DIETARY POTENTIALS OF *ALBIZZIA, ADANSONIA, DELONIX* AND *SESAMUM* SEED PROTEIN FOR MAINTENANCE OF LABORATORY MICE

BY

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CERTIFICATION

This thesis entitled "DIETARY POTENTIALS OF *ALBIZZIA, ADANSONIA, DELONIX* AMD *SESAMUM* SEED PROTEIN FOR MAINTENANCE OF LABORATORY MICE" by KAGA, BENEDICT ISHAYA meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to Scientific knowledge.

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DECLARATION

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

Signed by the author.

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DEDICATION

TO

Mrs Paulina I. Kaga

Abel M. Kaga

Gideon K. Kaga

Jerry Kaga

Godiya L. Kaga

Peninnah N. Kaga

To all the Kaga's

Above all to God Almighty for his love, protection and spiritual guidance during this work.

ABSTRACT

Three types of wild seeds, namely Albizzia lebbeck, Adansonia digitate Delonix regia as well as Sesamum were chemically analysed for their proximate composition, amino acids and mineral elements using standard methods of analyses. Results of the proximate composition showed that all the seeds had good keeping qualities, Since they had low moisture content. Fermented Delonix was richest in protein, ash, carbohydrates and nitrogen free extract than Sesamum, Adansonia and *A* lbizzia respectively. Sesamum seeds had the best energy and fat contents followed by Adansonia, Delolix and Albizzia. White Sesamum had more crude protein than Black Sesamum. Adansonia whole seeds were highest in fibre while Delonix kernel had the least amount

Fermentation was observed to give a dark brownish, attractive colour and pleasant smelling aroma to the dried fermented kernels of Delonix. Furthermore, fermentation increased lysine, threonine, valine, phenylalanine, cystine, aspartic acid, serine, glycine and alanine and mineral elements such as sodium, calcium, zinc, Iron and copper in fermented Delonix. Albizzia had the poorest amino acid profile. Adansonia had the highest amount of leusine, glutamic acid and proline. Sesemum seeds had the highest arginine, histidine, Methionine, Isoleucine and tyrosine.

The feeding trial experiment gave more feed consumption, better weight gain, feed conversion and protein efficiency ratios for mice on Delonix than the control diet. In addition, the mice fed on Delonix diet littered while those of the control diet did Dot Hie investigation provided useful information on the proximate and amino acid

constituents of Adansonia, Delonix and Black Sesamum. The possibility of incorporating fermented Delonix seeds in animal feeds and their processing into food seasoners were suggested.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 STATEMENT OF THE RESEARCH PROBLEM

The problem of malnutrition, particularly of profein, is a very knoty one in most developing countries of the World. These countries, including Nigeria, are located in the warm humid tropics where the level of animal protein intake is very low compared to the advance countries. The Food and Agricultural Organization (FAO) (1981) observed that developed nations consumed about 60 grams of animal protein per capita per day while Nigeria consumes only 5.5 grams per capita per day. This is low when conpared with the recommended minimum of 20 grams. It is also observed that the level of animal protein included in the diet of the average Nigeria is 9% while that of his counterpart in Newzeland is 11%, Denmark 10%, United States of America and United Kingdom 12% (Ademosun, 1973). This is due to the fact that most Nigerians depend on mainly plant proteins from grains, cereal and legumes.

Reid (1970) predicted that by the year 2000 A.D., there will be insufficient grains for all forms of livestock production generally, due to insufficient local production which resulted into undue competion between man and animals. However, it is morally unjustifiable to use grains for feeding laboratory animals when human needs have not been met.

The FAO (1991) identified population pressure rising at 3.3% annually, poor food security and poor management of natural resources as the bane of the African food crisis. Hence, the food demand of Africans have been estimated to be growing at an alarming rate of 3.5% annually. It is therefore, pertinent to investigate as many as possible conventional and unconventional food sources in the quest for nutrient rich

food materials in order to ameliorate food crisis. That is why serious considerations are being given to the formulation of cheap rations from unconventional and unpopular food materials that are of little or no direct value as human food. This would remove the unhealthy competition between man and livestock for the same food.

Also, the high demand for livestock for human consumption and laboratory animals for medical, veterinary, agricultural, biological and educational research is for ever increasing because of the teaming number of human and animal populations and the upsurge of reseacchers and students in these fields of study. However, insufficient feed ingredients have always been the limiting factors in the livestock and laboratory animal production outfit. Therefore, developing countries, including Nigeria have often resorted to the use of pods and seeds of wild plants because of their availability and relative abundance.

It is hoped that the effective utilization of these seeds would reduce competition for conventional grain, cereals and seed legumes as well as boost the production of all types of animal feeds. This kind of shift towards such alternative sources of feed ingredients would provide the much needed nutritional information on the use of wild seed legumes in animal feeds and eventually in human food. Therefore, this research was undertaken to examine the nutritive value and dietary potentials of Delonix regia (flamboyant) Adansonia digitata (Baobab) Albizzia lebeck in camparison to Sesamum as protein suppliers in the diet for laboratory mice.

1.2 Research Objectives

The research objectives are:

(1) To determine the proximate and amino acid composition of Delonix, Adansonia and Albizzia in comparison with Sesamum and Parkin seeds.

(2) To determine the mineral composition of Delonix seeds.

(3) To determine the effect of fermentation on the nutrient composition of Delonix regia seeds.

(4) To incorporate Delonix regia seeds in a feeding trial experiments using mice.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 EDUCATIONAL IMPORTANCE OF LABORATORY ANIMALS.

The educational importance of laboratory animals cannot be over emphasized. These animals are required for human, veterinary, physiological, and educational research. Apart from identifying new sources of nutritional information, the feeding of the African human population and animals, providing dietary treatment of diseases and the support of qualitative studies on nutrition, the keeping and maintenance of laboratory animal deserved a serious attention. There are no statistical figures to ascertain the number of laboratory animals used in our tertiary institutions, it is however, obvious that the practical aspect of biology has been neglected due to lack of experimental animals. From the above, the importance of laboratory animals in scintific studies led to keeping and rearing laboratory animals particularly rats, mice and rabbits.

In Nigeria the materials for breeding laboratory animals are not readily available. the cost of suitable apparatus and space for keeping and maintenance of laboratory animals are the main obstacles. Therefore, efforts must be made to provide these facilities. One way of tackling the problem is the exploitation of wild seed proteins in the laboratory animal feeds.

2.2 Protein Sources

Investigations on proteins began much earlier by Beccare in 1845 when he seperated gluten from wheat flour. Marked advances were made by other researchers like Osborne (1891), Rittanses (1962) and Haikewal (1969).

Since the lack of economic source of animal protein is acute, vegetable or plant protein is increasingly being resorted to inspite of the fact that the best quality proteins comes from animal source (Sikka *et al.*, 1979). This is because it is possible to formulate satisfactory diets to meet the requirements for boh man and livestock from appropriate plant mixture (Ega, 1986). Corn protein is low in lysine while soyabean is high in lysine. If the correct amount of soya bean and corn were mixed together, all the essential amino acids requred by either pig or rat may be provided without further supplementation (Maynard *et al.*, (1979). This kind of experiment have been done successfully in several places; for instance soyogi produced by the Federal Institute for Industrial Research, Oshodi, Lagos. Soyogi has been found to meet the daily protein requirement for children. The protein daily requirement for the human male adult(70kg); female adult (58kg) and children (12kg) have earlier been reported to be 40 grams, 32 grams and 12 grams respectively (Harper, 1973).

Kapu *et al.* (1987) reported that consumption of majority of locally available foods in Nigeria is limited by the complete lack of adequate information on their chemical and biological values. They determined the physical properties and nutrient composition of Gmelina aborea fruits. Their findings revealed that the flesh of Gmelina" fruit contained more moisture, crude lipid, ash, crude fibre and caloric value than the nuts of the stone of the fruits respectively. However, the crude protein, carbohydrate and dry matter were higher than those of the flesh of the fruit.

In an attempt to provide dietary information on protein sources, Ega, (1986) evaluated the biochemical composition and amino acids of roots of sweet cassava and taro cocoyam corns, Parkia and Tamarindus seeds. She also fed rats with diets compounded from mixtures of sweet cassava and cocoyam, fermented Parkia and Tamarindus proteins. Her findings showed that Parkia and Tamarindus protein were

rich in lysins but defficient in methionine and could be fortified with other local grains rich in methionine. Furthermore, her findings revealed that Parkia contained more amino acids than Tamarindus. Consequently, Parkia and Sesamum complementary diets were formulated and tested by Onabid (1992) on the growth and reproductive performance of laboratory rats, and it was found that the diet containing 10% Parkia and 10% Sessamum protein promoted adequate growth and reporduction in the rats.

