REPLACEMENT VALUE OF JACKBEAN MEAL FOR SOYBEAN MEAL IN NILE TILAPIA (*Oreochromis niloticus*) DIETS

BY

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CERTIFICATION

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DEDICATION

This thesis is humbly dedicated to Allah and to those who show mercy to their fellow men, animals and plants.

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ABSTRACT

Replacement value of jackbean meal for soybean meal as a dietary component for Tilapia, *Oreochromis niloticus* was evaluated. Four isonitrogenous and isocaloric (30% crude protein) diets were fed to *Oreochromis niloticus* fingerlings (6.36 ± 0.07g) in a glass tank for 70days. The soybean meal in the diet was replaced at the rate of 20, 30 and 40% to serve as test-diet D20, D30 and D40 respectively while a diet without jackbean meal served as control (CTR). There were no significant differences ($P > 0.05$) in specific growth rate, weight gain, % weight gain and protein efficiency ratio between the fish fed control diet and diet D20 . Diet D40 differed significantly ($P < 0.05$) from diet CTR, D20 and D30. However, there was no significant difference ($P > 0.05$) in the protein efficiency between the fish fed control diet and test diets. Significant ($P < 0.05$) variation occurred in nutrient (Protein) digestibility of fish fed control diets and test diets. However, there were no significant differences in lipid and organic matter digestibility of fish fed control diets and test diets. The profitability of feeding fish with the test diet was positive (as attested to by profit index). The incidence costs of producing 1kg of fish with the control diet and test diets D20, D30 and D40 showed that it was cheaper to produce 1kg of fish with diets D20 and D30. The results showed that it is technically feasible, biologically and economically effective to replace soybean meal up to 30% with jackbean in the practical diets of *Oreochromis niloticus*.

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CHAPTER ONE

INTRODUCTION

Pulses or legumes belong to the family Leguminosae which contains several members that are second in abundance to the Gramineae (the cereals). They are important and economic sources of protein, energy and other nutrients in the diets of most of the developing countries (Igene, 1999). Grain legumes are cheapest and readily available protein source and provide better nutrition in sufficient quantity as an alternative to poor availability of animal protein in most of the developing countries (Nigeria inclusive) whose reliance is placed on cereal grains, starchy root and tuber for their energy and protein needs (Aletor and Aladetimi, 1989). Most legumes contain 20 – 50% crude protein with a variation attributed to difference in species, varieties, geographical location and soil condition. The protein has been reported to contain high level of lysine but low level of methionine which can be compensated when combined with cereal protein which contains appreciable levels of the sulphur containing amino and to produce a mixture of enhanced value (Apata, 1990). Furthermore, grain legumes contain significant amount of phosphorus and other essential minerals needed for proper growth and development of the body. Except oilegenous seeds such as groundnut, soybean, etc. Legumes have low oil content and contain in most cases more than 50% carbohydrate mainly starch which is easily absorbed and metabolized; other carbohydrate, are in the form of dietary fibre (Igene, 1999).

Soybean products and other legumes form the bedrock of not only the fish feed industries but poultry and livestock industries (Apata, 1990). However, there

is currently high level of competition between man and livestock for available grain legumes with the result that only the lefts over of the conventional legumes are available for animal feeding. In Nigeria, grain legumes consumption is in various forms. They are used in soups, snacks, boiled dishes and constitute the base material in local recipes like steam pudding (moin-moin) and baked beans (Akara). The resultant remnant cannot but be scarce and expensive (Igene, 1999).

Soybean products have high protein contents and the best protein quality among plant protein feedstuffs used in fish feed (Davies *et al* 1999). It has been reported to replace fishmeal (partially or totally) in the diets of many aquaculture species (Lovell 1988, Lim and Akinyama, 1992). However, Fasakin *et al* (1999) reported that most conventional protein feedstuff used in fish feed formulation in developing countries such as fishmeal, soybean meal and groundnut cake are scarce, competitive and their cost are usually beyond the reach of fish farmers and producers of aquafeed. This phenomenon according to Balogun (1988) has hindered the expansion and profitability of aquaculture enterprise in many developing countries and has to encourage the need to look for cheaper alternative protein source for the development of low-cost feed that can replace this conventional feedstuffs without reducing the nutritional quality of the diets. In order to meet the wide gap between supply and demand of conventional grain legumes in Nigeria and other developing countries, there has to be (1) the need to increase the production of the edible legume and (2) to redirect attention on the neglected or unexploited legumes which are rarely consumed by man with suitable processing to an appreciable extent for fish consumption. Legume in this group can be called lesser-

known legumes (Aletor and Aladesinmi, 1989) and include among others pigeon pea (*Cajanus cajan*), the Jackbean (*Canavalia spp*), lima beans (*Phaseolus lunatus*) and winged bean (*Psophocarpus tetragonololobus*).

Jackbean (*Canavalia ensiformis*), a lesser known edible and indigenous legume, has shown potential as energy source and protein source for livestock because of its high yields and protein contents (Esonu *et al*, 1999). Under optimal agronomic management conditions, dry seed yield of up to 2.5 tons/ha have been reported in Zimbabwe (Addison, 1957), Mexico (Herrera *et al*, 1981) and Northern Nigeria (Okonkwo and Udedibie, 1991). The seeds are white, relatively large, about 2.3 cm in diameter and contain up to 30% crude protein (Esonu *et al*, 1996) and 60% carbohydrate (Udedibie, 1990). Although it is low in methionine and tryptophan (like most proteins of plant origin), it is relatively high in lysine (Bressani *et al*, 1987, Ranjaram and Janardhanam, 1992). The principal factors which determine the nutritive value of legume protein are

(i) The protein content

(ii) The essential amino acid composition of the protein.

(iii) The availability of the amino acid for tissue metabolism.

Jackbean has comparable nutrient density as the conventional grain legumes. It has been reported to have similar amino acid profile with other legumes (Ologhobo, 1992). As with other tropical legumes, raw; unprocessed seed of the jackbean contain toxic substances which limits its use as human food or animal feed (Udedibie, 1990, Nakatsu *et al*, 1996), the most important being trypsin inhibitors and Concanavalin A (Con. A). It appears therefore that for the *Canavalia* seeds to

be rendered acceptable as fish feeds, method(s) or suitable processing techniques must be devised that can completely and economically inactivate the endogenous trypsin inhibitor and thermostable Con. A.

Various methods have been used to inactivate the endogenous trypsin inhibitors and Con.A. These include heat treatment alone (Udedibie and Nwaiwu, 1988, Kesler *et al*, 1990, Udedibie and Madubuike 1988, Udedibie and Nkwocha 1990), treatment with urea followed by heating (Montilla *et al*, 1981, Udedibie and Nwocha 1990, Udedibie and *et al*, 1994). It was concluded that heat treatment done is insufficient to allow C. *ensiformis* to be incorporated in commercial diets and produce satisfactory growth rate. It was reported by Udedibie and Carlini 1998a that trypsin inhibitors are of little nutritional consequence, if the seed is properly and adequately heated and complete inactivation of con.A in the seed can be easily achieved if the seed is broken into pieces (3 – 6 parts) and cooked for 1hour or pressure cooked for 15 mins (Udedibie and Carlini, 1998b).

Nile Tilapia (*Oreochromis niloticus*) is a suitable fish for culture because it has rapid growth rate, high tolerance to low water quality, efficient food conversion, ease of spawning, resistance to diseases and have a good consumer acceptance.

JUSTIFICATION

Greater emphasis is placed on research into use of alternative feedstuff in livestock production, due to the ever-increasing prices of conventional legume grains such as soybean in Nigeria. The high cost of soybean and other conventional legumes is due to their use in other animal feeds and infant formulae, hence competition is also on the increase. Jackbean is widely available and have high

nutrient value and good agronomic characteristics and is underutilized. Because of its large yield and the protein content, there is justification for it replacing soybean meal in fish diet as this will reduce over dependence on the well-known legume seeds such as groundnut cake and soybean meal as protein sources and may eventually result to a decrease in the cost of fish feeding.

OBJECTIVES

The general objective is to evaluate the effect of replacing soybean meal with processed jackbean meal in the diet of *Oreochromis niloticus* using growth performance and nutrient utilization of Nile tilapia as indices. The specific objectives are:

(1) to characterize cracked and cooked (processed) jackbean seed with respect to the proximate composition.

(2) to determine the digestibility of the processed jackbean meal in the diet of *Oreochromis niloticus*.

(3) to carry out the incidence of cost of feeding processed jackbean meal based diets to tilapia.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 JACKBEAN (*Canavalia ensiformis*)

The genus *Canavalia* comprises of a small group of some 48 species, which are distributed throughout tropics among which is *Canavalia ensiformis* commonly called jackbeans. These are rigorous, exceptionally productive large seeded tropical lesser-known legumes (Udedibie, 1990).

Canavalia ensiformis has a potential as economic crop in view of its excellent agronomic characteristic (Udedibie and Nwaiwu, 1988) requires no staking if planted on open land. It can grow continuously and regenerates under severe soil and harsh climatic conditions. The excellent germinating and rigorous initial growth make the establishment of the jackbean relatively easy. The crude protein content of the ripe seed ranges from 26 to 31% but the proteins are relatively low in methionine and tryptophan (Bressani *et al.* 1987).

2.2 NUTRITIVE VALUE OF JACKBEAN

The crop has significant potential on the far east countries diet because the young green and pod are cooked and eaten as vegetables (Purseglove, 1982). The plant itself, being a legume, is valuable for restoring soil fertility throughout its growth, it sheds copious leaves which decay rapidly and enrich the soil. It is used as high protein food and forage crops for many countries by native of South Western United State, Mexico, Central America countries, Brazil and West Indies (Sauer and Kaplan, 1969).It is used in Nigeria as an ornamental plant grown near houses and allowed to trail on walls and trees (Udedibie, 1990). It has been a useful specie in tropical soil

reclamation efforts because of its deeply penetrating root system, its nitrogen fixation capabilities and its tolerance of a wide range of soil acidity and salinity conditions (Echo 1999). In Indonesia, steamed flowers and leaves are used as flavouring. Jackbean seeds sometimes are roasted and used as a coffee substitute. It is also reported (Akpapunam and Sefa-Dedeh, 1997) that properly processed *C. ensiformis* may be used as an alternative in the preparation of dishes made with cowpea, peanut, pigeon pea and soybean. It was further reported that industrial products such as protein concentrates and isolates, starch, flakes, grits and flour can be produced from the bean.

2.3 CULTIVATION OF JACKBEAN

Jackbean is grown from seed. If grown as a green manure, the seeds usually are broadcast; if grown as a bush variety pulse or food crop, the seeds may be planted 2.5 – 5cm apart, in rows 60 – 90cm apart. Twining varieties require support for best yield. Jackbean thrives on warm, humid environment with a long growing season like that found in the lowland tropics. Jackbean has been grown, however at elevations up to 1800m. The plant thrives in full sunlight but tolerates somewhat shady environment. Jackbean tolerate acid soil conditions ranging from pH 5 to pH 6. They also tolerate waterlogged soil or high salinity soil better than most other pulse species. (Echo 1999)

Green immature pods may be harvested 90 – 120 days after planting. Mature seeds may be harvested 180 – 300 days after planting. The seeds should be harvested by hand to prevent shelling (Echo, 1999).

Jackbean is relatively free of pests and diseases. The thick coat resist seed pest strongly. Root rot by the fungus *Colletotrictium lindemuthianum* has been a problem. Stem-borers and leaf eating beetles pose problems in early stage of growth. Moth caterpillars (*Laphygma sp.)* and pod weevils (*Sternechus sp.)* are reported as major pest on jackbean in Brazil (Echo 1999).

2.4 NUTRIENT COMPOSITION OF JACKBEAN

2.4.1 Protein and Amino Acid Composition

Raw jackbean contained 28.5% crude protein as reported by Udedibie (1990). This value agreed very closely with earlier values of 26% reported by Borchers and Ackerson (1950), 30% by Molina *et al*, (1974). The crude protein value of the heat-treated sample dropped to 25% an indication that during the cooking or autoclaving some nitrogenous substances in the raw jackbean were solubilised and removed (Udedibie 1990).

The amino acid composition of jackbean reported by Ologhobo (1992), Bressani *et al*, (1987), D'Mello *et al* (1985), Wyss and Bicjel (1988) revealed jackbean to be deficient nutritionally primarily in methionine and tryptophan. However it is rich in lysine (Bressani *et al*, 1987, Ranjaram and Janardhanam 1992). Furthermore, Ologhobo (1992) made comparison between amino acid composition of some legumes including jackbean, the results revealed similarity in amino acid composition of jackbeans to that of other legumes.

It is however to be noted that protein and amino acid composition of various legumes varies with the species, varieties, geographical source degree of maturity of

the seeds, genetic factors, soil fertility especially sulphur fertilization and also the condition of handling after the harvest; processing and storage procedures.

