EFFECTS OF OLEANDER SEED MEAL BASED DIET ON GROWTH PERFORMANCE, HAEMATOLOGY AND TISSUE COMPOSITION OF *Clarias gariepinus* FINGERLINGS

BY

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ALABI BISOLA BRIDGET

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF FISHERIES AND AQUACULTURE

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NIGERIA.

DECLARATION

I, ALABI BISOLA BRIDGET hereby declare that this project was written by me and it is a record of my research work. It has not been presented before in any reputable presentation elsewhere. All borrowed ideas were duly and properly acknowledged. Under the supervision of Dr. T.O BABALOLA

ALABI,BISOLA BRIDGET

22 - 03 - 2019

DATE

CERTIFICATION

This is to certify that this project work was carried out by ALABI, BISOLA BRIDGET with MATRIC NUMBER FAQ/13/0989 in the Department of Fisheries and Aquaculture of Federal University, Oye-Ekiti, under the supervision of DR. T.O BABALOLA. The thesis has been read and approved as meeting the requirements for the award of Bachelor of Fisheries and Aquaculture.

PROJECT SUPERVISOR DATE

HEAD OF DEPARTMENT DATE

DEDICATION

This project is dedicated to Almighty God, the beginning and the end, who has preserved my life during the course of my five years programme. It is also dedicated to my parents, Mr. and Mrs. Alabi, my siblings (Anjola-Oluwa and Goodluck), and to everyone who as contributed positively and immensely to my project work, you all are no doubt my greatest source of inspiration, without your support and encouragements I could not have completed this programme.

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My deepest and utmost appreciation goes to Almighty God, the giver of life and breath, for His all-sufficient mercy and graceful grace, who has made it possible for me to attain another milestone. I tender my unalloyed praises to HIM for protecting me throughout the course of my five years degree programme.

I write with huge respect and appreciation also to my amiable supervisor, Dr. T.O Babalola, for his unending fatherly care, support, assistance, guidance and knowledge he equipped me with during my project work and my entire stay in the university. I forever remain appreciative to the HOD, Dr. J.B Olasunkanmi, my able Professors and all other indefatigable lectures for the maximum support and precious time created to guide and put me through my project work, I pray God reward you all respectively.

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ABSTRACT

An 8-weeks feeding trial of different percentage inclusion of *Thevetia peruviana* seed meal in the diet of *Clarias gariepinus* was conducted. The experimental diets were Ctrl0TSM, 5TSM,10TSM 15TSM and 20TSM percentage replacement of plant protein in the control diet. The experimental design is a complete randomize design in which there were three replicates for each of the treatment. And the fish were stocked 10 per each tank, fed experimental diet two times a day of 5% body weight over eight weeks. Growth performance, haematology and tissue composition were measured to assess diet effects. At the end of the study, there was significant difference ($p<0.05$) between the weight gained, average daily weight gained, feed intake, FCR, PER, FER, SGR, of fish fed percentage inclusion of *Thevetia peruviana* seed meal to the control experimental diet, with better growth values recorded in fish fed 20TSM *Thevetia peruviana* seed meal inclusion, however a bit high percentage of mortality was recorded as increase in the percentage inclusion but low mortality in 20% inclusion of the oleander seed meal, similarly in the haematological parameters there were significant difference ($p< 0.05$) also in the values recorded in white blood cell, packed cell volume, red blood cell, hemoglobin concentration and MCH, but similarities ($p>0.05$) occurred in the neutrophil, lymphocyte, MCV and MCHC of haematological parameters of fish fed the experimental diets. The inclusion of Oleander seed meal up to 20% in the diets of *Clarias gariepinus*, had a positive effect on growth and haematology of the fish.

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CHAPTER ONE

1.0. INTRODUCTION

Aquaculture has been driven by social and economic objectives such as nutrition improvement in rural areas, generation of supplementary income, diversification of income activities, and the creation of employment (Osigbo*et al.*, 2014), and putting into consideration of the anthropogenic activities, over fishing, use of unregulated fishing gear, destruction of natural habitat with the use of bottom trawls and disposal of effluent into the natural habitat, this and many more, which have led to the migration and/or loss of some species in such habitat, and putting in mind the high demand of fish which the natural habitat cannot be able to meet and the continuous increasing demand population, in other to meet the demand of fish, this therefore has made aquaculture a way out in meeting fish demand in the country, for developing countries like Nigeria, where in emphasis is on oil, Aquaculture can generate significant employment, enhance the socioeconomic status of the farmer as well generate foreign exchange (Oluwasola and Ajayi, 2013), if necessary improved technologies are adopted such as nutrition technology. Despite the promising nature of aquaculture to increase productivity of fish in the country, significant improvements are yet to be noticed (Olaoye *et al.*, 2016) as a result of high cost of fish feed. Catfish has been known to be a prolific and easy to raise fish, it is hardy and can survive in most condition. This is the reason why catfish culture is popular in Nigeria.

Fish occupy an important position in improving the nutrition of millions of people in the world as a cheap source of animal protein in the diet of human. Fish is also a good source of vitamins, minerals, fatty acids and other micronutrients essential to a healthy diet (Ovi and Raji, 2006). However, in other to make this available for all, productivity should meet the target increase population, this can only be achieved if the cost of production is reduced. The cost of production

can be reduced if conventional protein (fishmeal, soybean meal, groundnut cake) feed stuff which are scare, expensive and in high competitive use with human and other livestock, are replace partially or completely by a non-conventional feedstuff. Attempts have been made with many researchers on the utilization of non-conventional feed stuff as alternative protein sources in the diets of fish. One of the promising underutilized plant protein source that can be used in animal diet is *Thevetiaperuviana* commonly known as Yellow Oleander.*Thevetiaperuviana*, belongs to the family of Apocynaceae and commonly called yellow oleander, lucky nut tree, be-still tree and milk bush. (Ibiyemi *et al,* 2002), *Thevetia peruviana* (yellow oleander) is mostly grown as ornamental tree in gardens along road side and there is no current human use for it as dietary or commercial demand which makes it cheap compared to other conventional protein concentrate (Taiwo*et al.*,2004). *Thevetiaperuviana*have the advantage of being able to grow in harsh conditions (Ibiyemi *et al.* 2002), it can survive well in both rain and dry season or where there is no water, as it does not require the use of fertilizers and it fruits profusely. According to Nair *et al.* (1982), the seed contains about 35% protein, Ibiyemi *et al.*(2002) and Ofoegbu and Kelle (2016)in a different study reported 37% crude protein in the seeds. However, Atteh *et al.*(1995) reported 47.5% protein in the seed and this is comparable in quality to soybean meal while Oluwaniyi *et al.* (2007), reported that the crude protein content of the defatted seed ranges from 42.79 – 47.50% of the seed cake while crude lipid ranges from 4.40 to 4.80%. *Thevetiaperuviana* seed can be made into cake and used as a protein supplement in the diet of livestock if the oil which constitute about 40-60 % is appropriately extracted from it

The anti-nutritional factors in the seed has reduced its utilization in animal feed. These include cardiac glycosides, phenols, terpinoids, oxalates, phytic acid and saponins (Oji and Okfor 2000; Bandara *et al.* 2010), Several glycosides have been extracted from various parts of *Thevetia*

peruviana plant, with the major one being thevetin which is responsible for the bitterness and very low palatability of the seed (Oluwaniyi *et al.* 2011).

Attempt has however been carried out to remove the toxic substance present in the seed with satisfactory result by Oluwaniyi and coleague (2011). The glucoside content was reduced by 95% when the seed was detoxified using acid detoxification followed by alcoholic extraction of the glycoside. Furthermore, direct alcohol detoxification led to a 98% reduction in the glycoside content of the seed meal. Feeding alcohol detoxified *Thevetiaperuviana* seed meal to cockerel produced comparable growth performance with the control diet at 50% replacement of soybean meal in the diet (Oluwaniyi *et al.* 2011). Similarlydetoxification of the seed improved amino acid profile, specifically the percentages of essential, aromatic, sulphur and basic amino acids increased (Akinpelu and Amao 2017).

There is paucity of information on the utilization of *Thevetia peruviana* as a feed ingredient in fish diet. This study was initiated to evaluate the effects of *Thevetia peruviana* seed meal as alternative protein supplement in the diet of *Clarias gariepinus* fingerlings on growth performance, haematology and tissue proximate composition.

1.1 PROBLEM STATEMENT

Protein sources use in formulating fish feeds are scarce because it is been consumed by both human and animals thereby making it expensive and not easily accessible by farmers.

1.2 JUSTIFICATION

Thevetiaperuviana seed is under- utilized due to it poisonous nature but can be useful if it is detoxified. it is rich in protein and essential amino acids. This study will give information on the

effect of replacing soy-bean meal with *Thevetia peruviana* seed meal in the diet of *Clarias gariepinus* fingerlings.

1.3 OBJECTIVE

The objective of this study is to:

To determine the effect of replacing soy-bean meal with *Thevetia peruviana* seed meal on the growth performance, hematology and tissue composition of *Clarias gariepinus* fingerlings

1.4 HYPOTHESIS

Null hypothesis (HO): there is no significant difference in the growth performance and haematology of *C. gariepinus* fed diet containing *Thevetiaperuviana* seed meal as substitute to soybean meal and those fed control diet.

