 ± 0.24	7.76 ± 0.04	5.98 ± 0.07	6.78 ± 0.30
Total Dissolved Solids (ppm)	60 ± 0.33	63 ± 0.03	57 ± 0.31	61 ± 0.21
Electrical Conductivity (µS)	121 ± 0.12	124 ± 0.42	105 ± 0.71	120 ± 0.06

CHAPTER FIVE

DISCUSSION

The proximate analysis of Treatments 1 to 8 contributed to the choice of the Treatments that made it to the feeding trial stage. It was observed that the raw seeds had low lipid content (5.08 – 6.87%). This is desirable as rancidity in seeds stored for long periods will not occur. Apart from the oil legumes, other legumes have been reported to be low in oil content ranging from 1-5%. However, oilseeds have a range of lipid contents from about 18% in soyabean to as high as 43% in groundnut (Ene-Obong and Carnovale, 1992). This implies that *A. lebbeck* is not an oil seed. Also, the moisture content of *A. lebbeck* is low (2.47 – 5.29%). This implies that the shelf life for this seed will likely be long. The ash content (4.20 – 7.84%) of this seed is higher than that of other legumes which has been reported to range between 3.0 and 4.8% (Elegbede, 1998). While the carbohydrate content (32.28 – 37.47%) is comparable with that of other legumes ranging from 23% in groundnut to 66% in Bambara groundnut (Ene- Obong and Carnovale, 1992).

Fibre is the portion of the plant that provides it with structural strength and form. Generally, vegetative parts, especially the stem possess high fibre content. Seed hulls and/or coats often contain fibre. Therefore, the high fibre content of the *Albizzia* seeds may have been due to the inclusion of the seed coat during milling. Fibre is not required in the diet of non-ruminants like fish. This informed the choice of the boiled seeds over the other treatments since the former had reduced fibre composition. Although the crude protein levels of the toasted and roasted seed samples were appreciably high, ranging from 29.73 (toasted 2 hours) to 33.19 (roasted traditionally), their higher fibre contents could pose a distinct deterrent to their use in aquaculture diets. The high nitrogen free extract (NFE) may have served as an adequate energy source for the fish.

Phytochemical screening of the seed showed the presence of saponins, oxalates and phytic acid which were reduced by heat processing methods, however, the levels of cyanogenic glycoside and tannin were low and not affected by heat. Saponins are glycoside components often referred to as “natural detergent” because of their foamy nature (Seigler, 1998). Saponins in seeds have been known to posses both beneficial and deleterious properties depending on their concentration in the sample (Oakenful and Sidhu, 1989; Seigler, 1998). Seigler (1998) reported that saponins have anti-carcinogenic properties, immune modulation activites and regulation of cell proliferation as well as health benefits such as inhibition of the growth of cancer cells and cholesterol lowering activity.

Nevertheless, saponins in fish nutrition, are regarded as antinutrients known to cause haemolysis of blood. This implies that if found in high quantities they may lead to the rupture or destruction of blood vessels. Saponins are thermal sensitive. Shi *et al*. (2004) reports that portions of saponin dissolved in water are lost during soaking or blanching. Therefore, from the values obtained this statement is in conformity as the raw sample was higher than the toasted and roasted samples. Also, the boiled samples were the least in concentration of the anti-nutritional factor.

William and West (1967) reported that oxalate is a rapid irritant poison, and generally a fatal termination precludes any possibility of treatment. The survival rate of the fish seem to suggest that levels of up to 2.80 mg/g which was observed in the raw sample is still within acceptable limits and so will not detrimentally affect fish. They also reported that salts of oxalic acid occur in other plants such as sorrel and rhubarb. Both plants are eaten by humans even though they contain oxalate.

Phytate is not broken down in monogastric animals (*e.g*., poultry, fish and swine). An option to enhance greater utility of feedstuff that may contain this anti-nutrient is by boiling. From the results obtained from this study, boiling significantly reduced the levels of phytic acid in the samples. This finding agrees with Abu *et al*. (2005) who observed that boiling lima bean (*Phaseolus lunatus*) reduced the level of phytic acid in the seed samples. The levels of cyanogenic glycoside in all the samples were not significantly different ($P>0.05$) however, they were all in the permissible level of 10-20mg/100g (Abu *et al*., 2005).

Tannin reduces protein solubility by forming a complex with protein thereby causing a reduction in digestibility. It also causes depressed growth rates, damage to the mucosal lining of the digestive tract, alteration in the excretion of certain cations and increased excretion of essential amino acids (Webmaster, 2006). Feedstuffs that have high level of tannins are astringent or have a bitter or unpleasant taste. From the result of the analysis for tannin, the low levels in *Albizzia* seed meal would suggest that the seed meal may have been palatable or that the other components used to make the experimental feed may have masked its effects thereby allowing for feed intake. The levels of tannins in Treatments 1 - 8 were not significantly different from one another, therefore the presence of other anti-nutritional factors may have caused the differences noted in feed conversion ratio across the diets. Webmaster (2006) postulates that for monogastrics, levels of tannin from 3 to 7% can cause death. As the levels of tannins in the seed samples were very low, it is assumed that they were not detrimental to the utilization of the feed.

Another likely reason as to why the tannin levels were very low (0.002-0.003mg/l) in all the treatments may have been due to the fact that not all tannins are extractable. Insoluble tannins are therefore not quantified. Hagerman and Klucher (1986) reported that insoluble tannins may have equal or greater biological activity than those that are more easily extracted. Another reason may have been due to the drying of the experimental diets as drying reduces the solubility of tannins in feedstuff and, hence their ability to complex proteins (Hagerman and Klucher, 1986).

The levels of the amino acids analysed in the raw sample of *A. lebbeck* is similar to the values obtained by Kaga (1999) with a few exceptions such as valine and isoleucine which were higher in the present study. These differences may be attributable to seasonal differences or other ecological variables during pod collection.

Albizzia seed protein is enhanced in feeding value for *Clarias* juveniles by sufficient heat treatment as can be observed in the trend of increased growth rate from diet 1 to diet 4. There may also be some changes in the protein molecule itself due to heat application which increases the availability of the essential amino acids. *Albizzia* seed protein is not the only one that is improved in digestibility by cooking since in the case of soyabean several investigators have demonstrated that heating soyabean meal rather severely not only increases its acceptability to fish, but also improves the availability of nutrients (Eyo, 1999). This is first accomplished by denaturing the protein and thus making it more digestible, and also by detoxifying natural toxicants. Several studies, however, indicate that when soyabean meal is heated, its performance is still below the expected considering its amino acid composition. The same may be said of *Albizzia* since the seeds that were boiled for 30 minutes ranked highest in amino acid array but could not translate to better weight gain for the fish and ranked second in that regard.

The proteins of some feeds, on the other hand, decrease in nutritive value when subjected to heat. Experimental evidence seems to indicate that when heating damages a protein, the damage is likely due to a destruction of lysine. Lysine, in fact, is rather easily damaged, and some evidence indicates that even mild drying of some proteins of animal origin may be detrimental. Of the proteins that are ordinarily fed to livestock, only the proteins of soyabean products appear to be improved by heating. The others are more likely to be damaged, primarily by destruction of lysine (NRC, 1993).

It would appear that D1 must contain some anti-growth factors other than the ones investigated or that the *Clarias* juveniles could not utilize the free amino acids present since the meal was in its raw state. Growth was significantly reduced when the control diet was fed to the mudfish juveniles. It is believed that part of the problem may be due to the phytate content of the seed meals. No other obvious physiological defects were noted in the juveniles fed the control diet. Compounds other than phytates present in the meal are also capable of binding trace nutrients.

An increase in growth was observed with each increment in heat-exposure time of the *Albizzia* seed meals. *Clarias gariepinus* juveniles fed diet 1 exhibited a reduction in mean weight gain, specific growth rate and percentage live weight gain. These could be attributed to reduced feed intake by the fish, because of the raw content of the seed meal in the diet which may have resulted in lower protein intake. A probable reason as to the under utility of the control diet may have been due to problems relating to palatability due to the inclusion of raw seed meal in the experimental diet.