2.4.2 Carbohydrate

Most dry grain legumes contain between 68 and 78g/100g of total carbohydrate fraction in the form of fibre and are present in less available forms (Igene, 1999). Olomu (1995) explained that the carbohydrate fraction of legumes are made of essentially readily digestible starch and sucrose, the content being between 11.70% - 60.00%.Analysis of the raw jackbean revealed the content of carbohydrate to be 60% (Udedibie, 1990). Echo (1999) reported carbohydrate contents of jackbean to be between 45 – 57%. Akpapunam and Sefa-Dedeh (1997) reported carbohydrate content of 55% to jackbean.

2.4.3 Crude Fibre

The dietary fibre also known as "unavailable carbohydrate" has been defined by Trowell (1972) as the skeletal remains of plant cells that are resistant to hydrolysis by the enzymes of man. It includes cellulose, hemicellulose, pectin and lignin. Crude fibre is a component of the dietary fibre as validated by Paul and Smithgate (1978). Karmath and Belavady (1980) reported the total dietary fibre of legume as 15.3 to 25% while Norton *et al* (1985) reported 11.7 to 25%. Analysis of the raw jackbean revealed the crude fibre content of 7.8 (Udedibie 1990), 7.4% (Fagbenro 1998 unpublished). The crude fibre value of cooked jackbean appeared quite high for a grain feed and thus was attributed to presence of thick and tough coat of the seed (Udedibie 1990).

2.4.4 Fats and Fatty acids

Legume seeds are low in oil with the exception of the oleaginous seed (e.g. soybean, winged bean and groundnut) the values are usually less than 3% (Apata 1990). Legume seeds fats have Oleic and linoleic fatty acid as the main unsaturated and palmitic acid as the saturated fatty acid. Fats from legume seeds, with low linoleic acid levels are of high nutrient value and are readily digestible (Apata 1990). Udedibie 1990 reported a value 3.1% for ether- extract of raw jackbean seed, *Canavalia* meal ether extract value of 2.9% was recorded by Fagbenro (1998, Unpublished).

2.4.5 Minerals

Legumes are relatively high in Calcium, Iron, Magnesium, Phosphorous and Potassium and low in Sodium. These values for 10 different kinds of legumes as reported by meiners *et al* (1976b) are as follow: Calcium 35 – 181 mg/100g, Magnesium 90- 200 mg/100, Phosphorous 348 to 522 mg/100g, Iron 2.2 to 9.56 mg/100g and Sodium 1.66 to 12.69 mg/100g. Oke (1967) reported values of 0.05 - 0.3%, 0.308 – 0.944% and 18 to 69 ppm respectively for Calcium, Phosphorous and Iron for some Nigerian pulses. Udedibie 1990 reported the value of some mineral contents of raw jackbean seed, a value of 0.08% Calcium, 0.62% phosphorous, 0.09% Magnesium and 0.06% Manganese way recorded. Arkroyd *et al* (1982) reported that legume grains contain reasonable amount of Calcium and Iron but that Calcium content varies with variety climate, cultural method and mineral content of the soil.

2.4.6 Vitamins

Legume seeds are good sources of thiamine and niacin. They are generally rich in the B- vitamins but do not contain any vitamin C. (Olomu 1995), although vitamin C is formed during germination. Apart from germinated seeds, small amount of it are in some species, but the vitamins get oxidized with long storage. Prolong cooking/heating and the habit of bulk – cooking have been reported also to be implicated in removing any vitamin C they contain (Apata 1990).

2.5 TOXIC AND ANTINUTRITIONAL FACTORS IN JACKBEAN

There is no doubt from the discussion above that legumes are good sources of nutrients for fish nutrition. Those nutrients can be used for growth, repairs and replacement of old and worn out cell tissue and organs and for the provision of energy. The use of legume, to a very large extent, is however, limited by the presence of toxic and antinutritional factors. In reviewing the toxic and antinutritional factor in the jackbean or any other potential feedstuff, it is only the toxicity associated with oral ingestion of the factor that has any nutritional significance. This is so because a factor, which may be toxic, if injected into the body may not be toxic if orally administered (Udedibie and Carlini, 1998a). Jackbean contains toxic and antinutritional factors, which limits it use as human or animal feeds among which are trypsin inhibitors, canavanine specialized secondary biomolecules, urease activity canatoxin and ancanavalin A.

2.5.1 Trypsin Inhibitors

Trypsin and chemotrypsin inhibitors have been implicated in reducing protein digestibility and in pancreatic hypertrophy (Liener, 1976). They are

polypeptides which form well-characterized stable complexes with trypsin and chemotrypsin on a one to one molar ratio obstructing their binding sites and disrupting their enzymatic action. Protease Inhibitors are widely distributed in plants, particularly legumes.

The existence of trypsin inhibitors in the raw jackbean seed was first reported by Orru and Demel (1941) and confirmed by Borchers and Ackerson (1950). However, the unique effect of heat in improving the nutritive value of legume seeds, first demonstrated by Osborne and Mendel, 1917 has since been demonstrated in the jackbean by Borchers and Ackerson (1950) who also observed that trypsin inhibitors in the jackbean seed are heat labile. Babar *et al* (1988) have confirmed this by complete inactivation of trypsin inhibitors in the jackbean seed and meal following – 24 hours of soaking in water prior to cooking for just 20 min. Dry heat is however not effective as moist heat for destruction of antitryptic activities of the seed (Babar *et al* 1988, Bressani and Sosa, 1996; Carlini and Udedibie, 1997).

Trypsin Inhibitors are therefore of little antinutritional consequence, if the seed is properly and adequately heated.

2.5.2 Canavanine

The alkaline toxic amino acid, canavanine (2-amino, 4-guanidinooxy – butyric acid), a naturally occurring structural analogue of L – arginine was originally isolated from jackbean by Kitagawa and Tomiyawa, (1929). It is however important to observe that canavanine has also been reported to be present in over 500 species of the Leguminosae (Bell 1978) canavaniae constitutes about $50gKg^{-1}$

of the dry weight of dry jackbean (Rosenthal, 1972). Swaffer *et al* 1994 observed that canavanine is active as an arginine antagonist only in the condition of low arginine concentration. A similar observation has also been made by Horowitz and Sob (1948) and recently, Enneking *et al* (1993) and Michelangeli and Vargas (1994) also demonstrated that canavanine reduces feed intake of non-ruminants but this is observable only at the equivalent of about 300g/kg dietary level of raw jackbean. Udedibie and Carlini (1998a) observed that nutritionally speaking, 300g/kg dietary level of any raw legume grains are high in toxic and antinutritional factors. Furthermore the effects of feeding pure canavanine as was done by Enneking *et al* (1993) and Michelangeli and Vargas (1994) may be different from its effect as component of a feedstuff because of differences in release rate in the gut. Canavanine in raw jackbean has also been reported to rapidly disappear during germination of the seed (Bell, 1960 Rosenthal, 1970; Nakatsu *et al* 1996), but recent trials in Nigeria (Esonu, 1996) have demonstrated that jackbean seed still exhibit strong toxic effects in chicks even after sprouting prior to heating in the oven.

Canavanine solubilises in water (Obizoba and Obiano 1988; D' Mello and Walker, 1991) and converts to non-toxic cyclic deaminocanavanine on heating (Rosenthal, 1977b). It therefore seems doubtful if canavanine contribution to toxicity of the seed is significant especially after the seed has been made to sprout and then heated or processed and included in a diet that is adequate in arginine. Arginine deficiency in balanced fish diet in rare.

2.5.3 Specialized Secondary Plant Biomolecules

The primary adaptive role of specialized secondary plant biomolecules is the repulsion of herbivores, either through direct toxicity or some indirect mechanism like distastefulness. Secondary compounds founds in seeds seems to be no exception to this generalization. The food specificity of insects has been reported to be based solely upon the presence or absence of the secondary plant biomolecules (Fraenkel, 1959).

Phytochemical studies in Nigeria and in Brazil (Udedibie and Nwaiwu, 1988; Oliveria 1997) have shown that raw unprocessed jackbean seeds contains saponins, cyanogenic glycosides, terpenoids, alkaloids and tannic acid. This may partially explain total freedom from insect attack which jackbean plants enjoy. But Janzen *et al* (1977) have observed that the effects of these compounds are dosage – dependent and their concentration in the seed have also been reported to drop below detectable level following one hour of cooking or 15 minutes autoclaving (Udedibie and Nwaiwu, 1988).

2.5.4 Urease

Urease, the first enzyme to be ever crystallized and shown to be a protein by Sumner (1926), is very active in the jackbean. Urease has been shown to be highly toxic when injected into rabbit (Tauber and Kliener 1931). But the ubiquity of Urease in virtually all plants particularly the family, Leguminosae and cucurbitaceae, has also been reported (Hogan *et al*, 1983). Crystalline Urease is, however, lacking in haemoglutinin activity (Sumner and Howell, 1935). It should be noted too that urease is abundantly produced in the rumen by the rumen microflora as a

very important component of nutritional ecology of the animal. It therefore cannot be considered as an anti nutritional factor.

2.5.5 Canatoxin

The isolation of another toxic but non-haemoglutinating protein (canatoxin) from jackbean was also reported by Carlini and Guimaraes (1981). Canatoxin induces convulsion preceding the death of experimental animals when administered by interperitoneal injection but exhibit no toxic effect if orally administered (Carlini and Guimaraes 1991). It is highly unstable in the low pH medium of the stomach.

Therefore, even though, canatoxin can be of some value(s) as a pharmacologically biochemical tool (Carlini and Guimaraes, 1991), it cannot be classified as an anti nutritional factor in the jackbean.

2.5.6 Concanavalin A (Con A)

Concanavalin A (Con A), the most studied of the plant lectins, first isolated and crystallized by Sumner and Howell (1936), seems to be the most nutritionally important toxic and antinutrional factor in the raw, unprocessed, jackbean seed. Lectins are carbohydrates-binding protein that are resistant to digestion when ingested (Putszai, 1989) and Con A represents about 20% of the protein of the seed (Dalkin and Bowles, 1983). In addition to its ability to aggluginate the erythrocytes of numerous animal species, clumb certain bacteria and precipitate glycogen and starch from solution, Con A negatively affects nutrients utilization by other mechanisms. it binds to the glycoproteins and glycolipids of the digestive tract mucosa (Hague 1975; Jaffe, 1980), inhibits the activity of the enzyme of the brush border of the enterocytes (Rosenthal, 1972) interfere with the adherence of

enterobacteria to the intestinal wall (Jayne-Williams, 1973) and possibly has several side effects on immune functions, protein metabolism, enzyme activities and hormonal regulation (Putszai, 1989). It induces severe reduction in feed intake of non-ruminants under ad libitum feeding regime (Liener, 1953; Leon *et al* 1991; Larue – Achagiotis *et al,* 1992) and has ever been implicated in the pathogenesis of coelias disease (Kolberg and Sollid 1985) Con A. ordinarily, is heat labile but somehow seems to be protected against heat destruction as a component of the seed *(Carlini and Udedibie 1997).*

From the foregoing, it would appear that of all toxic and antinutritional factors so far identified in the jackbean. Con A seems to be the one that has the strongest defiance for treatment. This property has continued to render the jackbean unacceptable as human and or animal feed. It seems to follow, therefore, that in order to render the jackbean acceptable as human food and or fish feed, economically feasible method(s) of eliminating it from the seed must be devised.

Table 2.1: The principal anti-nutritional contents of some tropical legumes seeds

Major group	Anti-nutritional components	Legume species
Proteins	Lectins	*Psophocarpus tetragonolobus* *Phaseolus lunatus* *Canavalia ensiformis* *Vigna unguiculata* *Lablab purpereus*
	Proteinase inhibitors	*Arachis hypogea* *Cajanus cajan* *Vigna unguiculata* *Phaseolus lunatus* *Psophocarpus tetragonolobus* *Cicer arietinum* *Lablab purpureus* *Cyamopsis tetragonoloba* *Canavalia ensiformis* *Lythyrus sativa* *Vicia sativa*
Non protein aminoacid	Nauroclathyrogens	*Vicia sativa* *Lythyrus sativa* *Lythyrus cicera*
	Canavanine	*Canavalia ensiformis* *Gliricidia sepium* *Robina pseudoacasia* *Indogotera spicata* *Sesbania sesban* *Vicia villosa*
	Mimosine	*Leucaena leucocephala*
Carbohydrate	Galactomannan gums	*Cyamopsis tetragoloba* *Leucaena leucocephala*
Polyphenolic compounds	Tannins	*Psophocarpus tetragonolobus* *Vigna unguiculata* *Leucaena leucocephala*
Glycosides	Cyanogens	*Phaseolus lunatus* *Vigna unguiculata* *Cajanus cajan* *Vicia sativa* *Lablab purpereus*
	Saponins	*Arachis hypogea* *Vigna radiata* *Cyamopsis tetragonoloba*
Alkaloids	Erythrina alkaloids	*Erythrina species*
Metal –binding agents	Phytic acid	*Psophocarpus tetragonolobus* *Vigna unguiculata* *Lablab purpereus*

D'Mello (1992)

2.6 JACKBEAN PROCESSING TECHNIQUES

Udedibie and Carlini (1998a) reported that jackbean has been processed in various ways by different investigators with conflicting results indicating no more than partial detoxification. The improvement in its nutritive value through toasting was first reported by Borchers and Ackerson (1956). However toasting could improve its nutritive value for broiler chicks only to the extent of 100g/kg dietary inclusion (Udidibie *et al,* 1994). Partial detoxification by heat treatment of the seed, mainly by cooking and autoclaving (D'Mello *et al* 1985, D'Mello *et al,* 1989, Udedibie and Madubuike 1988, Kessler *et al,* 1990, Udedibie and Walker 1991) and by extrusion cooking (Aguirre – Montoya, 1988; Brassani and Sosa 1990; Leon *et al* 1991; Melcion *et al* 1991) claimed complete elimination of haemoglutinating activity of the jackbean through extrusion cooking, feeding trials with the product indicated only partial detoxification since it still depressed growth of the experimental cockerels. Kesler *et al* 1990 concluded that autoclaving alone is insufficient to allow *C. ensiformis* to be incorporated in commercial diets and produce satisfactory growth rates.