2.3 Leave and Seed Proteins

Since protein malnutrition is very acute and worrisome, a lot of work has been done on leaves and seeds as source of protein in different human dishes and animal feeds. Umoh and Oke, (1974) determined the protein contents of Irvinga, Citrillus and Parkia Seeds. They found that thse oilseeds were highly digestible. Ekpenyoung *et al.*, (1977) fortified maize flour based diets with blends of cashew nuts, Parkia and Sesamum oilmeal and compared their relative efficiency with those of commercial baby foods (Farlene, Cerelac, Farex and Cow and gate). Promotion of growth ranged from 0.75 - 4.75g/day for the seeds while those of commercial baby food ranged from 2.64 - 4.5g/day. The values of cashew nuts and Parkia, 4.73 and 4.61g/day respectively, were the best blends compared to those of 4.5g/day for Cow and gate and theose of Cerelac food which is superior to those of Farlene and Cerelac, 3.41 and 2.64g/day respectively. A similar research was conducted on Pumkin seeds (Cucurbita pepo) by Fagbemi *et al.*, (1992). Their findings was also on the chemical and functional properties of full fat marrow pumkin seed flour which contained a high percentage of crude protein and fats. Similarly, Edwyne *et al.* (1980) worked an the dietary potentials of Nestle leaves on young pigs, Guineapigs, mice and rats. The

leaves prorided 66% of the dietary protein and supported normal growth. The study was later extended on chicks receiving the whole nestle leaves meals, as their sole dietary protein. The chicks growth was half the rate of chicks given a standard commercial diet. Bressani and Elias (1977) reported that the seeds of Phaseolus vulgaris possessed a high protein content but suffered a disadvantage nutritionally by the presence in the seeds of anti physiological activity such as lectins and trypsin inhibitors, even though, these do not pose a problem in practice as man rarely consume raw legumes and that activity is virtually destroyed by the usual cooking procedures. However, rats fed with heat treated Phseolus meals as a source of dietary protein showed a poor protein utilization in comparison to that of standard protein as casein. Apart from anti-nutritional factors being present in Phaseolus which adversely affect the protein utilization, the protein perse has been found to be resistant to digestion in rat feeding trials (Puztai,*et al.*, 1974). The poor digestibility of legume protein has been attributed to the presence of resistance "core" which have a high cystine content and trypsin inhibitors which have been shown not to release cystine when incubated with protease (Adquist *et al.*, 1966).

A similary study was conducted in india using sunflower seeds to evaluate protein quality of mixtures based on sunflower meal through rat growth studies by chandrasekhar and Kanjana (1976). It was discovered that the sunflower meal was a concentrated source of protein, thus the need to test its suitability to be included in diets. They went further to evaluate the sunflower meal, Bengal gram flower and Sessamum seeds on school children. The increase in height and weight of children fed on sunflower mixtures were significantly higher (<0.01) than the control group.

2.4 Wild Plant Legumes

Although wild plant legumes have not been completely exploited, it is a well known fact that they are a potential source of protein for human and animal feeding. Gliricida sepium (Cacahuanano) is an aboreal legume widely distributed in Mexico. It contains 40% protein and 24% fat. The lysine and tryptophan contents are as high as 4.4g and 1.3g/16gN respectively but they are defficient in sulphur amino acids (Felker, 1976). Also six protein fractions as high as (24-30%) crude protein have been seperated from Leucaena leucocephala grown in Indonesia. The protein concentrates were prepared from juice obtained when leucaena leoves macerated in water and the fibrous materials removed by filteration under pressure. The leaves were found suitable for feeding monogastric animals while the cytoplasm fractions were considered suitable for human consumption after removing the mimosine which is a toxic amino acid (3-5%) (Felker, 1976). Infact, plant legumes are very inportant from a nutritional point of view, because they are widely available vegetable food that contain good quantities of protein and B-vitamins. They usually supplement very well the predominantly carbohydrate diet of the cereal, root or plantain eater of African (Lathan, 1979). Leguminous plants also bring fertility to the soil through the nitrogen fixing bacteria of the roots and can produce more protein from an hactare of land than any cereal (Nicholls, 1951) some legumes have the advantage of being able to obtain nitrogen from the air and also add some to the soil, where as most other plants take the nitrogen from the soil and do not replace it (Lathan, 1979).

According to Altschule, (1962) pregnant and lactating mothers subsist on a definitely subnutritional diet consisting mainly on dahl (Legumes). Legume seeds have

the highest protein content of all plants. Dried beans contain at least 20% protein and there are some species which have over 30% protein especially soya beans.

In central and North America nutritional studies have been done on about 46 wild plant species such as Cassia, Lupinus and Crotolaria species which supported live weight gains of experimental rats. The diets were also found to be palatable and non-toxic. Physiological toxicity is normally reduced through processing such as steaming, leaching, as well as germination of seeds Felker *et al.*, (1977).

The Winged bean, Psophocarpus tetragonobolus is one of the under utilized seed proteins. It is a high protein leguminous crop adopted to the humid tropics (NAS, 1981). The crop has a great potenital as a source of human food because its flowers, leaves, inmature pods, seeds and tubers are all edible and very rich in protein (srinkantha and Erdman, 1978). The Winged beans has significantly ($P<o.05$) higher crude protein, fat and ash content than the commonly consumed cowpea, and in overall composition most closely resemble the soya beans (Giami *et al.*, 1992).

The Jack and Sword beans also serve as protein source to many houses in different parts of the world. Roasted Jack and Sword beans are used as adulterant of coffee, the ripe dry beans are usually eaten after prolonged cooking or boiling for 3 hours, the outer coat removed and soaked in water for 2 days followed by fermentation for 4 day prior to cooking (Duke, 1981). There is little information on the nutritional status of Jack and Sword beans, none the less, it is known that the legumes contain toxic materials which include tannin, trypsin inhibitor and protease inhibitor. These toxic and antinutritional factors must be eliminated prior to consumption, since, the nutritive value of a food depends on its composition, the bioavailability of its essential nutrient and the cancentration of its toxic and antinutritional factors.

2.5 Biological Characteristics of *Delonixregia* (flamboyant)

Delonix regia (Hook)

Family	Leguminesae
sub family	Casalpinioideae.
English	flamboyant
Hausa	Furen Kallo
Yoruba	Sekeseke

Delonix is a Greek word meaning conspicuously clawed petals. Keay (1989) gave a detailed biological characteristics of Delonix as leaves twice pinate, leaflets less than 12mm long, very numerous flowers with long stalks in racemes. There are about 20 pairs of branches on leaves (pinnae) each 7 - 13 cm long. Leaflets opposite. Flowers are conspicuous and scarlet. This handsome ornamental tree is known as the flame of the forest in Nigeria it is a fast growing tree usually with very widely spreading branches producing a broad flat crown. The leaves are borne profusely in short, upright terminal racemes. There are 5 large valvate lobes and 5 large scarlet petals with the one mottles red and white. There are 10 free stamens. The fruits are large flat pods up to about 60cm long by 5 - 7.5cm broad, almost black when ripe, hard and woody hanging conspicuously for n long time on the tree and eventually split open. There are numerous elongated seeds about 2.5cm long arranged lengthwise across the pod. The tree is widely cultivated and self sown throughout the tropics, a very rare native of Madagascar.

Biobaku (1994) investigated the performance of rabbit fed on raw and cooked Delonix seeds. The result showed that raw Delonix bean seeds were significantly

(P<0.01) lower in protein content than the cooked Delonix. However, he did not ferment the seeds nor determine the amino acid contents.

2.6 Biological Characteristics of *Adansoniadigitata* (Baobab).

Adansonia digitata (L)

Family : Bombacaceae.

English: Baobab

Hausa : Kuka

Yoruba : Ose

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Keay (1989) also gave a clear description of Adansonia trees with alternate compound leaves and leaflects arranged digitately. Stipules are present but soon, fall off. Flowers are arranged with a toothed calx. There are 5 sepals and numerous stamens. The ovary is superior. The fruits of the Nigerian representative of the family split open releasing numerous seeds except Adansonia which contain numerous seeds but do not, split open. The fruit contain acid pulp and pendulous on stalk about 30 cm long. Adansonia digitata was named after Michael Adanson, an 18thC Botanist.

A small tropical genus represented by one species in Nigeria is wide spread in the drier regions of Africa. The tree is planted or preserved in Savannah may extend into the forest regions. The Adansonia tree has irregular trunk that tappered conspicuously to the top. It is extremely broad at the base and quite narrow at the top. The tree is about 24 meters high and may attain a girth of 12 meters or more. Branches are short and thick. Leaves are compound digitate with 5.7 leaflets and 5.6cm long by 2.5cm broad. The Margin is entire or slightly toothed. It flowers between May to July, the calx are deeply lobed and very hairy on both sides. Fruiting starts from November to March. Seeds are spherical or ovoid, with a woody shell covered on the outside.

Seeds are numberous embeded in a dry acid pulp. The wood is soft and light. Addy, (1978) conducted research on the nutritive value of leaves and fruits of Adansonia and found the calcium content of ten-grams semi-dry leaves met about 41% of the daily requirement for children, however, the availability of this nutrients is limited by the presence of oxalates, although the possiblity of oxalate poison is remote. He observed that other toxic substances, such as phytic acid and hydrocyanic acid present in the seed and leaves were not likely to constitute a health hazard to those who make culinary use of them, since most of these toxic substances were check by heat. He went futher to recommend the use of Adansonia seeds for human consumption.

