EVALUATION OF KOLANUT POD HUSK AS SUBSTITUTE FOR MAIZE IN THE DIET OF AFRICAN CATFISH (*Clarias gariepinus*)

BY

RABIU HALIMAT OLATOKUNBO

(FAQ/12/0472)

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF FISHERIES AND AQUACULTURE

IN PARTIAL FULFILLMENT OF REQUIREMENT FOR THE AWARD OF BACHELOR OF FISHERIES AND AQUACULTURE (B.FAQ)

IN THE FACULTY OF AGRICULTURE,

FEDERAL UNIVERSITY OYE-EKITI,

NIGERIA.

DECLARATION

I declare this work entitled "**EVALUATION OF KOLANUT POD HUSK AS A SUBSTITUTE FOR MAIZE IN THE DIET OF AFRICAN CATFISH** (*Clarias* ***gariepinus***) has been carried out by me in the department of fisheries and aquaculture under the supervision of Dr. J. B Olasunkanmi.

Rabiu Halimat Olatokunbo

Signature

18/12/2017

Date

CERTIFICATION

This is to certify that RABIU HALIMAT OLATOKUNBO with matric no FAQ/12/0472 carried out this project work in the department of Fisheries and Aquaculture of Federal University, Oye Ekiti.

DR. J. B. OLASUNKANMI (B.Sc,M.Sc,PGDE,PhD(Ibadan)
SUPERVISOR

10/01/2018
DATE

DR. T. O. BABALOLA
HEAD OF DEPARTMENT

DATE

DEDICATION

This project is dedicated to God Almighty the helper in ages to come whom in his infinite mercy made this project a successful one both in provision of life, financially , mentally and academically and also to my parent.

ACKNOWLEGEMENTS

To my ever Faithful Father, The One that knew me before I was born, My Rock and shield, the author and the finisher, I render all the gratitude to ALMIGHTY GOD for been all there at all times.

I feel pleasure to express my sincere gratitude to my supervisor Dr J.B Olasunkanmi for his fatherly advice, relentless effort, guidance, supervision, encouragement, warm-hearted intelligence, contribution, moral attention and understanding during the period of this project.

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ABSTRACT

The evaluation of Kolanut pod husk (KPHM) as substitute for maize in the diet of African catfish (*Clarias gariepinus*) was investigated for period of eight (8) weeks. KPHM was substituted for maize at 0% (control (T1)), 25% (T2), 50% (T3), 75% (T4) and 100% (T5) in the five different diets .*C. gariepinus* fingerlings (average Weight 3.02g to 3.07g) were randomly distributed into plastic tanks with 10 fish/tank in triplicate treatments and were fed twice daily at 8:00am and 4:00pm per day. Data obtained were subjected to statistical analysis using one-way ANOVA. Results showed that weight gain, specific growth rate and protein efficiency ratio was significantly higher in the control diet and this progressively decreased as inclusion level increased. The best FCR value was recorded in control and worst in T5. Pack cell volume (PCV), Red blood cell (RBC), haemoglobin (Hb), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Lymphocyte values were significantly higher ($p<0.05$) in T3 .White blood cell (WBC) was significantly higher ($p<0.05$) in T5 and lowest in control. Neutrophil was significantly higher ($p<0.05$) in T4 while lowest value was recorded in T2. In conclusion, the result of this study on KPHM as a feed stuff (major energy source) in fish diets revealed that kola pod husk contain sufficient energy to compare favourably with maize, however, the best inclusion level obtain in this study is 25% KPHM.

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CHAPTER ONE

1.0 INTRODUCTION

The major recurrent cost in fish production is feed; this alone has progressively taken the larger share of the cost of production so much that total feed cost accounts for over 60% of total production cost. Tiamiyu *et al.* (2007) reported that feed may account for between 60 – 80% of production cost for fish hence, the need to focus on least cost feed through the use of unconventional feed stuff. Conventional feed ingredients used in fish production are becoming increasingly expensive due to competition from humans; therefore efforts have been channelled to investigating other alternative feed ingredient which are unconventional in nature and are not edible by man as possible replacement for conventional feed ingredients in the diet of fish (FAO, 2002).

The fact that the availability of the world's raw materials is dwindling as population grows exponentially, together with the real threat of global food shortages, contributes to a growing awareness of the need for conservation and the re-use of things which once would have been thrown away without a second thought. Agro-industrial by-products and crop wastes/ residues such as wheat offals, maize wastes, palm kernel cake, cassava peels, rice bran, cocoa pod husk, kolanut husk, kola testa etc. have proved to be valuable in replacing a certain proportion of maize in monogastric nutrition (Ogbonna and Adebowale, 2009). Considerable quantities of these crop residues and agro-industrial wastes suitable for use as feed or feed components are lost or under-utilized. Oluokun and Olalokun (2015) reported that Nigeria produced, 2 million metric tonnes of kolanut annually which represented 70% of world kolanut production, and majority of this kola were produced in the forest zone of south western Nigeria.

Kolanut seeds has found usefulness in some industries for the production of commodities such as drugs, wines beverages, liquid soap and formulation of animal feed just to mention a few. The husk and white shell on the other hand has been regarded as waste until recently, when its nutritive quality was reported. Kolanut husk was analysed for its nutritive value by Hamzat and Adeola (2008) for the formulation of poultry feeds. The waste was nutritive but concluded that it's not suitable for poultry feed due to its high fiber content. A study carried out by Arotupin *et al.* (2007) on microorganisms, proximate and anti-nutrient content of *Cola nitida* white shell also indicated the nutritive value of kolanut white shell, showing that these agro-wastes can find usefulness.

Kola tree is a tropical tree which belongs to the family Sterihaceace; it is mostly common in the rain forest region of West Africa. This crop is of socio-economic importance. There are over 40 kola species, out of which *Cola nitida* and *Cola acuminata* are of major economic and social importance in Nigeria (Egbe and Oladokun, 2009). They are used industrially for the manufacturing of different types of soft drink flavour; caffeine contained in kola nuts is useful as a fat burner (Blades, 2000).

Many Agricultural by-products that are considered as wastes in Nigeria have great potentials as animals feed ingredients if properly handled, processed and incorporated into rations. A good example of such wastes is kola pod husk (KPH). Nigeria is one of the largest producers of Kola and Kola pod husk, kola pod husk constitute 75% of the kola fruit. It has been reported to have 13% crude protein and energy of 2546.9kcal/kg (NRC, 2007) hence has high potential for consideration as possible substitute to conventional feedstuffs.

Kolapod husk is a by-product from processing the seeds Cola acuminata and Cola nitida and its nutritive quality has been reported (Oguntuga, 2010; Oluokun and Olalokun, (2015); Olubamiwa *et al.* 2000 ;(Babatunde and Hamzat,(2005) reported that kolanut husk meal (KHM) shared similarity with cocoa pod husk (CPH) but had higher crude protein and lower crude fibre contents than KPHN. The KHM contained (g/kg); 139 crude protein, 173crude fibre, 12.5crude fat, 58.5 ash (Oluokun and Olalokun, 2015) and 5562 kcal/kg gross energy (Babatunde and Hamzat, 2005). Oguntuga (2010 reported 0.02-0.08%

theobromine (a toxic alkaloid) in KHM. The cellulose content is 8.1-9.0%, carbohydrate 52.4%, caffeine 3.2% and nicotine 0.2(Opeke, 2012).

The paucity of information on the nutritive values of Kola pod husk in the nutrition of African catfish necessitated this research which seeks to evaluate the growth performance and nutrient utilization of African catfish fed varying levels of Kola pod husk as replacement for maize.

1.1 RESEARCH PROBLEMS

In order to sustain the high growth of the aquaculture industry, it is imperative to increase fish feed production (Francis *et al.*, 2001) because fish feed accounts for 60-80% of variable cost of production (New, 1993).

However the scarcity of these plants sources and competition from other sectors for such conventional crops for livestock and human consumption as well as industrial use make their costs too high and places them far beyond the reach of fish farmers or producers of aqua-feeds (Ayinla, 1988; Fasakin *et al.,* 1999; Omitoyin, 2007). Therefore, in an attempt to attain a more economically sustainable and viable production, research interest has been directed towards the evaluation and use of unconventional or lesser utilized energy sources.

Previously feedstuffs like maize (*Zea mays*), sorghum (*Sorghum bicolor*), soybeans (*Glycine max*), groundnut (*Arachis hypogea*) and fishmeal were used in preparing fish pellets. However, now they face serious competition from man and his livestock as food sources in Nigeria. Since the use of maize in fish feed formulation is not cost effective, efforts are being directed globally towards discovering unconventional, cheaper, readily available and highly digestible alternative energy sources of feedstuff for fish. However attempts at searching for these unconventional sources as supplement or total replacement is a difficult task (Ayinla, 1988). In Nigeria one of the plant energy source that have been extensively investigated for possible use as ingredient for fish feed formulation are plaintain peel (Agbabiaka,et.al 2013), Sweet potato peel (Olukunle, 2006), cassava leaf (Bichi 2010), sweet potato peel (Omoregie et.al 2009) etc.

In view of all the aforementioned, this research intends to explore the potentials of using graded levels of *kola pod husk* as energy sources in the diet of *Clarias gariepinus* fingerlings

1.2 JUSTIFICATION

The African catfish, *Clarias gariepinus* is widely cultivated in Nigeria because of its ability to consume supplementary feeds, good conversion of feed to flesh, resistance to diseases, ability to reproduce in captivity, fast growth rate and tolerance to a wide range of environmental condition (Ayinla, 2013). The economic importance of this species has increased tremendously in recent years as a result of its extensive use in aquaculture (de Graaf and Janseen, 2011).