Martinez – Palacious *et al* (1988) reported that *Canavalia* seed meal is potentially useful as a partial substitute for fish meal in tilapia diet. He however, concluded that caution must be exercised when using this protein source because of the presence of residues of non-thermolabile toxins (Con. A) in the meal. It is clear that the non thermolabile toxins such as the amino acid L – canavanine causes high mortality of fish. (Martinez – Palacious *et al,* 1988), D' Mello *et al,* 1985 reported

that it is abundantly clear that the autoclaving procedure offers inadequate protection against severe growth – retarding properties.

The best improvement in the nutritive value of the seed seems to have been obtained through soaking in urea solution for about a week prior to cooking for an hour (Montilla *et al,* 1981; Udedibie and Nkwocha, 1990) but the process is cumbersome. The efficacy of dry urea treatment was recently reported by Udedibie *et al* 1994, although, dry urea treatments appears very convenient feeding trials with the products therefrom show that it is not so good as the wet Urea treatment. Two-stage cooking, the method used in some villages in Nigeria in cooking some poisonous local feedstuffs, has also been applied to jackbean. This process, which involves removing the cooking water after one hour and continuing the cooking for another 30 - 40 minutes with fresh water, resulted in a product which at a level of dietary inclusion of 200g/kg produces toxic effects (Udedibie *et al,* 1996). The interpretation of the result of these totals have mostly been based on inference rather than on accurate laboratory analytical data. Recently, concluded series of analytical studies (Udedibie and Carlini, 1998) have shown that none of these methods mentioned above could completely eliminate Con A from the seed. Con A in the jackbean requires 3hours of cooking at 96^{0}C or soaking in water for 72 hours followed by I hour of ordinary cooking or 15 minutes of pressure cooking for complete inactivation (Udedibie and Carlini, 1998a). It can however be easily and completely inactivated if the seed is broken (3 – 7 parts per seed) and cooked for just 1 hour pressure cooked for 15 min. Udedibie and Carlini ,(1998b) concluded that in addition to the 2 promising moist heat processing methods briefly mentioned

(soaking in water for 72 hours, cracking prior to cooking for an hour), possibility may still exist for the development of better and more convenient methods of eliminating Con A from the seed.

2.7 THE NILE TILAPIA: *Oreochromis niloticus*

Oreochromis niloticus belongs to the family Cichlidae which contain at least 14 species with their colour varying slightly depending on species. It is the most common species grown in various ponds, it is robust, hardy and easy to handle. They breed easily in various fish ponds and are able to withstand high water temperature. They can easily be identified by the vertical brown stripes on its caudal fin which are absent in other tilapia. The tilapia spot which is always located at the base of the second half of the dorsal fin is very prominent. It is a native of Africa, hence found in almost every river and lake. The males grow faster than the females and reach a bigger size in ponds but growth rates reduces with age. (Bardach *et al.* 1972). Shiau and Hung, (1989) reported that tilapia has become predominantly cultured fish species in Taiwan. Tilapia *(Oreochromis niloticus)* is a suitable fish for culture because of simplicity of rearing, hardiness, versatility, undemanding feed requirement, firm flesh texture and neutral flavour (Fagbenro, 2002). All these characteristics make tilapia a suitable fish for culture.

Oreochromis niloticus do well in very enriched water. The dietary requirement of *Oreochromis niloticus* for growth, reproduction and other normal physiological function are similar to other warm water fish specie. They need to consume protein, minerals, vitamins and energy sources. *Oreochromis niloticus* adults are omnivorous but feed predominantly on phytoplankton and can utilize

blue-green algae, Juveniles consume a wider range of food items Jauncey and Ross (1982).

2.7.1 Digestive Enzymes in Tilapia

The length of the entire intestine of a tilapia is between 5 and 8 time the length of the fish (Caulton, 1976; Pauly, 1976; Ross and Jauncey, 1981). The intestine is differentiated into an anterior short thin walled duodenum and a very long posterior section which has a smaller diameter (Bowen, 1982).

Enzymes responsible for the digestion of lipids (lipases) carbohydrates (amylase) and proteins (protease) all occur in the pyloric ceca or intestina mucosa of fish (Phillip 1969). Digestion is a two step process with distinct gastric and intestinal component. The pH of the stomach fluid of an actively digesting tilapia is frequently as low as 1.25 (Moriarty, 1973; Bowen 1976; Cauton, 1976) and values as low as 1.0 have been recorded (Payne, 1978). The acid in the stomach decomposes chlorophyll and lyses blue-green algal cell wall, which makes subsequent intestinal digestion possible by allowing enzymes access to the algal cell contents (Moriarty, 1973).

In Tilapia, secretion of gastric acid stops at the end of the daily feeding period and the pH increases to the range of 5 to 7 (Bowen, 1932). In the intestine, carbohydrate is digested in the first and second quarter while protein digestion is completed in the first quarters. Amino acid absorption appears to continue throughout most of the intestine in Tilapia (Bowen 1980). Trypsin, chemotrypsin, amylase and esterace have been measured from the intestine of tilapia; *Oreochromis niloticus, O. mossambicus* (Fish, 1960; Nagase; 1964; Moriarty, 1973). In addition,

the intestinal pH of tilapia was found to be maintained by bile salt secreted from the bile duct (Fish, 1960; Nagase, 1964). Fish (1960), finds that a predominantly herbivorous tilapia have amylase activity distributed throughout the gastro intestinal tract. It should be noted that tilapia posses set of pharyngeal teeth that are used to break food particles prior to digestion (Bowen, 1962).

2.8 NUTRIENT REQUIREMENT OF TILAPIA

2.8.1 Quantitative and Qualitative Protein Requirement of Tilapia

The amount of protein required in the diet is directly influenced by the amino acid composition of the diet (NRC, 1983). Dietary protein is always considered to be of primary importance in fish feeds as the protein requirement of fish are higher than that of land animal and protein sources account for more than half of the total feed costs. The level of dietary protein producing maximum growth of fish depend upon, the energy content of the diet, the physiological state of the animal (age, reproductive state and environmental factors), the protein quality (i.e. its amino acid profile and availability) and the level of feed intake (Jauncey and Ross, 1982).

The optimum protein level for tilapias is between 25 and 40% (Jauncey and Ross, 1982). Luquet (1991) recommended optimum dietary protein requirement of *Oreochromis niloticus* to be between 30 and 35%. The qualitative requirements for tilapias have only been determined for *Tilapia zilli* (Mazid *et al*, 1978) which required the same ten essential amino acid as other fish. It is unlikely therefore that other tilapia species will differ in their qualitative requirements. These essential amino acid must be present in the feed at the levels required by the fish in order for protein synthesis to occur (Jauncey and Ross, 1982).

2.8.2 Energy Requirement

Generally fish have a lower dietary energy requirement because they do not have to maintain a constant body temperature and they exert relatively low energy in maintaining position and to move in water than do mammals and birds on land (Tucker, 1969; Lovell, 1984) because they excrete most of their nitrogenous waste as ammonia instead of urea or uric acid thus loss less energy in protein catabolism and excretion of nitrogen waste (Goldstein and Furster, 1970).

Providing the optimum energy level in diet for fish is important because an excess or deficiency of useful energy can result in reduced growth rates for energy needed for maintenance and voluntary activities must be satisfied before energy is available for growth (Halver, 1989). The design of practical feed is a compromise between a protein level that produces good growth with little conversion to energy and an energy level that gives high rates of protein synthesis but that does not result in undesirably high levels of carcass lipid. The three classes of compounds which provide dietary energy for fish are protein, lipid and carbohydrate. Stickney and Andrew (1970) and Garling and Wilson (1977) reported that tilapia utilize these effectively as energy sources.

2.8.3 Lipid Requirement

Dietary lipids are important source of energy and essential fatty acid for normal growth and survival of warm water fish species. Dietary lipid provide the vehicle for absorption of fat soluble vitamins and lipid especially phospholipid and sterol ester play a vital role in the structure of biological membrane at both cellular and sub-cellular level. Lipid are also important in the favour and textural properties

of the feed consumed by warm water fishes as well as similar properties in the fish themselves (NRC, 1983). Tilapia do not appear to utilize high levels of dietary lipid as effectively as salmonids or carp (Jauncey, 1979). In order to maximize protein utilization for growth dietary lipid level between 6 and 10% appear optimal (Jauncey and Ross, 1982). The essential fatty acid requirements of fish vary from species to species, appearing to be principally influenced by the natural diets of species in question and environmental temperature. To be certain of satisfying the EFA requirements of Fish specie, in general, it is probably wise to provide level of 1% ω6 (linoleic) and 1% ω3 (linolenic) in the diet. As EFA deficiency may result in reduced growth, poor feed conversion, tail fin erosion, fatty livers and erratic and pronounced responses to any kind of shock and stress (Jauncey and Ross 1982).

2.8.4 Carbohydrate Requirements

There is no actual requirement for dietary carbohydrate as fish are capable of synthesizing carbohydrates, from both protein and lipid sources (Jauncey and Ross, 1982). However, according to (Jauncey and Ross, 1982) carbohydrate are included in feeds as protein-sparing energy sources, bulking agents and as binders. Fish seems to resemble diabetic higher animals in their utilization of dietary carbohydrate and this means that the level of digestible carbohydrate in the feed should be restricted.

2.8.5 Vitamins Requirements

These are organic compounds required in trace amount in the function of most forms of life but of which organism are unable to synthesize. Vitamins

requirement are affected by size, age, growth rate, environmental factors and nutrient inter-relationship.

The levels of each vitamin in a pelleted diet should be higher than the required level because certain vitamins may be destroyed during manufacture and storage (Hilton *et al* 1977, Jauncey and Ross, 1982).

The recommendation as to the dietary level of vitamins premix composition to be included in a diet that will satisfy vitamin requirement of tilapia assuming no natural food is available is 2% (Jauncey and Ross, 1982).

2.8.6 Minerals Requirement

Fish require the same mineral as warmblooded animal for tissue formation and various metabolic processes (Lovell, 1984). Besides Calcium and Phosphorus, other minerals known to be required by fish include Magnesium, Iron, Iodine, Zinc, Copper and Manganese (Ketola 1977, Ogino and Yuang, 1980). So also Sodium, Potassium, Chloride ion and Chromium. Suggested level of Calcium and Phosphorus requirement for tilapia is 3.0 and 6.0 respectively. The recommendation as to dietary inclusion of mineral premix in a tilapia diet is suggested to be 4% (Jauncey and Ross, 1982).

2.9 DIGESTIBILITY

Yudkin (1985) reported that the word Digestibility has a different meaning to different people; to a layman, a food is considered digestible if it is unlikely to cause pain or discomfort after it is eaten, to another people it is believed that digestibility is referred to as the speed with which food is broken down by the digestive juice and this is attested to by an in-vitro experiment by adding digestive

to a good and measure the rate at which digestive juice disappear. The third meaning is the one used especially by the nutritionist, it refers to how much of the food is absorbed by measuring the composition of the stool/faeces to see how much of the food has been absorbed.

In practice, it is difficult to measure true digestibility of a food since faeces are complex mixture containing undigested food remains, dead cells, slough from intestinal wall, waste product voided with bile and each component contribute a certain amount of energy content of the faeces. It is not usual to make correction for the non-food faecal component and hence the digestibility value reported in this literature review is apparent digestibility (Jobling, 1983).

According to Smith, 1989, several methods have been devised to get the faecal sample; fish can be sacrificed and a faecal sample removed from the lower large intestine; faecal sample can also be gotten by gentle pressure on the outside of the fish; a sunction method has also been used; Cho *et al* (1982) have developed a system in which faecal matter is rapidly flushed from the fish holding tank and into a setting column. Faecal samples are periodically removed from the bottom of the column.

Anti-nutritional factors in the diet are known to interfere with the digestibility and utilization of nutrients. Other factors affecting digestibility are temperature, length of the alimentary canal, proportion of nutrient in the diet and meal size. Even within a given specie fed a fixed dietary formulation, factors such as fish size, temperature and feeding regime may affect digestibility measurement.