Alternative hypothesis (HA): there are significant differences on the growth performance and haematology of fish fed diets containing *Thevetia peruviana* seed meal as a substitute for soy-bean meal and those fed control diet.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. History and Origin of *Thevetia peruviana*

Thevetia peruviana (yellow oleander) a dicotyledon was discovered during the 16th century and belong to the Dogbane an *Apocynaceae* family which is a native of tropical America that is mexico, west indies and central America, it grows wild in the humid zone of West Africa including Nigeria, but widespread in American, Asian and African continents, but it is now widely naturalized in the tropical and subtropical regions of the world, it is believed to be more than 2000 years old,*Thevetia* Genus; is called so many common names such as yellow oleander, according to Chinese mythology, the plants on seed set are supposed to bring good luck to homes, hence named 'lucky nut', also known as be-still tree and milk bush and in Yoruba Olomi-Ojo (Akintelu and Amoo 2016; Dibakar and sanjay 2011; Sahoo*et al.* 2009; Shepherd 2004; Ibiyemi*et al,* 2002; Linnaeus 1753), named after the French monk F. Andre Thevet (1502-1592) (Burrkilol, 1985).

Thevetia peruviana was one of the earliest plant that was discovered and contained a milky sap with a compound called thevetin that is used as a heart stimulant but its natural form is extremely poisonous as are all parts of plants, especially the seeds (Gupta *et al.,* 2011; Bandara *et al.*, 2010; Kareru *et al.*, 2010 and Eddlestone*et al.*, 2000). The plant is perennial with its leaves being linear, narrow Sword-like and green, with its shrub reaching a height as high as about 3 to 3.9 meters. In Nigeria, *Thevetia peruviana* has been grown for over 50 years as an ornamental plant in homes, schools and churches by missionaries and explorers (Ibiyemi et al., 2002), this made this plant almost in every part of the country, and readily available, also considering the fact that that it is a perennial plant.

2.2. Description

*Thevetia peruviana*is a tree, with a diffusely branched and dense crown, its leaves are dark green, glossy, linear and spirally arranged growing about 13-15cm in length (Bandara *et al.* 2010), it is believed that there are two varieties of the plant, one having a yellow flower (yellow oleander) and the other having purple flower (nerium oleander) to dull orange or peach, clustering at the tip of the twig with any of it being in a funnel-like shape, with 5 petal lobes that are spirally twisted. The fruit with diameter 4-5cm, longitudinally and transversely divided which grows as globular or triangular drupe, and with fleshy mesocarp, are usually green turning yellow, and then black when it is ripe and contain 1-4 seeds per kernel which can produce all the year round providing a steady supply of seeds ranging between 400 – 800 fruits per annum depending on the rainfall pattern and plant age, The plants bears sap of milky white juice in all organs (Bandara *et al.* 2010, Usman *et al.* 2009, Shepherd, 2004).

2.3. Composition and nutritive value of *Thevetia peruviana* (Oleander seed meal)

The plant has a highly nutritive value, couple with the fact of having so many medicinal value, which includes the high usefulness of its oil, the meal contains lipids, carbohydrates, vitamins, minerals and protein which have essential amino acid which can be compared with that of conventional feed stuff such as soybean meal which is the standard plant protein feed stuff.

The level or range of crude protein or crude lipid present in the *Thevetia peruviana* meal depends on the method of lipid extraction used, according to Usman *et al.* (2009) the seed has 60 – 65% oil which after extracting the oil is left with cake, which has a protein concentration ranging between 30 – 37% protein, but in an earlier report of Oluwaniyi *et al.* (2007), they reported that the crude protein content of the cake after being defatted ranges between 42.79-47.50 % while

crude lipid ranges from 4.40 to 4.80 %, which signify more level of oil extraction or deoiling, also they further reported in the search that the value of the protein further increase after it was detoxify using two methods of detoxification, the first gave them 44.45 ± 0.05 and the second method gave 53.60 ± 0.22, while the crude lipid reduced respectively. The determined proximate and amino acid composition of Oleander seed meal suggests that the plant protein could be potentially used for livestock feed, as the seeds of this plant have been reported to be rich in protein (Oluwaniyi *et al.* 2011), it is however characterized to have an edible oil if the thevetin which cause the bitterness can be removed (Ayinde *et al.* 2013).

2.4. Protein, amino acid composition of *Thevetia peruviana* seed meal

Though the research on the protein and amino acid composition on *Thevetia peruviana* seed meal are scanty but from the little research, it is believed that it has crude protein constituent such to the extent that it could be used to replace soy-bean meal in the diet of life stock, according to Ibiyemi *et al.* (2002); and Oluwaniyi *et al.*, (2007).

It was reported that *Thevetia peruviana* seed meal has as much crude protein as much as 30-35% crude protein, on the contrary Usman *et al.* (2009) reported 53.60% crude protein, while in the report of Oluwaniyi *et al.* (2011) they concluded it has up to 47.5% crude protein depending on the level of deioling and detoxification, and in the same vein detoxification of *Thevetia peruviana* seed meal led to an improved amino acid profile, with the detoxified meal having a higher percentage of essential, aromatic, Sulphur and basic amino acids but limiting in methionine, while the undetoxified meal had a higher proportion of the non-essential acid (Oluwaniyi *et al* 2011; El-Adawy and El-Kadousy 1995) and so, up to 50% soybean meal could be replace with *Thevetia peruviana* seed meal.

However according to Akintelu and Amoo (2017) essential amino acids required in human and animal diet were present in both raw and detoxified *Thevetia peruviana* seed meal by boiling with Glutamate acid being abundant in both raw (18.45%) and boiled (19.99%) sample respectively while the lowest of all in raw is Cysteine (1.18%) and in the boiled Histidine (1.25%) which they said was as a result of boiling.

Research on this subtopic are Just an handful of research as the best method to detoxify it in other to annex the potential nutrient may have led to few research works on the nutritional composition of the *Thevetia peruviana* seed meal which may have promote research on non-

dependency on most of the known conventional feed stuff, that would have promoted its industrial and domestic potentials (Nwozo *et al.* 2014).

Table 2.1. Amino acid profile of raw and detoxified *Thevetia peruviana* seed meal and soy-bean meal as the standard plant protein

Aminoacid(g/16gram of N)	Undetoxicated TSM	Alcohol detoxify TSM	Soybean meal
lysine	4.47	5.65	6.3
Histidine	1.62	1.65	2.7
Arginine	4.48	5.19	8.1
Aspartic acid	19.85	20.34	11.8
Threonine	2.61	2.67	3.9
Serine	3.93	4	5.2
Glutamic acid	14.21	15.67	17.9
Proline	4.24	4.49	5.9
Glycine	3.63	3.7	4.4
Alanine	4.49	4.56	4.3
Cysteine	1.69	1.69	1.6
Valine	4.01	4.01	5.1
Methionine	0.88	0.9	1.6
Isoleucine	2.94	2.97	7.9
Leucine	5.49	5.59	5.1
Trytophan	ND	ND	1.3

Source: Annongu and Joseph, 2008; Oluwaniyi *et al.* 2011

2.5.Oil composition of *Thevetia peruviana*

Thevetia peruviana seed oil has been investigated to produce biodiesel which has made so many research works in this direction, aside the oil being annexed for biodiesel production, the oil has also being investigated to have a medicinal value, the oil yellowish to golden colour oil when extracted and the best method of extracting the oil is through soxhlet extraction (Ofoegbu and Kelle 2013) , the oil plant can have an annual seed yield of 52.5l h^{-1} and about 1750 l of oil can be obtained from a hectare of waste land (Balusamy and Manrappan, 2007), according to Oluwaniyi *et al.* (2011); and Nwozo *et al.* (2014).

The plant produces seeds rich in oil ranging between 60—65% depending on level of extraction on dry matter bases, while according to Sahoo *et al.* (2009) he reported it having oil of 67% in the seed, with research showing that the oil has a very good replacement value for orthodox domestic vegetable oils, (Nwozo *et al* 2014), he further reported in their research report that the oil consist of 97.583% fatty acid and the most abundant is the monounsaturated Oleic acid (52%), and the others are saturated fatty acids Stearic acid (25%) and the other Palmitic acid. While according to Akintelu and Amoo (2017) the unsaturated fatty acids present in the oil include are palmitoleic acid, oleic acid, linoleic acid, linolenic acid and erucic acid, and Oleic acid was reported as still the most abundant unsaturated fatty acid followed by linoleic acid with values as follows, (54.75 % and 21.48 %) while for boiled (52.45 % and 18.95 %) respectively in each case, Ofoegbu and Kelle further work on the physical component of the oil and reported that the oil have a pH of 4.1, congealing temperature of -13^{0}C, burning with non-sooty, specific gravity of 0.9018g/cm @ 29°C and Viscosity of 126.1mpa.s at 28°C, confirming the presence of vitamin A, B2, B9, C and E.

However aside the usefulness of the oil earlier mentioned it has been researched to be useful in making paint with antifungal, antibacterial and anti-termite properties (Kareru *et al.*, 2010), Ibiyemi *et al,* (2002) also confirmed that the high saponification value of the oil makes it useful in the production of liquid soaps and shampoos.