However, Kola-pod husk is a by-product during Kolanut production and over 1.5million tones is disposed of in Nigeria annually, constituting environmental nuisance and costing the government so much money to render it physically, chemically and biologically harmless. Harnessing this by- products and utilizing it in the formulation and compounding of Livestock (especially fish) feed, will not only eradicate its environmental disturbance in the kola nut producing areas, but will remove the rivalry for maize between man and animals, thus bringing about a great reduction in the cost of producing livestock feed and hence reducing the cost of producing fish.

1.3 OBJECTIVES OF THE STUDY

General objective is to determine the growth response and feed utilization of *Clarias gariepinus* fingerlings fed with graded dietary levels of kola pod husk meal.

The specific objectives are to

Study the effects of feeding kola pod husk on the haematology of *C. gariepinus.*

Determine the potential replacement level of kola pod husk for maize in the diet of *C. gariepinus.*

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW

In the wild, fish obtain food naturally from aquatic environment; this food may be phytoplankton or zooplanktons, insects, seeds and small fish. However, under culture condition the natural feeds are not adequate for optimum growth therefore there is need for supplementary feeds to help fortify the naturally available diet with extra protein, carbohydrate, lipid, minerals and vitamin (Alatise and Okoye, 2009; Lim and Dominy,2012).

The quality and quantity of feed used in fish culture are the major factors in determining profitability because feed represents the largest single expenditure in semiintensive or intensive culture operations. Economical production depends on availability of least-cost nutritionally balanced diet (Lim and Dominy, 2012). In Nigeria the high cost of feed inputs is a major problem of fish farmers in intensive and semi-intensive fish farming culture system (Ayinla, 2007; Fagbenro and Davis, 2003). Nutrition is critical in fish production since it accounts for 40-80% of production cost (Igeofagha, 1979; Eyo, 1990; New, 2005; Prendagast *et al.*, 2013).

2.2 ORIGIN OF KOLA

The genus Kola of the family *sterculiaceae* is indigenous to tropical Africa and has its center of greatest diversity in the forest areas of West Africa (Russel, 2010). Kola is perhaps second only to palm oil in importance in the list of indigenous cash crops in West Africa. About 40 kola species have been described in West Africa. However, in Nigeria, the kola species of real importance are C.ola *acuminate* and Cola *nitida* (Quarcoo,2014;(Daramola,2013) Cola *acuminata* and Cola *nitida* are important economic crops in the forest areas of west and central Africa, carribean islands, mauritivs, Sri Lanka and Malaysia (Eijnatten,2015);(Oladokun, 2015). The cultivation of cola nitida in Nigeria began sometime in the 19th

century. The *nitida*—goro was observed not to be abundantly in the outta bush in 1854 while its cultivation was noted in Egba Division in 1902 and in Labochi and envious in 1901. From Agege, cola *nitida* cultivation presumably spread to the forest areas following first the course of the railway line into Abeokuta, Ibadan and Offa replacing the local kola *acuminata* and penetrating later along streams and river banks into the Guinea savannah and at present, south and Eastern state (Eijnatte, 2015).

Kola nuts have for hundreds of years served as an important article of internal trade in Nigeria and other parts of Africa (Nzekwu, 2011). It has been an item of trade in West Africa and in the Trans-saharan trade routes for many countries (Egbe and Sobamiwa, 2009). Kola nut is used as a masticatory stimulant by Africans and has numerous uses in social, religious, ritual and ceremonial functions by the natives in the forest region of Africa it is used during ceremonies related to marriage, child naming, installation of chiefs, funeral and sacrifices made to the various gods of Africa Mythology (Nzekwu, 2011) ;(Daramola, 2013). There is also increasing demand for its usage in pharmaceuticals industries and for production of soft drinks, wines, and candles (Beattie, 2012); Ogutuga, 2010). Its uses have inevitably created a high demand in excess of its production (Oladokun, 2015). Kola nut has been used as abuse for a now brand of chocolate and wine (Kola chocolate and kola wine) which were developed by CRIN researchers (Famuyiwa, 2007).

Cola *nitida* which is referred to as the true kola of commerce has featured in the internal trade of west Africa for a number of centuries. Kola nuts are common sight in the Nigeria markets, cities and villages. They are often sold by street vendors at bus stops and train depots. Many Nigerians consume Kola nuts regularly, for medicinal, stimulating and sustaining properties.

2.3 DESCRIPTION

Kola nitida is an evergreen tree growing to a height of 12 to 20 meters (39-66ft). The leaves of *Cola* species are simple, entire and narrowed or rounded towards the base. The arrangement of the leaves on the stem is alternate in some species and verticiliate, in whorls of 3 or 4, in others.

The flowers of both *C. nitida* and *C. acuminata* have a white or coloured perianth. Typically, trees bare two types of flowers; male, with anthers fused into a single column or hermaphrodite with one or two rings of anthers at the base of the superior ovary. After fertilisation, the ovary divides forming separate fruiting carpels or follicles, usually five to ten in number. Fruits are sessile, placed at the end of a short peduncle, from which they radiate in star-shaped fashion. As the fruit increases in weight, the stem hangs vertically and the follicles are borne horizontally or ascending in recurred fashion, containing one to ten seeds.

The nuts of a small number of *Cola* species, including *C. nitida* and *C. acuminata,* are good to eat though most species produce seed that is hard and inedible. Some *Cola* species are polycotyledonous, e.g. *C. acuminata.* The seed of the edible species is ovoid or ellipsoid, or angular by compression, varying in size up to *5* cm long and 3 cm in wide. Most of the seed consists of cotyledons to which the minute embryo is attached. In *C. nitida* there are two cotyledons and the seeds readily split into half whilst in *C. acuminata,* where there are three or four cotyledons, sometimes as many as six, the seed splits into a correspond number of pieces (Irvine, (2002); Keay, (2005); Russell (2013)).

The kola nut is a caffeine-containing nut of the genus *Cola*, primarily of the species *Cola acuminata* and *Cola nitida*. *Cola acuminata*, an evergreen tree about 20 metres in height, has long, ovoid leaves pointed at both the ends with a leathery texture. The trees have yellow flowers with purple spots, and star-shaped fruit. Inside the fruit, about a dozen round or square seeds develop in a white seed-shell. The nut's aroma is sweet and rose-like. The first taste is bitter, but it sweetens upon chewing. The nut can be boiled to extract the cola. This tree reaches 25 meters in height and is propagated through seeds. *C. nitida* and *C. acuminata* can easily be interchanged with other *Cola* species.

Kolapod husk is a by-product from processing the seeds Cola acuminata and Cola nitida and its nutritive quality has been reported (Oguntuga, 1975; Oluokun and Olalokun, 2015; Olubamiwa et al. 2000 ;(Babatunde and Hamzat, 2005) reported that kolanut husk meal (KHM) shared similarity with cocoa pod husk (CPH) but had higher crude protein and lower crude fibre contents than KPHN. The KHM contained (g/kg) ; 139 crude protein, 173crude fibre, 12.5crude fat, 58.5 ash (Oluokun and Olalokun, 2015) and 5562 kcal/kg gross energy (Babatunde and Hamzat,2005). Oguntuga (2010) reported 0.02-0.08% theobromine (a toxic alkaloid) in KHM. The cellulose content is 8.1-9.0%, carbohydrate 52.4%, caffeine 3.2% and nicotine 0.2(Opeke, 2012).

2.4 ECOLOGY OF KOLANUT

Originally a tree of tropical rainforest, it needs a hot humid climate, but can withstand a dry season on sites with a high ground water level. It may be cultivated in drier areas where ground water is available. *C. nitida* is a shade bearer, but develops a better spreading crown which yields more fruits in open places. Though it is a lowland forest tree, it has been found at altitudes over 300 m on deep, rich soils under heavy and evenly distributed rainfall. Regular weeding is a must and can either be done manually or by using herbicides. Some irrigation can be provided to the plants, but it is important to remove the water through an effective drainage system, as excess water may prove to be detrimental for the growth of the plant.

When not grown in adequate shade, the kola nut plant responds well to fertilizers. Usually, the plants need to be provided with windbreaks to protect them from strong gales. Kola nuts can be harvested mechanically or by hand, by plucking them at the tree branch. When kept in a cool, dry place, kola nuts can be stored for a long time.

2.5 HEALTH BENEFIT OF KOLANUT

Kola nuts may possess antimicrobial properties; Lowe *et al. 2014* published a study in the *Advances in Biological Chemistry* suggesting that the kola nut shows promising results against microbes. The fruits showed antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonae, Pseudomonas aeruginosa and Candida albicans when compared with antibiotics and antifungals Gentamicin and Nystatin. Although more research is still being conducted, kola nuts have been used to treat malaria, fever, ringworm, scabies, gonorrhea, dysentery, cough, chest order and vomiting.

Kola nuts may help fight cancer; Solipuram et al. 2009 measured the chemoprotective properties of kola nuts, which contained phytoestrogens and phytoandrogens. These nonsteroidal chemicals mimic or disrupt sex-linked hormones like estradiol and dihydrotestosterone to prevent hormone-dependent cancers like breast and prostate cancers.

Kola nuts could help improve your mental well-being; Kola nuts are comprised of two percent caffeine, a stimulant. This psychoactive substance is absorbed into the bloodstream and travels to the brain to block an inhibitory transmitter named adenosine. Once that occurs, brain cells increase in firing rate because of the build-up of excitatory neurotransmitters like norepinephrine and dopamine. This process could help improve memory, boost energy levels, and increase reaction times Odugbemi (2008)

Kola nuts could help you suppress your appetite; Umoren et al. 2009 conducted an animal study monitoring mice that ate kola nuts and body weights for 28 days. They found that mice that ate chow with kola nuts ate less total chow than mice that did not eat kola nut. Also, the mice's body weight significantly dropped when compared with kola nuts.