DETERMINATION OF DIGESTIBILITY COEFFICIENT

The nutritive value of any feed depend upon the ability of the animal to digest nutrient in the feed. Several assay techniques both direct and indirect have been used to estimate the coefficient of digestibility of dry matter and of specific component of the diet. The digestibility coefficient of a specific component of a diet can be measured by measuring acid insoluble ash in feeds and in faeces. This according to Halver *et al* (1993) is more reliable indicator of digestibility coefficient since the dietary ingredient (ash) are used and analysis of this component in the faeces collected uses simple gravimetric technique. Comparism of indigestible marker like chromic oxide, iron oxide, magnesium ferrite or dietary fibre and acid insoluble ash shows that acid insoluble ash is more responsible and more convenient to use (Halver *et al,* 1993).

Atkinson *et al* (1984) reported that Acid Insoluble Ash (AIA) gives result comparable to those with chromic oxide but a major advantage of endogenous marker over exogenous marker is that fish need not feed on marked food. On analyzing a sample of food and faeces for the nutrients and AIA.

Digestibility can then be calculated as

$$\text{Digestibility} = 100 - 100 \left(\frac{\text{Indicator in feed}}{\text{Indicator in faeces}} \times \frac{\text{\% nutrient in faeces}}{\text{\% nutrient in feed}} \right)$$

It is assumed that:

1. The faecal sample collected is a representative of that which would be voided naturally.
2. Loss by leaching is not significant.

3. Marker is not absorbed or retained by the test animal.
4. Marker does not interfere with the digestion and absorption of nutrient.
5. Faecal sample is not contaminated with uneaten feed.

Hepatosomatic Index (HSI)

The HSI is simply the proportion of liver weight to total body weight, expressed as a percent. Enlarged livers can occur in fish exposed to some trace metal and organic pollutants (Sorensen and Thomas, 1988; Spitsbergen *et al.* 1988, Wester and Canton, 1992), it is also known that HSI is also affected by diet and disease (Adams and McLean, 1985)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCE OF *CANA VALIA* SEED

The jackbean seeds were collected from National Root Crops Research Institute (NRCRI) in Vom, Plateau State. They were broken into coarse pieces (about 3 – 6 parts) using a grinding machine. The broken seeds were subjected to 1 hour ordinary cooking, the products were dried in the sun for three days and analyzed for its proximate composition.

3.2 PROXIMATE ANALYSIS

The cracked and cooked jackbean seeds and other practical feedstuffs used (soybean meal, maize and fishmeal) were analyzed prior to feed formulation. The proximate analysis of the diets, faeces and fish carcass were carried out according to the procedures of the AOAC 1990 in triplicate. A factor of 5.4 was used to convert nitrogen to crude protein for legumes and 6.25 for fish.

3.2.1 Moisture Contents

Moisture content was determined as the percentage loss in weight. A known weight of the sample in a weighed, dry and clean evaporating dish was oven dried at 80^0C for 24 hours. The sample was cooled in a dessicator and weighed. The process of drying, cooling and weighing was continued until a constant weight was obtained. The difference in weight represented moisture loss and was expressed as a percentage of the original sample weight.

Calculation

$$\text{Moisture contents of sample \%} = \frac{(y^0 - y^1)}{(y^0 - x)} \times 100$$

Where,

x = Weight of clean, dry, evaporating dish

y^0 = Weight of clean, dry, evaporating dish + sample

y^1 = Weight of clean, dry, evaporating dish + dry sample

3.2.2 Crude Protein

Digestion Stage: A known weight of the sample (W_1) was put into kjedahl flask unit. Added to it were 25ml of concentrated H_2SO_4 and a gram of copper catalyst. It was heated in a kjedahl heating unit until the colour changed to green. With the aid of distilled water, the digest was made up to 50ml and was put in a sample bottle and labeled.

Distillation Stage: 5ml of boric acid was pipetted into conical flask. Added to it were three drops of mixed indicator. This was placed below the receiver of the kjedahl distillation unit. 5ml of the labeled sample and 10ml of 40% NaOH were pipetted into the distillation unit. Heating continued until 50ml of the distilled sample was obtained.

***Titration* Stage:** 50ml of the distilled sample was titrated against 0.01m HCl until the end point was noted.

The percentage total nitrogen was then calculated

$$\text{\% Total Nitrogen} = \frac{T_1 \times M_a \times 0.014 \times V_1 \times 100}{W_1 \times V_2}$$

M_a = Molarity of acid

W_1 =Weight of the sample

V_1 = Initial volume put in a distillation unit

V_2 = Final volume obtained from the distillation

% crude protein = N x 5.4 (Legume)

3.2.3 Ether Extract

The lipid content was determined by subjecting the samples to a continuous extraction with petroleum ether using soxhilet apparatus according to A. O. A. C (1990). After extraction, the ether was evaporated and the weight of the material extracted was determined.

$$\text{Crude lipid content} = \frac{\text{Weight of sample} - \text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.2.4 Ash Content

Ash content was determined by subjecting the already dried samples with known weight to ignition into a muffle furnace at 550^{0}C for 3 hours. Samples was cooled in a dessicator and weighed. The process of heating, cooling and weighing was continued until a constant weight was obtained.

$$\text{Ash content of sample (\%)} = \frac{(y^1 - x)}{y^0 - x} \times 100$$

Where,

x = Weight of clean, dry, crucible

y^0 = Weight of crucible + sample

y^1 = Weight of crucible + ash

3.2.5 Crude fibre

Crude fibre was determined by the method described by A. O. A. C. (1990). The residue from ether extraction was subjected to successive treatment of boiling with dilute acid and dilute base, after which it was filtered and ignited in a furnace. The difference obtained between the weight of the unburnt and burnt fraction was calculated to be crude fibre.

$$\% \text{ Crude fibre} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Weight of residue}}$$

3.2.6 Nitrogen Free Extract

This was determined by difference

NFE = 100 – (% Crude protein + % Crude lipid + % Ash + % Crude fibre + % Moisture content)

3.2.7 Gross Energy

The gross energy content of the samples was determined by calculation based on the physiological value of 5.61kcal/g protein, 9.50kcal/g lipid, and 4.11kcal/g carbohydrate (Tacon, 1999).

3.3 EXPERIMENTAL DIETS FORMULATION

Four (4) Isonitrogenous (30% crude protein) were formulated containing fish meal, soybean meal which was substituted with jackbean meal at 0% (CTR), 20% (D20), 30% (D30), 40% (D40), cassava flour, fish oil and vegetable oil and vitamin-mineral premix. The feedstuffs were ground, weighed according to the values indicated on Table 3.1 and hot water was added to aid binding. This was then introduced into Horbart A – 200T pelleting and mixing machine to obtain a

homogenous mass and then passed through mincer to produce 0.8mm (long), 0.2mm (diameters) pellets which was immediately sundried. After drying for three days, the diets were kept in a refrigerator.

Table 3.1: Gross composition(g/100g of dry matter) of experimental diets at varying replacement levels of jackbean meal

	Control	D20	D30	D40
Fish meal	24.00	24.00	24.00	24.00
Jackbean meal	———	12.00	18.00	24.00
Soybean meal	33.00	26.00	22.00	19.00
Maize	25.00	20.00	18.00	15.00
Cod-liver oil	2.50	2.50	2.50	2.50
Vegetable oil	3.50	3.50	3.50	3.50
Vitamin/mineral premix	2.00	2.00	2.00	2.00
Cassava starch	10.00	10.00	10.00	10.00
Total	100	100	100	100

3.4 EXPERIMENTAL SYSTEM AND FISH

The feeding trial was conducted in glass tanks (70 x 90 x 40cm) filled with water from University reservoir. The water was allowed to stand for 3 – 10 hours before putting it into the tanks. *Oreochromis niloticus* (6.37±0.07g) were obtained from FUTA Fisheries and Wildlife fish farm and transported life to the department of fisheries and wildlife. The fish were acclimated in glass tanks for seven days while being fed on a commercial pelleted diet (30% crude protein). Fifteen fingerlings were stocked into each tank with three replications per treatment. Experimental diets were assigned randomly to the tanks and each group of fish was fed at 5% body weight per day in two equal proportion at 9 – 10.00 hrs and 16.00 –

17.00 hrs for 70 days. All the fish were removed from each tank every 14 days and batch- weighed. Data there from were recorded for statistical analysis and the amount of feeds to be fed were adjusted accordingly. Siphoning was done during the second, third and fourth week of the experiment to collect faecal samples using a hose pipe of diameter 15mm into a sieve of mesh size 350µm, then pooled and kept in a freezer. The faecal samples were oven dried in a Gallencamp oven (24^0C) and were analyzed for its proximate composition.

Mortality was monitored daily and recorded. At the beginning of the feeding trial and at the end two compost whole fish were sacrificed by decapitation for carcass analysis of its crude protein, lipid and Ash content.

3.5 WATER QUALITY MONITORING

Water temperature and dissolved oxygen were measured thrice in a week using combined digital YSI DO meter (YSI Model 9071 Jenway).

pH was monitored thrice weekly using an electronic pH meter (Mettler Toledo Model 320).

3.6 Acid Insoluble Ash (AIA)

AIA in feed and faeces was obtained by adding 25ml of 10% HCl to the weighed ash content. This was then covered with a watch glass and heated gently over a low flame for 5 minutes after which it was filtered and washed with hot distilled water until it was acid free. The residue was returned into the crucible and ignited until it was carbon free and was weighed. The AIA was calculated as

$$\% \text{AIA} = \frac{\text{Weight of AIA}}{\text{Weight of Ash}} \times 100$$

3.7 Digestibility Coefficient

The values obtained for AIA was used as indicator in the calculation of digestibility coefficient. The digestibility coefficient was calculated according to Halver, 1993 as

$$\text{Digestibility coefficient} = 100 - \left(100 \quad \frac{\%\ \text{AIA in feeds}}{\%\ \text{AIA in faeces}} \times \frac{\text{Nutrient in faeces}}{\text{Nutrient in feeds}}\right)$$

3.8 DIET PERFORMANCE EVALUATION

Growth performance and nutrient utilization of fish fed with the different dietary treatments were determined in terms of final individual weights, survival rate, specific growth rate (SGR %/day), % weight gain, Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Protein Productive Value (PPV).

$$\text{Weight gain (\%)} = \frac{100\ (\text{Final weight} - \text{Initial weight})}{\text{Initial weight}}$$

$$\text{SGR (\%/Day)} = \frac{100\ (\text{Log}_e\ \text{Final body weight} - \text{Log}_e\ \text{Initial body weight})}{\text{Time (in days)}}$$

$$\text{FCR} = \frac{\text{Dry weight of feed fed}}{\text{Fish weight gain}}$$

$$\text{PER} = \frac{\text{Fish weight gain}}{\text{Protein fed}}$$

$$\text{PPV} = \frac{\text{Protein in carcass}}{\text{Protein fed}}$$

3.9 HEPATOSOMATIC INDEX (HI)

This was carried out in the Fisheries and Wildlife Laboratory Obakekere, FUTA as follow; At the end of the feeding trial, two fishes were randomly picked from each tank and weighed individually, dissected and their livers removed and weighed individually. HI was calculated according to Fashakin *et al,* (1999).

$$\text{Hepatosomatic Index} = \frac{\text{Weight of the liver}}{\text{Weight of the fish}} \times 100$$

3.10 COST ANALYSIS

Economic analysis was carried out using profit index and incidence of cost as indices of profitability (Faturoti ,1989)

$$\text{Profit Index} = \frac{\text{Value of fish}}{\text{Cost of feed}}$$

$$\text{Incidence of Cost} = \frac{\text{Cost of feed consumed}}{\text{Kg of fish produced}}$$

Gross profit = value of 1 kg fish – incidence of cost

3.11 STATISTICAL PROCEDURES

Data resulting from SGR, FCR, PER, PPV, Weight gain during the experiment were subjected to one way analysis of variance (ANOVA), where the ANOVA revealed significant difference, Fishers' least significant difference (LSD) test was used to compare differences among individual treatment means.

CHAPTER FOUR

4.0 RESULTS

4.1 PROXIMATE COMPOSITION OF INGREDIENTS

The results of the proximate composition of the feed ingredients are presented in table 4.1 prior to the feed formulation.

Table 4.1: The proximate composition (g/100g) of raw and processed jackbean meal with other feed ingredients

PARAMETER	RAW JACKBEAN	PROCESSED JACKBEAN MEAL	MAIZE	FISH MEAL	SOYBEAN MEAL
Moisture	11.03	4.00	5.98	5.04	3.96
Protein	29.69	25.30	9.82	63.68	46.52
Lipid	3.36	4.55	4.30	10.61	3.58
Fibre	12.07	7.68	2.86	1.05	5.96
Ash	3.91	2.07	1.15	10.23	6.80
NFE (calculated)	39.94	56.41	75.89	9.39	33.18
*Gross energy kcal/g	362.37	415.85	405.86	495.48	431.92

*Gross energy in kcal/g is based on the physiological values of 5.66 kcal/g protein, 9.10 kcal/g lipids and 4.10kcal /g carbohydrate.

The crude protein and crude fibre of processed jackbean meal were lower than the values obtained for crude protein and crude fibre of raw jackbean meal. The crude protein of cracked and cooked jackbean meal, maize, fishmeal and soybean meal were 25.30, 9.82, 63.63 and 46.52% respectively. The lipid present in these ingredients were 4.55, 4.30, 10.61 and 3.58% respectively. The crude fibre of processed jackbean meal was 7.68%, that of maize 2.86%, and soybean meal

5.86%. The Nitrogen free extract of processed Jackbean meal is 56.41% that of maize, fishmeal and soybean meal are 75.89%, 9.39% and 33.18% respectively.