2.6.Utilization of *Thevetia peruviana* seed meal in animal nutrition

This is one of the reasons why this study holds water. However, there have been some research work on it utility in the inclusion in the diet of poultry and was confirmed that about 15% of *Thevetiaperuviana* seed meal can be used to replace soybean meal in the diet of animal, according to Usman *et al.* (2009) *Thevetia peruviana* seed meal treated with ethanol produce no mortality an gave satisfactory growth performance in the diet of poultry bird even at inclusion level up to 15% with good nutrient retention, in the same vein, Oluwaniyi *et al.* (2007) in her feed trial using aqueous alcohol detoxified *Thevetia peruviana* seed meal in the diet of cockerel reported the bird had good growth performance when the diet's soybean meal was replace with either 10% or at 15% which is equivalent to 50% replacement of soybean meal, with no mortality but diet formulated with acid detoxified seed cake gave a satisfactory performance only at 5% inclusion, while Oluwaniyi *et al.* (2011) further affirm their work 4 years after that 15% alcohol detoxify *Thevetia peruviana* seed meal can be added to the diet of animal and it will have satisfactory growth performance and no mortality due to initial fear of the toxin present in the cake.

2.7. Factors Affecting the Nutritional Quality of *Thevetia peruviana*(Oleander seed meal)

As fascinating as the nutrient composition of this seed is, which could bridge the enlarging gap of protein deficiency and meeting the world's demand for high quality protein crops for livestock. However the limitation of *Thevetia peruviana* feed for either man or animal use is however due to the presence of some anti-nutritional factors which are very deadly for either man or animal and the anti-nutritional factors of the seed includes cardiac glycosides, phenols, terpinoids, oxalates, phytic acid, saponins and aglycones found in them (orji and Okafor 2000; Eddlestonet *al.* 2000; Oluwaniyi *et al.* 2011). Several glycosides have been extracted from various parts of *Thevetia peruviana* plant, with the major one being thevetin (Perez-Amador *et al.* 1995, Huang *et al* 1965). This toxin is however responsible for the bitterness and led to very low palatability of the seed Oluwaniyi *et al.* (2011). Detoxification of the seed will make it an excellent and cheap source of protein for animal feed because it is readily available and there is no competition between animals and man for its use

2.7.1. Anti-nutritional factors

It is reported that raw *Thevetia*cake is toxic to livestock and man (Nayar, 1957; Inman, 1967; Ahlawat et al., 1994; Eddleston et al ., 2000), horses (Siemens et al , 1995), Singh and Singh (2002) reported that leaf, stem and bark extracts of the plant killed fish, and extracts together with seed kernel extract also caused poisoning symptoms and death of albino rats (Oji & Okafor, 2000), hence the need for further processing before it can be used as ingredient in livestock feeds (Sahooet *al* 2009), *Thevetia peruviana* seed kernels are rich in cardioa-ctive glycosides, triosides i.e. the aglycone these glycosides consists of three sugar units and major constitutional glycoside is thevetin which is a mixture of two triosides namely Thevetin A and Thevetin B (cereberoside)

(Ahmad 2017), Peruvoside, nerrifolin, thevetoxin, rivoside (Rajbhar and Kumar, 2014), others includes phenols, terpinoids, oxalates, phytic acid and saponins. are found in them (Orji and Okafor 2000; Eddlestonet *al.,* 2000; Oluwaniyi *et al.,* 2011).

2.7.2. Detoxification of the Anti-nutritional factors

Several attempts have been made to detoxify the seed cake by heat treatment using autoclaving and boiling which failed to produce the desired results, neither was fermentation method successful, as evident on feed trial by researchers (Taiwo *et al.* 2004; Oluwaniyi *et al.* 2007; Akintelu and Amoo 2017), However, method employed for the detoxification of *Thevetia peruviana* seed cakes are based on the polar nature of the toxins which enhance their extraction by polar solvents e.g. ethanol, methanol; and the susceptibility of the glycosides to hydrolysis which could give lower molecular weight sugar moiety and aglycone. According to Usman *et al.* (2009) they reported 95% reduction in the cardiac glycoside in *Thevetiaperuviana* seed meal treated acid detoxification while 98% reduction of the glycoside in ethanol treated sample, Oluwaniyi *et al* (2011) gave similar report by reporting that acid detoxification followed by alcoholic extraction of the aglycones gave a meal with 95% reduction in the glycoside content (from 42.7 to 2.15 g kg-1), while direct alcohol detoxification led to a 98% reduction in the glycoside content of the seed meal (from 42.7 to 0.83 g kg-1),with the second treatment method giving the best report outcome.

2.8. Protein and amino acid Requirement of Catfish

Protein requirement is the number one watch for nutrient in the diet of any animal including fish as the protein requirement determines what level of protein is to be administer in the formula of

the feed and would meet requirement for the growth of the fish, however would not lead to eutrophication in the pond and loss in the production of feed, and so it is very essential to have a ground knowledge in the production of feed, meeting the requirement of the fish, be it the type or age of the fish and at the same time be economical. However amino acid is the building block of protein and so the amino acid requirement of catfish should also be met as the deficiency in one will affect the other essential amino acid requirement of the catfish being met, and as amino acid is the building block of protein, then the protein requirement would not be met, the most limiting essential amino acid which could affect the rest is lysine and methionine which are limiting in plant protein are ensured it meet the requirement of the fish (catfish), and in high density cultured fish a lot of energy is expanded, and so the diet is formulate to meet this demand to be between 30-45% depending on the life stage of the catfish. Other requirement to ensure that protein requirement of Catfish is met is water parameter to be at optimal range of what is required by the fish (NRC 1993)

2.9. Haematology

Haematology of any animal, catfish inclusive is traceable to the diet of the fish, or its environment which could be due to stress or bad water quality parameters, according toArejinuwa *et al.* (2001). Haematological parameters is influenced significantly by dietary treatments, other factors that could affect haematological vale of catfish includes the age, sex size and physiological condition and therefore, the heaemtology parameter will enable nutritionist to know if the anti-nutritional factors do not affect the fish. According to Binukumari *et al.*, (2011) he said that haematological parameters measure can be used to provide physiological indices that may offer critical feedback, if the fish is doing well or not.

CHAPTER THREE

3.1. MATERIALS AND METHODS

The seed was gotten from Ikole in Ikole Local Government Area, Ekiti State. Five (5) kg of the dehulled seed was used. preparation of the *Thevetia peruviana* seed meal was done to remove the anti-nutritional factor, while the wet lab of the Department of Fisheries and Aquaculture, Federal University Oye-Ekiti was used for the feeding Trial and proximate analysis done in the laboratory of Fisheries and Aquaculture of the Federal University Oye-Ekiti, and the haematological analysis was carried out in the analytical laboratory of the General Hospital Ikole-Ekiti.

3.2. Dehulling and Deioling

The fruit seed were cracked opened to remove the soft seed kernels, crushed into paste, the paste was defatted first by mechanical processing followed by solvent extraction by soxhlet method using petroleum ether to obtain the oil. The defatted cake was then air-dried.

3.2.1. Detoxification

The solvent extraction of the defatted seed meal was done using 80% (v/v) aqueous alcohol mixture. The detoxification experiments were carried out as reported previously Oluwaniyi *et al.* (2007) and described by Finnigan and Lewis (1988). The extractions were performed at ambient temperature. Sample of the defatted *Thevetia peruviana* seed meal was placed in a flask and solvent (aqueous alcohol) was added to give the appropriate solvent to meal ratio. Extraction at 45 min was achieved using a magnetic stirrer. Alcoholic extraction was performed using aqueous

8:2 ethanol: methanol mixture, and detoxified samples were air-dried (at ambient temperature) to remove residual solvent in them.

3.3. Diet formulation

Five isonitrogenous (40% protein) and isocaloric (2821.75 k cal/kg ME) ration was formulated and compounded with different level inclusion of *Thevetiaperuviana*seed meal. Diet 2,3,4 and 5 having portion of *Thevetiaperuviana* seed meal at 5%, 10%, 15% and 20% respectively, while diet 1 has no *Thevetiaperuviana* seed meal as shown in the Table 3.1

The feed ingredient for each of the diet were weighed, ground and each diet were mixed together and pelleted using pelleting machine and 2mm die. The resulting pellet was sun dried, packed in a polythene bag and stored in a cool dry place until used.

Table 3.1 Percentage composition of experimental diet

INGREDIENT	0TSM	5TSM	10TSM	15TSM	20TSM
Fish meal	30.00	30.00	30.00	30.00	30.00
Groundnut cake	14.00	13.40	13.40	13.40	13.40
Maize	8.96	9.56	9.56	9.56	9.56
Wheat offal	5.00	5.00	5.00	5.00	5.00
Thevetiaperuviana seed meal (TSM)	0.00	5.00	10.00	15.00	20.00
Soy-bean meal	33.01	28.01	23.01	18.01	13.40
Vitamin/mineral premix	2.00	2.00	2.00	2.00	2.00
Vitamin c	0.03	0.03	0.03	0.03	0.03
Salt	0.50	0.50	0.50	0.50	0.50
Methionine	1.00	1.00	1.00	1.00	1.00
Binder	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Vegetable oil	3.00	3.00	3.00	3.00	3.00
TOTAL	100	100	100	100	100

Vit. A; 4000000iu, Vit. D3 800000iu, Tocopherois; 4000 iu, Vit k3; 800mg, folacin; 200mg,Vit b11.8mg Vit B2, 5mg, thiamine; 600mg riboflavin 1800mg, niacin 6000mg, calcium pantothenic 2000mg, pyridoxine 00mg, cyanocolabamin 4mg, biotin 3mg, magnesium 30000mg, zinc 20000mg, iron 8000mg, copper 20000mg, iodine 480mg cobalt 80mg; selenium 40mg, chlorine chloride 80000mg, manganese, 30000mg, BHT 26000mg, anticaking agent 6000.