The acceptance of the experimental feed is in conformity with the observation of Burgess (1989) that *Clarias gariepinus* is not specific in its food requirements. The absence of mortality during the experimental period may be attributed to the life stage of the fish (i.e.,juveniles), which enabled them to withstand stress during weighing.

Water quality parameters except dissolved oxygen conformed to the recommendation of Boyd and Lichtopher (1990) for warm water fishes. D1 and D2 recorded Mean DO of 4.94 ± 0.24mg/l and 3.76 ± 0.23mg/l respectively which is below the recommended level of 5mg/l or above. However, the ability of the experimental fish to withstand periods of critically low dissolved oxygen during the experiment agrees with the findings of Viveen *et al*. (1986) that *Clarias* can live in very poorly oxygenated waters and so can tolerate low dissolved oxygen levels in the water.

The carcass composition shows that the crude protein content in all the fish fed the experimental diet was higher than the Initial. However, only D3 and D4 were significantly different from the Initial. This suggests that there was a reasonable level of protein retention due to optimal utilization for growth (Alegbeleye *et al*., 2004).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

With regards to its composition, *Albizzia* seed meal appears to be a reasonably good feed component for *Clarias* juveniles. It contains about 30-38 percent protein, 4-8 percent ash, 5-7 percent lipid and about 32-37 percent carbohydrates. Analytical studies indicate that saponins, oxalates and phytic acid may be reduced by heat processing methods, however, the levels of cyanogenic glycoside and tannin are low and not affected by heat.

In conclusion, on the basis of growth performance, feed efficiency, protein utilisation, and body composition, diet 4, containing *Albizzia* seed meal which was boiled for one hour performed best, and so may effectively be used as a dietary protein source in the diet of *Clarias gariepinu*s juveniles. It may be postulated that from the outcome of the results of the study that the Null (H_0) hypotheses be rejected. However as regards the levels of cyanogenic glycoside and tannin, the Alternative (H_1) hypotheses should be accepted as there were no significant differences of these two anti-nutrients in the raw and processed seeds of *A. lebbeck.*

From the results of this study, the following are recommended:

i. For most aquaculture diets, *Albizzia* seed meal should not be used as a single protein source as it is deficient in lysine and the sulphur-containing amino acids, for example methionine. It is suggested that it be compounded with other protein feed-stuffs when it is used in fish rations.

ii. Due to its high crude protein level, low lipid level and low crude fibre when boiled, together with the fact that it is abundant in the Nigerian environment, its usage is encouraged for inclusion as a high protein source in fish feed formulations.

iii. There appears to be no work done as regards the lipid, vitamin and mineral components of *Albizzia* seeds. This represents a void in the knowledge of *A. lebbeck* seeds and offers an aspect for future study.

iv. The formulation of unconventional and unpopular food materials that are of little or no direct value as human food should be adopted / considered. This would reduce the competition between man and culture animals for the same food source.