The seeds which are roasted in some parts of Ghana and Malawi are often used to replace groundnuts as side dishes. The seeds can be made into cakes used in flavouring soup Dadawa cake) (Addy. 1987).

2.7 Biological Characteristics of *Albizzia lebbeck*.

Albizzia lebbeck (Linn),

Family - leguminoseae.

Subfamily - mimosoideae.

This is an introduced species (Keay, 1989). It can reproduce itself readily and may be found growing wild. The tree is easily grown from seeds. It is large straw - coloured and its very persistent found fruits hanging down in conspicious clusters. The tree could grow 15m to 50m high usually branching low down. The bark is greyish. This genus is represented throughout the tropics. All the Nigerian species are decidous with ascending widely spreading branches. The fruits are pendulous, elongated and flat, often clearly visible inside the pod and spaced uniformly along it whole length, each seed being attached to the margin by a hair like thread (pappus). It

flowers from November to February, heavily scented with the stamen, free above the corolla. Fruiting is from September to May remaining on tree untill after the new flowers appears. The foliage is commonly used as fodder in India and else where for cattle, and in Sudan goats eat the fallen leaves and flowers. The finely powdered bark contains saponin and can be used as soap. It contains 5 -15% tannin and is used in tanning. (Kay, 1989).

2.8 Biological characteristics of sessamum (Beniseed)

Family		Pediliaceae
Subfamily		Sesamum
	-	Indica (Black)
	-	Radiatum (White)
English	-	Beniseed
Hausa	-	Ridi
Kataf (ATyap	-	Tsuan.

According to purseglow (1968), the sessamum plant is either annual or perenial. The leaves are opposite and simple. The flower is hermophrodite and zygomorphic. The calx usually 5. corolla gamopetalous 5 lobed. Statement 4. anthers connivent in pairs. The seed has no endosperm with flattened cotyledon. Sessamum indicum is an importants oil seed crop that contains 37% oil. The seeds are used in soups and cakes. The oil is used in manufacturing margarine, as a substitute for olive oil, salad and cooking oil. It is used in soap and paints lubricant and illuminant It is also used as a vehicle for medicine, drugs and perfumes. In India, it is used as a ghee substitute and for anointing the hair and body. In South India, it is customary to annoint the body hair weekly due to its beneficial effects to health. Sesamum contains excellent protein used in human and livestock feeds. Fried seeds are eaten in soap, mixed with

sugar, scaterred on tops of bread, cakes and pastry. The young leaves are used as a soup vegetable in Nigeria (Southern Kaduna) various part of the plant are used as native medicine in Africa and Asia. The stem are burnt as fuel. (Purseglow 1968)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMAL

16 Newly weaned mice 21-25 days old were obtained from the department of Veterinary Parasitology and Entomology Ahmadu Bello University Zaria. the animals were weighed on arival using a tripple beam balance. the mice were distributed into groups of four males and four females per group in a locally made clay cages secured with wood and covered with a net of wire were used, the mice were characterised by a large genital papilla and greater distance between the anus and the genital papila. Petri dishes were used to serve the food inside the cage. Standard water bottles were used and wood shavings were obtained from the carpenter in Sabon Gari Market Zaria.

3.2 POD TREATMENT AND SEED FERMENTATION

Ten pods of *Delonix* and *Albizzia* each were randomly collected from 30 trees in Samaru and Sabon Gari area of Zaria. The whole pods of *Delonix* collected were assembled in a pool of water for three days for easy splitting near the library of the department of Biological Sciences, Ahmadu Bello University, Zaria. The pods were broken open manualy on the forth day to remove the seeds for boiling, fermentation and subsequent analyses. Ten kilograms of Delonix seeds were boiled for 8hrs to soften the hard testa. The seeds were allowed to cool and then washed with tap water. The seeds were pressed with hand to seperate the kernel from the seed coat. The method of fermentation was similar to that of Fetuga, (1974), Simon, (1976), Eka, (1980), and Ega, (1986). The kernel were put in a small bagco bag and covered with plastic container which was allowed to stand for three days in one corner of the laboratory to

ensure fermentation of the kernel. after three days, the kernel were sun dried for four days and oven dried at 60°C. The dried kernel were ground in the laboratory to obtain the flour for ration formulation and analyses. The pods of Albizzia were sun dried and pounded in a mortar to remove the seeds. The seeds were taken to the laboratory mill for grinding into flour which was stored for analyses.

Adansonia seeds were got from Fulani women after they had used the pulp of the fruit for their "Fura da nono". The seed were sun dried and oven dried at 60°C, grounded in a laboratory mill for analyses. While fermented parkia and Sesamum seeds were bought from Samaru and Sabon Gari Markets in Zaria.

3.3 Chemical Analyses of Seeds

The proximate composition of the seeds was determined. Moisture, dry matter, total organic matter, Ash, crude lipid and crude fibre were determined as described by A.O.A.C. (1980) (Appendix I).

The total nitrogen was determined using the Micro Kjeldahl method as described by Pearson (1976) (Appendix I). Protein was calculated by multipplying by 6.25 as conversion factor. Total carbohydrate was determined by difference method (Schneider and Flat, 1975). The Nitrogen Free Extract was derived according to Crampton and Harris (1969). The calorific values were determined by multiplying the percentage of carbohydrate, fat and protein by atwater factors 4,9, and 4 kilo calaries/100 grams respectively. The sum total was taken as the value for each sample (Winberg, 1971). The amino acids were determined using the methods described by A.O.A.C.(1980). The samples were oven dried to constant weights, defatted, hydrolysed and evaporated in a rotary evaporator and then loaded into the Technicon Sequential multisample amino acid analyser (TSM). The individual amino acid calculated from the resultant graph. Duplicate analyses of samples were carried out in each case on dry matter basis and the means were used to calculate the standard error and test of significance (Appendix II). The mineral elements were analysed using the atomic spectrophotometer (AAS), Perkin. Eloner model 2903; the flame photometer Corning - Eel model and spectronic 20, (A.O.A.C, 1980) (Appendix III). Both calorimetary and absorption spectrophotometry were based on Beer - lambert's Law.

Log 10
It = abc where 10 = Intensity of light

It = Intensity of transmitter

a = Molar absorptivity

b = Concentration

c = Thickness of median.

3.5 Diet Formulation

Among the wild seeds of Albizzia, Adansonia and Delonix, the Delonix seeds with the highest protein composition from the experiment were chosen for use in the feeding trial experiment. The amount of fermented Delonix seed protein incorporated into the experimental diet was calculated as shown in Appendix IVa, while the control diet consisted of equal amounts of Parkia and Sesamum seeds mixed proteins (Samba and Ega, 1994 and 1995). The calculation is also shown in Appendix IVa. These seeds contributed 20% of the protein in both control and experimental diets (Table 3.1). The common ingredients for control and the experimental diets were yellow maize, table salt, Mineral and Vitamin premixes. (appendix IVC). There was no binder used, the seed were ovendried at 60°C for 24hrs at room temparature.

Table 3.1 **Percentage Composition of experimental diets (%).**

Components	Control diet	Fermented Delmix diet
Yellow maize	55.77	67.66
Delonix	-	31.59
Parkia/ Sesamum	21.74 21.74	- -
Salt	0.5	0.5
Vitamin & Mineral premixes	0.25	0.25
Total protein	100% 25%	100% 26%

3.6 Experimental Procedure

A male and female mice were paired in each of the four cages per feed. This means that each feed was replicated. The mice were feed daily with 5.00-6grams. of feed moulded in form of pellets and placed in an oven set at 60°C to dry for twenty four hours before feeding to the mice. This trentment was to prevent scattering and wastage of feed by the mice since the feed was put in petri dishes and placed inside the cages for easy access.

Water was supplied outside the cages using standard bottles, both feeds and water were supplied ad libitumm. The experiment was started after one week of adjustment in the cage and lasted for a period of eight weeks. The cages were cleaned daily and the wood shaving removed whenever they were wet or dirty to avoid fungal growth. The bottles were washed daily and fresh tap water supplied. The experiment was carried out at normal room temperature. Daily feed consumed were recorded for each feed. The weight of each mouse was taken twice per week for each feed.

The following biological indices were then calculated.

(a) Weight gain or loss = Weight of animal at the end of the experiment minus (-) weight of animal before the experiment.

(b) Feed Conversion Ratio (F.C.R)

$$\frac{\text{Total feed consumed per mouse}}{\text{weight gain or loss.}}$$

(c) Proteim Efficiency Ratio (P.E.R)

$$= \frac{\text{Weight gain/loss per mouse}}{\text{Protein consumed.}}$$

Student t - test was employed to determine whether there was a significant difference between the control diet and the experimental diet in terms of feed *consumed, weight gain,* FCR *and* PER.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Analyses

Table 4.1 shows the proximate composition of Albizzia (AB), Adansonia (AD), Delonix (DR), Black Sesamum (BS), and white Sesamum (WS). The results showed that (AB) had the highest moisture content (14.49%), closely followed by BS, AD, WS fermented Delonix regia (FDR) seed kernel of Adansonia (Ad-T) and lastly seed kernel of Delonix regia DR-T (5.58%.).