Kola nuts could help you burn fat; a basal metabolic rate is the minimal rate of energy your body burns when not exercising or moving around. The amount of energy burned is measured in calories. When your metabolic rate increases, you burn more calories at rest. One of the main ingredients in fat burning supplements is caffeine. Research shows that caffeine can help boost your metabolic rate by 3-11 percent Adam *et al.* (2011)

Kola nuts can be used as a diuretic; Theobromine, formerly known as xantheose, is another abundant stimulant in kola nuts. This bitter chemical increased urine production which can help treat heart failure, liver cirrhosis, hypertension, influenza, water poisoning, and certain kidney diseases Ruxton (2008)

Kola nuts can help improve blood circulation to your body; Caffeine, theobromine, and kolanin can stimulate the heart rate, thus increasing circulation. This action can help boost oxygenation to certain parts of the body like the skin, organs, and brain. Increased oxygen levels in the brain have been linked with increased concentrating and higher cognition. James (2011)

In Benin Republic, fresh nut of C. nitida are chewed as aphrodisiac and the pounded fruit is used to treat ocular problem (Natabuo degbe).

2.6 USES OF KOLANUT POD HUSK

The kola pod husk is used to manufacture detergents (Oguntuga 2000).

The kola pod husk provides some essential materials for cloth drying (Famuyuwa, 1987).

Kola pod husk as also been utilized in the production of liquid soap Olubamiwa *et a*l., 2002).

Table 1; PROXIMATE COMPOSITION OF KOLA POD HUSK

Nutrient composition	%
Protein	12.26
Lipid	0.3
Fibre	23.2
Moisture	13.5
Ash	8.70
Carbohydrate	42.04

Table 2: AMINO ACID PROFILE OF KOLAPOD HUSK

	(%)
Taurine	0.08
Hydroxyproline	0.36
Aspartic acid	0.93
Threonine	0.40
Serine	0.39
Glutamic acid	0.92
Proline	0.47
Lanthionine	0.02
Glycine	0.44
Alanine	0.46
Cysteine	0.17
Valine	0.53
Methionine	0.14
Isoleucine	0.37
Leucine	0.61
Tyrosine	0.28
Phenylalanine	0.38
Hydroxylysine	0.01
Lysine	0.53
Histidine	0.22
Arginine	0.42
Tryptophan	0.04

(Source; Hamzat and Adeola; 2011)

Table 3: MINERAL COMPOSITION OF KOLAPOD HUSK

Mineral	%
Calcium (%)	0.66
Phosphorus (%)	0.16
Magnesium (%)	0.25
Potassium (%)	2.36
Sodium (%)	0.009
Sulphur (%)	0.15
Chloride ion (%)	0.44
Iron (ppm)	1122.00
Zinc (ppm)	36.00
Copper (ppm)	13.00
Manganese (ppm)	76.00
Molybdenum (ppm)	9.40
Cobalt (ppm)	3.55

(Source; Hamzat and Adeola; 2011)

2.6 TAXONOMY AND IDENTIFICATION OF *C. GARIEPINUS*

The African catfish *C. gariepinus* belong to the family of Clariidae and it is the widest cultured species in this family. It is characterized by the ability to grow on a wide range and tolerance to low dissolved oxygen adverse aquatic condition (Hulisman and Richter 1987; Fagbenro *et al* 1993).

They are found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems. The African catfish was introduced all over the world in the early 1980s for aquaculture purposes, so it is found in countries far outside its natural habitat, such as Brazil, Vietnam, Indonesia, and India.

The African catfish is a large, eel-like fish, usually of dark gray or black coloration on the back, fading to a white belly. In Africa, this catfish has been reported as being second in size only to the vundu of the Zambesian waters, although Fish Base suggests the African catfish surpasses that species in both maximum length and weight (Senanan et al., 2004). *C. gariepinus* has an average adult length of 1–1.5 m (3 ft 3 in–4 ft 11 in). It reaches a maximum length of 1.7 m (5 ft 7 in) TL and can weigh up to 60 kg (130 lb). These fish have slender bodies, flat bony heads, notably flatter than in the genus *Silurus*, and broad, terminal mouths with four pairs of barbels. They also have large accessory breathing organs composed of modified gill arches. Also, only the pectoral fins have spines.

2.7 NUTRIENT REQUIREMENTS OF CATFISH (*Clarias gariepinus)*

2.7.1 Energy requirement of *Clarias gariepinus*

Energy is one of the most important parts of the diet. Feeding standards for many animals are based on energy needs. Feed intake for catfish may be more a function of how much feed the catfish are allowed to have rather than energy concentration in the feed. Although catfish feed intake may not be strictly regulated by dietary energy, balance of dietary energy is important when formulating catfish feeds. This is true mainly because a lack of non-protein energy in the diet will result in the more expensive proteins being used for energy. Also, if dietary energy is too high, catfish may not eat as much, resulting to fewer intakes of essential nutrients. Too high of a dietary energy/protein ratio may lead to higher body fat, which may reduce dressed yield and shorten shelf life of frozen products

Estimates have been made by measuring weight gain or protein gain of catfish fed diets with a known amount of energy. Energy requirements reported for catfish, which have generally been expressed as a ratio of digestible energy (DE) to crude protein (DE/P), range from 7.4 to 12 kilocalorie/ gram (kcal/g). Based on current knowledge, a DE/P ratio of 8.5 to 9.5 kcal/g is adequate for use in commercial catfish feeds. Increasing the DE/P ratios of catfish diets above this range may increase fat deposition and reduce processed yield, and if the energy value is too low the fish will grow slowly. Carbohydrates and lipids (fats and oils) are the major energy sources for catfish.

2.7.2 Protein and Amino Acids of *Clarias gariepinus*

Protein comprises about 70 percent of the dry weight of fish muscle. A continual supply of protein is needed throughout life for maintenance and growth. Catfish, like other animals, need nitrogen and certain amino acids rather than protein as such. Usually the most economical source of these elements is a mixture of proteins in feedstuffs. Using protein for energy is expensive, so catfish feeds should be balanced to ensure adequate levels of nonspecific nitrogen, amino acids, and non-protein energy are supplied in the right amount. The requirements for proteins and amino acids have been studied in catfish for many years, but there is still a debate as to which level of dietary protein is most cost effective. How much protein is needed for the most economical gain may differ as the cost of feed ingredients varies. Also, it is difficult to set a level of protein that is best for all situations because of the factors that affect the dietary protein requirement of catfish. These include water temperature, feed allowance, and fish size, amount of non-protein energy in the diet, protein quality, natural food available, and management practices.

For greatest profits the optimum dietary protein level should be changed as fish and feed prices change. But in practice, most catfish producers feed a diet with the same amount of protein throughout the growing season. Commercial catfish feeds used for growing food fish typically contain either 28 or 32 percent protein. Diets containing lower levels of protein are adequate for maximum growth but may increase body fat. Catfish fry and small fingerlings require diets with more protein. Fry diets used in the hatchery should contain 45 to 50 percent protein, and fingerlings (less than 20 lbs/1,000) should be fed a 35 percent protein diet.

Catfish feeds generally contain a mixture of plant and animal proteins, but amino acid requirements of catfish can be met with a mixture of plant proteins alone. Major protein sources used in catfish feeds include soybean meal, cottonseed meal, meat and bone/blood meal, and fish meal. Although meat and bone/blood meal is a good source of protein for catfish, we do not recommend using beef products in catfish feeds because of the possibility it may thought of as being related to mad cow disease.

2.7.3 Minerals requirement of *Clarias gariepinus*

Apparently catfish need the same minerals for metabolism and bone development that other animals need. Catfish also require minerals for a balance between body fluids and their environment. They can absorb some of the minerals from the water. Fourteen minerals are considered to be essential to catfish.

Although mineral studies with fish are difficult to do, we know the mineral amounts they need, and we know the signs of mineral of deficiency. Phosphorus is particularly important in fish feeds because fish require a fairly large amount of it. Feedstuffs, especially those from plants, are poor sources of phosphorus, and fish do not get enough phosphorus from pond water. As a result, catfish feeds are usually supplemented with phosphorus. Dicalcium and defluorinated phosphates are commonly used as phosphorus supplements in catfish feeds.

Catfish feeds are typically supplemented with a trace mineral premix with enough of all essential trace minerals (minerals required at very low levels) to meet or exceed dietary requirements of catfish.

A trace mineral premix may not be needed in catfish feeds that contain 4 percent or more animal protein.

2.7.4 Vitamins requirement of *Clarias gariepinus*

Vitamins are a heterogeneous group of organic compounds essential for the growth and maintenance of animal life. The majority of vitamins are not synthesized by the animal body or at any rate sufficient to meet the animal's needs. they are distinct from the major food nutrients(proteins, lipids and carbohydrates) in that they are not chemically related to one another, are present in very small quantities within animal and plant foodstuffs, and are required by the animal body in trace amounts.