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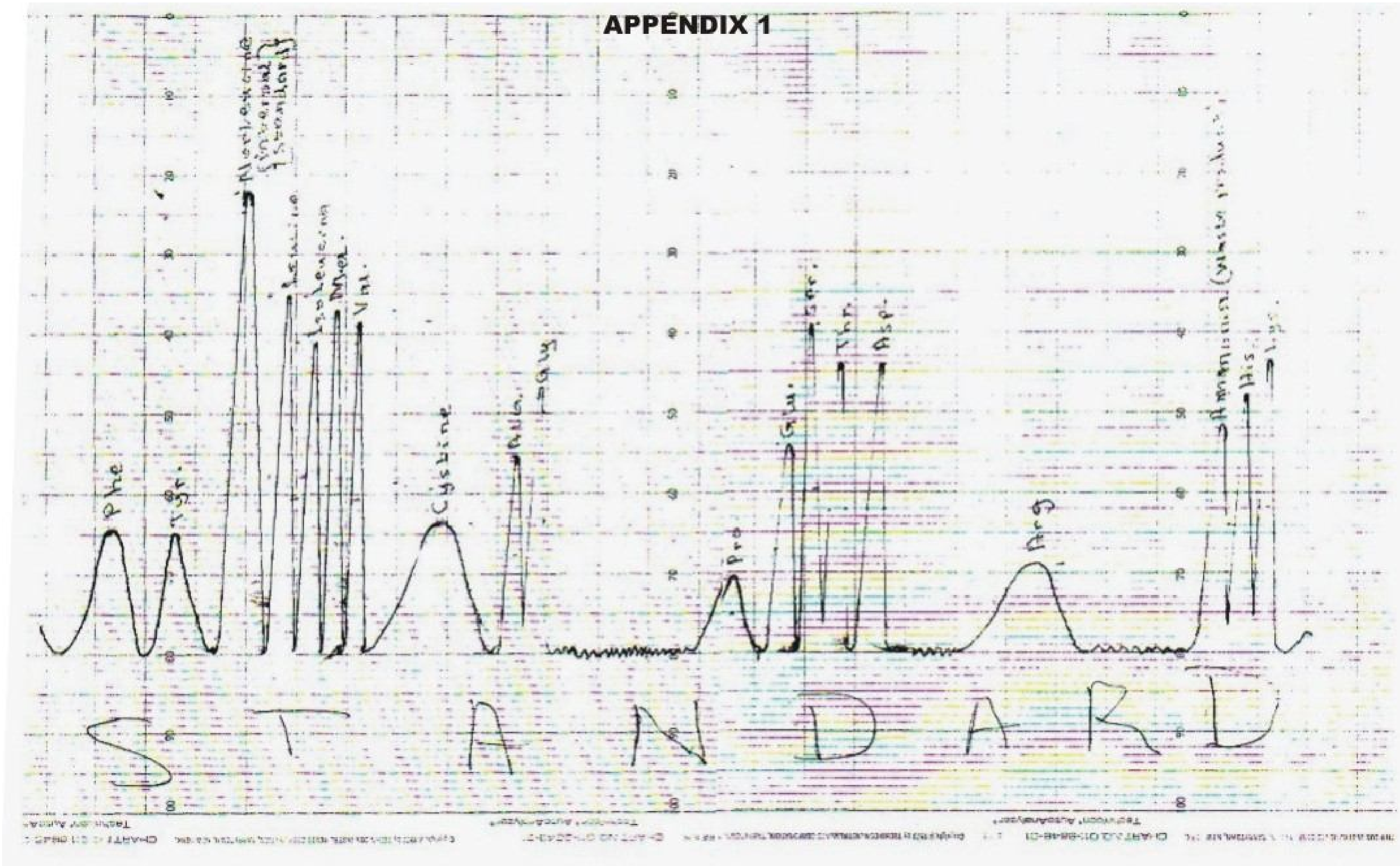
APPENDIX 1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Leucine
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Met.
Val.
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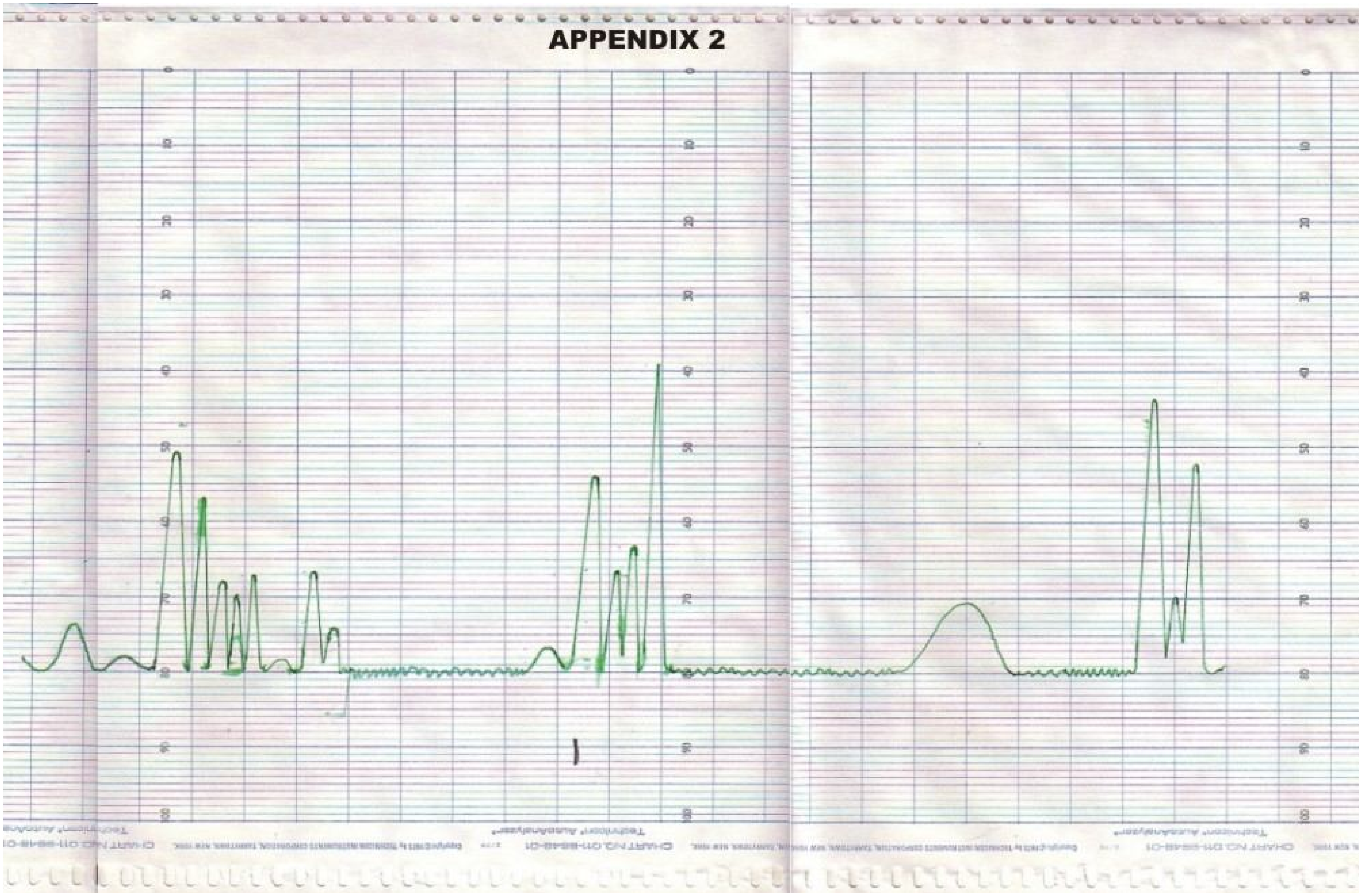
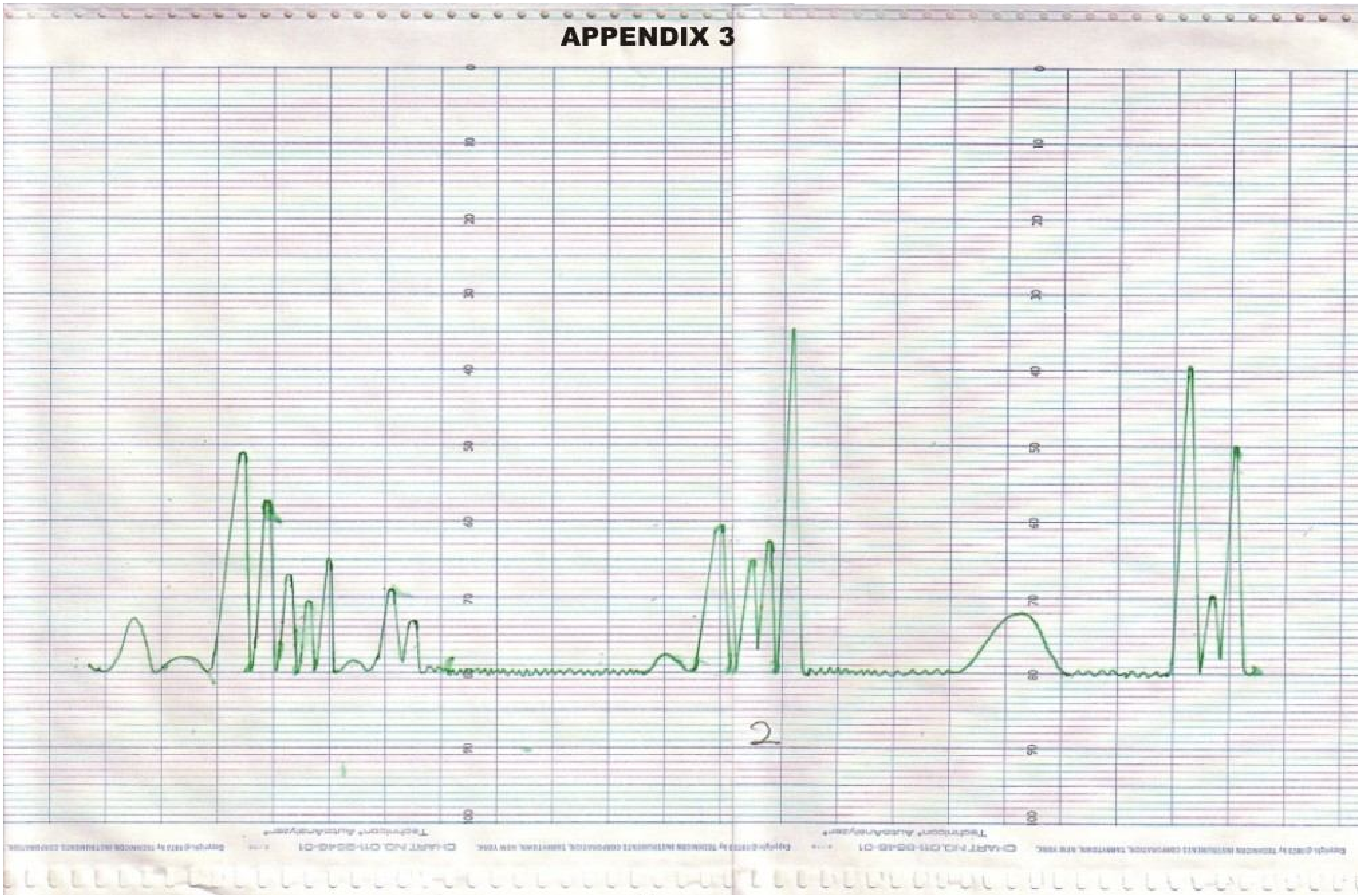
Lys
STANDARD