BS had the highest amount of organic matter (96.96%) followed closely by WS then AD-T, AB, AD and lastly DR (91.01%). The highest amount of crude protein was got from FDR (63.31%)followed by DR-T, WS, AD-T, AB, BS and lastly AD (18.81%). The crude lipid and calories were highest in BS,WS respectively (41.36%,35.02%) (2255.39, 2173.97) followed by DR-T, AD, AD-T, FDR and lastly AB. The crude fibre values were highest in AD (18.61%) follow by AB, FDR, DR, BS, WS, AD-T and lastly DR-T (3.97%). Carbohydrate and Nitrogen Free Extract (NFE) (55.72, 50.71%) had the same trend for DR, followed by AD, AB then AD-T, BS, FDR, DR-T and lastly WS with the least values (4.85, 0.18%). It was observed that AD and AB which had the highest values for carbohydrate (45.95%) and NFE (27.37%), and also the least calories (1345.65Kca/100gm) since they had the least fat content, while BS and WS with the highest fat content (41.36g 35.05%) also had the highest calorific values (2255.39,2193.97, Kcal/100gm). The highest Ash content was found in FDR (6.0%)followed by DR, AD, AB, AD-T, WS and BS (3.15%) respectively.

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TABLE 4.1 PROXIMATE COMPOSITION OF ALBIZZIA, ADANSONIA, DELONIX AND SESAMUM SEEDS (%)

NUTRIENTS 6	AB	AD	AD-T	DR	DR-T	FDR	BS	WS
Moisture cont	14.19	10.15	6.85	6.76	5.58	8.86	13.98	9.33
Dry matter	85.51	89.15	93.15	93.24	94.42	91.14	86.02	90.04
Ash Content	4.72	5.19	4.62	8.38	5.57	6.09	3.15	3.34
T. O. M.	94.18	94.8	95.04	94.01	94.10	93.32	96.34	96.31
Crude protein	34.95	18.81	46.63	22.12	50.03	63.31	24.94	47.19
Crude lipid	11.62	19.17	21.15	7.01	30.58	16.99	11.36	35.02
Crude fibre	10.99	18.61	4.02	5.01	3.97	5.18	4.89	4.67
Carbohydrate by difference	34.22	46.57	20.75	55.72	8.14	4.75	16.57	4.85
Nitrogen free extract	23.24	27.37	16.73	50.71	4.17	-0.43	11.68	0.18
Energy value K cal/100gm	381.26	322.21	159.87	374.49	508.80	460.15	538.28	523.62
Energy value k/joules/100g	1597.46	1345.65	1926.86	1569.11	2131.87	1928.03	2255.39	2193.97

KEYS

AB = Albizzia whole seed
AD = Adansonia whole seed
BS = Black Sesanum Seeds
Ad-T = Adansonia kernel
DR = Delonix whole seed
T.O.M. = Total Organic Matter
Dr-T = Delonix kernel
FDR = Fermented Delonix Kernel
Cal. = 4.19 joules

4.2 Amino Acid Profile

The amino acid profile revealed that both DR and AD were highest in both essential and none - essential anino acids while AB had the poorest amino acid content (Table 4.2). The essential amino acid, lysine (6.83g/16gN) of DR was twice as high as that of AB, BS, or WS (3.37g/16gN) and only slightly higher than that of AD(6.15g/16gN). Histidine was highest in BS (2.9g/16gN)followed by WS, then AD, DR and lastly AB. (1.83g/16gN).

Arginine was highest in WS (12.0g/16gN) and then BS followed by DR, AD and AB repectively. Four Sulphur amino acids, threonine, cystine, methionine and phenylalanine were highest in DR followed by BS, WS, AD and lastly AB. AD was the highest in leusine (8.9g/16gN) followed by WS and BS than DR and AB (1.64g/16gN). Isoleusine was highest in BS (3.17g/16gN) followed by AD, WS, DR and lastly AB (0.11g/16gN). Glutamic acid, a non - essential amino acid was the highest occuring amino acid in all the samples. Aspartic acid, serine, Alanine and Valine were highest in DR except Tyrosine and proline which were the least occuring non-essential amino acid. DR was followed by WS and BS respectively in their contents of aspartic acid, serine and valine. BS had the highest Glycine (6.95g/16gN) followed by DR, WS, AD and AB (0.17g/16gN), while the highest proline occured in AD (5.47g/16gN) followed by WS, BS, AB and lastly DR which was completely destroyed.

TABLE 4.3 AMINO ACID COMPOSITION OF ALBIZZIA, ADANSONIA, DELONIX AND SESAMUM SEEDS (g/16gN)

Amino Acid	AB	AD	DR	BS	WS	FAO/WHO
Lycine	3.24	6.15	6.83	3.34	3.37	4.2
Histidine	1.83	2.4	2.19	2.92	2.89	-
Arginine	1.73	8.98	9.25	9.58	12.06	-
Aspertic Acid	2.64	3.65	11.5	9.52	6.37	-
Threonine	1.75	3.47	5.18	4.16	4.14	2.8
Serine	1.75	4.91	9.79	5.35	6.22	-
lysine	4.06	21.55	18.99	18.84	20.99	-
Proline	1.49	5.47	-5.13	4.26	5.23	-
Alycine	0.17	3.79	5.34	6.59	4.63	-
Alanine	1.16	3.48	5.99	3.95	3.55	-
Cystine	0.49	1.29	1.72	1.66	1.36	4.2
Valine	0.31	2.99	4.75	3.89	4.18	4.2
Methionine	1.57	2.26	2.59	2.66	2.33	4.2
Isoleucine	0.11	2.39	2.33	3.17	2.55	4.2
Leucince	1.34	8.91	6.03	8.3	8.56	4.8
Tyrocine	1.64	2.95	2.9	3.88	2.98	2.8
Phenylalanine	2.28	4.3	4.48	3.12	4.21	2.8
Amino Acid	AB	AD	DR	BS	WS	FAO/WHO

4.3 Mineral Composition of Delonix regia (flamboyant) %

Table 4.3 shows the mineral composition of Delonix regia seeds. The concentration of potassium in the kernel(2.5%) was twice the concentration of potassium in the whole seeds (1.27%.). When the kernels were fermented the percentage composition of of potassium (0.41%.) decreased significantly (P< 0 .05) Sodium concentration in the whole seed and the kernels were the same (0.16gm), but after fermentation it increased to (0.19%). Calcium in the kernels (0.99%) was higher than the whole seeds (0.81%). Fermentation also increase calcium in the kernels

(1.09%). Similarly, Magnesium in the kernels was higher than the whole seed (0.38%),but decreased during fermentation (0.04%).

Zinc and Manganese concentrations were the same in both whole and kernels (0.04% - 0.019 ppm). However, fermentation increased zinc concentration (0.6%) while manganese concentration was lowered (0.0016ppm). Iron and copper concentration were higher in the whole seed (0.16%, 0015ppm) than the kernels (oo.15, 0012ppm) , but fermentation increased both elements in the kernels (00.19ppm,0.0020ppm) significantly ($P < 0.05$). Phosphorus were concentrated in the kernels (1.09%) than the whole seeds (0.48%). Fermentation of the kernels decreased the phospharus concentration (1.02%`).

TABLE 4.3 MINERAL COMPOSITION OF DELONIX (%)

ELEMENT	*WHOLE SEED*	*SEEDNUT*	*FERMENTED SEEDNUT*
Potassium	1.27	2.56	0.41
Sodium	0.16	0.16	0.19
Phosphorus	0.48	1.09	1.02
Calcium	0.81	0.99	1.09
Magnesium	0.38	0.46	0.04
Zinc	0.04	0.04	0.06
Manganese	0.0019	0.0019	0.0016ppm
Iron	0.16	0.15	0.19
Copper	0.0015	0.0012	0.0020ppm

4.4 Feeding Trial Experiment

Table 4.4 gives the summary of feed consumed, weight change, feed conversion ratio, (FCR), and protein efficiency ratio (PER) of the mice. During the first two days of the experiment, the mice were restless, but by the third day, they had calmed down in their cages as they become acclimatized to the feeding regime. It was observed that the mice on the experimental diet were pregnant on the 4th week of the experiment. This corresponded to a substantial increase in weight gain of the female mouse after five weeks on the experimental diet (Tables 4.5 and 4.6). While the mouse on the control diet did not become pregnant. The mice on the experimental diet delivered three pups in the 7th week of the experient. On the seconday it was noticed that two of the pups had been eaten by the male mouse, so at the male mouse was removed.