3.4. EXPERIMENTAL FISH

One hundred and fifty *Clarias gariepinus* Fingerlings were obtained from a reputable farm within the vicinity and was acclimatized in a randomly selected 15 aquaria tank for 14 days before the commencement of the feeding trial, during which the fish where feed vital feed, after then starved for a day to aid acceptance of introduced feed and then place on feeding trial as stated above i.e. feeding with a diet comprising *Thevetiaperuviana* seed meal at level 0%, 5%, 10%, 15%, 20%. Feeding was done by 5% body of the fish. Fish were weighed every two weeks to determine growth performance.

3.5. EXPERIMENTAL DESIGN

There were five (5) treatment groups with three replicate each. Fifteen (15) tanks were used, the fish were acclimatized for two (2) weeks and the fish was fed and the weight taken, the treatment was replicated and ten fish stocked randomly into each of the 15 aquarium tank after which feeding was suspended for 24 hours before feeding trial to aid appetite for introduced treatment diet. The experimental design used is complete randomized design (CRD)

3.6. EXPERIMENTAL PROCEDURE

One hundred fifty *Clarias gariepinus* fingerlings was purchased from a reputable farm within the vicinity of Ikole and used during the experiment. The fish were acclimatized for two weeks, during the period of acclimatization the fish were fed at 5% body weight twice daily with a formulated diet of 40% crude protein. At the end of the acclimatization period, the fish were randomly selected and stocked into 15 rectangular aquaria holding 10 fishes. The trial lasted 8 weeks and was conducted in fifteen plastic tanks, the tank was properly placed in the wet laboratory of Department of Fisheries and Aquaculture of Federal University Oye-Ekiti, and properly monitored to ensure good water quality state. Feeding trial three tanks were randomly assigned to each diet groups for the eight weeks' experiment, at the start of the experiment, 10 fish were batch weighted and stocked into each tank. During the feeding period, fish were fed the experimental diet of 5% body weight twice a day at 08:00 and 16:00 respectively, One hour later the uneaten feed was siphoned.

3.7. CHEMICAL ANALYSES

Chemical Analysis of feed were analyzed for proximate composition: moisture, ash, crude protein, crude lipid, crude fibre and nitrogen free extract according to the methods Proximate composition of feed was determined using Association of Official Analytical Chemistry (A.O.A.C, 2003) method, Samples of experimental diets were taken to the Laboratory of Department of Fisheries and Aquaculture for proximate analysis using the methods described by A.O.A.C. Crude protein by the Kjedahl procedure, ether extract by subjecting the samples to petroleum ether extraction at 60-100^{0}C using the soxhlet extraction apparatus. Dry matter by oven drying the samples at 105^{0}C over a 6- hour period. Crude fiber by boiling the samples under flux in weak sulphuric acid (0.255N H2S04), then in a weak sodium hydroxide (0.312N

NaOH) for 1 hour. The residues which consist of cellulose, lignin and mineral matter were dried and weighed. The ash content was determined by igniting a weighed sample in a Muffle furnace at 550^0C. The nitrogen free extract (NFE) was obtained by the difference after the percentages of the other fractions were subtracted from 100%.

3.7.1.GROWTH AND NUTRITIONAL ANALYSIS

Growth and nutritional analysis were determined using the following formula

- Specific growth rate=$\{[In(W_2)-In(W_1)\ /T] \times 100\}$
- Feed conversion ratio=$\frac{feedintake}{weightgained}$
- % mortality =number of dead fish/number of fishes stocked X $100\frac{X}{Y} \times 100$

3.8 HAEMATOLOGY

Red blood cell count (RBC), Haemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBC) and white cell differential count was determined by the methods of baker and silverton (1985), while the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) was determined from RBC, Hb, and PCV (Hamening 1992).

Using

- MCV (Fl)= PCV/RBC (10^6µl-1)
- MCH (pg)= [Hb(gdl-1) X10] /RBC (106 µl-1)
- And MCHC (gl-1) = [Hb(gdl-1)X 10]/PCV

3.8.1.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 using haematocrit reader (Kelly, 1979).

3.8.2. 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).

3.8.3. 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.

3.8.4. 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 were 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 STATISTICAL ANALYSIS

The data collected was subjected to analysis of variance (ANOVA) and significant ($p<0.05$) difference found, was subjected to Duncan's multiple range test (Duncan, 1955) ranked using SPSS version 16.0 (SPSS 2008) and SAS. The data presented as mean ± S.E.M. of three replicate groups.

The statistical model used was a one-way analysis of variance.

$Yij = \mu + Bi + Eij$

Yij = the jth observation of the ith. Treatment (*Thevetiaperuviana* seed meal)

μ= the overall estimate of population mean

Bi= the effect of the ith treatment

Eij = the random error.

CHAPTER FOUR

4.1 PROXIMATE COMPOSITION OF EXPERIMENTAL DIET

Proximate composition of experimental diet is presented on table 4.1, there were significant difference ($p<0.05$) in all the moisture content of the feed, with highest recorded in control (8.19 ± 0.01) and lowest in 5TSM (7.61 ± 0.01), there were no significant difference ($p>0.05$) in protein content of 5TSM and 15TSM, which was significantly different ($p<0.05$) from diet of 20TSM, control and 10TSM respectively, equally there were no significant difference ($p>0.05$) in the lipid of 5TSM, 15TSM and 20TSM, and significantly different ($p<0.05$) in the diet of 10TSM and Control respectively, with highest lipid content recorded in control (20.25 ± 0.04) and lowest in 10TSM (17.56 ± 0.02). Also, there were no significant difference ($p>0.05$) in the fiber content of diet of 5TSM and 10TSM respectively but were significantly different ($p<0.05$) from the diet of control,15TSM and 20TSM respectively with highest fiber content in diet recorded in control diet (4.58 ± 0.01) and lowest in 20TSM (2.50 ± 0.08). However, there were significant difference ($p<0.05$) in the ash content of the diet in 10TSM, 15TSM and 20TSM respectively, but no significant difference ($p>0.05$) was observed in the control and 5TSM diet respectively. Finally, no significant difference was observed in the diet of control, 15TSM and 20TSM, likewise in 5TSM and 10TSM respectively, with highest NFE recorded in 5TSM (24.18 ± 0.02) and lowest NFE in 15TSM (23.00 ± 0.14).

Table 4.1: Proximate composition of the experimental diet

Parameters	Ctrl	5TSM	10TSM	15TSM	20TSM
Moisture	8.19 ± 0.01^{d}	7.61 ± 0.01^{a}	7.91 ± 0.02^{b}	8.26 ± 0.04^{e}	8.00 ± 0.01^{c}
Crude Protein	40.49 ± 0.07^{b}	40.71 ± 0.01^{c}	40.94 ± 0.00^{d}	40.73 ± 0.02^{c}	40.30 ± 0.00^{a}
Lipid	20.25 ± 0.04^{c}	18.08 ± 0.01^{b}	17.56 ± 0.02^{a}	18.04 ± 0.09^{b}	17.94 ± 0.08^{b}
Fiber	2.50 ± 0.08^{a}	3.81 ± 0.01^{b}	3.90 ± 0.01^{b}	4.16 ± 0.01^{c}	4.58 ± 0.01^{d}
Ash	5.52 ± 0.01^{a}	5.62 ± 0.02^{ab}	5.72 ± 0.01^{bc}	5.81 ± 0.01^{c}	6.12 ± 0.07^{d}
NFE	23.04 ± 0.01^{a}	24.18 ± 0.02^{b}	24.00 ± 0.02^{b}	23.00 ± 0.14^{a}	23.06 ± 0.10^{a}

Mean ± S.E with different super script are significantly different from each other

4.2 Proximate composition of Clarias gariepinus fed Thevetiaperuviana seed meal

Proximate composition of *Clarias gariepinus* fingerlings fed Oleander seed meal is presented on table 4.3: moisture composition of the fish fed the experimental diet shows no significant difference in diet of control and 15TSM,5TSM and10TSM respectively, but were significantly different from diet of 20TSM, while highest moisture content was recorded in 20TSM (70.96 ± 0.02), and lowest in 5TSM (70.10 ± 0.05), also no significant difference ($p>0.05$) occurred in protein content of fish fed 10TSM and 15TSM, but were significantly different ($p<0.05$) from those fed 5TSM, 20TSM and control diet, with highest crude protein recorded in the flesh of fish feed control diet (19.77 ± 0.14) and lowest in body flesh of fish fed 5TSM (16.06 ± 0.17). No significant difference ($p>0.05$) occurred in the lipid content of flesh of fish fed 5TSM and 15TSM, 10TSM and 20TSM respectively, significantly different was observed in ($p<0.05$) that of control, while highest lipid was recorded in control (1.31 ± 0.02) and lowest in 5TSM (0.86 ± 0.01). Furthermore, there were significant difference ($p<0.05$) in fiber content of all the fish fed the experimental diet, with highest in control (0.86 ± 0.01) and lowest in 20TSM (0.45 ± 0.01). Similarly, in the NFE,significant difference ($p<0.05$) occurred in the flesh of the fish fed the experimental diet, where highest occurred in 5TSM (8.56 ±0.14) and lowest in 20TSM (3.20 ± 0.23). However, there were no significant difference ($p>0.05$) in the flesh of fish fed 15TSM and 20TSM, but were significantly different from control,5TSM and 10TSM.