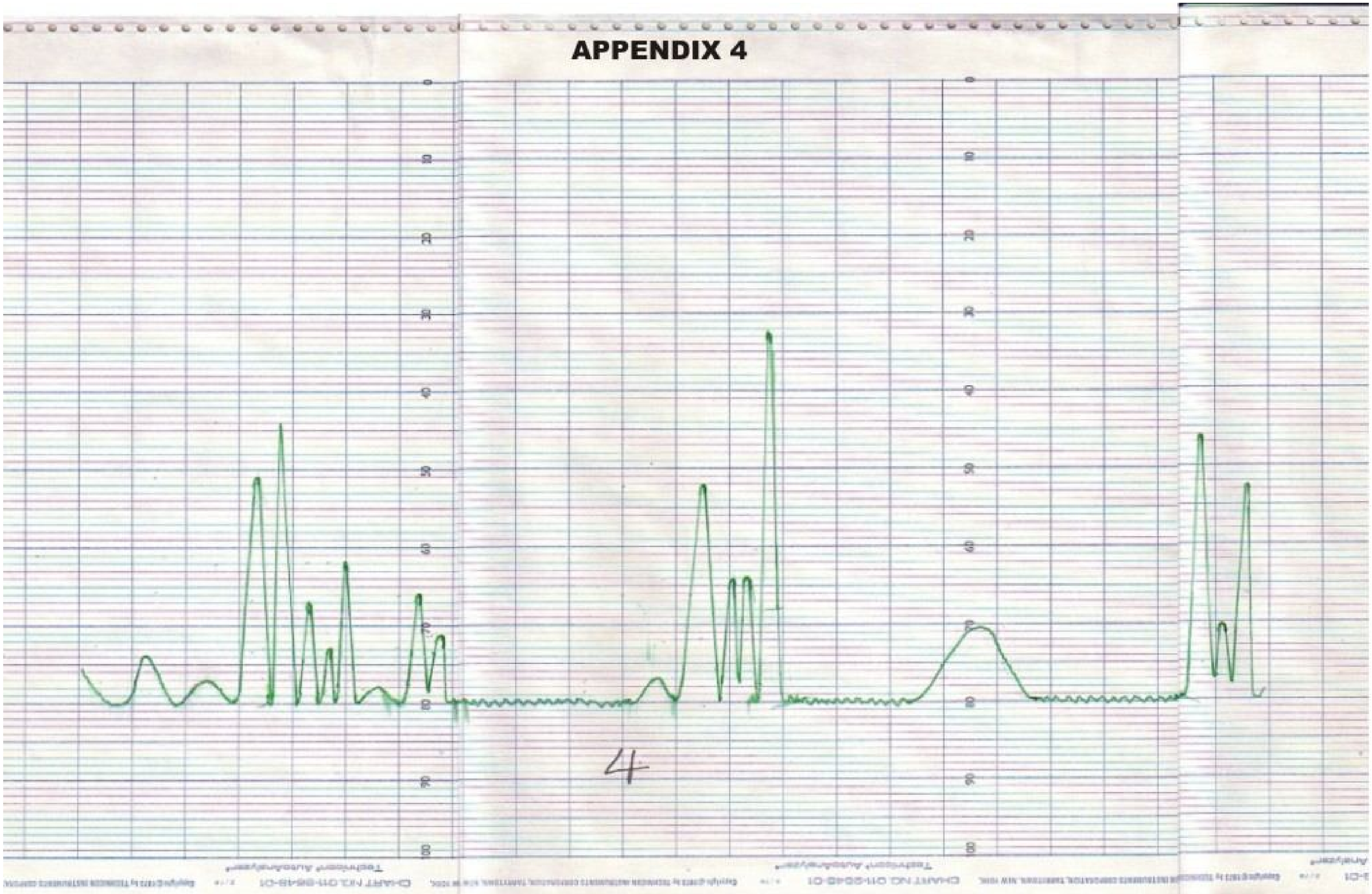
APPENDIX 2



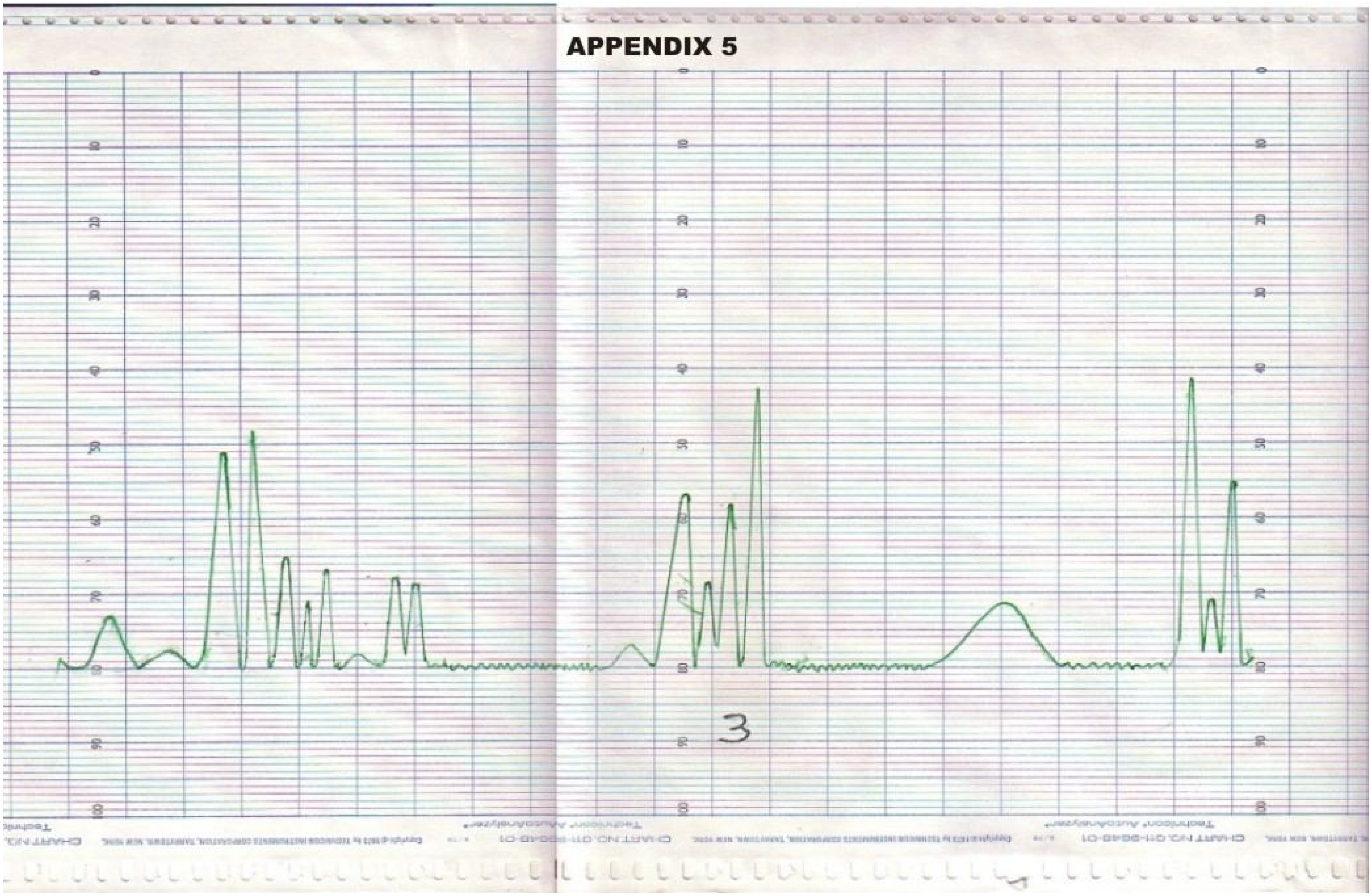
APPENDIX 3



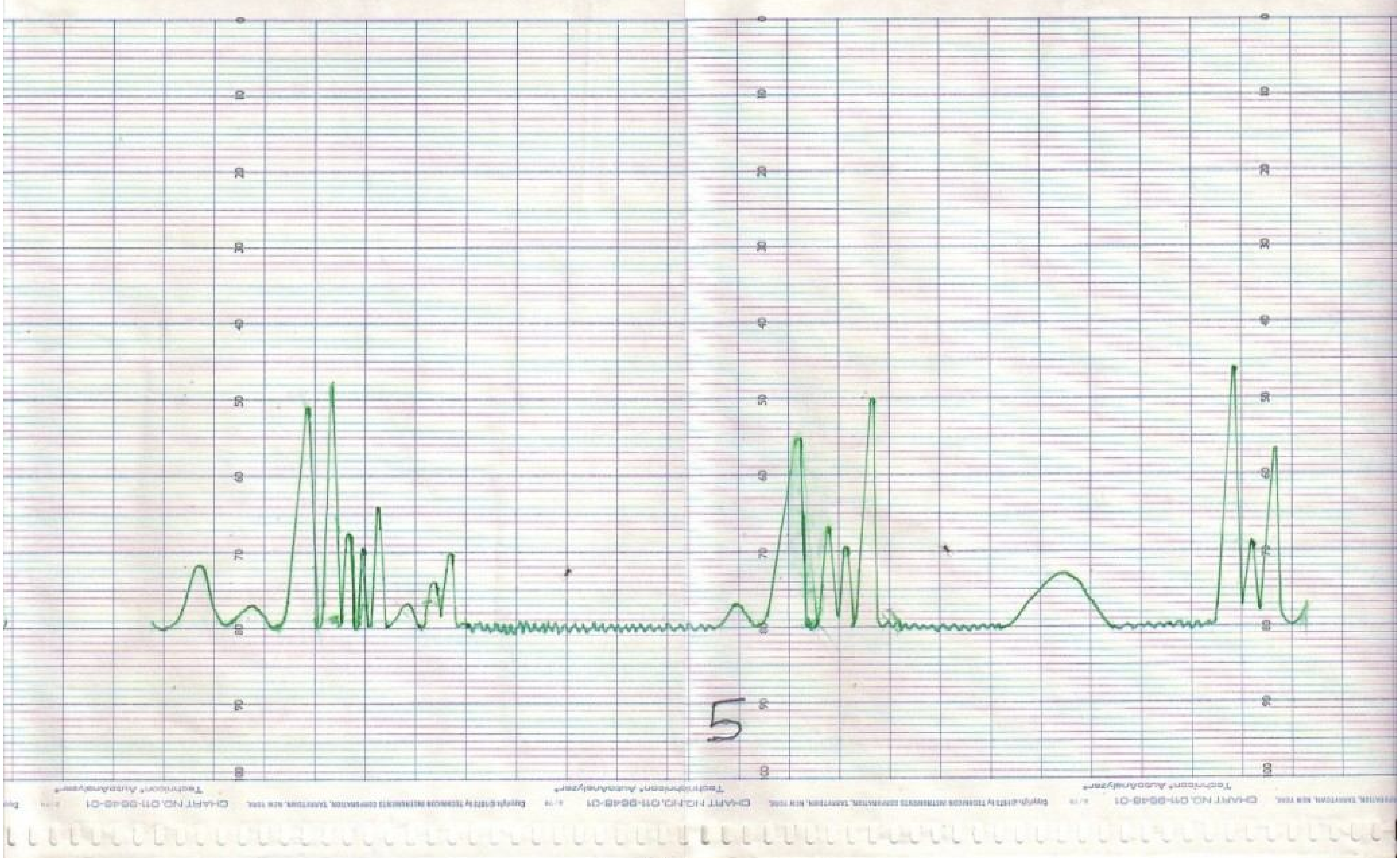
APPENDIX 4



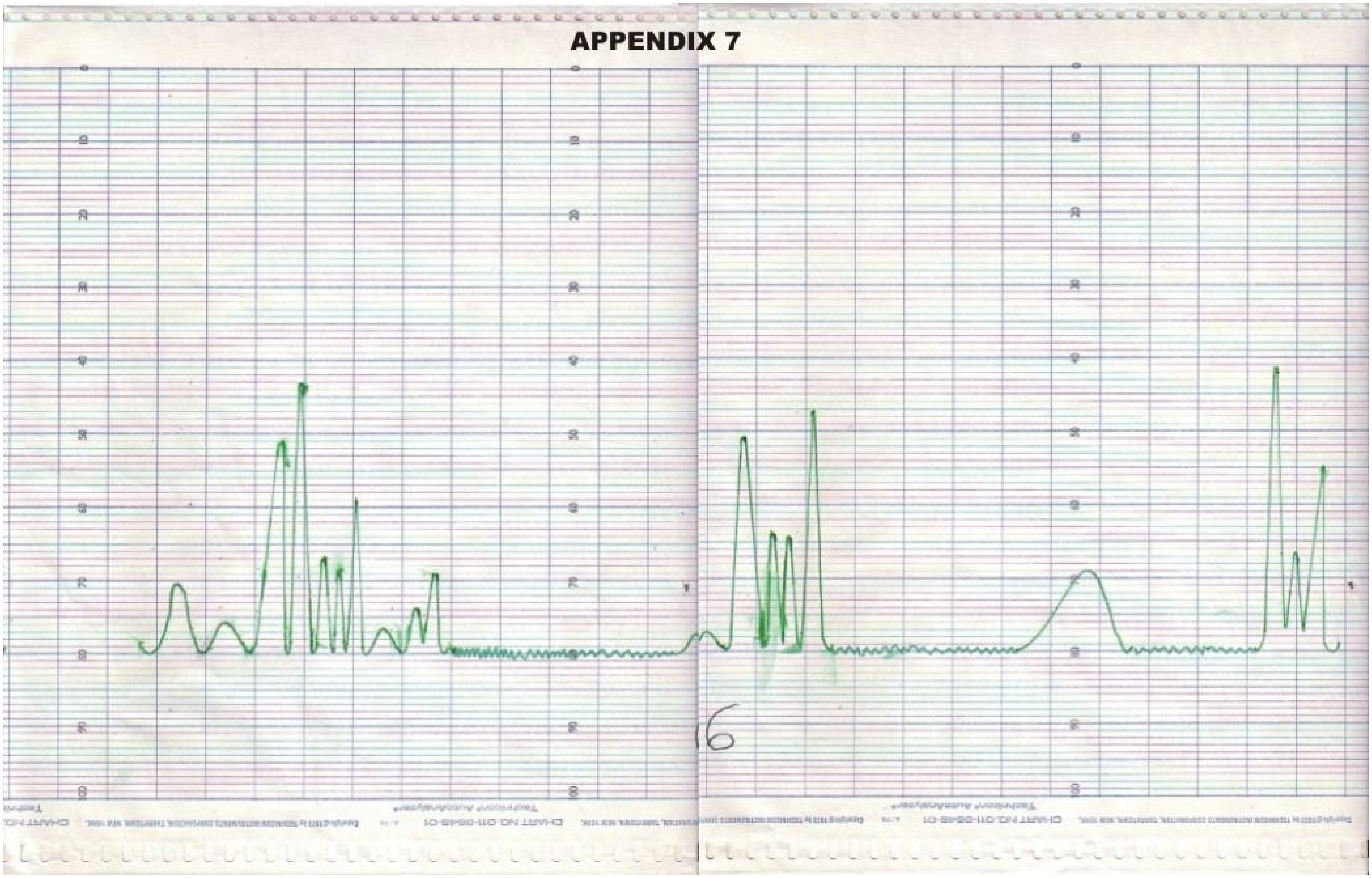
APPENDIX 5



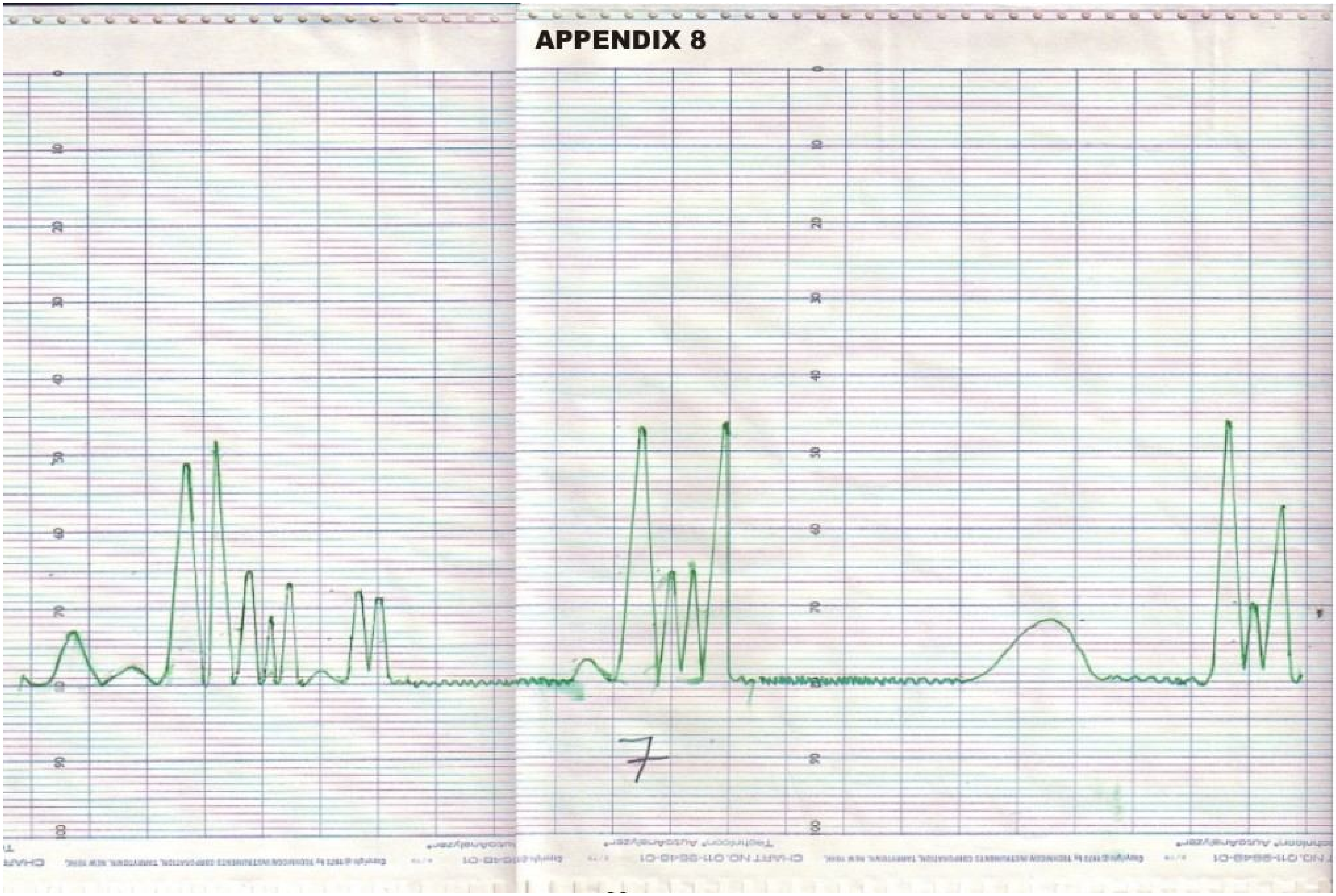
APPENDIX 6



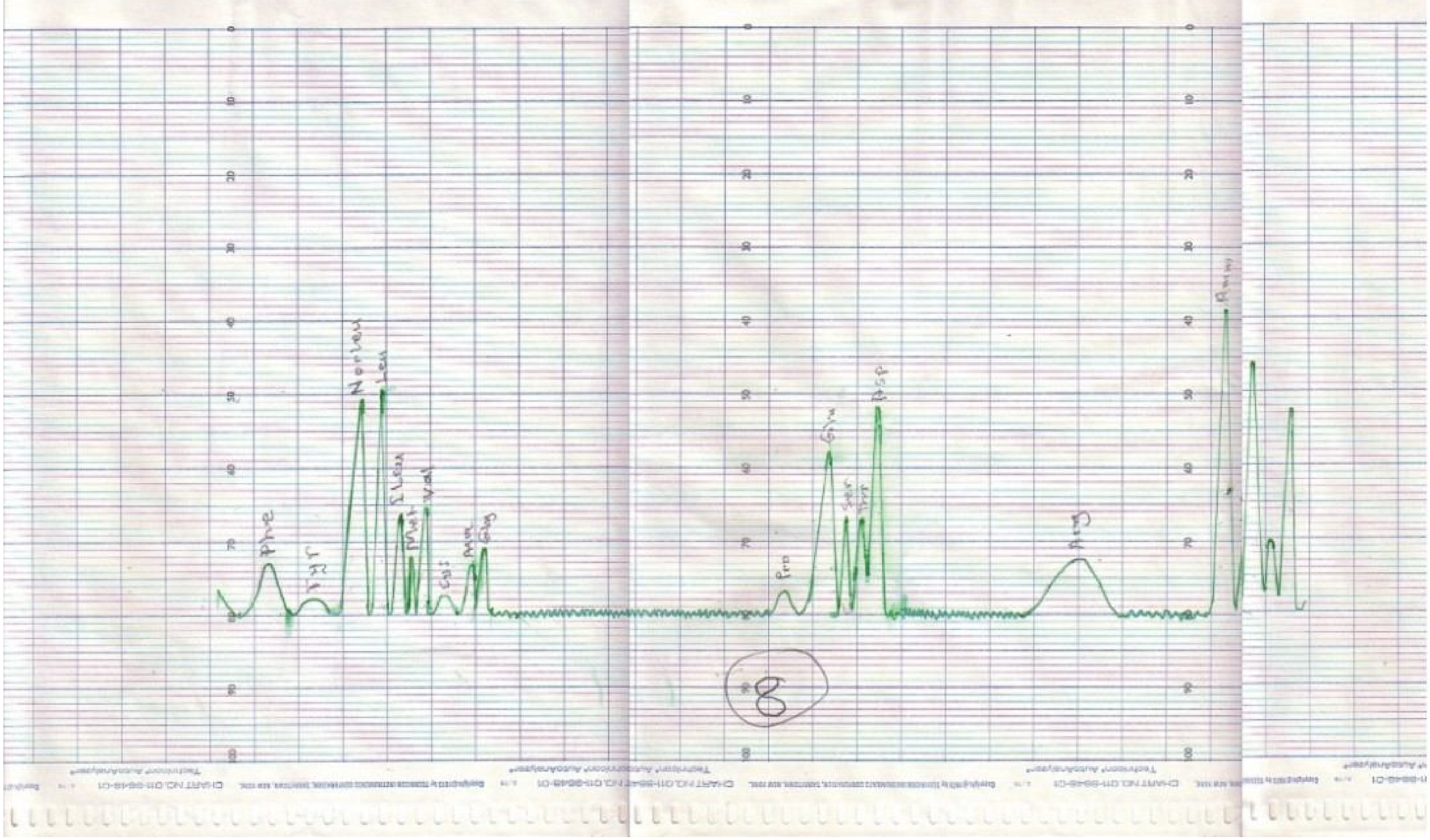
APPENDIX 7



APPENDIX 8



APPENDIX 9



Phe
Tyr
Norleu
Leu
I Leu
Met
Val
Cys
Amm
Gly
Pro
Glu
Ser
Thr
Asp
Arg
8

DATA OF AMINO ACID PROFILE OF THE DIFFERENTLY PROCESSED SEEDS OF *A. lebbeck*

AMINO ACID ANALYSIS
TECHNICON TSM – 1

STANDARD RUN

NOTE: 1: µmoles AA = 0.025

2: $NE_{std} = \frac{NH \times W\ (nLEU)}{NH \times W\ (AA)}$