The experimental diet was consumed more than the control diet as the mean daily and weekly feed consumption of the control diet was significantly ($P < 0.05$) lower than that of the experimental diet. The weekly weight gain of the rats fed on the exerimental diet was twice the weekly weight change of those on control diet (Tables 4.5 and 4.6). Both the feed conversion and protein efficiency ratios of the experimental diet were significantly ($P < 0.05$) better than those of the control diet.

TABLE 4.4: FEED CONSUMPTION, WEIGHT CHANGE, FCR AND PER OF EXPERIMENTAL ANIMALS (%)

Experimental period in weeks	Feed consumed	Weight change	Feed consumed	Weight change
1	1.19	1.78	5.08	7.18
2	1.90	3.86	1.95	6.22
3	1.88	1.05	5.21	8.60
4	1.71	1.72	6.29	9.80
5	5.00	5.17	5.85	10.51
6	1.18	5.72	5.30	11.99
7	3.89	6.11	6.62	12.66
8	3.14	8.26	3.65	15.43
Total	31.28	11.12	39.61	82.12
Mean	1.285	5.18	1.96	10.30
FCR	6.62		3.85	
PER	0.63		1.21	

TABLE 4.5

GROWTH RATE OF MICE ON *DELONIX* DIET (GRAMS).

CAGE	WEEKS								
	0	1	2	3	4	5	6	7	8
1	10.30	13.73	13.18	15.70	16.69	13.34	16.10	19.90	19.10
2	4.88	6.50	11.38	13.03	15.63	15.54	16.83	15.97	23.15
3	7.20	14.79	13.95	17.00	17.43	21.49	21.75	22.50	24.90
4	7.98	11.80	13.68	18.88	19.93	22.03	23.49	26.53	24.80
Mean	7.59	11.71	12.80	16.15	17.42	18.10	17.41	21.22	22.99

TABLE 4.6

GROWTH RATE OF MICE ON CONTROL DIET (GRAM

CAGE	WEEKS								
	0	1	2	3	4	5	6	7	8
1	13.30	11.38	13.30	17.68	19.33	20.33	20.45	21.40	20.65
2	7.30	11.58	13.99	11.03	10.09	10.60	9.65	10.50	13.93
3	7.00	9.30	10.40	12.03	14.30	14.07	15.95	16.73	15.95
4	7.45	9.65	12.90	10.69	10.39	11.92	12.08	13.80	14.65
Mean	8.81	10.48	12.65	12.86	13.53	14.23	14.54	15.51	16.30

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Composition

The moisture content of the kernels of Adansonia and all the portions of Delonix seeds were found to be lower than the moisture safe limite of plant materials suggested by Ranjhan and Krishan (1980); it appears that these seeds could be stored for a long time without growing mould or lowering their quality. Although the moisture content of Albizzia and Black Sesamum were significantly ($p<0.05$) higher than those of Delonix and White Sesamum, their values were slightly less than that of the safe storage limit of 15%. The generally lower moisture content of kernels than whole seeds agreed with an earlier report by Ega *et al.* (1988) on Parkia and Tamarindus seeds and their kernels which had the same pattern as shown by this study. The inverse relationships between the moisture content and dry matter, ash and organic matter contents are clearly demonstrasted by all portions of Delonix seeds which had high dry matter and ash with correspondingly low moisture, and organic matter. Conversely, Albizzia with the highest moisture content had the lowest Dry matter while Sesamum with the lowest ash had the highest organic matter.

Despite the high protein in all portions of Delonix seeds, the seed coat should be removed before incorporation into monogastric animal feeds. The fat content of fermented Delonix was lower than that of the whole seeds possibly because of the high heat employed during cooking which was reported to be responsible for the removal of some volatile compounds such as fatty acids (Jacobs, 1958). Both the protein and fat values obtained in this study were as high as those reported by Biobaku (1994) for Delonix seeds. Although, Sesamum had more fat than Adansonia and Delonix, both

seeds contained high enough fat contents for them to be considered as oil seeds, as their fat contents were much higher than those in Parkia or Tamarindus seeds (Ega, 1986). Furthermore, the high protein and fat contents of Black and White Sesamum and Delonix contributed to the high energy values recorded for them while Albizzia and Adansonia had lower energy values, because of their lower protein and fat contents, even though their carbohydrates and NFE were high.

Due to high fibre content in Adansonic and Albizzia whole seeds, it is suggested that their kernels could be used for monogastric animal feeding while the whole seeds could be employed in ruminant nutrition. A similar pattern had earliear been reported for fibre in Parkia and T amarindus seeds by Ega *et al.* (1988). Also, fermented Delonix kernels which contained lesser carbohydrate and NFE than the whole seeds, indicated that the carbohydrates were utilized by microbes during fermentation thereby depleting them. This pattern was also noted to be similar to the effect of cooking and fermentation in other legume seeds used by Akinrele *et al.* (1970); Akindele (1978). Okoh and Eka, (1978) and Ega, (1986). The lowered carbohydrate content might not be a limitation, since cooking and fermentation are usually employed only to increase the protein quality of grain legumes while cereals and root crops are the main carbohydrate suppliers.

5.2 Amino Acid Profile

The amino acid profiles of Delonix and Adansonia reported in this research were much higher than those of Parkia and Tamarindus seeds reported by Ega (1986). Although, Albizzia had the poorest amino acid profile. It was better than the cocoyam and cassava amino acid profile determined by Ega (1986). Even the amino acid profile of Butyrospermum (Ega 1997) was much better than that of Albizzia. All the wild seeds contained higher amounts of virtually all amino acids than root crops, since leguminous

plants are known for nitrogen fixation. The high content of lysine and arginine shown in Delonix and Adansonia compares well with those reported by Altschul, (1962); Aykroyd and Doughty, (1964); Fetuga *et al.*, (1974); Abdi and Sahib, (1976); Felker and Bandursk, (1977); and Nierle and El-Baya (1978).

The essential amino acids of Delonix and Adansonia seeds were also well above the FAO/WHO (1973) reference values except for Methionine and Isolencine which were below the reference values. With the exception of threonine, Leucine and phenylalanine which were higher, the rest of the essential amino acids of Black and White Sesamum were below the FAO/WHO (1973) reference values.

Glutamic acid, Aspartic acid, and leucine constitute the highest form of amino acids in all samples, thus maintaining the trend which is in agreement with that obtained for some plant proteins such as leaf extract from grasses (Oke, 1973) and seeds of Adansonia (Addy, 1978); groundnuts and soya bean meal (Jefferies *et al.*, 1978). The amino acid of Delonix and Adansonia could be used to supplement cereal stapples since various investigations had shown that cereal and legume proteins are nutritionally complementary to each other (Akinrele, 1971, Oke, 1975; Aremu, 1991; Samba and Ega, 1994 and 1995). Furthermore, the high amount of lysine, histidine and arginine are typical of seed legumes as reported by previous researchers such as Fetuga, *et al.* (1974) Eka, (1980) Ikeoma, (1984) and Ega, (1986).

5.3 Mineral composition of Delonix regia

The high concentration of potassium, phosphorus, calcium, magnesium and Iron in Delonix seeds shows that the seeds contained very vital or essential mineral elements which were required by the mice and other animals for bone formation, active transport and oxygen exchange. The high composition of phosphorus and Iron in fermented Delonix agrees with the findings of Pomeranz and Meloan, (1978), Who reported that these elements were present in relatively adequate concentrations in legumes. Similarly, the values of potassium and calcium in Delonix were higher than the values reported for Parkia and Temarindus by Ega, (1986). Sodium and Potassium are important in the Ionic balance of tissue fluids and sodium - potassium pump in impulse transmission (Allen, 1974). The calcium content of Delonix seeds which was next to potassium in abundance, was higher than the value reported for Tamarindus seeds by Ega, (1986). Also calcium is known to play a part in the synthesis of cell plates affecting cell permeability (Gauch, 1972). Furthermore, correct calcium, magnesium, phosphorus, copper and manganese balance is required for skeletal and nerve functions in animal tissues (Allen, 1974). In addition, the determined value of mangnesium in Delonix was higher than that of African Walnut (Itam *et al.*, 1983) and Tetracarpidium conophorus (Sallau, 1989). Both the Magnesium and zinc content of Delonix fell within the remmended range (WHO, 1973) for laboratory animals, other livestock and even infants and lactating women.

Zinc is required by animals for proper hair growth, in peptidases and carbonic anhydrase enzymes and for the prevention of rough and thickened skin in animals. Copper, in conjunction with Iron, which are essential catalyst of cellular metabolic reactions involving cytochromes, haemoglobin synthesis, maintenance of myelin and bone formation (Allen, 1974), is inadequate in Delonix seeds. This low copper content

agrees with an earlier report by Ledoux *et al.*, (1991) that copper concentration was generally low in plant materials and its bioavailability for incorporation into rations was a serious problem in livestock nurtrition. Finally, to ensure the availability of most of the essential mineral elements in Delonix seeds, it appeared that the removal of the seed coat after cooking but before fermentation, further increased the concentrations of sodium, zinc Iron and copper.