Table 4.2: Proximate composition of *Clarias gariepinus* fed *Thevetiaperuviana* seed meal

Parameters	Ctrl	5TSM	10TSM	15TSM	20TSM
Moisture	70.60 ± 0.08^{b}	70.10 ± 0.05^{a}	70.19 ± 0.06^{a}	70.55 ± 0.02^{b}	70.96 ± 0.02^{c}
Protein	19.77 ± 0.14^{d}	16.06 ± 0.17^{a}	17.06 ± 0.02^{b}	17.63 ± 0.29^{b}	19.00 ± 0.26^{c}
Lipid	1.31 ± 0.02^{c}	0.86 ± 0.01^{a}	$0.92\ 0.01^{b}$	0.89 ± 0.02^{ab}	0.92 ± 0.01^{b}
Fiber	0.45 ± 0.01^{a}	0.53 ± 0.01^{b}	0.62 ± 0.01^{c}	0.72 ± 0.01^{d}	0.86 ± 0.01^{e}
Ash	3.57 ± 0.02^{a}	3.86 ± 0.02^{b}	4.02 ± 0.03^{c}	5.01 ± 0.01^{d}	5.04 ± 0.01^{d}
NFE	4.30 ± 0.10^{b}	8.56 ± 0.14^{e}	7.18 ± 0.06^{d}	5.20 ± 0.33^{e}	3.20 ± 0.23^{a}

Mean ± S.E with different super script are significantly different from each other

4.3: Effect of *Thevetia peruviana* seed meal seed meal based diet on growth performance of *Clarias gariepinus* fingerlings.

Result of growth performance of *Clarias gariepinus* fingerlings fed *Thevetiaperuviana* seed meal diet is presented on Table 4.1, initial weight of the *Clarias gariepinus* fingerlings samples was similar ($p>0.05$) in all the experimental treatment with highest (6.35 ± 0.80) in 15TSM and lowest (5.85 ± 0.64) in 10TSM. Similarly, there were no significant differences ($p>0.05$) in the result of the final weight, weight gain and average daily weight gain of the fish fed control diet and *Thevetiaperuviana* seed meal diets 5% TSM, 10%TSM, and 15%TSM, but were significantly different ($p<0.05$) from the final weight of *Clarias gariepinus* fed 20TSM. Feed intake of *Clarias gariepinus* fingerlings fed control diet and 5TSM, 10TSM and 20TSM were similar ($p>0.05$) but were significantly different ($p<0.05$) from feed intake of fish fed 15TSM, with highest (11.34 ± 0.92) feed intake observed in fish fed 15TSM and lowest (6.10 ± 0.07) in fish fed 5TSM. Protein efficiency ratio was similar ($p>0.05$) in *Clarias gariepinus* fingerlings fed Control diet, 5TSM, 10TSM, and 15TSM, but was significantly higher ($p<0.05$) in fish fed 20TSM. The feed conversion ratio of fish fed 15TSM (1.80 ± 0.23) was the highest and was significantly different ($p<0.05$) from that of *Clarias gariepinus* fingerlings fed Control diet, 5TSM, 10TSM and 20TSM diets. Feed efficiency ratio of *Clarias gariepinus* fingerlings fed Control diet, 5TSM and 20TSM, were similar ($p>0.05$) but was significantly different ($p<0.05$) from those fed 10TSM and 15TSM which were similar ($p>0.05$). Specific growth rate of *Clarias gariepinus* fingerlings fed shows similarity ($p>0.05$) in all experimental diet of control diet and *Thevetiaperuviana* seed meal-based diet respectively, with highest specific growth rate (1.67 ± 0.07) observed in fish fed 20%TSM and lowest (1.13 ± 0.14) in fish fed 5%TSM based diet respectively. Significant differences were not observed ($p>0.05$) the relative growth rate of

Clarias gariepinus fingerlings fed the experimental diets. Mortality rate of *Clarias gariepinus* fingerlings fed control, 5TSM, 20TSM and 10TSM diets were similar ($p>0.05$) but was significantly different ($p<0.05$) from that of fish fed 15TSM. Initial length of*Clarias gariepinus* fingerlings used for the study shows similarity ($p>0.05$) for fish fed control diet, 5TSM, and 20TSM but was significantly different ($p<0.05$) from that of fish fed 10TSM and 15TSM. There was no significant difference ($p>0.05$) in the final length of *Clarias gariepinus* fingerlings fed 5TSM, 10 TSM, 15 TSM and 20TSM, however, they were significantly different from that of fish fed the control diet.

Table 4.3: Effect of *Thevetia peruviana* seed meal diets on growth performance of *Clarias gariepinus* fingerlings

Parameter	Ctrl 0TSM	5TSM	10TSM	15TSM	20TSM
Initial weight	5.92 ± 0.84^{a}	6.30 ± 0.36^{a}	5.85 ± 0.64^{a}	6.35 ± 0.80^{a}	6.00 ± 0.25^{a}
Final weight	12.09 ± 0.78^{a}	11.84 ± 0.41^{a}	12.45 ± 0.20^{a}	12.91 ± 0.33^{ab}	14.16 ± 0.45^{b}
Weight gain	6.17 ± 0.52^{a}	5.54 ± 0.66^{a}	6.60 ± 0.68^{ab}	6.56 ± 1.10^{ab}	8.61 ± 0.37^{b}
Average daily weight gain	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}	0.12 ± 0.01^{ab}	0.12 ± 0.02^{ab}	0.15 ± 0.01^{b}
Feed intake	6.96 ± 0.37^{ab}	6.10 ± 0.07^{a}	8.39 ± 0.44^{b}	11.34 ± 0.92^{c}	8.13 ± 0.47^{b}
Protein Efficiency ratio	0.15 ± 0.01^{a}	0.14 ± 0.02^{a}	0.16 ± 0.02^{ab}	0.16 ± 0.03^{ab}	0.21 ± 0.01^{b}
Feed conversion ratio	1.13 ± 0.04^{a}	1.14 ± 0.14^{a}	1.31 ± 0.19^{a}	1.80 ± 0.23^{b}	0.95 ± 0.06^{a}
Feed Efficiency ratio	88.42 ± 2.84^{b}	90.74 ± 10.77^{b}	79.87 ± 12.31^{ab}	57.13 ± 6.46^{a}	106.58 ± 6.99^{b}
Specific Growth rate	1.31 ± 0.20^{a}	1.13 ± 0.14^{a}	1.47 ± 0.18^{a}	1.29 ± 0.26^{a}	1.67 ± 0.07^{a}
Relative Growth rate	210.48 ± 24.22^{a}	189.47 ± 15.22^{a}	218.84 ± 27.51^{a}	210.28 ± 0.13^{a}	255.89 ± 9.96^{a}
Mortality	6.67 ± 6.67^{a}	6.67 ± 6.67^{a}	23.33 ± 3.33^{ab}	40.00 ± 5.77^{b}	16.67 ± 3.33^{a}
Initial Length	8.62 ± 0.30^{ab}	8.73 ± 0.18^{ab}	9.03 ± 0.26^{b}	9.13 ± 0.03^{b}	7.90 ± 0.47^{a}
Final length	12.11 ± 0.68^{c}	9.48 ± 0.56^{a}	11.52 ± 0.30^{bc}	11.62 ± 0.74^{bc}	10.97 ± 0.49^{ab}

Mean ± S.E with different super script are significantly different from each other

4.4: Haematological analysis of *Clarias gariepinus* fingerlings fed *Thevetia peruviana* seed meal