3: $S = NE_{std} \times MOL. \times weight \times \mu MAA_{std}$

1	2	3	4	5	6	7	
AMINO ACID	NET Height (mm)	NH/2 (mm)	WIDTH at NH/2 (mm)	NH x W	NEstd	MOLECULAR WEIGHT	S
Lysine	87	43.5	4.5	391.5	2.830140485	182.650	12.92
Histidine	77	38.5	5.0	385	2.877922078	209.63	15.08
Ammonia						53.49	
Arginine	26.5	13.25	20	530	2.090566038	174.20	9.10
Aspartic acid	86	43	5	430	2.576744186	133.11	8.57
Threonine	86.5	43.25	5	432.5	2.561849711	119.12	7.63
Serine	98	49	4	392	2.826530612	105.00	7.42
Glutamic acid	62	31	5.5	341	3.249266862	147.13	11.95
Proline	23	11.5	9.5	218.5	5.070938215	115.13	14.60
Glycine	79	39.5	4.5	355.5	3.11673699	75.09	5.85
Alanine	59	29.5	4.5	265.5	4.173258004	89.09	9.29
Cystine	39	19.5	19	741	1.495276653	240.30	9.10
Valine	99.5	49.75	3.5	348.25	3.181622398	117.15	9.32
Methionine	103	51.5	4	412	2.689320388	149.21	10.03
Isoleucine	93	46.5	4.5	418.5	2.647550777	131.18	8.63
Leucine	107	53.5	4.5	481.5	2.303534304	131.18	7.55
Norleucine	138.5	69.25	8	1108		131.18	
Tyrosine	36	18	9	324	3.419753086	181.18	15.50
Phenylalanine	37.5	18.75	12	450	2.462222222	165.19	10.17

$$E.g\ NE_{std}\ [Lysine] = \frac{138.5 \times 8}{87 \times 4.5} = \frac{1108}{391.5} = 2.830140485$$

$$S\ [Lysine] = 2.830140485 \times 182.650 \times 0.025 = 12.92$$

APPENDIX 11

AMINO ACID ANALYSIS
TECHNICON TSM – 1
SAMPLE 1

(*Albizzia* – RAW)