5.4 Feeding TrialExperiment

The positive response of mice fed with fermented Delonix supported the result of the rich proximate, mineral and amino acid compositions found in the seeds. Furthermore, fermentation of the seeds had greatly increased the protein, and consequent performance of the mice on the Delonix feed which supported growth and reproduction.

Since the mice on fermented Delonix consumed more feed, and their feed conversion (FCR) and protein efficiency ratios (PER) almost double those of the control mice it was not suprising that more weight gain was recorded for the experimental mice than the control.

The cooking and fermentation of Delonix seed also confirmed the claim by jaffe, (1975) that cooking of legumes seeds improved dry matter digestibility, protein quality (Labelle and Hackier, 1975) and weight gain of Animals (Jaffe and Letter, 1968). Therefore the higher weight gain, FCR and PER recorded could be attributed to cooking and fermentation which possibly destroyed the antinutritional element usualy present and enhanced the protein performance in the experimental mice. These similar explanations were ealier advanced by Fetuga *et al.* (1974) for fermented Parkia and Ega *et al.* (1988) for fermented Parkia and Tamarindus protein.

Although Biobaku (1994) used rabbits in the assay of raw and cooked *Delonix* seeds, he similarly recorded weight gain and found that the rabbits on cooked seeds did better than those on the raw ones. In this investigation where the seeds were fermented after cooking, the mice not only gained weight but also reproduced. Similarly, Samba and Ega, (1994 and 1995) using fermented Parkia and Sesamum seed proteins, obtained weight gains and reproduction in rats. The dried fermented seeds were attractively dark brown and pleasant smelling, therefore, the posibility of further processing into flavour cubes and use in spicing human food should be research into.

5.5 Summary and Conclusion

According to the proximate analyses, all the wild seeds of Albizzia, Adansonia, Delonix and Sesamum had good storage qualities since their moisture contents were below the highest safety limit. Fermented Delonix kernels, followed by whole seeds, had the highest amounts of crude protein, Ash, carbohydrates and nitrogen free extracts than both White and Black Sesamum, Adansonia and Albizzia respectively. Black Sesamum was richest in fat and energy than White Sesamum followed by Adansonia and lastly Albizzia, Adansonia whole seeds had the highest fibre content followed by Albizzia while the least fibre content was found in Delonix seeds.

The advantages of fermenting Delonix include the production of pleasant smelling attractive seeds of higher protein quality. Although the proximate and mineral composition of raw and cooked seeds of Delonix had been reported by Biobaku (1994), this study provides first hand information on the amino acid profile of Delonix, Adansonia and Albizzia seeds.

Delonix seeds were richest in essential amino acids such as lysine, threonine, valine, cystine and phenylalanine, and non - essential amino acids such as aspartic acid,

serine, glycine, and alanine while proline was lowest. Black Sesamum had the highest amounts of histidine, methionine, Isolencine, and tyrosine. The white Sesamum was rich only in arginine while Adansonia seeds had the highest leucine, glutamic acid and proline. Albizzia seeds had the poorest amount of all amino acids.

The mineral composition of Delonix seeds had shown that the kernels contained more potassium, phosphorus, calcium and Magnesium than the whole seeds. Furthermore, fermentation increased sodium, calcium, zinc, Iron, and copper while potassium, phosphorus, Magnesiurm and Manganese were decreased.

The feeding trial experiment showed that Delonix seeds gave a higher weight gain than the control diet. The feed conversion and protein efficiency ratios were also significantly ($P<0.05$) higher. The fact that the mice also reproduced on fermented Delonix indicated that the protein was of high quality. On account of the rich nutrient composition of Delonix seeds, further feeding trial experiments are suggested in order to determined the suitablility for flavouring human food, like dawadawa cubes, since the aroma of the fermented Delonix was similar to that of fermented Parkia which is used in seasoning soup.

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APPENDICES

1.0 CHEMICAL ANALYSIS

The chemical composition of the seeds were determined using the methods of the Association of Official Analytical Chemist (A.O.A.C.), (1980) in Post Graduate Laboratory, University. of Jos. The chemical components analysed were moisture content, ash, lipid, crude protein, crude fibre, and amino acids. These were determined in triplicates - with the help of the Chief Technologist Mr. Ojobe T.O.

2.0 DETERMINATION OF MOISTURE CONTENT AND DRY MATTE

The weighed samples were labelled and placed in pre-weighed crucibles. The samples in crucibles were then dried in Gallen Kamp hot box humidity-oven model OV-620 at 105°C for forty eight hours. The weights were taken at intervals of six hours after the first twenty four hours. The samples were dried and cooked in an air-tight dessicator and later weighed directly on electronic meter balance. The samples were dried until constant weights were obtained. The moisture content was obtained from the weight loss of the fresh sample:

i Wt of crucible with fresh sample - wt of crucible with dry sample = moisture content.

ii Dry Matter Weight = constant weight after drying

iii $$\% \text{ Dry Matter} = \frac{\text{Dried sample wt}}{\text{fresh sample weight}} \times 100$$

iv $$\% \text{ moisture content} = \frac{\text{Wt of H20 wt}}{\text{fresh sample wt}} \times 100$$

where wt = weight

3.0 DETERMINATION OF ASH

Each dry sample was ground into a homogenous powder with laboratory electric grinder (Glen Creston) and transfered into labelled sample bottles for further chemical analysis. 1.5 grams was removed from each of the homogenous sample powder labelled and placed in a pre-weighed porcelain crucible. The crucibles and their contents were ignitted in an ashing Carbolite (GLM) Muffle furnace maintained at 600^{0}C for twenty four hours. The white ash obtained was then cooled in the oven to 100^{0}C and later transferred to a dessicator and cooled further for another twelve hours. The crucibles and contents were then weighed. The heating, cooling and weighing were repeated at four hours intervals until no further changes in colour and weight occured.

The percentage ash content was estimated.

$$\% \text{ Ash} = \frac{\text{Weight of Ash}}{\text{wt of sample powder taken}} \times 100$$

4.0 TOTAL ORGANIC MATTER

The Total organic matter (T.O.M) was determined by the procedures of linghstein and Oginsky (1965)

The percentage of the Total Organic Matter in the seed samples was determined by subtracting the ash weight from the dry sample weight then dividing organic matter weight by dry sample weight multiply by 100. Organic matter consist of fats, protein and carbohydrates.

$$\text{T.O.M.} = \frac{\text{Wt of dry matter - Ash} \times 100}{\text{wt of dry matter}}$$

5.0 DETERMINATION OF LIPID CONTENT (SoxhletContinousMethod)

The lipid content was determined using the Soxhlet Continous Extraction method of A.O.A.C (1980). From each sample 5 grams was put into a known weighed extraction thimble and weighed. The thimble was plugged with fat free cotton wool and the contents were placed in the central syphon portion or extractor of the Soxhlet apparatus. A 250 ml ground glass-joint flask was weighed and 40ml of analytical grade (60-80°C) Diethyl ether plus 40ml analytical grade Petroleum Ether were poured into the flask.

The flask was connected to the Soxhlet syphon and condenser with the flask sitting in the heating mantle. The mantle was then connected to the electric supply and heating was slowly increased until the solvent boiled. The vapour of the boiling solvent which condensed in the Soxhlet syphon were the sample was placed and the lipid was dissolved.

At a certain level, the solvent with the dissolved lipid ran back into the 250cml ground - glass-joint flask. The heating and the extraction was continued for 8 hrs. At the end of the 8 hrs, the timble was removed and placed in an oven at 105°C for 3 hrs distillation. The glass flask was cooled in the descicator and weighed. The flask and the content were replaced in the oven for another 30 minutes and reweighed.

This was repreated until a constant weight was obtained. The increase in weight of the flask was taken as the weight of the lipid or crude fat extracted .

$$\% \text{ lipid or crude fat} = \frac{\text{weight loss of sample}}{\text{weight of sample.}}$$

The defatted material was preserved for protein analysis.

100°c to a constant weight. The paper and content were incinerated to an ash at a dull red heat.

The weight of the ash was subtracted from the increase of weight on the paper due to the insoluble matter. The difference was reported as the fibre content.

$$\% \text{ crude fibre} = \frac{Y-X \times 100}{Z}$$

where X = weight of the ashless filter paper.

Y = weiht of Ash after incinerating.

Z = weight of powdered sample

9.0 DETERMINATION OF CRUDE PROTEIN

0.5 grammes of each sample powder was accurately weighed on a filter paper. The filter paper and samples were transferred into small kjehdal flask containing 0.8g of catalyst mixture and anti bumping granules. To another flask 0.165 grams of glycine was added as standard. 10ml of concentrated sulphuric acid was added to each of the flasks. The flasks were warmed gently at first to minimise frothing then the heat was increased until the solution was cleared.

9.1 Digestion was carried out for six hours. Boiling was continued for a further 30 minutes, after which the solution were allowered to cool. The content of the flask was transferred into 1000ml volumetric flask and the volume made up to 100ml mark with distilled water.