Result of Haematological parameters of *Clarias gariepinus* fingerlings fed *Thevetia peruviana* seed meal diets is presented on Table 4.2: white blood cell ($p>0.05$) in Control diet, 5TSM, and 10TSM, was significantly different ($p<0.05$) from 15TSM and 20TSM, with the highest (10368.33 ± 900.28) in 20TSM while lowest (2833.33 ± 202.76) in control, Neutrophi l observed showed that there were no significant difference ($p>0.05$) in all the experimental diet with highest (32.33 ± 4.33) in 10TSM fed fish while lowest (18.00 ± 1.73) in control fed fish diet. Also there was no significant difference ($p>0.05$) recorded Lymphocyte of *Clarias gariepinus* fingerlings fed control diet and *Thevetiaperuviana* seed meal (5TSM, 10TSM, 15TSM and 20TSM) base diet, with highest recorded in control (82.00 ± 1.73) and lowest in 10TSM (67.67 ± 4.33).Packed cell volume of the fish shows no significant difference ($p>0.05$) in 10TSM and 15TSM, and in Control diet and 5TSM respectively, but was significantly different from record of packed cell volume of fish fed 20TSM. Also the red blood cell (RBC) of fish fed the experimental diet shows that there were no significant difference ($p>0.05$) in red blood cell of fish fed 10TSM and 15TSM, likewise in that of control diet and 5TSM respectively, but were significantly different ($p<0.05$) in red blood cell of fish fed 20TSM, with highest (3.47 ± 0.09) in RBC of fish fed control diet and lowest in RBC of fish fed 20TSM (1.73 ± 0.12).Haemoglobin of *Clarias gariepinus* fed Control diet and 5TSM,10TSM and 20TSM were not significantly different ($p>0.05$),but were significantly different ($p<0.05$) from Haemoglobin of fish fed 20TSM, with highest haemoglobin in control diet fed fish (14.89 ± 0.29) and lowest in 15TSM (11.11 ± 0.95) fed fish. Mean corpuscular haemoglobin of fish as recorded were not significantly different($p>0.05$) in control and 5TSM, fed fish, but were significantly different ($p<0.05$) from

10TSM, 15TSM and 20TSM fed diet fish. Mean corpuscular volume (MCV) of *Clariasgariepinus*fingerlings ($p>0.05$) in control and 5TSM diet fed fish, are significantly different ($p<0.05$) from MCV of fish fed 10TSM, 15TSM and 20TSM diet with highest (158.29 ± 4.28) in 20TSM and lowest (128.88 ± 0.74) in MCV control feed diet. Mean corpuscular haemoglobin concentration of the fish shows no significant effect ($p>0.05$) for all fish fed the experimental diet fed either the control diet or *Thevetiaperuviana* seed meal diets.

Table 4.4: Haematological analysis of fish (*Clarias gariepinus*) fingerlings fed *Thevetia peruviana* seed meal

Parameter	Ctrl 0TSM	5TSM	10TSM	15TSM	20TSM
White blood cell($x10^3$mm)	2833.33 ± 202.76^{a}	3233.33 ± 145.30^{a}	4133.33 ± 185.59^{ab}	5266.67 ± 290.59^{b}	10368.33 ± 900.28^{c}
Neutrophill (%)	18.00 ± 1.73^{a}	28.33 ± 10.93^{a}	32.33 ± 4.33^{a}	30.67 ± 3.48^{a}	23.00 ± 2.08^{a}
Lymphocyte(%)	82.00 ± 1.73^{a}	71.67 ± 10.93^{a}	67.67 ± 4.33^{a}	69.33 ± 3.48^{a}	77.00 ± 2.08^{a}
Packed cell Volume(%)	44.67 ± 0.88^{c}	41.67 ± 1.45^{c}	34.00 ± 1.15^{b}	33.33 ± 2.85^{b}	27.33 ± 1.20^{a}
Red blood Cell($x10^{12}$mm/L)	3.47 ± 0.09^{c}	3.17 ± 0.15^{c}	2.40 ± 0.12^{b}	2.30 ± 0.31^{b}	1.73 ± 0.12^{a}
Haemoglobin concentration(g/L)	14.89 ± 0.29^{c}	13.89 ± 0.49^{c}	11.33 ± 0.38^{b}	11.11 ± 0.95^{b}	9.11 ± 0.40^{a}
Mean corpuscular Haemoglobin(pg)	4.30 ± 0.0^{a}	4.39 ± 0.05^{ab}	4.73 ± 0.07^{bc}	4.89 ± 0.23^{cd}	5.28 ± 0.14^{d}
Mean corpuscular Volume (fl)	128.88 ± 0.74^{a}	131.71 ± 1.48^{ab}	141.86 ± 2.02^{bc}	146.66 ± 6.77^{cd}	158.29 ± 4.28^{d}
Mean corpuscular Haemoglobin concentration (g/l)	33.33 ± 0.00^{a}	33.33 ± 0.00^{a}	33.33 ± 0.01^{a}	33.33 ± 0.00^{a}	33.33 ± 0.01^{a}

Mean ± S.E with different super script are significantly different from each other

4.5DISCUSSION

The aim of all food nutritionist is to reduce the inclusion of conventional protein source with non-conventional protein feed source, as feed source such as fishmeal, groundnut cake, and soybean meal which constitute a substantial part of formulated feed for diverse livestock. Proximate composition of *Thevetia peruviana* seed meal are relatively rich in protein value with the formulate diet meeting the requirement of *Clarias gariepinus* fingerlings of 40% crude protein which is similar to the report of Adebayo *et al.* (2016), with moisture, ash, fiber, lipid and NFE still within the recommended range (NRC 1993).

In the present study, the growth performance of fish improved with the replacement of soybean meal with detoxified *Thevetiaperuviana*seed meal. Improve growth performance have been previously reported in cockerel when 50% of soybean meal was replaced by detoxified TSM by Oluwaniyi *et al.*, (2011)., which is contrary to previous report (Taiwo *et al.* 2004). As a result of the treatment, there was increase in weight gain, average daily weight gain, feed intake, protein efficiency ratio, specific growth rate and relative growth rate across the diet which has part inclusion of *Thevetia peruviana* seed meal, with perform more that feed fed control diet, with fish fed 20*Thevetia peruviana* seed meal performing better than all, under the above mention parameters similar to the report of Oluwaniyi *et al.* (2011) and Usman *et al.* (2009), the result of this study on nutritional and growth performance of *Clarias gariepinus* fed *Thevetia peruviana* seed meal as partial replacement of protein plant protein source indicated that up to 20% replacement with *Thevetia peruviana* seed meal can be included in the diets of *Clarias gariepinus*, Oluwaniyi *et al.* (2011) and Usman *et al.* (2009)revealed in their report that 15% replacement of *Thevetia peruviana* seed meal in the diet of livestock performed positively, but Taiwo et al. (2004) gave negative report majorly due to the mode of treatment method used, but

5% inclusion was advised in his report, but in this report up to 20TSM inclusion had better growth performance as against all other experimental diet, but performed below other experimental fish samples in parameters obtained for final length and feed conversion ratio but increase in PER as the growth increased and vice versa. Such observation may be related to the fact that FCR decreases while PER increases with increased feeding rate as reported by Pechsiri and Yakupitiyage, (2005), but still are within required range, and might be caused by unforeseen circumstances such as genetic makeup, mortality was observed in all the experimental unit, which or growth retardation (Oluyemi and Nelson 2016), mortality was however observed in all the experimental diet, with may be due to physiochemical parameters which are uncontrollable such as temperature. Dried fish had higher crude protein than the fresh fish. Increase of crude protein in dried samples may be due to the dehydration of water molecule present between the proteins thereby causing aggregation of protein and thus resulting in the increase in protein content of dried fishes (Adebayo *et al.* 2016), crude protein of the fish after the experimental period has an average of 19.00%, which was similar to the report of Adebayo et al. 2016, Fagbenro *et al.,* (2010) and differ from the report of Oladipo and Bankole (2013) who reported 17.50%, the difference recorded may be due to their ability to metabolize and utilize essential nutrients from their diets (Adewoye and Omotosho 1997), the nature and quality of nutrient present in the experimental diet (Adebayo *et al., 2016*)

Haematology of any animal, catfish inclusive is traceable to the diet of the fish, or its environment which could be due to stress or bad water quality parameters, according to Arejinuwa *et al.,* (2001) which holds high value 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 all within the recommended physiological ranges reported for *Clariasgariepinus*.

CHAPTER FIVE

5.1 CONCLUSION

Conventional protein plant source are good sources of protein source in the diet of livestock, but they are expensive, scarce which cause high competitive demand, *Thevetia peruviana* plant is one of the cheapest close substitute,its nutrient profile is close to that of other plant protein source. Therefore, from the research the results obtained from this study, *Thevetia peruviana* seed meal could be used to partially replace soy-bean meal up to 20% level in *Clarias gariepinus* diets without any negative effects on the growth and feed efficiency. It will equally bring aboutreduction in the cost of feed and fish production.