MODEL. DNA 0209

Wt. of sample hydrolysed = 0.3876

%N (fat free) = 5.16

Volume loaded: Basic = 10µl

Acidic/Neutral = 5µl

Width @

Concentration (g) 100 protein = NH x NH/2 x S_{std} x C

Where S. std = NF_{std} x Mol. weight x μMAA_{std}

B = 0.001038952727

A/N = 0.002077905455

$$C = \frac{\text{Dilution x 16}}{\text{Sample Wt (g) x N\% x 10 x Vol. Loaded}}$$

÷ NH x W (nleu)

1	2	3	4	5	6 =(2 x 4 x 5 x C)
AMINO ACIDS	NET HEIGHT (mm)	NH/2 (mm)	WIDTH NH/2 (mm)	S.std	Concentration (g/100) protein) or g/16gN
Lysine	60	30	4	12.92	3.22
Histidine	30	15	4	15.08	1.88
Ammonia					
Arginine	18	9	18	9.10	3.06
Aspartic acid	66	33	3.5	8.57	4.11
Threonine	32	16	3.5	7.63	1.78
Serine	31	15.5	3.5	7.42	1.67
Glutamic acid	53.5	26.75	6	11.95	7.97
Proline	7.5	3.75	7	14.60	1.59
Glycine	21	10.5	4	5.85	1.02
Alanine	15.5	7.75	4	9.29	1.20
Cystine	6	3	7	9.10	0.79
Valine	35	17.5	3	9.32	2.03
Methionine	19.5	9.75	2.5	10.03	1.02
Isoleucine	33	16.5	3.5	8.63	2.07
Leucine	73	36.5	3.5	7.55	4.01
Norleucine	70	35	5.5		
Tyrosine	5.5	2.75	10	15.50	1.77
Phenylalanine	16	8	8	10.17	2.70

APPENDIX 12

AMINO ACID ANALYSIS
TECHNICON TSM – 1
SAMPLE 2

(*Albizzia* – BOILED 15 MINS)

MODEL. DNA 0209

Wt. of sample hydrolysed = 0.3711

%N (fat free) = 5.39

Volume loaded: Basic = 10µl

Acidic/Neutral = 5µl

Concentration (g) 100 protein = NH x Width @ NH/2 x S_{std} x C

Where S. std = NF_{std} x Mol. weight x μMAA_{std}

B = 0.001038842092

A/N = 0.002077684183

$$C = \frac{\text{Dilution} \times 16}{\text{Sample Wt (g)} \times \text{N\%} \times 10 \times \text{Vol. Loaded}} \div \text{NH} \times \text{W (nleu)}$$

1	2	3	4	5	6 =(2 x 4 x 5 x C)
AMINO ACIDS	NET HEIGHT (mm)	NH/2 (mm)	WIDTH NH/2 (mm)	S.std	Concentration (g/100) protein) or g/16gN
Lysine	56.0	28.0	4	12.92	3.01
Histidine	27.0	13.5	4	15.08	1.69
Ammonia					
Arginine	18.0	9.0	18	9.10	3.06
Aspartic acid	72.0	36.0	3.5	8.57	4.49
Threonine	26.0	13.0	3.5	7.63	1.44
Serine	32.0	16.0	3.5	7.42	1.73
Glutamic acid	60.5	30.25	6	11.95	9.01
Proline	7.0	3.5	7	14.60	1.49
Glycine	24.0	12.0	4	5.85	1.17
Alanine	15.0	7.5	4	9.29	1.16
Cystine	7.5	3.75	7	9.10	0.99
Valine	39.0	19.5	3	9.32	2.27
Methionine	25.0	12.5	2.5	10.03	1.30
Isoleucine	30.0	15.0	3.5	8.63	1.88
Leucine	78.5	39.25	3.5	7.55	4.31
Norleucine	70	35	5.5		
Tyrosine	7.5	3.75	10	15.50	2.41
Phenylalanine	20.0	10.0	8	10.17	3.38

APPENDIX 13
AMINO ACID ANALYSIS
TECHNICON TSM – 1
SAMPLE 3
(*Albizzia* – BOILED 30 MINS)

MODEL. DNA 0209

Wt. of sample hydrolysed = 0.3466

%N (fat free) = 5.77

Volume loaded: Basic = 10µl

Acidic/Neutral = 5µl

Width @

Concentration (g) 100 protein = NH x NH/2 x S_{std} x C

Where S. std = NF_{std} x Mol. weight x μMAA_{std}

B = 0.001039022341

A/N = 0.002078044683

$$C = \frac{\text{Dilution x 16}}{\text{Sample Wt (g) x N\% x 10 x Vol. Loaded}}$$
÷ NH x W (nleu)

1	2	3	4	5	6 =(2 x 4 x 5 x C)
AMINO ACIDS	NET HEIGHT (mm)	NH/2 (mm)	WIDTH NH/2 (mm)	S.std	Concentration (g/100) protein) or g/16gN
Lysine	60.5	30.25	4	12.92	3.25
Histidine	32	16	4	15.08	2.01
Ammonia					
Arginine	26	13	18	9.10	4.42
Aspartic acid	78.5	39.25	3.5	8.57	4.89
Threonine	38	19	3.5	7.63	2.11
Serine	38.5	19.25	3.5	7.42	2.08
Glutamic acid	70	35	6	11.95	10.43
Proline	6.5	3.25	7	14.60	1.38
Glycine	26	13	4	5.85	1.26
Alanine	15	7.5	4	9.29	1.16
Cystine	8.5	4.25	7	9.10	1.13
Valine	50.5	25.25	3	9.32	2.93
Methionine	28.5	14.25	2.5	10.03	1.49
Isoleucine	31.5	15.75	3.5	8.63	1.98
Leucine	89	44.5	3.5	7.55	4.89
Norleucine	70	35	5.5		
Tyrosine	9.5	4.75	10	15.50	3.06
Phenylalanine	23.5	11.75	8	10.17	3.97

APPENDIX 14
AMINO ACID ANALYSIS
TECHNICON TSM – 1
SAMPLE 4
(*Albizzia* – BOILED 1 HR)

MODEL. DNA 0209
Wt. of sample hydrolysed = 0.3436

%N (fat free) = 5.82
Volume loaded: Basic = 10μl
Acidic/Neutral = 5μl

Width @
Concentration (g) 100 protein = NH x NH/2 x S_{std} x C
Where S. std = NF_{std} x Mol. weight x μMAA_{std}

B = 0.001039089886
A/N = 0.002078179772

$$C = \frac{\text{Dilution} \times 16}{\text{Sample Wt (g)} \times N\% \times 10 \times \text{Vol. Loaded}}$$
÷ NH x W (nleu)

1	2	3	4	5	6 =(2 x 4 x 5 x C)
AMINO ACIDS	NET HEIGHT (mm)	NH/2 (mm)	WIDTH NH/2 (mm)	S.std	Concentration (g/100) protein) or g/16gN
Lysine	55	27.5	4	12.92	2.95
Histidine	25	12.5	4	15.08	1.57
Ammonia					
Arginine	19	9.5	18	9.10	3.23
Aspartic acid	81	40.1	3.5	8.57	5.05
Threonine	35	17.5	3.5	7.63	1.94
Serine	35	17.5	3.5	7.42	1.89
Glutamic acid	79.5	39.75	6	11.95	11.85
Proline	6.5	3.25	7	14.60	1.38
Glycine	22.5	11.25	4	5.85	1.09
Alanine	14	7	4	9.29	1.08
Cystine	9	4.5	7	9.10	1.19
Valine	48	24	3	9.32	2.79
Methionine	17	8.5	2.5	10.03	0.89
Isoleucine	47	23.5	3.5	8.63	2.95
Leucine	92	46	3.5	7.55	5.05
Norleucine	70	35	5.5		
Tyrosine	9	4.5	10	15.50	2.90
Phenylalanine	22.5	11.25	8	10.17	3.80