Approximately 15 vitamins have been isolated from biological materials; their essentiality depending on the animal, the growth rate of the animal, feed composition and the bacteria synthesizing capacity of the gastrointestinal tract of the animal. In general, all animals display distinct morphological and physiological deficiency signs when individual vitamins are absent from the diet. Craig and Helfrich (2002) reported that vitamin C is the most important since it's a powerful antioxidant and helps in the immune system of fishes. The fat soluble vitamins A, D, E, K) perform useful function in fish body Vitamin A (retinol) is important in vision; vitamin D (cholecalciferols) ensures bone integrity (Craig and Helfrich, 2002). Deficiency of almost any vitamin can result into increased susceptibility to disease and retard growth (Robert, 2006).Fish feeds are generally supplemented with a vitamin premix that contains all essential vitamins in sufficient quantities to meet the requirement and to compensate for losses due to feed processing and storage. Vitamins present in feedstuffs are usually not considered during feed formulation because their availability is not known, but they certainly contribute to the vitamin nutrition in catfish.

2.7.5 Lipid and fatty acids requirement of *Clarias gariepinus*

Dietary lipids are important sources of energy and fatty acids that are essential for normal growth and survival of fish. Although fish have a low energy demand, and is thus susceptible to deposition of excessive lipid (Earle, 2004). Lipids do have a role as carriers for fat soluble vitamins and steroids.

Lipids are important in the structure of biological membranes at both the cellular and sub cellular levels. They are components of hormones and precursors for synthesis of various functional metabolites such as prostaglandins, and are also important in the flavour and textural properties of the feed consumed by the fish (NRC, 1993).The use of lipids (fats and oils) in feeds is desirable because lipids are highly digestible sources of concentrated energy containing about 2.25 times as much energy as does an equivalent amount of carbohydrate (Eyo, 2002). Fish in general require fatty acids of longer chain length and a higher degree of unsaturation than mammals. Fish appear to have the ability to synthesize most of their fatty acids. Nutritionally, there may be no 'best' level of dietary lipid except that needed to provide essential fatty acids.

2.7.7 Carbohydrates requirement of *Clarias gariepinus*

Carbohydrates represent a broad group of substances which include the sugars, starches, gums and celluloses. The common attributes of carbohydrates are that they contain only the elements carbon, hydrogen ,oxygen ,and that their combustion will yield carbon dioxide plus one or more molecules of water. Carbohydrates make up three fourths of biomass of plants but are only present in small quantities in the animal body as glycogen, sugars and their derivatives. No dietary requirement for carbohydrates has been demonstrated in fish.

However, carbohydrates present a cheap energy source that would 'spare' the catabolism of other components such as protein and lipids to energy. Warm water fish can use much greater amounts of dietary carbohydrates than cold water and marine fish species (NRC, 1993).The utilization of carbohydrates by catfish appears to differ depending on the complexity of the carbohydrate. Starch or dextrin (partially hydrolysed starch) is used more efficiently by catfish than are sugars such as sucrose or glucose (Edwin and Meng, 2012). It has generally been thought that tilapia and certain other fish resemble diabetic animals by having insufficient insulin for maximum carbohydrate utilization (Dupree and Hunter2010).

However information has shown that insulin levels are about the same as those found in mammals, which indicates fish are not diabetic. Glucose is highly digestible in tilapia but a large portion is excreted (Edwin and Meng, 2012). Although catfish use carbohydrates effectively, there is no dietary requirement for carbohydrates. Carbohydrates are important dietary components as an in expensive energy source as precursors for various metabolic intermediates such as non-essential amino acids and fatty acids (Dupree and Hunter, 2010) and as an aid in pelleting practical catfish feeds, and in reducing the amount of protein used for growth (Dupree and Hunter, 2010).

2.8 Taxonomy classification *Clarias gariepinus*

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Clariidae

Genus: *Clarias*

Species: ***C. gariepinus***

(Source; Gruntar and Fink 2004)

Plate 1: *Clariasgariepinus*

Source: (Burchell, 1822)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The research was conducted at the wet laboratory of the department of Fisheries and Aquaculture Federal University Oye-Ekiti, Ikole campus.

3.2 Collection and Processing of kola pod husk

Kola pod husks were obtained in Ikole local government area of Ekiti state, sun dried until a constant weight is obtained. The dried husks were milled and sieved to powder._Kola pod husk meal was collected and it was store in an air tight container. The kola pod husk meal was thereafter analysed for proximate composition according to AOAC (2000)

3.3 Fish diet formulation and processing

All the experimental diets in the study were formulated using Pearson Square method. 40% crude protein diets were prepared to meet protein requirement for the fish with KPH replacing maize meal at varying level Diet1 {Control (KPH 0%) Diet 2 (KPH 25%), Diet 3 (KPH 50%), Diet 4 (KPH 75%), Diet 5(KPH 100%)} as shown in Table 4. The feed ingredients for each of the Diet were weighed, ground, mixed thoroughly and warm water was added then stirred to form consistent dough which was passed through a 2mm die pelleting machine. The pellets produced were sundried to constant weight; the dried pellets were stored in in an air tight polythene bag and kept in a cool dry place.

Table 4: Percentage composition of experimental diet

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fishmeal	30	30	30	30	30
Kola pod husk meal	0	2.66	5.32	7.98	10.64
Maize	10.64	7.98	5.32	2.66	0
Groundnut cake	15.79	15.81	15.66	15.36	15.12
Soybean meal	30.53	30.19	30.17	30.07	30.01
Wheat offal	4.52	4.84	5.01	5.41	5.71
Methionine	1	1	1	1	1
Vitamin/mineral premix	2	2	2	2	2
Salt	0.5	0.5	0.5	0.5	0.5
Vitamin c	0.015	0.015	0.015	0.015	0.015
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Starch	2	2	2	2	2
Vegetable oil	3	3	3	3	3
Total	100	100	100	100	100

3.4 Experimental design and feeding trials

One hundred and fifty fingerlings of *Clarias gariepinus* were gotten from a private farm in Ikole Ekiti, Ekiti state. The fish were randomly distributed into 15 plastic tanks with 10 fish per tank. Each treatment was triplicate. The fish was acclimatized for ten (10) days.

Fish were starved for 24hous before the experiment diets was introduced to the fish. The fish were fed 5% of their body weight daily for 8weeks, 8am in the morning and 4pm in the evening. Fish in each treatment was weighed on weekly basis.

3.5 Growth performance and nutrients utilization parameters

3.5.1 Growth performance parameters

Fish response to treatments was measured using mean weight gain, average daily growth, specific growth rate, percentage weight gain, survival rate.

Mean weight gain

(MWG) = W1 – W0

Where W1 = Finial Weight

W0 = Initial Weight

Specific growth rate

$$(SGR) = \frac{\log \text{ of } W1 - \log \text{ of } w0}{T1 - TO} \times 100$$

Where: -W1 and w0 = final and initial weights of fish respectively

T1 and T0 = final and initial time of the experiment respectively

Average daily growth

$$(ADG) = \frac{\text{Mean weight gain}}{\text{Experimental period (in days)}}$$

% Weight gain

$$(WG) = \frac{\text{Mean weight gain}}{\text{Initial Mean weight}} \times 100$$

Mortality

$$(M) = \frac{\text{Number of fish dead at the end of the experiment}}{\text{Initial number of fish stocked}} \times 100$$

Survival Rate

$$(SR) = \frac{\text{Initial number of fish stocked} - \text{Mortality}}{\text{Initial number of fish stocked}} \times 100$$

Feed conversion Ratio

$$(FCR) = \frac{\text{Total weight of diet fed (g)}}{\text{Total Weight of fish (g)}}$$

3.6 Nutrients utilization parameters

Nutrient utilization was measured using food conversion ratio, protein efficiency ratio and productive protein valve as described by Adepoju (2008)

Feed conversion efficiency

$$(FCE) = \frac{\text{Weight gain}}{\text{Feed intake}} \times 100$$

Protein efficiency ratio

$$(PER) = \frac{\text{Mean weight gain (g)}}{\text{Crude protein intake (g)}}$$

Protein Intake

$$(PI) = \frac{\text{Total feed consumed} \times \text{\% Protein in feed}}{100}$$

Economic conversion ratio

(ECR) = Feed intake X Feed Conversion Ratio

3.7 Water quality assessment

Water quality parameters were monitored throughout the period of the experiment. Temperature, pH, dissolved oxygen, ammonia and nitrite will be measured.

3.8 Sample collection

At the end of feeding trial, fish blood samples were collected with heparinised bottle. Blood samples were obtained from the caudal vein of fish from each tank. Blood serum analysis were collected into bottles (EDTA Bottle)

3.9 Haematological Assessment

Haematological examination of the fish was carried out at the beginning and the end of the experiment in order to investigate the possible effect(s) on the feed on fish. Haematological parameters such as Haemoglobin, Red blood Cell (RBC), White blood Cell (WBC), Packed Cell Volume (PCV), Mean corpuscular (MCV), Mean corpuscular haemoglobin concentration (MCHC) were determined.

3.9.1 Packed cell volume (PCV): The heparinized capillary tubes were 3/4 filled with whole blood and one end sealed with plasticine. The tubes were centrifuged for 5 min in a micro-haematocrit centrifuge at 12,000 rpm. The PCV was read by the use of haematocrit reader (Kelly, 1979)

3.9.2 Red blood cell (RBC) and white blood cell (WBC) counts: The RBC and total WBC counts were carried out by use of the Neubauer improved counting chamber as described by Kelly (1979). For red blood cell counts, blood was diluted 1:200 with Dacies fluid (99 mL of 3% aqueous solution of sodium citrate; and 1 mL of 40% formaldehyde) which keeps and preserves the shape of the red blood cell for estimation in the counting chamber (Kelly, 1979).

3.9.3 Total white blood cell counts: For white blood cell counts, the dilution was 1:20 using 2-3% aqueous solution of acetic acid to which tinge of Gentian violet was added. Thin blood smears were stained with Wright-Giemsa stain (Schalm *et al.*, 1975). A total of 100 white blood cells were enumerated and differentiated.