5.2 RECOMMENDATIONS

Given the nutritional potential of the*Thevetia peruviana* meal, I recommend that:

- Up to 20% of TSM be included in the diet of *C. gariepinus* fingerlings.
- Research on the possibility of total replacement of soy-bean meal in the diet of *C. gariepinus* be conducted.
- More *Thevetia peruviana* seed should be planted to improve the production and availability in the market

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The ANOVA Procedure

Class Level Information

Class	Levels	Values
TREATMENT	5	1 2 3 4 5

Number of observations 15

The ANOVA Procedure

ependent Variable: MOISTURE MOISTURE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.43460000	0.35865000	50.51	<.0001
Error	10	0.07100000	0.00710000		
Corrected Total	14	1.50560000			

R-Square	Coeff Var	Root MSE	MOISTURE Mean
0.952843	0.119554	0.084261	70.48000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	1.43460000	0.35865000	50.51	<.0001

The ANOVA Procedure

ependent Variable: CP CP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	26.53244000	6.63311000	55.86	<.0001
Error	10	1.18753333	0.11875333		
Corrected Total	14	27.71997333			

R-Square	Coeff Var	Root MSE	CP Mean
0.957160	1.924600	0.344606	17.90533

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	26.53244000	6.63311000	55.86	<.0001

The ANOVA Procedure

ependent Variable: LIPID LIPID

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.42084000	0.10521000	147.49	<.0001
Error	10	0.00713333	0.00071333		
Corrected Total	14	0.42797333			

R-Square	Coeff Var	Root MSE	LIPID Mean
0.983332	2.721634	0.026708	0.981333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	0.42084000	0.10521000	147.49	<.0001

The ANOVA Procedure

pendent Variable: FIBRE FIBRE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.31404000	0.07851000	206.61	<.0001
Error	10	0.00380000	0.00038000		
Corrected Total	14	0.31784000			

R-Square	Coeff Var	Root MSE	FIBRE Mean
0.988044	3.055421	0.019494	0.638000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	0.31404000	0.07851000	206.61	<.0001

The ANOVA Procedure

ependent Variable: ASH ASH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.59910667	1.39977667	846.64	<.0001
Error	10	0.01653333	0.00165333		
Corrected Total	14	5.61564000			

R-Square	Coeff Var	Root MSE	ASH Mean
0.997056	0.945170	0.040661	4.302000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	5.59910667	1.39977667	846.64	<.0001

ependent Variable: NFE NFE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	56.72786667	14.18196667	121.08	<.0001
Error	10	1.17126667	0.11712667		
Corrected Total	14	57.89913333			

R-Square	Coeff Var	Root MSE	NFE Mean
0.979771	6.011201	0.342238	5.693333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	56.72786667	14.18196667	121.08	<.0001

ependent Variable: MOISTURE1 MOISTURE1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.79502667	0.19875667	188.69	<.0001
Error	10	0.01053333	0.00105333		

Corrected Total	14	0.80556000

R-Square	Coeff Var	Root MSE	MOISTURE1 Mean
0.986924	0.405993	0.032455	7.994000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	0.79502667	0.19875667	188.69	<.0001

The ANOVA Procedure

ependent Variable: CP_feed CP feed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.72126667	0.18031667	60.78	<.0001
Error	10	0.02966667	0.00296667		
Corrected Total	14	0.75093333			

R-Square	Coeff Var	Root MSE	CP_feed Mean
0.960494	0.134034	0.054467	40.63667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	0.72126667	0.18031667	60.78	<.0001

The ANOVA Procedure

ependent Variable: LIPIDfeed LIPIDfeed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	13.72550667	3.43137667	345.67	<.0001
Error	10	0.09926667	0.00992667		
Corrected Total	14	13.82477333			

R-Square	Coeff Var	Root MSE	LIPIDfeed Mean
0.992820	0.542209	0.099633	18.37533

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	13.72550667	3.43137667	345.67	<.0001

11

The ANOVA Procedure

ependent Variable: FIBREfeed FIBREfeed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.31280000	1.82820000	502.25	<.0001
Error	10	0.03640000	0.00364000		
Corrected Total	14	7.34920000			

R-Square	Coeff Var	Root MSE	FIBREfeed Mean
0.995047	1.591884	0.060332	3.790000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	7.31280000	1.82820000	502.25	<.0001

12

The ANOVA Procedure

ependent Variable: ASHfeed ASHfeed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.62896000	0.15724000	45.45	<.0001
Error	10	0.03460000	0.00346000		
Corrected Total	14	0.66356000			

R-Square	Coeff Var	Root MSE	ASHfeed Mean
0.947857	1.021921	0.058822	5.756000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	0.62896000	0.15724000	45.45	<.0001

13

The ANOVA Procedure

ependent Variable: NFEfeed NFEfeed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.96337333	0.99084333	58.06	<.0001
Error	10	0.17066667	0.01706667		
Corrected Total	14	4.13404000			

R-Square	Coeff Var	Root MSE	NFEfeed Mean
0.958717	0.557145	0.130639	23.44800

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREATMENT	4	3.96337333	0.99084333	58.06	<.0001

The ANOVA Procedure

Duncan's Multiple Range Test for MOISTURE

OTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.0071

Number of Means	2	3	4	5
Critical Range	.1533	.1602	.1643	.1668

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	70.96000	3	5
B	70.60000	3	1
B			
B	70.55000	3	4
C	70.19000	3	3
C			
C	70.10000	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for CP

)TE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.118753

Number of Means	2	3	4	5
Critical Range	.6269	.6551	.6717	.6824

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	19.7667	3	1
B	19.0033	3	5
C	17.6267	3	4
C			
C	17.0667	3	3
D	16.0633	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for LIPID

OTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.000713

Number of Means	2	3	4	5
Critical Range	.04859	.05078	.05206	.05289

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	1.31333	3	1
B	0.92667	3	5
B			
B	0.91667	3	3

	B			
C	B	0.88667	3	4
C				
C		0.86333	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for FIBRE

)TE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.00038

Number of Means	2	3	4	5
Critical Range	.03546	.03706	.03800	.03860

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	0.86333	3	5
B	0.72333	3	4
C	0.62000	3	3
D	0.53333	3	2
E	0.45000	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for ASH

OTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.001653

Number of Means	2	3	4	5
Critical Range	.07397	.07730	.07926	.08051

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	5.04333	3	5
A			
A	5.01333	3	4
B	4.02333	3	3
C	3.86333	3	2
D	3.56667	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for NFE

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.117127

Number of Means	2	3	4	5
Critical Range	.6226	.6506	.6671	.6777

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	8.5767	3	2
B	7.1833	3	3
C	5.2000	3	4
D	4.3033	3	1
E	3.2033	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for MOISTURE1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.001053

Number of Means	2	3	4	5
Critical Range	.05904	.06170	.06326	.06426

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	8.26000	3	4
B	8.19333	3	1
C	7.99667	3	5
D	7.91000	3	3
E	7.61000	3	2

Duncan's Multiple Range Test for CP_feed

OTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.002967

Number of Means	2	3	4	5
Critical Range	.0991	.1035	.1062	.1079

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	40.94333	3	3
B	40.73333	3	4
B			
B	40.71000	3	2
C	40.49333	3	1
D	40.30333	3	5

Duncan's Multiple Range Test for LIPIDfeed

)TE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.009927

Number of Means	2	3	4	5
Critical Range	.1813	.1894	.1942	.1973

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	20.25333	3	1
B	18.07667	3	2
B			
B	18.04333	3	4
B			
B	17.94000	3	5
C	17.56333	3	3

The ANOVA Procedure

Duncan's Multiple Range Test for FIBREfeed

OTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.00364

Number of Means	2	3	4	5
Critical Range	.1098	.1147	.1176	.1195

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	4.58000	3	5
B	4.16000	3	4
C	3.90000	3	3

	C			
	C	3.81000	3	2
	D	2.50000	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for ASHfeed

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.00346

Number of Means	2	3	4	5
Critical Range	.1070	.1118	.1147	.1165

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TREATMENT
	A	6.11667	3	5
	B	5.81000	3	4
	B			
C	B	5.71667	3	3
C				
C	D	5.61667	3	2
	D			
	D	5.52000	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for NFEfeed

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.017067

Number of Means	2	3	4	5
Critical Range	.2377	.2484	.2547	.2587

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREATMENT
A	24.1767	3	2
A			
A	23.9667	3	3
B	23.0633	3	5
B			
B	23.0400	3	1
B			
B	22.9933	3	4

The ANOVA Procedure

Class Level Information

Class	Levels	Values
TSM	5	1 2 3 4 5

Number of observations 15

The ANOVA Procedure

ependent Variable: INITIALWEIGHT INITIALWEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.33584000	0.33396000	0.28	0.8815
Error	10	11.74333333	1.17433333		
Corrected Total	14	13.07917333			

R-Square	Coeff Var	Root MSE	INITIALWEIGHT Mean
0.102135	18.07517	1.083667	5.995333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	1.33584000	0.33396000	0.28	0.8815

The ANOVA Procedure

pendent Variable: FINALWEIGHT FINALWEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	10.07004000	2.51751000	3.74	0.0414
Error	10	6.74013333	0.67401333		
Corrected Total	14	16.81017333			

R-Square	Coeff Var	Root MSE	FINALWEIGHT Mean
0.599044	6.470208	0.820983	12.68867

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	10.07004000	2.51751000	3.74	0.0414

The ANOVA Procedure

ependent Variable: WEIGHTGAIN WEIGHTGAIN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	15.96609333	3.99152333	2.66	0.0956
Error	10	15.00966667	1.50096667		
Corrected Total	14	30.97576000			

R-Square	Coeff Var	Root MSE	WEIGHTGAIN Mean
0.515438	18.29659	1.225139	6.696000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	15.96609333	3.99152333	2.66	0.0956

The ANOVA Procedure

pendent Variable: ADG ADG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00510667	0.00127667	2.74	0.0897
Error	10	0.00466667	0.00046667		
Corrected Total	14	0.00977333			

R-Square	Coeff Var	Root MSE	ADG Mean
0.522510	18.20433	0.021602	0.118667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	0.00510667	0.00127667	2.74	0.0897