9.2 Distillation: The contents of the flasks were transferred into the distillation apparatus. A recieving flask was set up containing approximately 25ml of boric acid and 4 drops of screened methyl red. The digest was made alkaline by addition of 40% sodium hydroxide solution. The mixture was distilled for 40 minutes. Care was taken

to ensure that ammonia did not escape into the atmosphere. The ammonia was boiled over into the recieving flask.

9.3 Titration: The distillate was titrated with 0.IN sulphuric acid. This was reported for all samples.

Calculations: The percentage crude protein was calculated as percentage Nitrogen (%N) x 6.25. The (%N) was calculated as follows: $NH_3 + H_2S0_4 \rightarrow NH_4SO_3$

= 1ml of H_2S0_4 = 1ml of 0.02m Nitrogen (N)

= 0.00028g N.

$$\% \text{ crude protein} = \frac{M \times 0.014 \times D \times T \times 6.25 \times 100}{W \times L}$$

Where:

M	=	Molarity of acid.
0.014	=	Nitrogen content per 100ml
D	=	Dilution factor
6.25	=	Protein conversion factor (1mg nitrogen = 6.25 Protein)
W	=	Weight of sample powder
L	=	Volume of liquor distillate

10.0 DETERMINATION OF CALORIFIC VALUE.

Conversion factors as described by Winberg (1971) were used to derive calorific values as follows

a. % cabohydrate was multiplied by 4.0 K cal/100grams

b %Lipid (Fat) was multipliedd by 9.0 K cal/100grams

c %Protein was multipliedd by 4.0 K cal/100grams

All the three (a,b and c) were added to give calorific value for each sample.

Appendix 2

1.0 DETERMINATION OF AMINO ACID PROFILE

The amino acid profile of the seeds samples were determined using methods described by spackman *et al.*(1958) with the help of the chief technologist P.G. Laboratory University of Jos. Mr. Ojobe, T.O. The samples were dried to constant weights, defatted, hydrolysed, evaporated in a rotary evaporator and then loaded into the Technicon sequential multisample Amino Acid Analyser (TSM).

2.0 DETERMINATION OF DRY WEIGHT

Three filter papers (No.1- type) were accurately weighed on a chemical balance. 1.5g of sample was added to the weighed filter paper. The sample was dried in the rotary evaporator. The weight of the dried sample and the filter paper were noted. The difference between the weight of the filter paper with sample and that of the filter paper only gave the weight of the dry sample which was used for the analysis.

3.0 DEFATTINGOF THE SAMPLES

1.5 grams of seed samples were weighed into extraction thimble and the fat was extracted with chloroform/methanol mixture using soxhlet extraction apparatus as described by A.O.A.C (1980). The extraction lasted for 8 hrs.

4.0 HYDROLYSIS OF THE SAMPLES

0.5g of each defatted sample was transferred into a glass ampoule 7ml of 6NHCL was added and oxygen was expelled by passing Nitrogen into the amploule.

(This is to avoid possible oxidation of some amino acids during hydrolysis). The glass ampioule was then sealed with bursen burner flame and put in an oven preset at 105°C ± 5°C for 22 hours. The ampoule was allowed to cool before being broken at the tip to release the content which was filtered using a Whatman filter paper No 1.

5.0 EVAPORATION OF THE SAMPLE FILTERATES

The filterate was transferred into 250ml round bottom flask of the rotary evaporator where it was evaporated to dryness at 40°C under suction. The residue was subsequently washed twice with 10ml of de-ionized water and evaporated to dryness each time. The residue was picked up in 3ml of 0.0IN HCL and preserved in a refregirator at - 6±4°C for loading into an amino acid analyser when required.

6.0 LOADING OF SAMPLES INTOECHNICON-SEQUENTIAL MULTISAMPLE AMINO ACID ANALYSER (TSM)

1ml of the hydrolysed samples and the standard mixture were loaded with equal volume of internal standard solution (Norleucine) using a micropipette. 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 TSM sampler. The front coloumn was used for separation of basic amino and the rear column for separation of the acidic and nentral amino acids.

Buffer solutions were connected to the systems via peristatic valve.

7.0 PREPARATION OF BUFFERS

(a) Buffer No.1 (PH 3.25,0.25N Na^+/0.lm citrate/ 6% methyl (cellosolve). 400ml of stock solution was measured and pured into a 4 litre beaker equiped with

stirrer. About 3 litres of distilled water was added and agitated. 240ml methyl cellosolve and 1.0ml thiodiglycol were added after which 6N hydrochloric acid was used to titrate the solution to PH.3.25. It was then transferred to a volumetric flask and made up to total volume of 4 litres.

(b) Buffer No 2. (PH.5.25, 0.37 N Na^+/0.ml. This was prepared as buffer I except that methl cellosolve was omitted and that it was titrated to PH.4.25.

(c) Buffer No 3 (PH.5.25, 0.37 N Na^+/0.1ml citrate). This buffer was also prepared in the same way as the second one except that 40.0g sodium chloride was added. When the analysis was started, the buffers were delivered to the columns through the sampler from two high pressure pumps and selected by the buffer-changing valve (the valve sequences, the buffers required in the steps wise elution of amino acids and sequences, the flow of effluent from either column into the analytical system or to waste).

The buffer washed the column, 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 the bubble system, into the analytical system. Here they were mixed with hydrin solution supplied through the proportioning pump. The reaction mixture now formed passed through capillary tube immersed in a 100°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 continuosly as it passed over a sensitive photoelectric unit. The sighnals 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.

8.0 METHOD OF CALCULATING AMINO ACID VALUE FROM THE CHROMATOGRAM PEAKS

The net height of individual peaks were measured. The half-height of the peak on the chart was located and the width of the peak at the half-height was accurately measured and recorded. The approximation of the area of each peak was 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 formular.

NE = Area of norleucine peak

Area of each amino acid.

From the norleucine equivalent above, a constant S was calculated for each amino acid in the standard mixture.

S Standard = NE standard x Mol. weight x NM AA Standard

APPENDIX 3

1.0 DETERMINATION OF MINERAL ELEMENTS

The mineral elements were analysed using the Atomic Absorption Spectrophotometer (AAS) Perkin - Elmer Model 2903, the Flame photometer *Carning-Eel model and Spectronic 20* respectively (A.O.A.C, 1980) with the help of the Chief Technologist Institute of Agricultural Research, Mr. L. Lawal Ahmadu Bello University - Zaria. Both calorimetry and absorption spectrophotometry were based on Beer - Lambert's law.

$$\underline{LOg^{10}} = abc$$

$$It$$

Where 10 = Intensity of incident light

It = Intensity of transmitted light

a = Molar absorptivity

b = Concentration

c = Thickness of medium

The ASS was used for the analysis of calcium, copper, Iron, magnesium and zinc.

Flame photometer was used for sodium and potassium, while spectronic 20 was used for phosphorous.

2.0 DETERMINATION OF CALCIUM, IRON, MAGNESIUM, COPPER AND ZINC

250mg of each sample powder was weighed into a procelain dish. The sample was heated carefully over a burnsen burner with the crucible cover until the smoke had

ceased. Then the cover was removed and the content roasted in a muffle furnace for 8 hours at 500°C. After ashing, the crucible was allowed to cool in desicator. The Ash was extracted with 20% analytical grade hydrochloric acid. The solution was made up to 50ml with more 20% hydrochlaric acid.

3.0 DILUTION OF STOCK SOLUTION

The standard solutions of sodium, calcium, magnesium, Iron, copper and zinc were used for the analysis. Each standard solution contained 1000 ppm of each element. The stock solutions were diluted to give 2, 4, 6, 8 and 10 ppm of each element except magnesium. For magnesium the solutions were further diluted to give 0.2, 0.4, 0.6, 0.8, and 1.0 ppm due to the sensitivity of magnesium lamp.

Procedure

0.2ml of each stock solutions was taken in a graduated pipette and made up to 100ml with distilled water in a volumetric flask. This dilution gave 2 ppm. Other dilutions were made as follows.

0.4ml made up to 100ml gave 4ppm

0.6ml made up to 100ml gave 6ppm

0.8ml made up to 100ml gave 8ppm

1.0ml made up to 100ml gave 10ppm

472295

QK
898.P8

Magnesium A further dilution was made as follows 5.00ml of solution containing 2ppm was made up to 50ml with distilled water to obtain 0.2 ppm.

5.00ml of solution containing 4 ppm was mayle up to 50ml with distilled water to obtain 0.4 ppm. Similarly 5.00ml of 6 pp m ade up to 50ml gave 0.6ppm. 500ml of 8 ppm made up to 50ml gave 0.8ppm. 5.00ml of 10ppm made up to 50ml gave 1.0ppm.

Phosphrous

1ml stock phosphorus contained 0.05mg phosphorous 0,1,2,3,4, and 5ml of the stock phosphorous solution were pipetted into six 50ml volumetric flasks. The flasks were made up to mark with distilled water. The solutions contained 0,5,100,150,200 and 250mg phosphorous.