3.9.4 Haemoglobin (Hb) estimation: The cyanmethemoglobin method as described by Schalm *et al.* (1975) and Kelly (1979) was used in the determination of haemoglobin concentration. Well-mixed blood of 0.02 mL was added to 4 mL of modified Dabkin's solution (potassium ferricyanide, 200 mg; potassium cyanide, 50 mg; potassium dihydrogen phosphate 140 mg. The volume was made up to 1 L with distilled water at pH of 7.0. The mixture was allowed to stand for 3 min and the Hb concentration was read photometrically by comparing with a cyanmethemoglobin standard with a yellow-green filter at 625 nm.

3.9.5 Mean corpuscular volume

MCV (fl) = $\frac{\text{PCV (\%)}}{\text{RBC } (10^{-6}\mu l^{-1})}$

3.9.6 Mean corpuscular haemoglobin

MCH (pg) = $\frac{[\text{Hb } (gdl^{-1})]}{\text{RBC } (10^{-6}\mu l^{-1})}$

3.9.7 Mean corpuscular haemoglobin concentration

MCHC (gdl^-1) = $\frac{[\text{Hb } (gbl^{-1})]}{\text{PCV (\%)}}$

3.10 Statistical Analysis

The data were subjected using one way analysis of variance (ANOVA) and the significant differences in means was determined by applying Duncan's Multiple Range test. The tests use SPSS Software, version 20 (2011). Statistical significance effect on the parameters to be measured was set at $p \leq 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate Composition of African Catfish Fingerlings Fed Kola Husk Meal

Table 5 shows the proximate composition of fish fed kola husk meal. Moisture content, lipid and crude protein were significantly different ($p<0.05$) in catfish fingerlings fed all dietary groups. Moisture content was highest in fish fed T5 and lowest in control. Lipid content highest were recorded in T2 and lowest in T5 and crude proteins were highest in fish fed control and lowest were recorded in the fish fed T5. Ash content was similar ($p > 0.05$) T2 and T5 but differ significantly ($p < 0.05$) in the ash content recorded in the control, T3 and T4.

Fish fed all dietary treatments had significantly different fibre and nitrogen free extract. African catfish fingerlings fed the control diet had the least fibre while fish fed T5 had the highest. Fish fed T5 had the highest NFE while the least was recorded in fish fed T2.

Table 5: Proximate Composition of African Catfish Fingerlings Fed Kola Husk Meal

Parameters	T1	T2	T3	T4	T5
Moisture Content (%)	70.08 ± 0.04^{a}	70.36 ± 0.00^{b}	70.51 ± 0.01^{c}	70.98 ± 0.00^{d}	71.11 ± 0.01^{e}
Lipid (%)	5.80 ± 0.04^{c}	6.68 ± 0.01^{e}	6.30 ± 0.06^{d}	5.10 ± 0.06^{b}	4.20 ± 0.06^{a}
Crude Protein (%)	18.70 ± 0.06^{e}	18.50 ± 0.06^{d}	17.60 ± 0.06^{c}	16.70 ± 0.06^{b}	15.87 ± 0.03^{a}
Ash (%)	3.30 ± 0.06^{b}	3.70 ± 0.06^{d}	3.50 ± 0.06^{c}	2.91 ± 0.06^{a}	3.80 ± 0.06^{d}
Fibre (%)	0.51 ± 0.01^{a}	0.58 ± 0.01^{b}	0.71 ± 0.01^{c}	0.90 ± 0.01^{d}	0.93 ± 0.01^{e}
Nitrogen Free Extract (%)	1.61 ± 0.09^{c}	0.08 ± 0.01^{a}	1.38 ± 0.07^{b}	3.40 ± 0.01^{d}	4.09 ± 0.09^{e}

Mean ± S.E with different superscripts are significantly different at $p < 0.05$

4.2 Growth and Nutritional Responses of African Catfish Fed Kola Husk Meal

Growth and nutritional responses of African catfish fed different levels of kola husk meal were measured and estimated. And the overall performance after the 56 days feeding trial is presented on table 6. It reveals that the initial weight of fish were not significantly different ($p>0.05$).

From each other, amount of feed consumed experimental fish were significantly different ($p<0.05$) for all the treatments. Fish fed control diet had the highest feed intake (8.11 ± 0.03) while the lowest (6.24 ± 0.10) was recorded in catfish fed 100% kola husk meal (T5). At the end of the 56 days feeding trial final weight and weight gained of various dietary groups differ significantly ($p < 0.05$). Fish feed control diet had the highest final weight and weight gained (10.00 ± 0.08 and 6.95 ± 0.07) respectively. Nevertheless, the final body weight and weight gained in fish fed T2 and T3 were higher and different ($p < 0.05$) from T4 and T5.

Experimental fish showed differences ($p < 0.05$) in the feed conversion ratio with the highest (1.64 ± 0.01) recorded in fish fed T5 and lowest 1.17 ± 0.01 recorded in the control. Results indicated that fish fed T2 had the lowest FCR among diets containing kola husk meal but was however similar ($p < 0.05$) with FCR recorded in T3. Specific growth rate of fish fed trial diets were statistically different ($p < 0.05$) with the highest (2.28 ± 0.01) recorded in the control and lowest (1.90 ± 0.01) in *C. gariepinus* fingerlings fed T4. Protein efficiency ratio was highest (0.17 ± 0.01) in control and lowest (0.10 ± 0.01) in fish fed KHM100. Similar ($p > 0.05$) PER were however estimated for fish fed T4 and T5.

Table 6: Growth and Nutritional Performance of African Catfish fed Kola Husk Meal

Parameters	CTRL	T2	T3	T4	T5
Initial Weight (g)	3.05 ± 0.02^{a}	3.02 ± 0.01^{a}	3.04 ± 0.02^{a}	3.04 ± 0.02^{a}	3.02 ± 0.02^{a}
Final Weight (g)	10.00 ± 0.08^{e}	8.61 ± 0.12^{d}	8.15 ± 0.05^{c}	7.21 ± 0.03^{b}	6.82 ± 0.03^{a}
Weight Gained (g)	6.95 ± 0.07^{e}	5.59 ± 0.12^{d}	5.12 ± 0.06^{c}	4.17 ± 0.05^{b}	3.80 ± 0.03^{a}
Feed Intake (g)	8.11 ± 0.03^{e}	7.65 ± 0.05^{d}	7.13 ± 0.12^{c}	6.66 ± 0.04^{b}	6.24 ± 0.10^{a}
Feed Conversion Ratio	1.17 ± 0.01^{a}	1.37 ± 0.02^{b}	1.39 ± 0.01^{b}	1.59 ± 0.01^{d}	1.64 ± 0.01^{d}
Specific Growth Rate	2.28 ± 0.01^{e}	2.13 ± 0.01^{d}	2.08 ± 0.01^{c}	1.96 ± 0.01^{b}	1.90 ± 0.01^{a}
Protein Efficiency Ratio	0.17 ± 0.01^{d}	0.14 ± 0.01^{c}	0.13 ± 0.01^{b}	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}

Mean ± S.E with different superscripts are significantly different at $p < 0.05$

4.3 Haematological Parameters of African Catfish fed Kola Husk Meal

Haematological parameter of *C. gariepinus* fed diets containing different levels of kola husk meal is presented on Table 7. Fish fed control and T5 had similar ($p > 0.05$) PCV and Hb. PCV was highest (22.00 ± 0.58) in T3 and lowest (18.00 ± 0.58) in the control. Similar trend was observed in the Hb content where fish fed T3 had the highest haemoglobin (9.70 ± 0. g/dl) while the lowest 6.40 ± 0.06g/dl was recorded in the control. Red blood cells were similar ($p > 0.05$) in the control and T5. Catfish fingerlings fed T3 however had the highest (1.67 ± 0.03) RBC. Mean corpuscular volume of *C. gariepinus* blood fed trial diets were similar ($p > 0.05$) in all the dietary treatments. However, T3 had the highest (137±6.42fL) MCV while the least (120.53 ±7.14fL) was recorded in fish fed the control diet.

Furthermore, the mean corpuscular haemoglobin differ significantly ($p < 0.05$) in all the dietary treatments. MCH was lowest (41.67 ± 0.56) in T2 and highest (58.3 ± 1.51) in T3. Mean corpuscular haemoglobin concentration were similar ($p > 0.05$) in fish fed the control, T2, T4 and T5; they were however different ($p < 0.05$) from the value recorded for T3. White blood cell of test organisms increased as the level of kola husk meal increases. Thus, T5 had the highest white blood cell while the lowest was recorded in the control. Percentage neutrophil and lymphocytes differs significantly ($p < 0.05$) in all the diets. However, percentage lymphocytes in catfish fingerlings fed the control and T5 showed similarities ($p > 0.05$).