4.2 PROXIMATE COMPOSITION OF THE DIETS

The results of the proximate composition of the experimental diets are presented in Table 4.2.

Table 4.2: Proximate composition (g/100g) of experimental diets

	DIETARY TREATMENT			
	CTR	D20	D30	D40
PROTEIN	30.18	30.24	30.55	30.88
LIPID	10.39	9.76	16.52	10.41
FIBRE	5.58	5.65	5.70	5.69
ASH	6.04	5.41	5.26	5.30
*NFE	39.56	40.84	39.61	38.96
MOISTURE	8.25	8.10	8.36	8.76
TOTAL	100	100	100	100
ENERGY (KJ/g)	18.01	18.00	18.15	18.09
**AIA	3.05	3.08	3.11	3.36

* NFE – Nitrogen Free Extract

**AIA – Acid Insoluble Ash

The percentage crude protein varied from 30.18 in CTR diet to 30.88% in diet D40. From the lipid determination, diet D30 had the highest value of 10.52 and diet D20 had the lowest value of 9.76%. The highest percentage crude fibre was 5.70 in diet D30, with lowest value of 5.58 in control diet, the percentage ash

content ranged from 6.04 in diet CTR to 5.41% in diet D30. The moisture content varied from 8.10 in diet D20 to 8.76% in diet D40. The % AIA in the diet ranged from 3.05 in diet CTR to 3.36 in diet D40. Diet D20 had the highest value of NFE, while the lowest value of NFE was recorded in diet CTR. The gross energy value ranged from 430.53 in D20 to 434.42% in diet D30.

4.3 GROWTH PERFORMANCE OF *Oreochromis niloticus* FED PROCESSED JACKBEAN MEAL AS A REPLACEMENT FOR SOYBEAN MEAL

4.3.1 Mean Weight Gain

The mean weight gain and other growth parameters are presented in Table 4.3. The highest mean weight gain of 30.52g was obtained from fish fed diet CTR and the least weight gain was obtained from the fish fed diet D40. However, there was no significant difference ($P>0.05$) in the mean weight gain of the fish fed diet CTR and D20. A significant difference ($P<0.05$) occurred in the weight gain of the fish fed diets CTR, D20 and D30, D40. There was significant difference ($P<0.05$) in the mean weight gain of the fish fed diet D40 and others. The graph of weight gain against time is presented in fig. 4.1.

4.3.2 Percentage Weight Gain

The percentage weight gain of the fish fed diet CTR had the highest value while the lowest value of percentage weight gain was recorded for the fish fed diet D40. There existed a significant difference ($P<0.05$) in the percentage weight gain of the fish fed control diet and test diets D20, D30 and D40. However, there was no significant difference ($p>0.05$) in the percentage weight gain of fish fed diet CTR and diet D20.

4.3.3 Feed Conversion Ratio (FCR)

The FCR increased with increasing level of jackbean meal in the diets. The highest feed conversion ratio was recorded in the fish fed diet D40 with a value of 1.64% and the lowest FCR was obtained from diet CTR with a value of 1.37%. There was a significant difference ($P<0.05$) in the FCR of the fish fed the control diet and diets D20, D30 and D40. However, there was no significant difference ($P>0.05$) in the FCR of fish fed diets CTR and D20.so also no significant different existed between the FCR of the fish fed diet D20 and D30.

4.3.4 Specific Growth Rates (SGR)

The SGR decreased with increasing level of jackbean meal inclusion. The highest value of 2.51% /day was obtained in control diet while the lowest value of 2.03 %/day was obtained in diet D40. There was a significant difference ($p<0.05$) in the SGR of fish fed the different diets. However, there was no significant difference ($p>0.05$) in the SGR of the fish fed diet CTR and diet D20.

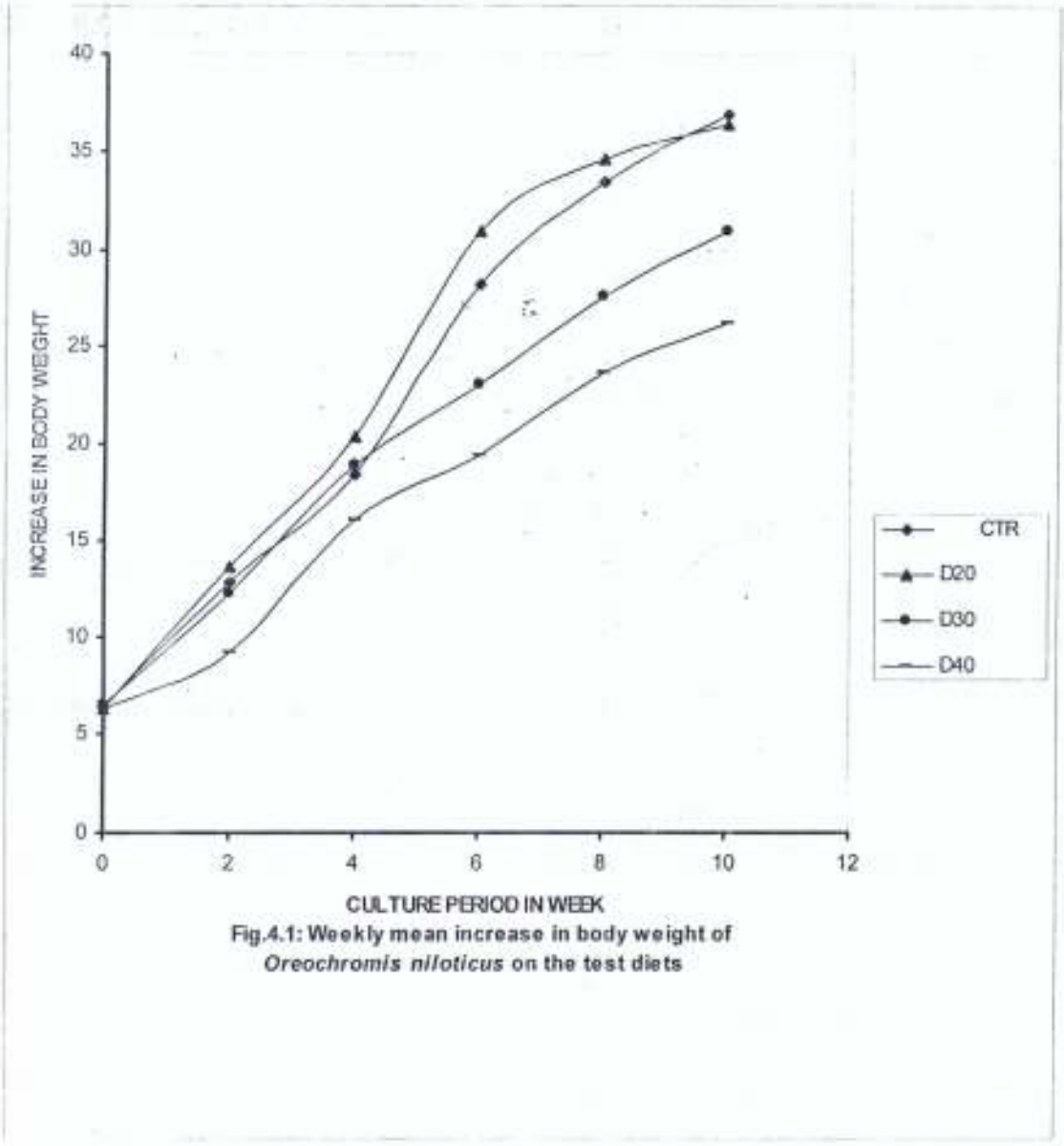

Fig.4.1: Weekly mean increase in body weight of *Oreochromis niloticus* on the test diets

CTR – Diets containing 0% Jackbean meal replacement level

D20 – Diets containing 20% Jackbean meal replacement level

D30 – Diets containing 30% Jackbean meal replacement level

D40 – Diets containing 40% Jackbean meal replacement level

Table 4.3: Growth performance of *Oreochromis niloticus* fed diets containing Processed jackbean meal at varying replacement level

		TEST DIETS		
	CONTROL	D20	D30	D40
Initial Weight* (g)	6.37±0.12	6.31±0.04	6.48±0.36	6.34±0.24
Final Weight* (g)	36.89±0.07	36.34±0.75	30.89±0.21	26.25±1.16
Weight gain (g)	30.52^{a}±0.05	$3.008±1.09^{a}$	$24.41±1.38^{b}$	$19.91±1.29^{c}$
Weight gain* (%)	$479±10.02^{a}$	$476±11.90^{a}$	$377±13.13^{b}$	$314±30.04^{c}$
Total food intake*(g)	$41.78±0.19^{a}$	$44.83±0.95^{b}$	$37.03±0.40^{c}$	$32.80±0.57^{d}$
Feed conversion ratio*	$1.37±0.05^{c}$	$1.48±0.06^{bc}$	$1.52±0.07^{ab}$	$1.64±0.08^{a}$
Specific growth rates* (%/day)	$2.51±0.02^{a}$	$2.50±0.03^{a}$	$2.23±0.04^{b}$	$2.030.10^{c}$

*Means of triplicate values

Means of the same superscript in the same row are not significantly different ($p>0.05$)

4.4 PROXIMATE COMPOSITION OF THE EXPERIMENTAL FISH

The proximate composition of the initial and the final fish samples fed both the control diet and the test diets are presented in Table 4.4.

Table 4.4: Proximate composition (g/100g wet weight) of the experimental fish before and after the experiments

	Initial	CTR	D20	D30	D40
PROTEIN	13.65±0.13	17.20 ± 0.33^{a}	17.13 ± 0.09^{ab}	16.70 ± 0.28^{bc}	16.50 ± 0.24^{c}
LIPID	5.10±0.24	9.70±0.24	9.43±0.29	8.98±0.19	8.94±0.08
ASH	2.75±0.36	3.97 ± 0.27^{a}	3.88 ± 0.10^{a}	3.46 ± 0.05^{b}	3.89 ± 0.11^{a}
MOISTURE	78.42±0.52	69.13±0.21	69.56±0.13	70.86±0.03	70.67±0.36

* Means of triplicate

Means of the same superscript in the same row are not significantly different ($p>0.05$)

The results showed that the fish at the beginning of the experiment had 13.65% crude protein, 5.1% lipid, 2.75% ash and 78.42% moisture. The crude protein, lipid and ash content increased after the feeding trial with a corresponding decrease in the moisture content. There was no significant difference ($P > 0.05$) in the crude protein of the fish fed diet CTR and D20. Also there was no significant difference ($P > 0.05$) in the carcass crude protein of the fish fed D20, and D30 and between diets D30 and diets D40.

The highest crude lipid was obtained from fish fed control diet with a value of 9.70%, the lowest value of crude lipid was obtained from the fish fed diet D40, however, there was no significant difference ($P>0.05$) in the crude lipid content of the fish samples fed various dietary treatment.

The percentage ash content ranged from 3.46% in fish fed diet D30 to 3.97% in fish fed diet CTR. There existed a significant difference ($P<0.05$) in the ash content of the fish fed the different dietary treatment however no significant difference ($P>0.05$) existed in the ash content of the fish fed diet CTR, D20 and D40.

4.5 NUTRIENT UTILIZATION

The various values for the protein intake, protein efficiency ratio (PER) and protein productive value (PPV) are presented in Table 4.5.

Table 4.5: Nutrient utilization of *Oreochromis niloticus* fed diets with jackbean meal at varying replacement levels.

	CONTROL	TEST DIET		
	CTR	D20	D30	D40
Weight gain (g)	30.52 ± 0.05^{a}	30.03 ± 1.09^{a}	24.41 ± 11.38^{b}	19.91 ± 1.29^{c}
Protein intake* (g)	$12.50\pm.008^{b}$	13.40 ± 0.41^{a}	11.11 ± 0.13^{c}	9.84 ± 0.14^{d}
PER*	2.44 ± 0.01^{a}	2.24 ± 0.11^{ab}	2.19 ± 0.10^{b}	2.03 ± 0.09^{b}
PPV %*	28.37±2.44	25.89±1.52	27.48±2.85	29.27±2.24
Survival rate (%)	97.35±0.41	98.39±0.13	96.14±0.09	97.07±0.11
Hepatosomatic index*(%	1.21±0.50	1.29±0.22	1.24 ± 0.21	1.26 ± 0.22

* Means of triplicate

Means of the same superscript in the same row are not significantly different ($p>0.05$)

Protein Efficiency Ratio (PER)

Fish fed diet D20 had the highest value of 13.40% protein intake while that fed diet D40 had the lowest value of 9.84% protein intake. The protein efficiency ratio (PER) ranged from 2.03 in the fish fed diet D40 to 2.44 in the fish fed diet CTR. There was significant difference ($p<0.05$) in the PER of the fish fed the various dietary treatments. However, there existed no significant difference ($p>0.05$) in the PER of fish fed diet D20 and CTR, Fish fed diets D20, D30 and D40 were not significantly different ($P>0.05$) in their PER.