Phosphorous stock solution

0.219g of potassium dihydrogen phosphate KH2PO4) was weighed and dissolved in 700ml distilled water, 25 grams of ammonium - meta venadate was weighed and dissolved in 500ml hot distilled water (600C). The entire ammonium molybdate solution was mixed with the ammonium - meta - venadate solution, the resulting solution was added to the potassium dihydrogen phosphate in a two litre volumetric flask. 700ml perchloric acid was added to the two volumetric flask content. The solution was allowed to cool down. The complex mixture was made up to two litre with distilled water. This stock solution contained 1000ppm phosphorous. 1 OMS

Instrumentation

The atomic absorption spectrophotometer was put on for 30 minutes before the Acetylene gas knob was opened. The gas was ignited and the standard solutions were introduced at the aspirator. The optical density was ready at the wave length of each element.

If linear equipment was confirmed to be working properly. The sample was introduced and the concentration was read at the galvanometer against the distilled water.

This was used for the determination of calcium, copper, Iron, and magnesium.

Spectronic 20 was put on for 30 minutes. Baton solution was added to the diluted phosphorus solution. The mixture was left for about 15 mintes for colour

development. The colour was then read at 385nm wavelength. The phosphorus content was calculated as:

$$\text{Mg/100 phosphorus} = \frac{\text{Mg P (from graph Dilution factor x 100}}{\text{1,000,000 x wt of sample.}}$$

Sodium and potassium

The photometer was used. The stock solutions were used to set the sensitivity control of the flame photometer to obtain a scale reading of just under 100 divisions.

Distilled water was used to obtain zero reading. A calibration curve of concentration against scale reading was prepared.

Reading using the sample solutions were taken and concentration were read off from the calibration curves.

Statistical Analysis

At the end of the experiment all data were subjected to two-way analysis of variance (ANOVA) and the F-value (ratio) was determined for the seeds. The 2 - tail test used was given by pearson product moment correlation co-efficient (r). The maximum error acceptable was 5 percent ($P<0.05$)

APPENDIX 4A.

a. Calculation of Parkia and Sesamum protein level at 20%.

Parkia and Beniseed were mixed in equal proportions 50:50 to provide 20% protein.

From analysis fermented parkia contains 46.0 grams. while white Sesamum contains 47grams of proteins.

So 100 grams of parkia/beniseed ⟹ 46.0g protein

∴ 20 grams of protein will be obtained from:

$$\frac{100 \times 20}{46} = \frac{2000}{46} = 43.48 \text{ grams}$$

43.48 grams of parkia/Beniseed gives 20% of protein so 21.74 grams of parkia and 21.74 grams of Beniseed were incoporated to give 20% protein.

b. Fermented Delonix regia seed contein 63.31 grams of protein.

100grams of Delonix gives 63.31 grams protein. 20grams protein

∴ 20 grams of protein will be obtained from

$$\frac{100 \times 20}{63.31} = 31.57 \text{grames of Delonix}$$

Therefore 31.57 grams delonix regia were incorated into the diet to give 20% protein require by the mice.

APPENDIX 4B

A. Weight gian/loss is given by weight of animal at the end of the experiment minus (-) weight of animal before the experiment.

Weight gain of the control diet =

Final weight	39.64
Initial weight	8.84
Weight gain =	30.80 grams

Weight gain of the experiment diet

Final weight	82.42
Initial weight	7.64
Weight gain	74.78 grams

B. Feed convertion Ratio (FCR)

$$\frac{\text{Total feed consumed}}{\text{Weight gain}} = \frac{34.28}{5.178}$$

Control Diet = 6.62

experimental diet (FCR)

$$\frac{\text{Total feed consumed}}{\text{Mean weight gain}} = \frac{39.64}{10.303}$$

FCR = 3.85

C Protein efficiency ratio (PER)

$$\frac{\text{Mean weight gain}}{\text{protein consumed}} = \frac{20}{100} \text{ of } 34.28$$

$$= 6.856 \text{ grams of protein}$$

$$\text{Therefore} \quad \text{PER} = \frac{4.285}{6.856} = 0.625$$

PER for control diet = 0.625

For experimental diet:

$$\text{protein consumed} = \frac{20}{100} \times 41.42$$

$$\text{Therefore, PER for experimental DIET} = \frac{10.303}{8.284}$$

$$= 1.24$$

APPENDIX 4C

COMPOSITION OF PFIZER AGRICARE - MIXED 0.25% GROWERS

Vitamins: Premix.

Vitamin	A.	-	Retional (Anti - Xerophthalmic
Vitamin	D_3	-	Calcopherols
Vitamin	E	-	Tocopherols
Vitamin	K	-	Phylloquinmie (Antihnemorrhagic)
Vitamin	B_{12}	-	Cobalamin
Vtamin B_2		-	Riboflavin

Panthothetic Acid

Nicotinic Acid - Anti pellagric

Chlorine Chloride

Folic Acid - Folacin

Minerals premix

Phosphorus

Calcium

Iodine

Copper

Manganese

Zinc

Iron

Selenium - With vitamin E ensures fertility

Coxistac	-	protent lonophore, anti-Coccidial for effective control of Conccidiosis, optimum feed effeciency.
Terymycin	-	Broad spectrum feed antibiotic and growth promotant protection against dieases.
Anti-Caking Agent	-	To prevent Hardening
Anti-Oxidant	-	To prevent Oxidation

APPENDIX 5A

Mean of the weekly feed consumption of the control and fermented Delonix diet (grams)

Week	Control Diet	Fermented Delonix diet
1	3.41	5.08
2	4.90	4.95
3	4.88	5.24
4	4.71	6.26
5	5.00	5.88
6	4.48	5.30
7	3.39	5.62
8	3.43	3.65

X	4.285	0.5185
S^2	0.51803	0.58223
S.E	0.437	

Control VS experimental diet

$$t = \frac{X_1 - X_2}{S/\sqrt{1/n_1 + 1/n_2}} \qquad \frac{4.285 - 5.185}{S/\sqrt{(1/8 + 1/8)}} = 0.9$$

$$t = 1.6971$$

$$t = \frac{4.285 - 5.185}{1.5457\sqrt{(1/8 + 1/8)}} = \frac{0.9}{24.7312}$$

(0.05, df=14) t = 0.0364. tcal > ttab. S*

APPENDIX 5B

Mean of mean weekly weight change of the control and fermented Delonix diet

Week	Control diet	Fermented Delonix diet
1	1.78	7.19
2	3.86	6.22
3	4.05	8.60
4	4.71	9.80
5	5.71	10.54
	[illegible]	11.99
7	6.11	12.66
8	8.33	15.43
$\bar{X}$	4.955	10.3025
S^2	1.884	3.0313
S.E	0.6661	1.0712

Control VS experimental diet

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S\sqrt{1/n1+1/n2}} \qquad \frac{4.955 - 10.3025}{4.915\sqrt{1/8 + 1/8)} = 9.9$$

$$t = \frac{4.955 - 10.3025}{1.5457\sqrt{1/8+1/8)} = \underline{5.34}$$

t cal (df=13) = 1.8384
t tab (0.05,df14) = 2.145
t cal t tab therefore NS but t tab (0.01,df14)
= 1.761 S.

APPENDIX 6A

Mean daily consumption of the control diet (grams)

DAYS	WEEK							
	1	2	3	4	5	6	7	8
1	-	7.04	4.40	5.04	5.09	4.33	5.00	3.03
2	-	4.43	4.48	4.45	4.65	4.25	2.60	3.40
3	-	3.05	4.64	4.95	4.90	3.55	2.80	2.93
4	-	4.55	5.83	4.71	5.50	4.26	3.00	3.58
5	-	5.52	4.49	4.98	6.01	5.06	3.09	3.35
6	5.43	4.46	5.46	4.64	6.12	4.90	3.98	3.93
7	1.55	5.25	-	4.21	2.69	5.00	3.28	3.80
S	6.98	34.29	29.30	32.98	34.96	31.35	23.75	24.02
X	3.49	4.90	4.88	4.71	5.00	4.48	3.39	3.43

APPENDIX 6B

Mean daily consumption of the fermented Delonix diet (grams)

DAYS	WEEK							
	1	2	3	4	5	6	7	8
1	-	4.95	5.04	6.08	7.19	4.88	6.33	4.64
2	-	5.62	3.93	4.80	5.83	5.66	5.34	4.33
3	-	5.17	5.46	6.86	6.18	5.38	5.88	4.33
4	-	3.99	4.99	6.15	5.38	4.73	5.00	3.88
5	-	3.75	5.73	6.76	5.56	5.15	7.10	3.93
6	5.60	3.00	6.09	6.90	5.44	5.50	5.40	4.50
7	4.55	4.67	-	6.48	5.38	5.78	4.28	3.80
S	10.15	31.15	31.44	44.06	40.96	37.08	39.33	25.53
X	5.08	4.45	5.24	6.29	5.85	5.30	5.62	3.65