Table 8: Haematological Parameters of African Catfish fed Kola Husk Meal

Parameters	Ctrl	T2	T3	T4	T5
PCV	18.00 ± 0.58^{a}	20.33 ± 0.33^{b}	22.00 ± 0.58^{c}	21.00 ± 0.58^{bc}	18.33 ± 0.33^{a}
Hb (g/dL)	6.40 ± 0.06^{a}	6.80 ± 0.06^{b}	9.70 ± 0.06^{d}	7.83 ± 0.03^{c}	6.50 ± 0.06^{a}
RBC (*10^12/L)	1.50 ± 0.06^{a}	1.63 ± 0.03^{bc}	1.67 ± 0.03^{c}	1.53 ± 0.03^{abc}	1.40 ± 0.03^{a}
MCV (fL)	120.53 ± 7.14^{a}	124.67 ± 3.93^{a}	132 ± 1.74^{a}	137.23 ± 6.42^{a}	131.60 ± 7.71^{a}
MCH (Pg)	42.60 ± 1.64^{ab}	41.67 ± 0.56^{a}	58.3 ± 1.51^{d}	51.37 ± 1.00^{c}	46.60 ± 2.12^{b}
MCHC (g/dL)	36.00 ± 1.47^{a}	33.47 ± 0.79^{a}	44.17 ± 1.44^{b}	37.37 ± 1.16^{a}	35.50 ± 0.72^{a}
WBC (*10^9/L)	123.80 ± 0.06^{a}	136.70 ± 0.06^{b}	138.50 ± 0.06^{c}	144.37 ± 0.09^{d}	145.87 ± 0.28^{e}
Neutrophils (%)	4.00 ± 0.58^{bc}	3.00 ± 0.58^{ab}	2.00 ± 0.58^{a}	5.00 ± 0.58^{bc}	4.00 ± 0.58^{c}
Lymphocytes (%)	96.00 ± 0.58^{ab}	97.00 ± 0.58^{bc}	98.00 ± 0.58^{c}	95.00 ± 0.58^{a}	96.00 ± 0.58^{ab}

Mean ± S.E with different superscripts are significantly different at $p < 0.05$

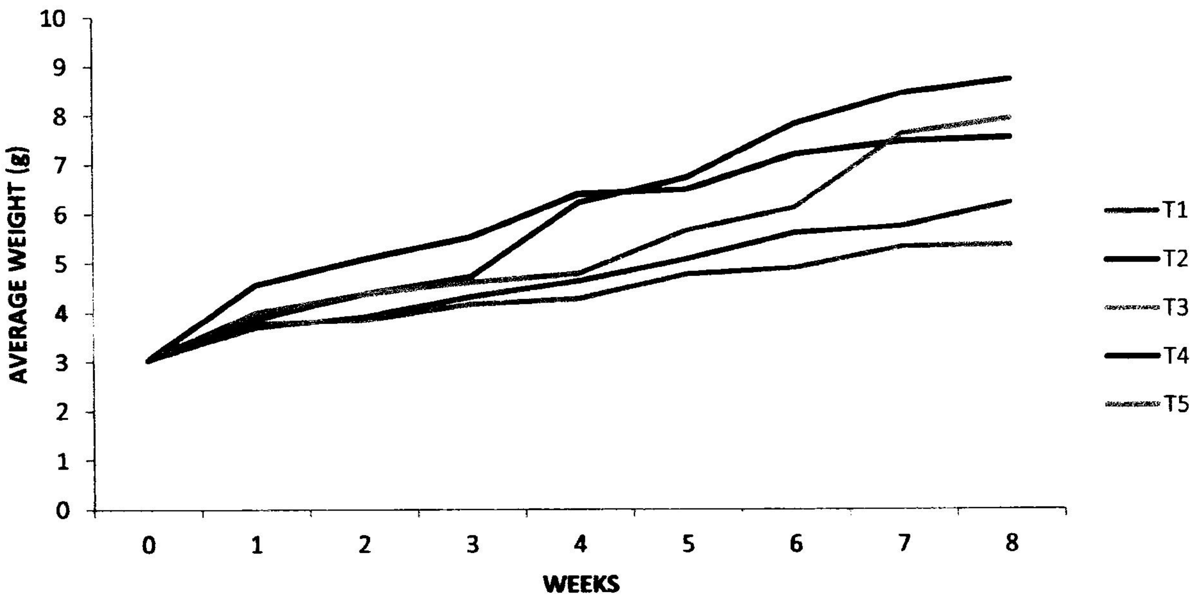

Figure 1: Graph of fish showing the growth pattern on weekly basis

4.4 DISCUSSION

The proximate composition of *kola pod husk* meal in the present work was in contrast with Mebudu (2015) but comparable with those reported in other unconventional feedstuff such as Mustafa and Alamin (2012) for watermelon seed, *Arislem et.al 2012.*. According to Hamzat and Adeola (2011) nutritional component of unconventional feed vary with their composition, origin and processing methods also the differences in chemical composition with the other study may be an indication of environmental factors such as season, geographical location and stage of maturity play a minor role in determining nutritive value of kola pod husk.

The result of growth and nutrient utilization obtained in this study also contrasts with the findings of Osineye (2008) who reported that Tilapia can be fed 100% KPH without significant ($p>0.05$) effect on growth and nutrient feed utilization. Haematological components of blood are valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (Oyawoye and Ogunkunle, 1998). All the haematological parameters measured in this study were within the recommended physiological ranges reported for *C. gariepinus*. Blaxhall and Daisley (1999) reported the essence of using haematocrit to detect anaemic condition in fishes. . Pack cell volume and haemoglobin values reported in this study were within the range of 15 to 50% reported by Pietse et al. (2001). White blood cell of test organisms increased as the level of kola husk meal increases, Thus, KHM100 had the highest white blood cell while the lowest was recorded in the control. Mean corpuscular haemoglobin concentration were higher than the range (20.82 to 26.60 pg) reported by Anyanwu et al., (2011) for *Heteroclarias* fed sweet potato peel meal..

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The result of this study on KPH as a feed stuff (major energy source) in fish diets revealed that kola pod husk contain sufficient energy to compare favourably with maize however the best inclusion level obtain in this study is 25% KHM. With these results fish feed can be produced at a relatively cheaper cost and thus profit of fish farmers can be increased.

5.2 RECOMMENDATIONS

The result of this study on KPH as a feed stuff (major energy source) in fish diets revealed that 25% maize substitution with KHM can be included in the diet of *Clarias gariepinus*

More research should be carried out on the digestibility of fish fed kola pod husk as a substitute for maize in the diet of *Clarias gariepinus.*

More research should be carried out on the economic analysis of kolanut pod husk as substitute for maize in the diet of *C. gariepinus*

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Appendix 1

Proximate composition of fish fed kolanut pod husk

Oneway

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MC	CTRL	3	8.8333	.05774	.03333	8.6899	8.9768	8.80	8.90
	KHM25	3	9.0733	.06429	.03712	8.9136	9.2330	9.00	9.12
	KHM50	3	9.3000	.10000	.05774	9.0516	9.5484	9.20	9.40
	KHM75	3	9.5000	.10000	.05774	9.2516	9.7484	9.40	9.60
	KHM100	3	9.6000	.10000	.05774	9.3516	9.8484	9.50	9.70
	Total	15	9.2613	.29880	.07715	9.0959	9.4268	8.80	9.70
Lipid	CTRL	3	17.0733	.06429	.03712	16.9136	17.2330	17.00	17.12
	KHM25	3	18.0167	.01528	.00882	17.9787	18.0546	18.00	18.03
	KHM50	3	18.3000	.10000	.05774	18.0516	18.5484	18.20	18.40
	KHM75	3	19.2000	.10000	.05774	18.9516	19.4484	19.10	19.30
	KHM100	3	19.6000	.10000	.05774	19.3516	19.8484	19.50	19.70
	Total	15	18.4380	.92729	.23942	17.9245	18.9515	17.00	19.70
CP	CTRL	3	43.8333	.05774	.03333	43.6899	43.9768	43.80	43.90
	KHM25	3	42.0400	.01000	.00577	42.0152	42.0648	42.03	42.05
	KHM50	3	41.3000	.10000	.05774	41.0516	41.5484	41.20	41.40
	KHM75	3	40.8000	.10000	.05774	40.5516	41.0484	40.70	40.90
	KHM100	3	40.2833	.00577	.00333	40.2690	40.2977	40.28	40.29
	Total	15	41.6513	1.28013	.33053	40.9424	42.3602	40.28	43.90

Ash	CTRL	3	10.1000	.10000	.05774	9.8516	10.3484	10.00	10.20
	KHM25	3	11.6000	.10000	.05774	11.3516	11.8484	11.50	11.70
	KHM50	3	12.1000	.10000	.05774	11.8516	12.3484	12.00	12.20
	KHM75	3	8.9333	6.35164	3.66712	-6.8450	24.7117	1.60	12.70
	KHM100	3	13.6000	.10000	.05774	13.3516	13.8484	13.50	13.70
	Total	15	11.2667	2.92689	.75572	9.6458	12.8875	1.60	13.70
Fibre	CTRL	3	9.1000	.10000	.05774	8.8516	9.3484	9.00	9.20
	KHM25	3	9.1300	.01000	.00577	9.1052	9.1548	9.12	9.14
	KHM50	3	9.1700	.01000	.00577	9.1452	9.1948	9.16	9.18
	KHM75	3	9.2700	.01000	.00577	9.2452	9.2948	9.26	9.28
	KHM100	3	9.2700	.01000	.00577	9.2452	9.2948	9.26	9.28
	Total	15	9.1880	.08257	.02132	9.1423	9.2337	9.00	9.28
NFE	CTRL	3	11.0600	.21633	.12490	10.5226	11.5974	10.88	11.30
	KHM25	3	10.1400	.17692	.10214	9.7005	10.5795	9.98	10.33
	KHM50	3	9.8300	.21000	.12124	9.3083	10.3517	9.62	10.04
	KHM75	3	12.2967	6.35432	3.66867	-3.4883	28.0817	8.42	19.63
	KHM100	3	7.6467	.29006	.16746	6.9261	8.3672	7.36	7.94
	Total	15	10.1947	2.88536	.74500	8.5968	11.7925	7.36	19.63

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
MC	Between Groups	1.175	4	.294	39.203	.000
	Within Groups	.075	10	.007		
	Total	1.250	14			
Lipid	Between Groups	11.969	4	2.992	435.353	.000
	Within Groups	.069	10	.007		
	Total	12.038	14			
CP	Between Groups	22.895	4	5.724	1.220E3	.000
	Within Groups	.047	10	.005		
	Total	22.942	14			
Ash	Between Groups	39.167	4	9.792	1.212	.365
	Within Groups	80.767	10	8.077		
	Total	119.933	14			
Fibre	Between Groups	.075	4	.019	8.971	.002
	Within Groups	.021	10	.002		
	Total	.095	14			
NFE	Between Groups	35.386	4	8.847	1.090	.412
	Within Groups	81.168	10	8.117		
	Total	116.554	14			

Post Hoc Tests

Homogeneous Subsets

MOISTURE

Duncan

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
CTRL	3	8.8333			
KHM25	3		9.0733		
KHM50	3			9.3000	
KHM75	3				9.5000
KHM100	3				9.6000
Sig.		1.000	1.000	1.000	.187

Means for groups in homogeneous subsets are displayed.