Protein Productive Value (PPV)

The protein productive value (PPV) varied from 27.45% in the fish fed diet D30 to 29.27% in the fish fed diet D40, there was no significant difference ($P > 0.05$) in the PPV of the fish fed various dietary treatment.

SURVIVAL RATE

The highest survival rate was obtained with diet D20. There was a high percentage of survival among all groups of fish.

HEPATOSOMATIC INDEX (HSI)

There was no significant difference ($P > 0.05$) in the HSI of the fish fed test diets with control diet.

4.6 PROXIMATE COMPOSITION OF THE FAECES

Table 4.6 shows the results of the proximate analysis of faecal samples of fish from each dietary treatment.

Table 4.6: Proximate composition (g/100g) of the faecal samples

	CONTROL	TEST DIETS		
	CTR	D20	D30	D40
PROTEIN	8.82±0.26^{b}	10.97±1.89^{ab}	12.01±1.35^{a}	14.01±1.5^{a}
LIPID	3.11±0.70	3.05±0.71	2.74±0.43	3.30±0.19
FIBRE	3.92±0.16	4.16±0.15	4.31±0.24	4.48±0.28
ASH	12.40±0.38^{c}	14.11±0.28^{a}	14.12±0.14^{a}	13.98±0.09^{b}
NFE	60.80±1.09^{a}	57.80±2.11^{a}	56.94±1.30^{a}	54.18±1.28^{b}
MOISTURE	10.95±1.11	9.91±1.17	9.88±1.13	10.05±1.15
TOTAL	100	100	100	100
ENERGY (KJ/g)	13.73±2.83	13.70±6.57	13.67±1.69	13.89±0.17
AIA (%)	8.90±1.58	9.78±1.96	9.12±0.92	8.94±1.39

Means of the same superscript in the same row are not significantly different ($p>0.05$)

The highest crude protein of faecal samples was obtained from fish fed diet D40. least crude protein was obtained from fish fed diet CTR. There was a reduction in crude protein values when compared to the diets. There was no significant difference ($P > 0.05$) between the crude protein values of the faecal samples of the fish fed diet CTR, D20 and D30. So also no significant differences ($P > 0.05$) existed in the crude protein of the faecal samples of fish fed diet D20, D30 and fish fed diet D40.

Faecal samples collected from the fish fed diet D40 contained the highest value of ether extract, fish fed diet D30 had the lowest value of ether extract, there

was no significant difference ($P > 0.05$) in the ether extracts of the faecal samples of the fish fed various dietary treatments.

The highest crude fibre value was obtained in the faecal sample of the fish fed diet D40. An increase in crude fibre value was observed in the faecal sample of the fish fed with diet CTR, D20, D30 and D40 respectively. However, there was no significant difference ($P > 0.05$) in the crude fibre value of the faecal sample of the fish fed various dietary treatments.

The ash content of the faecal samples of the fish fed diet D30 had the highest value of 14.12 while the lowest value was recorded for the faecal samples of the fish fed diet CTR. There was a significant difference ($P<0.05$) in the ash content of the faecal samples of the fish fed the different diets however there was no significant difference ($P>0.05$) in the ash content of the fish fed diet D20 and diet D30.

Faecal samples collected from the fish fed diet CTR contained the highest value of nitrogen free extract. The lowest value of NFE was obtained in the faecal sample of the fish fed diet D40. There was no significant difference between faecal samples of the fish fed diet CTR and diet D20 and D30. However, a significant difference ($P<0.05$) occurred between the NFE value of the faecal samples of the fish fed these diets and diet D40.

The highest gross energy value was obtained in the faecal samples of the fish fed diet D40 while the lowest gross energy value was obtained in the faecal sample of the fish fed diet D20. There was no significant difference ($P > 0.05$) between the gross energy of the faecal sample of the test diet and the control.

The highest value of the percentage AIA was recorded in faecal samples of the fish fed diet D20 while the lowest value of the percentage AIA was recorded in the faecal sample of the fish fed diet CTR.

4.7 APPARENT DIGESTIBILITY COEFFICIENT (ADC) OF THE NUTRIENTS IN DIETS

Table 4.7 shows the results of the apparent digestibility coefficient of the nutrient in the diets CTR, D20, D30 and D40 fed to ***Oreochromis niloticus.***

Table 4.7: Apparent digestibility coefficient (%) of the nutrient in the diets fed to *Orechromis niloticus.*

	CTR	D20	D30	D40
CRUDE PROTEIN (%)	89.98 ± 0.30^{a}	88.57 ± 1.55^{ab}	86.59 ± 1.50^{bc}	82.96 ± 2.49^{c}
LIPID (%)	89.70 ± 2.30^{a}	90.08 ± 2.17^{a}	91.1 ± 1.403^{a}	87.97 ± 0.72^{b}
ENERGY (%)	73.87 ± 0.23^{b}	76.02 ± 0.48^{a}	74.32 ± 0.14^{b}	71.12 ± 0.19^{c}
ORGANIC MATTER (%)	64.67 ± 6.02^{a}	67.33 ± 5.93^{a}	65.52 ± 3.56^{a}	61.53 ± 5.71^{a}

Means of the same superscript in the same row are not significantly different ($p>0.05$)

Apparent Digestibility Coefficient of protein

The highest apparent crude protein digestibility was contained in the diet CTR. The diet D40 had the lowest crude protein digestibility. There was a significant difference ($P < 0.05$) in the apparent crude protein digestibility coefficient of fish fed the different diet. However, there was no significant difference ($p > 0.05$) between diet CTR and D20. There was no significant difference in the apparent crude protein digestibility coefficient of the fish fed diets D20 and D30.

Apparent Digestibility Coefficient of lipid

The highest crude lipid digestibility coefficient was obtained in diet D30 while the lowest crude lipid digestibility is obtained in diet D40. There was no significant difference ($p>0.05$) in the crude lipid digestibility among the different diets.

Apparent Digestibility Coefficient of Energy

The highest energy digestibility was obtained in the diet D20 while the lowest was obtained in diet D40. There was a significant difference ($P<0.05$) in the energy digestibility coefficient of the different diets. However, there was no significant difference ($P > 0.05$) in the energy digestibility of the diet CTR and D30.

Apparent organic matter Digestibility Coefficient

The highest apparent organic matter digestibility (AOMD) was obtained in diet D20 and diet D40 had the lowest, there is no significant difference ($P > 0.05$) in the AOMD among the different dietary treatment.

4.8 WATER QUALITY ANALYSIS

The mean values of the physico-chemical parameters of the experimental tanks monitored during the experimental period are presented in the table 4.8. The temperature averages 26.10 – 26.58^{0}C in all tanks while the pH ranged from 6.92 – 7.20. The dissolved oxygen content of the tanks varied from 6.29 – 6.78 ppm.

Table 4.8: Mean values of water quality parameters in the experimental tanks

	CONTROL	TEST DIETS		
	CTR	D20	D30	D40
Temperature (^{0}C)	26.20 ± 0.21	26.58 ± 0.32	26.10 ± 0.15	26.35 ± 0.32
Dissolved oxygen mg/l)	6.78 ± 0.29	6.56 ± 0.18	6.69 ± 0.54	6.79 ± 0.64
pH	7.11 ± 0.35	7.20 ± 0.11	6.92 ± 0.53	7.04 ± 0.27

4.9 INCIDENCE OF COST ANALYSIS

Table 4.9a and 4.9b presented the cost of producing 1kg of each diet fed to *Oreochromis niloticus* and incidence of cost analysis respectively.

There was a reduction in the cost of producing 1kg of diets with the increasing replacement level of soybean meal with jackbean meal.

The incidence of cost analysis which is the cost of producing 1kg of fish showed that it was cheaper to produce 1kg of fish with diets D20 and D30. Diet D40 had the highest value of incidence of cost. However, feeding the fish with the control diet and the test diets left some profit margin.

Table 4.9a: Cost (₦) of producing 1kg of diets fed to *Oreochromis niloticus*

FEED INGREDIENTS	COST(₦)/KG	CTR	D20	D30	D40
Fishmeal	125	30	30	30	30
Jackbean meal*	2.25	-	0.27	0.41	0.50
Soybean meal	45	14.4	11.70	9.90	8.50
Maize	36	8.64	7.20	6.48	5.40
Cod-liver oil	600	15	15	15	15
Vegetable oil	80	2.8	2.8	2.8	2.8
Vitamins/mineral premix	300	6	6	6	6
Starch	15	1.50	1.50	1.50	1.50
Processing**		8.33	7.45	7.21	6.92
Cost/kg of feed(₦)		92.17	81.92	79.29	76.67

*5% of the cost of soyabean

**10%of total cost of feed

Table 4.9b: Incidence of cost analysis of producing *Oreochromis niloticus*

	CONTROL	TEST DIETS		
Parameters	CTR	D20	D30	D40
Cost of feed consumed (₦)	3.85	3.67	2.97	2.52
Weight gain of fish (kg)	0.0305	0.030	0.0244	0.0199
Value of fish (₦)	4.58	4.51	3.66	2.97
Profit index ₦ / kg	1.19	1.23	1.23	1.17
Incidence of cost (₦)	126	122	123	127
Profit/kg of fish (₦)	24	28	27	23

Value of fish based on ₦ 150/kg

$$\text{Incidence of cost} = \frac{\text{cost of feed consumed}}{\text{kg of fish produced}}$$

$$\text{Profit Index} = \frac{\text{Value of fish}}{\text{Cost of feed consumed}}$$

Gross Profit = Value of 1 kg fish – incidence of cost

CHAPTER FIVE

5.0 DISCUSSION

The proximate composition of cooked jackbean meal ingredient agreed with the work of Udedibie, (1990) who reported 25% crude protein for the heat-treated sample of jackbean seed. The values reported for crude lipid, crude fibre were closely in consonance with the earlier work of, Udedibie 1990; Montilla *et al* 1981; Monilla *et al,* 1974; Borchers and Ackerson 1950. The exceptional high crude fibre content of the jackbean meal may be attributed to the presence of a thick and tough coat of the seed. The reduction in crude protein and crude lipid of the processed jackbean meal relative to raw jackbean meal might have been as a result of solubilisation and removal of some protein during the cooking process. Adeparusi (1994) reported a decrease in crude protein, lipid and crude fibre content of cooked pigeon pea when compared raw pigeon pea; the decrease was attributed nutrient leaching caused by cooking. The same observation was reported in Udedibie (1990) for the same seedmeal.

The proximate compositions of soybean meal, corn and fishmeal were in line with the values recorded by NRC (1983). The variations obtained from the results may be attributed to environmental and or experimental variation.

The crude protein of 30% used in the formulation of the experimental diets in this study for *Oreochromis niloticus* were similar to the recommended optimum dietary protein requirement levels (30 – 35%) of the fish, (Luquet, 1991). The result from the proximate analysis of the diets clearly revealed the diet to be isonitrogenous, isolipidic and isocalorific, the lipid requirement of *Oreochromis*

niloticus was met by the quantity presented in the feed, this is because the recommendation as to the dietary lipid levels that should be included in tilapia diets for maximum protein utilization as reported by Jauncey and Ross (1982) was between 6 and 10%.

The growth performance of *Oreochromis niloticus* fed processed jeakbean meal showed a significant difference ($P < 0.05$) to that of control. This difference was not significant ($p>0.05$) at lower replacement level (20%) to that control. The result agreed with the result of Osigwe *et al* (2003),who fed the same seed meal to *Clarias gariepinus*, it was concluded that processed jackbean meal up to 20% in a diet did not differ significantly ($p>0.05$) to that of control. The result is also in consonance with the report of Martinez-Palacios *et al* (1988) that there was no significant difference ($p.>0.05$) in growth, weight gain, FCR, %WG between fish fed diets containing treated jackbean meal at 25% partial substitution with fishmeal. De-Silva and Gunasekera (1989) reported that the best of %WG, and FCR were observed at 25% level of plant protein ingredients fed to *Oreochromis niloticus* in substitution with fishmeal. Significantly reduced FCR, %WG were found in fish fed diet containing high level of jackbean meal a similar trend to that noted above was observed by Jackson *et al* (1982) for *Oreochromis mosambicus* fed with different level of plant protein. De-silva and Gunasekera (1989) also found that feeding high level of green gram, *Phaseolus aureus* to *Oreochromis niloticus* resulted in decreasing growth performance. Reduction in the availability and balance of amino acid in the diets containing high level of jackbean meal might have been responsible for the decreasing growth performance. However, the processing technique applied

had an impact on the elevation of the nutritional status of jackbean. Jackbean is reported to contain non-thermolabile toxins which is known to have several side effects on immune functions, protein metabolism, enzyme activities and hormonal regulation (Putzai, 1989). Udedibie and Carlini (1997) reported that the toxin (Con. A) specifically is heat labile but somehow seems to be protected against heat destruction as a component of the seed. The crack and cook processing method applied to jackbean rendered the seed to be nutritionally acceptable for fish. This result further confirms and attests to the earlier work of Udedibie and Carlini (1998a and b) that complete inactivation of Con. A can be achieved if the seed is broken (3 – 7 parts per seed) and cooked for just 1hour.