APPENDIX 15
AMINO ACID ANALYSIS
TECHNICON TSM – 1
SAMPLE 5
(*Albizzia* – TOASTED 1 HR)

MODEL. DNA 0209

Wt. of sample hydrolysed = 0.4073

%N (fat free) = 4.91

Volume loaded: Basic = 10μl

Acidic/Neutral = 5μl

Width @

Concentration (g) 100 protein = NH x NH/2 x S_{std} x C

Where S. std = NF_{std} x Mol. weight x μMAA_{std}

B = 0.001039042603

A/N = 0.002078085208

$$C = \frac{\text{Dilution x 16}}{\text{Sample Wt (g) x N\% x 10x Vol. Loaded}}$$

÷ NH x W (nleu)

1	2	3	4	5	6 =(2 x 4 x 5 x C)
AMINO ACIDS	NET HEIGHT (mm)	NH/2 (mm)	WIDTH NH/2 (mm)	S.std	Concentration (g/100) protein) or g16gN
Lysine	72.5	36.25	4	12.92	3.89
Histidine	25	12.5	4	15.08	1.57
Ammonia					
Arginine	19.5	9.75	18	9.10	3.32
Aspartic acid	109	54.5	3.5	8.57	6.79
Threonine	42	21	3.5	7.63	2.33
Serine	36	18	3.5	7.42	1.94
Glutamic acid	47	23.5	6	11.95	7.00
Proline	6	3	7	14.60	1.27
Glycine	16.5	8.25	4	5.85	0.80
Alanine	26.5	13.25	4	9.29	2.05
Cystine	3.5	1.75	7	9.10	0.46
Valine	36	18	3	9.32	2.09
Methionine	23	11.5	2.5	10.03	1.20
Isoleucine	32.5	16.25	3.5	8.63	2.04
Leucine	55	27.5	3.5	7.55	3.02
Norleucine	70	35	5.5		
Tyrosine	4.5	2.25	10	15.50	1.45
Phenylalanine	17	8.5	8	10.17	2.87

APPENDIX 16

AMINO ACID ANALYSIS
TECHNICON TSM – 1
SAMPLE 6

(*Albizzia* – TOASTED 2 HRS)

MODEL. DNA 0209

Wt. of sample hydrolysed = 0.4587

%N (fat free) = 4.36

Volume loaded: Basic = 10µl

Acidic/Neutral = 5µl

Width @

Concentration (g) 100 protein = NH x NH/2 x S_{std} x C

Where S. std = NF_{std} x Mol. weight x μMAA_{std}

B = 0.001038996364

A/N = 0.00207799273

$$C = \frac{\text{Dilution x 16}}{\text{Sample Wt (g) x N\% x 10 x Vol. Loaded}}$$

÷ NH x W (nleu)

1	2	3	4	5	6 =(2 x 4 x 5 x C)
AMINO ACIDS	NET HEIGHT (mm)	NH/2 (mm)	WIDTH NH/2 (mm)	S.std	Concentration (g/100) protein) or g16gN
Lysine	59.5	29.75	4	12.92	3.19
Histidine	21.5	10.75	4	15.08	1.35
Ammonia					
Arginine	21.0	10.50	18	9.10	3.57
Aspartic acid	90.0	45.0	3.5	8.57	5.61
Threonine	52.0	26.0	3.5	7.63	2.89
Serine	28.0	14.0	3.5	7.42	1.51
Glutamic acid	56.0	28.0	6	11.95	8.34
Proline	7.0	3.5	7	14.60	1.49
Glycine	27.0	13.5	4	5.85	1.31
Alanine	29.5	14.75	4	9.29	2.28
Cystine	4.0	2.0	7	9.10	0.53
Valine	32.5	16.25	3	9.32	1.89
Methionine	22.0	11.0	2.5	10.03	1.15
Isoleucine	36.0	18.0	3.5	8.63	2.26
Leucine	76.5	38.25	3.5	7.55	4.20
Norleucine	70	35	5.5		
Tyrosine	6.5	3.25	10	15.50	2.09
Phenylalanine	17.5	8.75	8	10.17	2.96

APPENDIX 17

AMINO ACID ANALYSIS
TECHNICON TSM – 1
SAMPLE 7

(*Albizzia* – TOASTED 3 HRS)

MODEL. DNA 0209

Wt. of sample hydrolysed = 0.4367

%N (fat free) = 4.58

Volume loaded: Basic = 10µl

Acidic/Neutral = 5µl

Width @

Concentration (g) 100 protein = NH x NH/2 x S_{std} x C

Where S. std = NF_{std} x Mol. weight x μMAA_{std}

B = 0.001038916366

A/N = 0.002077832731

$$C = \frac{\text{Dilution x 16}}{\text{Sample Wt (g) x N\% x 10 x Vol. Located}}$$

÷ NH x W (nleu)

1	2	3	4	5	6 =(2 x 4 x 5 x C)
AMINO ACIDS	NET HEIGHT (mm)	NH/2 (mm)	WIDTH NH/2 (mm)	S.std	Concentration (g/100) protein) or g/16gN
Lysine	66.0	33.0	4	12.92	3.54
Histidine	23.5	11.75	4	15.08	1.47
Ammonia					
Arginine	23.5	11.75	18	9.10	4.00
Aspartic acid	114.0	57.0	3.5	8.57	7.12
Threonine	39.0	19.5	3.5	7.63	2.16
Serine	38.0	19.0	3.5	7.42	2.05
Glutamic acid	67.0	33.5	6	11.95	9.98
Proline	7.0	3.5	7	14.60	1.50
Glycine	21.0	10.5	4	5.85	1.02
Alanine	33.0	6.75	4	9.29	2.59
Cystine	4.5	2.25	7	9.10	0.60
Valine	43.5	21.75	3	9.32	2.53
Methionine	17.0	8.5	2.5	10.03	0.89
Isoleucine	32.0	16.0	3.5	8.63	2.01
Leucine	87.0	43.5	3.5	7.55	4.78
Norleucine	70.0	35.0	5.5		
Tyrosine	7.0	3.5	10	15.50	2.25
Phenylalanine	15.0	7.5	8	10.17	2.54

APPENDIX 18

AMINO ACID ANALYSIS
TECHNICON TSM – 1
SAMPLE 8

(*Albizzia* - ROASTED TRADITIONALLY)

MODEL. DNA 0209

Wt. of sample hydrolysed = 0.4425

%N (fat free) = 4.52

Volume loaded: Basic = 10µl

Acidic/Neutral = 5µl

Width @

Concentration (g) 100 protein = NH x NH/2 x S_{std} x C

Where S. std = NF_{std} x Mol. weight x μMAA_{std}

B = 0.001038909094

A/N = 0.002077818187

$$C = \frac{\text{Dilution x 16}}{\text{Sample Wt (g) x N\% x 10 x Vol. Loaded}}$$

÷ NH x W (nleu)

1	2	3	4	5	6 =(2 x 4 x 5 x C)
AMINO ACIDS	NET HEIGHT (mm)	NH/2 (mm)	WIDTH NH/2 (mm)	S.std	Concentration (g/100) protein) or g16gN
Lysine	67	33.5	4	12.92	3.60
Histidine	24	12	4	15.08	1.50
Ammonia					
Arginine	23	11.5	18	9.10	3.91
Aspartic acid	98.5	49.25	3.5	8.57	6.14
Threonine	40.5	20.25	3.5	7.63	2.25
Serine	33	16.5	3.5	7.42	1.78
Glutamic acid	63	31.5	6	11.95	9.39
Proline	7.5	3.75	7	14.60	1.59
Glycine	14	7	4	5.85	0.68
Alanine	32	16	4	9.29	2.47
Cystine	4	2	7	9.10	0.53
Valine	31	15.5	3	9.32	1.80
Methionine	24.5	12.25	2.5	10.03	1.28
Isoleucine	29	14.5	3.5	8.63	1.82
Leucine	56.5	28.25	3.5	7.55	3.10
Norleucine	70	35	5.5		
Tyrosine	5	2.5	10	15.50	1.61
Phenylalanine	14.5	7.25	8	10.17	2.45