Lipid

Duncan

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
CTRL	3	17.0733				
KHM25	3		18.0167			
KHM50	3			18.3000		
KHM75	3				19.2000	

Crude Protein

Duncan

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
KHM100	3	40.2833				
KHM75	3		40.8000			
KHM50	3			41.3000		
KHM25	3				42.0400	
CTRL	3					43.8333
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

KHM100	3					19.6000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Ash

Duncan

Treatment	N	Subset for alpha = 0.05
		1
KHM75	3	8.9333
CTRL	3	10.1000
KHM25	3	11.6000
KHM50	3	12.1000
KHM100	3	13.6000
Sig.		.094

Means for groups in homogeneous subsets are displayed.

Fibre

Duncan

Treatment	N	Subset for alpha = 0.05	
		1	2
CTRL	3	9.1000	
KHM25	3	9.1300	
KHM50	3	9.1700	
KHM100	3		9.2700
KHM75	3		9.2700
Sig.		.103	1.000

Means for groups in homogeneous subsets are displayed.

Nitrogen Free Extract

Duncan

Treatment	N	Subset for alpha = 0.05
		1
KHM100	3	7.6467
KHM50	3	9.8300
KHM25	3	10.1400
CTRL	3	11.0600
KHM75	3	12.2967
Sig.		.096

Means for groups in homogeneous subsets are displayed.

APPENDIX 2

Growth and Nutritional Performance

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
IW	Ctrl	3	3.0533	.02887	.01667	2.9816	3.1250	3.02	3.07
	KHM25	3	3.0200	.00000	.00000	3.0200	3.0200	3.02	3.02
	KHM50	3	3.0367	.02887	.01667	2.9650	3.1084	3.02	3.07
	KHM75	3	3.0367	.02887	.01667	2.9650	3.1084	3.02	3.07
	KHM100	3	3.0200	.00000	.00000	3.0200	3.0200	3.02	3.02
	Total	15	3.0333	.02289	.00591	3.0207	3.0460	3.02	3.07
FI	Ctrl	3	8.1133	.04933	.02848	7.9908	8.2359	8.08	8.17
	KHM25	3	7.6533	.09018	.05207	7.4293	7.8774	7.56	7.74
	KHM50	3	7.1267	.20257	.11695	6.6235	7.6299	6.90	7.29
	KHM75	3	6.6567	.07572	.04372	6.4686	6.8448	6.57	6.71
	KHM100	3	6.2400	.17521	.10116	5.8047	6.6753	6.06	6.41
	Total	15	7.1580	.70383	.18173	6.7682	7.5478	6.06	8.17
FW	Ctrl	3	10.0000	.13229	.07638	9.6714	10.3286	9.90	10.15
	KHM25	3	8.6133	.21221	.12252	8.0862	9.1405	8.44	8.85
	KHM50	3	8.1533	.08505	.04910	7.9421	8.3646	8.09	8.25
	KHM75	3	7.2067	.04933	.02848	7.0841	7.3292	7.15	7.24
	KHM100	3	6.8233	.05508	.03180	6.6865	6.9601	6.76	6.86
	Total	15	8.1593	1.16529	.30088	7.5140	8.8046	6.76	10.15
WG	Ctrl	3	6.9467	.11547	.06667	6.6598	7.2335	6.88	7.08
	KHM25	3	5.5933	.21221	.12252	5.0662	6.1205	5.42	5.83
	KHM50	3	5.1167	.10599	.06119	4.8534	5.3800	5.02	5.23
	KHM75	3	4.1700	.07810	.04509	3.9760	4.3640	4.08	4.22
	KHM100	3	3.8033	.05508	.03180	3.6665	3.9401	3.74	3.84
	Total	15	5.1260	1.15720	.29879	4.4852	5.7668	3.74	7.08
FCR	Ctrl	3	1.1667	.02517	.01453	1.1042	1.2292	1.14	1.19
	KHM25	3	1.3700	.03464	.02000	1.2839	1.4561	1.33	1.39
	KHM50	3	1.3900	.02000	.01155	1.3403	1.4397	1.37	1.41
	KHM75	3	1.5967	.01155	.00667	1.5680	1.6254	1.59	1.61
	KHM100	3	1.6400	.02646	.01528	1.5743	1.7057	1.62	1.67
	Total	15	1.4327	.17834	.04605	1.3339	1.5314	1.14	1.67
SGR	Ctrl	3	2.2833	.01528	.00882	2.2454	2.3213	2.27	2.30

	KHM25	3	2.1333	.02517	.01453	2.0708	2.1958	2.11	2.16
	KHM50	3	2.0767	.01155	.00667	2.0480	2.1054	2.07	2.09
	KHM75	3	1.9567	.00577	.00333	1.9423	1.9710	1.95	1.96
	KHM100	3	1.9000	.01000	.00577	1.8752	1.9248	1.89	1.91
	Total	15	2.0700	.14046	.03627	1.9922	2.1478	1.89	2.30
PER	Ctrl	3	.1633	.00577	.00333	.1590	.1877	.17	.18
	KHM25	3	.1333	.00577	.00333	.1290	.1577	.14	.15
	KHM50	3	.1200	.00000	.00000	.1300	.1300	.13	.13
	KHM75	3	.1007	.00577	.00333	.0923	.1210	.10	.11
	KHM100	3	.1000	.00000	.00000	.1000	.1000	.10	.10
	Total	15	.1307	.02764	.00714	.1154	.1460	.10	.18
PCV	Ctrl	3	18.0000	1.00000	.57735	15.5159	20.4841	17.00	19.00
	KHM25	3	20.3333	.57735	.33333	18.8991	21.7676	20.00	21.00
	KHM50	3	22.0000	1.00000	.57735	19.5159	24.4841	21.00	23.00
	KHM75	3	21.0000	1.00000	.57735	18.5159	23.4841	20.00	22.00
	KHM100	3	18.3333	.57735	.33333	16.8991	19.7676	18.00	19.00
	Total	15	19.9333	1.75119	.45216	18.9636	20.9031	17.00	23.00
Hb	Ctrl	3	6.4000	.10000	.05774	6.1516	6.6484	6.30	6.50
	KHM25	3	6.8000	.10000	.05774	6.5516	7.0484	6.70	6.90
	KHM50	3	9.7000	.10000	.05774	9.4516	9.9484	9.60	9.80
	KHM75	3	7.8333	.05774	.03333	7.6899	7.9768	7.80	7.90
	KHM100	3	6.5000	.10000	.05774	6.2516	6.7484	6.40	6.60
	Total	15	7.4467	1.28167	.33092	6.7369	8.1564	6.30	9.80
RBC	Ctrl	3	1.5000	.10000	.05774	1.2516	1.7484	1.40	1.60
	KHM25	3	1.6333	.05774	.03333	1.4899	1.7768	1.60	1.70
	KHM50	3	1.6667	.05774	.03333	1.5232	1.8101	1.60	1.70
	KHM75	3	1.5333	.05774	.03333	1.3899	1.6768	1.50	1.60
	KHM100	3	1.4000	.10000	.05774	1.1516	1.6484	1.30	1.50
	Total	15	1.5467	.11872	.03065	1.4809	1.6124	1.30	1.70
MCV	Ctrl	3	1.2053 E2	12.36298	7.13777	89.8220	151.2447	106.30	128.60
	KHM25	3	1.2467 E2	6.80612	3.92952	107.7593	141.5740	117.70	131.30
	KHM50	3	1.3200 E2	3.01164	1.73877	124.5187	139.4813	129.40	135.30
	KHM75	3	1.3723 E2	11.11141	6.41517	109.6311	164.8356	125.00	146.70
	KHM100	3	1.3160 E2	13.35515	7.71060	98.4240	164.7760	120.00	146.20
	Total	15	1.2921 E2	10.49323	2.70934	123.3957	135.0176	106.30	146.70
MCH	Ctrl	3	42.6000	2.84781	1.64418	35.5257	49.6743	40.10	45.70
	KHM25	3	41.6667	.97125	.56075	39.2539	44.0794	40.60	42.50