The trend of results in nutrient utilization of *Oreochromis niloticus* fed the control diet and test diets D20, D30 and D40 relative to protein efficiency ratio and protein productive value is a pointer to the fact that the anti-nutritional factors in the seed have been completely inactivated by the processing method applied. This result agreed with the work of Fagbenro (1998, unpublished) that *Canavalia* meals can provide up to 33% of total dietary protein and can substitute for cowpea meal (on an equal basis of dietary protein and lipid content) to supplement dietary protein in dry grow out diets for Nile tilapia. The mortality was low (< 10%) and no abnormal fish behaviour was noted when the jackbean meals replaced soybean meal. HSI values did not differ significantly ($P > 0.05$) and did not show any trend relating to dietary treatment and nutritional disorder showing that there was no undue enlargement of the liver which could have happened if some anti nutritional factors had been present. The liver, metabolically, being the most vital organ,

regulating the intermediary metabolism, in all animals including fish (Walton and Cowey,1977). This result showed that the crack and cook processing method applied as a treatment to jackbean seed completely inactivated the originally thermolabile but thermostable component of the seed (Con. A). This result agrees with the recommendation made by Martinez-Palacios *et al* (1988) that "in the future, it will be necessary to develop improved methods for totally extracting the con. A from the seed so that the potential for using *Canavalia* meal as a partial substitute for fish diet is fully realized. The same recommendation was made by Mora and Parra (1987), Vierma and Montilla (1982) and Ellis and Belmar (1985), though for monogastric animals. Martinez-Palacios *et al* (1988) found that when processed jackbean meal was fed to *O. mossambicus* fry there was no significant difference ($p>0.01$) in growth and feed conversion rate between fish ingesting the diets containing processed jackbean meal and the control diet. Similarly, Montilla (1981); Udedibie (1990) and Udedibie and Nkwocha (1996) reported that the best improvement in the nutritive value of the seed seems to have been obtained through soaking in urea solution for about a week prior to cooking for an hour but the process is cumbersome; feeding trials with the product from dry urea treatment which appears convenient showed that it was not so good as the wet urea treatment. (Udedibie *et al*, 1994). Udedibie and Carlini (1998b) concluded after a series of analytical studies that none of the methods mentioned above could completely removed con. A from the seed. This implies that even minutes amount of con. A that remain in the processed jackbean meal can constitute a problem to the animals on *ad-libitum* feeding system. This is because con. A is resistant to proteolytic

digestion and therefore tends to accumulate in the animals by binding to the intestinal wall. The crack and cook processing technique applied to jackbean meal completely inactivated the Con A. in the seed meal, this is evident in the result in which there is no significant difference ($p>0.05$) in the nitrogen utilization of fish fed control diets and test diets.

The result of carcass analysis revealed general increase in carcass protein lipid and ash after the feeding trial coupled with the corresponding reduction in carcass moisture. This is an indication that protein synthesis occurred during the feeding trial as a result of energy sparing effect of lipid and other energy supplying nutrients in the diet. It is most likely that the carbohydrate present in jackbean meal was well utilized by *Oreochromis niloticus* this might have caused the higher carcass fat deposition as observed by Anderson *et al* (1984). Keembiyehetty and De-Silva (1993) also observed similar trend when *Phaseolus mungo* seed was fed to *Oreochromis niloticus*. The decline in carcass protein and lipid with increased levels of jackbean meal in the diet was probably due to reduced nutrient availability. The trend of results obtained for carcass composition in this study (Table 4.4) agreed with similar results reported for *Vigna catiang* and *Phaseolus mungo* seed (Keembiyehetty and De-Silva 1993 in *Oreochromis niloticus* diets; for jackbean meal (Martinez-Palacios *et al,* 1988) in *Oreochromis mossambicus* diets.

A general reduction in the nutrients of the faeces as compared to the diets revealed that some percentage of nutrients, are absorbed and made available for metabolism. It is the absorbed nutrients after digestion that is available for metabolism. The differential absorption of nutrient with increase level of jackbean

meal indicated the possibility that digestion, absorption and utilization of the nutrients in those diets might have been impaired. This result agreed with Adeparusi and Jimoh (2002), that there was a reduction in nutrient value of faecal samples of *Oreochromis niloticus* fed lima bean (*Phaseolus lunatus*) when compared to the diets.

The protein digestibility decreased with increasing dietary inclusion of jackbean meal, an indication to the possibility that certain percentages of nutrients (Protein) were not absorbed, hence, not available for metabolism. The protein digestibility of the experimental diets reported in this study is similar to that (84.7 ± 3.4%) reported by Fagbenro (1998) for the same seed meal fed to *Oreochromis niloticus* but lower than the values reported by Martinez-Palacious *et al* (1988); who fed the same seed meal to *O. mossambicus* the little variation could be attributed to variability of nutrients as well as differences in nutrient processing, experimental methodology and faeces sampling technique. (Jauncey, 1993). Thus, it can be said that *Oreochromis niloticus* utilized the processed jackbean meal effectively as similarly reported by Fagbenro (1998) of apparent crude protein digestibility ranging between 80.5 – 86.4% of *Oreochromis niloticus* fed various legume seed meal jackbean inclusive. Bressani *et al* (1987) reported the values of 76.4% and 78.7% respectively when cooked and roasted respectively. These results indicated the processing method applied to jackbean effectively removed the thermo-stable component of the seed meal otherwise the ADC crude protein of the seed meal would have been so low even below 50% (as reported in Adeparusi and Jimoh, 2002; Bressani and Elias, 1977; Jaffe, 1973).

The high lipid digestibility coeffiejent of jackbean meal fed at varying level of inclusion to *Oreochromis niloticus* also agreed with that reported by Adeparusi and Jimoh (2002) who fed lima bean meal to *Oreochromis niloticus* and for some plant protein sources fed to *Oreochromis mossambicus* (Hossain *et al*, 1992). A similar report was made by Cho and Slinger (1979) for rainbow trout. A range of 76 to 97% fat digestibility for various sources of fat has been reported for channel catfish (Lovell, 1977). Andrew *et al* (1978) reported that the ability to digest fat appears to be influenced by temperature and the level of fat in the diet. The apparent fat digestibility was improved by the crack and cook processing methods.

The high apparent digestibility coefficient of energy recorded in this study was similar to the values recorded by Fagbenro (1998) for the same seed meal fed to *Oreochromis niloticus*. A similar high value was reported by Fagbenro (1998, (Unpublished) of the same seed meal fed to *Oreochromis niloticus*. Crack and cook processing methods improved energy digestibility, a similar report by Lovell and Durve (1982) revealed that cooking improves the gross energy digestibility.

The organic matter digestibility coefficient reported in this study agreed with Fagbenro (1998) and Olvera *et al* (1988) but lower than the values reported in Martinez-Palacios *et al* (1988), the variation may be attributed to processing methods and/or experimental methodology.

The results of the physico-chemical parameters presented in this study fall within the acceptable range for tilapia culture (Balarin & Hatton, 1979).

The cost of producing a kg of feed reduced with increasing inclusion of jackbean meal, this is because of the relatively cheaper price of jackbean meal when

compared with soyabean meal .This is encouraging more so that the test diets (jackbean meal diets) gave a a positive growth performance when compared with control diets.

The incidence of cost analysis of feeding jackbean meal to *Oreochromis niloticus* clearly showed the impact of the cost of feed on the variable cost of fish production since maintenance of aquaculture depends on economic viability and relative profitability (Adeparusi and Balogun, 1999). The profit index reveals the profitability of the diets fed to *Oreochromis niloticus*. A profit index above 1 showed that it is profitable to feed the fish with the diets. Although there was a general increase in profit index observed with increasing level of jackbean meal except for diet D40; an indication of the quality of test diet up to 30% replacement level.

Profits remain the primary interest on most capital investment ,the gross profit and loan repayment where applicable, form the basis for operational evaluation (Faturoti,1989). Diet D20 gave the best economic returns using gross profit as an index of assessment followed by diets D30,CTR,D40.The economic implication of using the different dietary treatment might not be well appreciated since the margin is too small but it will be much more explicit when the magnitude of total cost and expected revenue of its large scale operation is critically and objectively considered (Faturoti , 1989). Adeparusi and Balogun ,(1999) reported profit margin as increasing when fishmeal was replaced by roasted pigeon pea meal in a diet fed to *Clarias gariepinus*.The reduced profit margin on D40 was due to lower rate of growth of fish fed on this diet.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

The results of the feeding trial in the study have evidently confirmed that crack and cook processing method completely eliminated the thermo-labile component of the *Canavalia* seed and it has shown in the growth performance, nutrient utilization and bioavailability (digestion, absorption and utilization) by *Oreochromis niloticus* fed the processed jackbean meal that it can conveniently replace soybean meal even at higher level of inclusion (40%). This is evident in the satisfactory results of FCR, PER, PPV, ADC, which fall within acceptable and profitable level of a prospective fish farmer.

Further experiments could be directed towards complete replacement of soybean meal or partial replacement of fish meal up to 50% by jackbean meal so as to assert the effectiveness of the meal as an alternative protein sources not only in tilapia diet but other culturable fish species.

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APPENDIX

PROTEIN IN FISH

	CTR	D20	D30	D40
i	17.60	17.24	16.30	16.25
ii	17.90	17.01	16.93	16.43
iii	17.10	17.14	16.87	16.82
SQUARE ROOT TRANSFORMATION				
I	4.20	4.15	4.04	4.03
ii	4.23	4.12	4.12	4.05
iii	4.14	4.14	4.13	4.10
Σx	12.57	12.41	12.29	12.18
X	4.19	4.14	4.09	4.06

LIPID IN FISH

	CTR	D20	D30	D40
i	9.25	9.14	9.24	8.83
ii	9.47	9.82	8.78	8.96
iii	9.82	9.32	8.92	9.03
SQUARE ROOT TRANSFORMATION				
I	3.04	3.02	3.04	2.97
ii	3.08	3.14	2.96	2.99
iii	3.13	3.05	2.99	3.01
Σx	9.32	9.21	8.99	8.97
x	3.08	3.07	3.00	2.99

ANOVA TABLE

	DF	SS	MS	FCAL	FTAB
atment	3	0.0280	0.0093	6.2	4.07
or	8	0.0121	0.0015		*
al	11	0.0401			

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.0212	0.007067	3.56	4.07
Error	8	0.0159	0.001988		ns
Total	11	0.0371			

LSD Value = 0.0729

CTR – D20 = 0.05 ns

CTR – D30 = 0.10 *

CTR – D40 = 0.13 *

D20 – D30 = 0.05 ns

D20 – D40 = 0.08 *

D30 – D40 = 0.03 ns

* There is significant difference (P < 0.05)

ns There is no significant difference (P > 0.05)

ASH IN FISH

	CTR	D20	D30	D40
I	4.36	3.99	3.48	3.97
Ii	3.75	3.92	3.38	3.96
iii	3.80	3.73	3.52	3.74
SQUARE ROOT TRANSFORMATION				
I	2.08	1.99	1.87	1.99
Ii	1.94	1.98	1.84	1.98
iii	1.95	1.93	1.88	1.93
Σx	5.97	5.90	5.59	5.90
X	1.99	1.97	1.86	1.97

ANOVA TABLE

SV	DF	SS	MS	Fcal	Ftab
Treatment	3	0.0289	0.00963	4.48	4.07
Error	8	0.0172	0.00215		*
Total	11	0.0461			

LSD Value = 0.087

CTR – D20 = 0.02 ns

CTR – D40 = 0.02 ns

CTR – D30 = 0.13 *

D20 – D40 = 0.00 ns

D20 – D30 = 0.11 *

D40 – D30 = 0.11 *

PROTEIN IN FAECES

	CTR	D20	D30	D40
i	9.19	13.61	13.92	12.64
ii	8.69	10.06	11.11	16.10
iii	8.59	9.25	11.01	13.28
SQUARE ROOT TRANSFORMATION				
i	3.03	3.69	3.73	3.56
ii	2.94	3.17	3.33	4.01
iii	2.93	3.04	3.31	3.64
Σx	8.90	9.90	10.37	11.21
x	2.97	3.30	3.46	3.74

ANOVA TABLE

SV	DF	SS	MS	Fcal	F_{tab}
Treatment	3	0.9283	0.309	5.24	4.07
Error	8	0.4705	0.059		*
Total	11	1.3988			

LSD Value = 0.4573

D40 – D30 = 0.28 ns

D40 – D20 = 0.44 ns

D40 – CTR = 0.77 *

D30 – D20 = 0.16 ns

D30 – CTR = 0.49 *

D20 – CTR = 0.33 ns

* There is significant difference ($P < 0.05$)

ns There is no significant difference ($P > 0.05$)

LIPID IN FAECES

	CTR	D20	D30	D40
i	3.68	3.66	2.13	3.60
ii	2.13	2.25	3.10	3.12
iii	3.53	3.23	2.98	3.28
SQUARE ROOT TRANSFORMATION				
I	1.92	1.91	1.46	1.90
ii	1.46	1.50	1.76	1.77
iii	1.88	1.80	1.73	1.81
Σx	5.26	5.21	4.95	5.48
x	1.75	1.74	1.65	1.83

NFE IN FAECES

	CTR	D20	D30	D40
i	59.48	54.82	55.13	54.74
ii	62.25	59.06	57.59	52.37
iii	60.64	59.50	58.10	55.33
TRANSFORMATION ($Sin^{-1}\sqrt{p}$)				
I	50.48	47.77	49.95	47.72
ii	52.09	50.22	49.37	46.36
iii	51.14	50.48	49.66	48.06
Σx	153.71	148.47	148.98	142.14
x	51.24	49.49	49.66	47.38

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.0482	0.016	0.46	4.07
Error	8	0.2834	0.035		ns
Total	11	0.3316			

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	22.57	7.52	7.9	4.07
Error	8	7.57	0.95		
Total	11	30.14			

LSD Value = 1.8352

CTR – D30 = 1.75 ns

CTR – D20 = 1.58 ns

CTR – D40 = 3.86 *

D30 – D20 = 0.17 ns

D30 – D40 = 2.28 *

D20 – D40 = 2.11 *

There is significant difference ($P < 0.05$)

s There is no significant difference ($P > 0.05$)

ASH IN FAECES

	CTR	D20	D30	D40
i	12.94	14.89	14.30	14.10
ii	12.12	14.35	14.11	13.98
iii	12.13	14.10	13.96	13.86
SQUARE ROOT TRANSFORMATION				
I	3.60	3.73	3.78	3.76
Ii	3.48	3.79	3.76	3.74
Iii	3.48	3.76	3.74	3.72
Σx	10.56	11.28	11.28	11.22
X	3.52	3.76	3.76	3.74

FIBRE IN FAECES

	CTR	D20	D30	D40
I	3.76	4.11	4.64	4.87
Ii	3.86	4.37	4.21	4.37
iii	4.16	4.01	4.07	4.20
SQUARE ROOT TRANSFORMATION				
I	1.93	2.03	2.15	2.21
Ii	1.96	2.09	2.05	2.09
iii	2.03	2.00	2.02	2.05
Σx	5.92	6.12	6.22	6.35
X	1.97	2.04	2.07	2.12

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.1233	0.0411	25.2	4.07
Error	8	0.013	0.00625		
Total	11	0.1363			

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.0329	0.01097	2.69	4.07
Error	8	0.0326	0.004075		ns
Total	11	0.655			

LSD Value = 0.1489

D20 – D30 = 0.00 ns

D20 – D40 = 0.02 ns

D20 – CTR = 0.24 *

D30 – D40 = 0.02 ns

D30 – CTR = 0.24 *

D40 – CTR = 0.22 *

* There is significant difference ($P < 0.05$)

ns There is no significant difference ($P > 0.05$)

ENERGY IN FAECES

	CTR	D20	D30	D40
I	330.55	336.25	324.81	329.87
Ii	324.58	320.25	328.19	335.17
Iii	330.61	326.73	328.58	332.88
Σx	985.74	983.23	981.58	997.92
X	328.58	327.74	327.19	332.64

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	54.7898	18.2633	0.8	4.07
Error	8	176.2699	22.0377		ns
Total	11	231.0597			

A O M DIGESTIBILITY

	CTR	D20	D30	D40
I	72.30	61.21	60.93	55.02
Ii	57.58	65.43	66.05	60.66
iii	64.12	75.36	69.60	68.92
TRANSFORMATION ($Sin^{-1}\sqrt{p}$)				
I	58.24	51.48	51.31	47.88
Ii	49.36	53.99	54.36	51.16
Iii	53.20	60.24	56.54	56.12
Σx	160.80	165.71	162.21	155.16
X	53.60	55.24	54.07	51.72

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	19.2634	6.42	0.39	4.07
Error	8	128.5891	16.07		ns
Total	11	147.8525			

* There is significant difference ($P < 0.05$)

ns There is no significant difference ($P > 0.05$)

A O M – APPARENT ORGANIC MATTER

PROTEIN DIGESTIBILITY

	CTR	D20	D30	D40
I	89.57	85.83	84.46	84.62
ii	90.13	89.52	87.60	80.41
iii	90.25	90.37	87.71	83.84
SQUARE ROOT TRANSFORMATION				
I	3.23	3.76	3.94	3.92
ii	3.14	3.24	3.52	4.43
iii	3.12	3.10	3.51	4.02
Σx	9.49	10.10	10.97	12.37
x	3.16	3.37	3.66	4.12

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	1.5605	0.52	8.67	4.07
Error	8	0.5153	0.06		*
Total	11	2.0758			

LSD Value = 0.4612

D40 – D30 = 0.46 ns

D40 – D20 = 0.75 *

D40 – CTR = 0.96 *

D30 – D20 = 0.29 ns

D30 – CTR = 0.50 *

D20 – CTR = 0.21 ns

ENERGY DIGESTIBILITY

	CTR	D20	D30	D40
i	73.71	75.40	74.53	71.36
ii	74.19	76.57	74.24	70.90
iii	73.71	76.10	74.21	71.10
TRANSFORMATION ($Sin^{-1}\sqrt{p}$)				
I	59.15	60.27	59.69	57.65
ii	59.47	61.05	59.50	57.35
iii	59.15	60.73	59.48	57.48
Σx	177.77	182.05	178.67	172.48
x	59.26	60.68	59.56	57.49

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	15.69	5.23	90.95	4.08
Error	8	0.46	0.0575		*
Total	11	16.15			

LSD Value = 0.45

D20 – D30 = 1.12 *

D20 – CTR = 1.42 *

D20 – D40 = 3.19 *

D30 – CTR = 0.30 ns

D30 – D40 = 2.09 *

CTR – D40 = 1.77 *

LIPID DIGESTIBILITY

	CTR	D20	D30	D40
i	87.86	88.19	93.10	87.00
ii	92.98	92.74	89.95	88.74
iii	88.36	89.58	90.34	88.16
SQUARE ROOT TRANSFORMATION				
I	3.48	3.44	2.63	3.61
Ii	2.65	2.69	3.17	3.36
Iii	3.41	3.23	3.11	3.44
Σx	9.54	9.36	8.91	10.41
X	3.18	3.12	2.97	3.47

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.3958	0.1319	1.08	4.07
Error	8	0.931	0.1226		ns
Total	11	1.3268			

HEPATOSOMATIC INDEX

	CTR	D20	D30	D40
i	1.28	1.30	1.28	1.28
ii	1.19	1.31	1.27	1.22
iii	1.17	1.25	1.18	1.28
SQUARE ROOT TRANSFORMATION				
I	1.13	1.14	1.13	1.13
ii	1.09	1.15	1.13	1.11
iii	1.08	1.12	1.07	1.13
Σx	3.30	3.41	3.33	3.37
x	1.10	1.14	1.11	1.23

ANOVA TABLE

SV	DF	SS	MS	F_{CAL}	F_{TAB}
Treatment	3	0.0040	0.00133	3.8	4.07
Error	8	0.0028	0.00035		ns
Total	11	0.0068			

* There is significant difference ($P < 0.05$)

ns There is no significant difference ($P > 0.05$)

PERCENTAGE WEIGHT GAIN

	CTR	D20	D30	D40
I	493.38	466.45	392.25	307.15
Ii	470.79	463.19	360.15	355.08
Iii	473.77	489.91	374.42	282.76
SQUARE ROOT TRANSFORMATION				
I	2.22	2.16	1.98	1.75
Ii	2.17	2.15	1.90	1.88
Iii	2.18	2.21	1.94	1.68
Σx	6.57	6.52	5.82	5.31
X	2.19	2.17	1.94	1.77

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.36	0.12	24	4.08
Error	8	0.04	0.005		*
Total	11	0.40			

LSD Value = 0.133

CTR – D20 = 0.02 ns

CTR – D30 = 0.25 *

CTR – D40 = 0.42 *

D20 – D30 = 0.23 *

D20 – D40 = 0.40 *

D30 – D40 = 0.17 *

* There is significant difference ($P < 0.05$)

ns There is no significant difference ($P > 0.05$)

PROTEIN PRODUCTIVE VALUE

	CTR	D20	D30	D40
i	25.70	27.83	23.45	26.53
ii	27.82	24.17	29.71	29.26
iii	31.60	25.66	29.27	32.02
TRANSFORMATION ($Sin^{-1}\sqrt{p}$)				
I	30.46	31.84	28.96	31.00
ii	31.83	29.45	33.03	32.75
iii	34.20	30.44	32.25	34.76
Σx	96.49	91.73	94.74	98.21
x	32.16	30.58	31.58	32.74

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	7.65	2.55	0.77	4.07
Error	8	26.36	3.30		ns
Total	11	34.01			

FOOD CONVERSION RATIO

	CTR	D20	D30	D40
I	1.37	1.39	1.47	1.63
ii	1.36	1.51	1.48	1.75
iii	1.37	1.54	1.62	1.55
Σx	4.10	4.44	4.57	4.93
x	1.37	1.48	1.52	1.64

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.12	0.04	6.55	4.07
Error	8	0.05	0.0061		*
Total	11	0.17			

LSD Value = 0.1471

D40 – D30 = 0.12 ns

D40 – D20 = 0.16 *

D40 – CTR = 0.27 *

D30 – D20 = 0.04 ns

D30 – CTR = 0.15 *

D20 – CTR = 0.11 ns

PROTEIN EFFICIENCY RATIO

	CTR	D20	D30	D40
I	2.43	2.41	2.27	2.06
ii	2.46	2.16	2.25	1.92
iii	2.44	2.16	2.04	2.16
Σx	7.33	6.73	6.56	6.14
x	2.44	2.24	2.19	2.05

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.24	0.08	6.15	4.07
Error	8	0.10	0.013		*
Total	11	0.34			

LSD value = 0.2146

CTR – D20 = 0.20 ns

CTR – D30 = 0.25 *

CTR – D40 = 0.39 *

D20 – D30 = 0.05 ns

D20 – D40 = 0.19 ns

D30 – D40 = 0.14 ns

* There is significant difference ($P < 0.05$)

ns There is no significant difference ($P > 0.05$)

SPECIFIC GROWTH RATE

	CTR	D20	D30	D40
i	2.54	2.54	2.28	2.17
ii	2.49	2.50	2.18	2.01
iii	2.50	2.47	2.22	1.92
SQUARE ROOT TRANSFORMATION				
I	1.59	1.59	1.51	1.47
ii	1.58	1.58	1.48	1.42
iii	1.58	1.57	1.49	1.39
Σx	4.75	4.74	4.48	4.28
x	1.58	1.58	1.49	1.43

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	0.046	0.015	15.33	4.08
Error	8	0.009	0.001		*
Total	11	0.055			

LSD Value = 0.0595

CTR – D20 = 0.00 ns

CTR – D30 = 0.09 *

CTR – D40 = 0.15 *

D20 – D30 = 0.09 *

D20 – D40 = 0.15 *

D30 – D40 = 0.06 *

WEIGHT GAIN

	CTR	D20	D30	D40
I	30.59	31.57	25.84	20.18
ii	30.46	29.20	24.85	18.21
iii	30.51	29.32	22.54	21.34
Σx	91.36	90.09	73.23	59.73
x	30.52	30.03	24.41	19.19

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	228.30	76.1	42.5	4.01
Error	8	14.32	1.79		
Total	11	242.62			

LSD Value = 2.519

CTR – D20 = 0.49 ns

CTR – D30 = 6.11 *

CTR – D40 = 11.33 *

D20 – D30 = 5.62 *

D20 – D40 = 10.84 *

D30 – D40 = 5.22 *

* There is significant difference ($P < 0.05$)

ns There is no significant difference ($P > 0.05$)

FEED INTAKE

	CTR	D20	D30	D40
C	42.00	43.10	37.60	33.10
ii	41.54	45.00	36.70	32.80
iii	41.80	45.20	33.10	31.80
Σx	125.34	133.30	107.40	97.70
x	41.78	44.43	35.80	30.57

ANOVA TABLE

SV	DF	SS	MS	FCAL	FTAB
Treatment	3	268.119	83.373	44.29	4.07
Error	8	15.0597	1.8825		*
Total	11	280.179			

LSD Value = 2.5833

D20 – CTR = 2.65 *

D20 – D30 = 8.63 *

D20 – D40 = 11.86 *

CTR – D30 = 5.98 *

CTR – D40 = 9.21 *

D30 – D40 = 3.23 *

PROTEIN INTAKE

	CTR	D20	D30	D40
I	12.60	12.90	11.30	9.90
ii	12.40	13.50	11.04	9.80
iii	12.50	13.60	11.00	9.50
Σx	37.50	40.00	33.34	29.20
x	12.50	13.33	11.11	9.73

ANOVA TABLE

SV	DF	SS	MS	FCAL	FT
Treatment	3	22.5484	7.5161	134.67	4.0
Error	8	0.4464	0.0558		*
Total	11	22.9948			

LSD Value = 0.4448

D20 – CTR = 0.83 *

D20 – D30 = 2.22*

D20 – D40 = 3.6 *

CTR – D30 = 1.39 *

CTR – D40 = 2.77 *

D30 – D40 = 1.33 *

* There is significant difference ($P < 0.05$)

ns There is no significant difference ($P > 0.05$)