	KHM50	3	58.3000	2.61534	1.50997	51.8031	64.7969	56.50	61.30
	KHM75	3	51.3667	1.73877	1.00388	47.0473	55.6860	49.40	52.70
	KHM100	3	46.6000	3.67560	2.12211	37.4693	55.7307	42.70	50.00
	Total	15	48.1067	6.71166	1.73294	44.3899	51.8235	40.10	61.30
MCHC	Ctrl	3	36.0000	2.55343	1.47422	29.6569	42.3431	33.20	38.20
	KHM25	3	33.4667	1.37961	.79652	30.0395	36.8938	31.90	34.50
	KHM50	3	44.1667	2.50067	1.44376	37.9547	50.3787	41.70	46.70
	KHM75	3	37.3667	2.01329	1.16237	32.3654	42.3680	35.50	39.50
	KHM100	3	35.5000	1.25300	.72342	32.3874	38.6126	34.20	36.70
	Total	15	37.3000	4.14849	1.07114	35.0026	39.5974	31.90	46.70
WBC	Ctrl	3	1.2380E2	.10000	.05774	123.5516	124.0484	123.70	123.90
	KHM25	3	1.3670E2	.10000	.05774	136.4516	136.9484	136.60	136.80
	KHM50	3	1.3850E2	.10000	.05774	138.2516	138.7484	138.40	138.60
	KHM75	3	1.4437E2	.15275	.08819	143.9872	144.7461	144.20	144.50
	KHM100	3	1.4587E2	.49329	.28480	144.6413	147.0921	145.30	146.20
	Total	15	1.3785E2	8.09867	2.09107	133.3618	142.3316	123.70	146.20
Neutro	Ctrl	3	4.0000	1.00000	.57735	1.5159	6.4841	3.00	5.00
	KHM25	3	3.0000	1.00000	.57735	.5159	5.4841	2.00	4.00
	KHM50	3	2.0000	1.00000	.57735	-.4841	4.4841	1.00	3.00
	KHM75	3	5.0000	1.00000	.57735	2.5159	7.4841	4.00	6.00
	KHM100	3	4.0000	1.00000	.57735	1.5159	6.4841	3.00	5.00
	Total	15	3.6000	1.35225	.34915	2.8512	4.3488	1.00	6.00
Lymph	Ctrl	3	96.0000	1.00000	.57735	93.5159	98.4841	95.00	97.00
	KHM25	3	97.0000	1.00000	.57735	94.5159	99.4841	96.00	98.00
	KHM50	3	98.0000	1.00000	.57735	95.5159	100.4841	97.00	99.00
	KHM75	3	95.0000	1.00000	.57735	92.5159	97.4841	94.00	96.00
	KHM100	3	96.0000	1.00000	.57735	93.5159	98.4841	95.00	97.00
	Total	15	96.4000	1.35225	.34915	95.6512	97.1488	94.00	99.00

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
IW	Between Groups	.002	4	.001	1.167	.382
	Within Groups	.005	10	.000		
	Total	.007	14			
FI	Between Groups	6.759	4	1.690	95.975	.000
	Within Groups	.176	10	.018		

	Total	6.935	14			
FW	Between Groups	18.860	4	4.715	313.359	.000
	Within Groups	.150	10	.015		
	Total	19.010	14			
WG	Between Groups	18.590	4	4.648	295.143	.000
	Within Groups	.157	10	.016		
	Total	18.748	14			
FCR	Between Groups	.439	4	.110	179.005	.000
	Within Groups	.006	10	.001		
	Total	.445	14			
SGR	Between Groups	.274	4	.068	302.132	.000
	Within Groups	.002	10	.000		
	Total	.276	14			
PER	Between Groups	.010	4	.003	131.167	.000
	Within Groups	.000	10	.000		
	Total	.011	14			
PCV	Between Groups	35.600	4	8.900	12.136	.001
	Within Groups	7.333	10	.733		
	Total	42.933	14			
Hb	Between Groups	22.911	4	5.728	660.885	.000
	Within Groups	.087	10	.009		
	Total	22.997	14			
RBC	Between Groups	.137	4	.034	5.722	.012
	Within Groups	.060	10	.006		
	Total	.197	14			
MCV	Between Groups	521.389	4	130.347	1.278	.342
	Within Groups	1020.120	10	102.012		
	Total	1541.509	14			
MCH	Between Groups	565.796	4	141.449	21.811	.000
	Within Groups	64.853	10	6.485		
	Total	630.649	14			
MCHC	Between Groups	200.340	4	50.085	12.336	.001
	Within Groups	40.600	10	4.060		
	Total	240.940	14			
WBC	Between Groups	917.644	4	229.411	3.866E3	.000
	Within Groups	.593	10	.059		
	Total	918.237	14			
Neutro	Between Groups	15.600	4	3.900	3.900	.037
	Within Groups	10.000	10	1.000		
	Total	25.600	14			
Lymph	Between Groups	15.600	4	3.900	3.900	.037
	Within Groups	10.000	10	1.000		

	Total	25.600	14			

Post Hoc Tests

Homogeneous Subsets

Initial Weight

Duncan

Trt	N	Subset for alpha = 0.05
		1
KHM25	3	3.0200
KHM100	3	3.0200
KHM50	3	3.0367
KHM75	3	3.0367
Ctrl	3	3.0533
Sig.		.124

Means for groups in homogeneous subsets are displayed.

Feed Intake

Duncan

Trt	N	Subset for alpha = 0.05				
		1	2	3	4	5
KHM100	3	6.2400				
KHM75	3		6.6567			
KHM50	3			7.1267		
KHM25	3				7.6533	
Ctrl	3					8.1133
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Final Weight

Duncan

Trt	N	Subset for alpha = 0.05				
		1	2	3	4	5
KHM100	3	6.8233				
KHM75	3		7.2067			
KHM50	3			8.1533		
KHM25	3				8.6133	
Ctrl	3					10.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Weght gain

Duncan

Trt	N	Subset for alpha = 0.05				
		1	2	3	4	5
KHM100	3	3.8033				
KHM75	3		4.1700			
KHM50	3			5.1167		
KHM25	3				5.5933	
Ctrl	3					6.9467
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Feed Conversion Ratio

Duncan

Trt	N	Subset for alpha = 0.05		
		1	2	3
Ctrl	3	1.1667		
KHM25	3		1.3700	
KHM50	3		1.3900	
KHM75	3			1.5967
KHM100	3			1.6400
Sig.		1.000	.346	.058

Means for groups in homogeneous subsets are displayed.

Specific Growth Rate

Duncan

Trt	N	Subset for alpha = 0.05				
		1	2	3	4	5
KHM100	3	1.9000				
KHM75	3		1.9567			
KHM50	3			2.0767		
KHM25	3				2.1333	
Ctrl	3					2.2833
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Protein Efficiency Rate

Duncan

Trt	N	Subset for alpha = 0.05			
		1	2	3	4
KHM100	3	.1000			
KHM75	3	.1007			
KHM50	3		.1200		
KHM25	3			.1333	
Ctrl	3				.1633
Sig.		.098	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

APPENDIX 3

Haematological Parameters

PCV

Duncan

Trt	N	Subset for alpha = 0.05		
		1	2	3
Ctrl	3	18.0000		
KHM100	3	18.3333		
KHM25	3		20.3333	
KHM75	3		21.0000	21.0000
KHM50	3			22.0000
Sig.		.644	.363	.183

Hb

Duncan

Trt	N	Subset for alpha = 0.05			
		1	2	3	4
Ctrl	3	6.4000			
KHM100	3	6.5000			
KHM25	3		6.8000		
KHM75	3			7.8333	
KHM50	3				9
Sig.		.218	1.000	1.000	

Means for groups in homogeneous subsets are displayed.

Means for groups in homogeneous subsets are displayed.

RBC

Duncan

Trt	N	Subset for alpha = 0.05		
		1	2	3
KHM100	3	1.4000		
Ctrl	3	1.5000	1.5000	
KHM75	3	1.5333	1.5333	1.5333
KHM25	3		1.6333	1.6333
KHM50	3			1.6667
Sig.		.071	.071	.071

Means for groups in homogeneous subsets are displayed.

MCV

Duncan

Trt	N	Subset for alpha = 0.05
		1
Ctrl	3	120.5333
KHM25	3	124.6667
KHM100	3	131.6000
KHM50	3	132.0000
KHM75	3	137.2333
Sig.		.092

Means for groups in homogeneous subsets are displayed.

MCH

Duncan

Trt	N	Subset for alpha = 0.05			
		1	2	3	4
KHM25	3	41.6667			
Ctrl	3	42.6000	42.6000		
KHM100	3		46.6000		
KHM75	3			51.3667	
KHM50	3				58.3000
Sig.		.663	.083	1.000	1.000

Means for groups in homogeneous subsets are displayed.

MCHC

Duncan

Trt	N	Subset for alpha = 0.05	
		1	2
KHM25	3	33.4667	
KHM100	3	35.5000	
Ctrl	3	36.0000	
KHM75	3	37.3667	
KHM50	3		44.1667
Sig.		.051	1.000

Means for groups in homogeneous subsets are displayed.

WBC

Duncan

Trt	N	Subset for alpha = 0.05				
		1	2	3	4	5
Ctrl	3	1.2380E2				
KHM25	3		1.3670E2			
KHM50	3			1.3850E2		
KHM75	3				1.4437E2	
KHM100	3					1.4587E2
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Neutrophil

Duncan

Trt	N	Subset for alpha = 0.05		
		1	2	3
KHM50	3	2.0000		
KHM25	3	3.0000	3.0000	
Ctrl	3		4.0000	4.0000
KHM100	3		4.0000	4.0000
KHM75	3			5.0000
Sig.		.249	.269	.269

Means for groups in homogeneous subsets are displayed.

Lymph

Duncan

Trt	N	Subset for alpha = 0.05		
		1	2	3
KHM75	3	95.0000		
Ctrl	3	96.0000	96.0000	
KHM100	3	96.0000	96.0000	
KHM25	3		97.0000	97.0000
KHM50	3			98.0000
Sig.		.269	.269	.249

Means for groups in homogeneous subsets are displayed.