The ANOVA Procedure

ependent Variable: FEED_INTAKE FEED INTAKE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	47.56636000	11.89159000	14.22	0.0004
Error	10	8.36073333	0.83607333		
Corrected Total	14	55.92709333			

R-Square	Coeff Var	Root MSE	FEED_INTAKE Mean
0.850507	11.17448	0.914370	8.182667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	47.56636000	11.89159000	14.22	0.0004

The ANOVA Procedure

pendent Variable: PER PER

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00926667	0.00231667	2.56	0.1043
Error	10	0.00906667	0.00090667		
Corrected Total	14	0.01833333			

R-Square	Coeff Var	Root MSE	PER Mean
0.505455	18.06654	0.030111	0.166667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	0.00926667	0.00231667	2.56	0.1043

The ANOVA Procedure

pendent Variable: FCR FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.27017333	0.31754333	4.78	0.0204
Error	10	0.66400000	0.06640000		

Corrected Total	14	1.93417333

R-Square	Coeff Var	Root MSE	FCR Mean
0.656701	20.36475	0.257682	1.265333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	1.27017333	0.31754333	4.78	0.0204

The ANOVA Procedure

ependent Variable: FER FER

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3936.616973	984.154243	4.48	0.0249
Error	10	2198.176267	219.817627		
Corrected Total	14	6134.793240			

R-Square	Coeff Var	Root MSE	FER Mean
0.641687	17.53589	14.82625	84.54800

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	3936.616973	984.154243	4.48	0.0249

The ANOVA Procedure

pendent Variable: SGR SGR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.50330667	0.12582667	1.29	0.3388
Error	10	0.97846667	0.09784667		
Corrected Total	14	1.48177333			

R-Square	Coeff Var	Root MSE	SGR Mean
0.339665	22.74391	0.312805	1.375333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	0.50330667	0.12582667	1.29	0.3388

The ANOVA Procedure

ependent Variable: RGR RGR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7079.36303	1769.84076	1.20	0.3705
Error	10	14792.62487	1479.26249		
Corrected Total	14	21871.98789			

R-Square	Coeff Var	Root MSE	RGR Mean
0.323673	17.72328	38.46118	217.0093

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	7079.363027	1769.840757	1.20	0.3705

The ANOVA Procedure

pendent Variable: _Mortality ortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2306.666667	576.666667	6.65	0.0070
Error	10	866.666667	86.666667		
Corrected Total	14	3173.333333			

R-Square	Coeff Var	Root MSE	_Mortality Mean
0.726891	49.87229	9.309493	18.66667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	2306.666667	576.666667	6.65	0.0070

The ANOVA Procedure

ependent Variable: INITIAL_LENGTH INITIAL LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.84086667	0.71021667	2.88	0.0799
Error	10	2.46966667	0.24696667		
Corrected Total	14	5.31053333			

R-Square	Coeff Var	Root MSE	INITIAL_LENGTH Mean
0.534949	5.723118	0.496957	8.683333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	2.84086667	0.71021667	2.88	0.0799

The ANOVA Procedure

pendent Variable: FINAL_LENGTH FINAL LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.98117333	3.74529333	3.79	0.0398
Error	10	9.88020000	0.98802000		
Corrected Total	14	24.86137333			

R-Square	Coeff Var	Root MSE	FINAL_LENGTH Mean
0.602588	9.064859	0.993992	10.96533

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	14.98117333	3.74529333	3.79	0.0398

The ANOVA Procedure

pendent Variable: WBC WBC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	111952006.7	27988001.7	47.04	<.0001
Error	10	5949683.3	594968.3		

Corrected Total	14	117901690.0

R-Square	Coeff Var	Root MSE	WBC Mean
0.949537	14.92824	771.3419	5167.000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	111952006.7	27988001.7	47.04	<.0001

The ANOVA Procedure

ependent Variable: NEU NEU

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	417.733333	104.433333	1.10	0.4066
Error	10	946.000000	94.600000		
Corrected Total	14	1363.733333			

R-Square	Coeff Var	Root MSE	NEU Mean
0.306316	36.74907	9.726253	26.46667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	417.7333333	104.4333333	1.10	0.4066

The ANOVA Procedure

pendent Variable: LYM LYM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	417.733333	104.433333	1.10	0.4066
Error	10	946.000000	94.600000		
Corrected Total	14	1363.733333			

R-Square	Coeff Var	Root MSE	LYM Mean
0.306316	13.22700	9.726253	73.53333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	417.7333333	104.4333333	1.10	0.4066

The ANOVA Procedure

ependent Variable: PCV___ PCV()

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	579.7333333	144.9333333	17.53	0.0002
Error	10	82.6666667	8.2666667		
Corrected Total	14	662.4000000			

R-Square	Coeff Var	Root MSE	PCV___ Mean
0.875201	7.942489	2.875181	36.20000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	579.7333333	144.9333333	17.53	0.0002

The ANOVA Procedure

pendent Variable: RBC__10___ RBC(*10^{12})

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.85733333	1.46433333	16.27	0.0002
Error	10	0.90000000	0.09000000		
Corrected Total	14	6.75733333			

R-Square	Coeff Var	Root MSE	RBC__10___ Mean
0.866811	11.47959	0.300000	2.613333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	5.85733333	1.46433333	16.27	0.0002

The ANOVA Procedure

ependent Variable: HB HB

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	64.41542667	16.10385667	17.51	0.0002
Error	10	9.19933333	0.91993333		
Corrected Total	14	73.61476000			

R-Square	Coeff Var	Root MSE	HB Mean
0.875034	7.949043	0.959132	12.06600

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	64.41542667	16.10385667	17.51	0.0002

The ANOVA Procedure

pendent Variable: MCH MCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.88860000	0.47215000	9.98	0.0016
Error	10	0.47293333	0.04729333		
Corrected Total	14	2.36153333			

R-Square	Coeff Var	Root MSE	MCH Mean
0.799735	4.610678	0.217470	4.716667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	1.88860000	0.47215000	9.98	0.0016

The ANOVA Procedure

pendent Variable: MCV MCV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1690.617507	422.654377	9.92	0.0017
Error	10	426.080867	42.608087		

Corrected Total	14	2116.698373

R-Square	Coeff Var	Root MSE	MCV Mean
0.798705	4.613674	6.527487	141.4813

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	1690.617507	422.654377	9.92	0.0017

The ANOVA Procedure

ependent Variable: MCHC MCHC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00013040	0.00003260	0.44	0.7794
Error	10	0.00074611	0.00007461		
Corrected Total	14	0.00087652			

R-Square	Coeff Var	Root MSE	MCHC Mean
0.148775	0.025915	0.008638	33.33125

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TSM	4	0.00013040	0.00003260	0.44	0.7794

The ANOVA Procedure

Duncan's Multiple Range Test for INITIALWEIGHT

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.174333

Number of Means	2	3	4	5
Critical Range	1.971	2.060	2.112	2.146

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	6.3500	3	4
A			
A	6.3033	3	2
A			
A	5.9233	3	1
A			
A	5.8500	3	3
A			
A	5.5500	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for FINALWEIGHT

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.674013

Number of Means	2	3	4	5
Critical Range	1.494	1.561	1.600	1.626

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	14.1600	3	5
A			
B A	12.9100	3	4
B			
B	12.4467	3	3
B			
B	12.0900	3	1
B			
B	11.8367	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for WEIGHTGAIN

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.500967

Number of Means	2	3	4	5
Critical Range	2.229	2.329	2.388	2.426

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	8.613	3	5
	A			
B	A	6.600	3	3
B	A			
B	A	6.557	3	4
B				
B		6.173	3	1
B				
B		5.537	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for ADG

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.000467

Number of Means	2	3	4	5
Critical Range	.03930	.04107	.04211	.04278

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	0.15333	3	5
	A			
B	A	0.11667	3	4
B	A			
B	A	0.11667	3	3
B				
B		0.10667	3	1
B				
B		0.10000	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for FEED_INTAKE

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.836073

Number of Means	2	3	4	5
Critical Range	1.663	1.738	1.782	1.811

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	11.3400	3	4
B	8.3900	3	3
B			
B	8.1267	3	5
B			
C B	6.9567	3	1
C			
C	6.1000	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for PER

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.000907

Number of Means	2	3	4	5
Critical Range	.05478	.05724	.05870	.05962

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	0.21333	3	5
A			
B A	0.16333	3	4
B A			
B A	0.16333	3	3

B				
B	0.15333	3	1	
B				
B	0.14000	3	2	

The ANOVA Procedure

Duncan's Multiple Range Test for FCR

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.0664

Number of Means	2	3	4	5
Critical Range	.4688	.4899	.5023	.5102

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	1.8000	3	4
B	1.3100	3	3
B			
B	1.1367	3	2
B			
B	1.1333	3	1
B			
B	0.9467	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for FER

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	219.8176

Number of Means	2	3	4	5
Critical Range	26.97	28.19	28.90	29.36

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	106.58	3	5
	A			
	A	90.74	3	2
	A			
	A	88.42	3	1
	A			
B	A	79.87	3	3
B				
B		57.13	3	4

The ANOVA Procedure

Duncan's Multiple Range Test for SGR

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.097847

Number of Means	2	3	4	5
Critical Range	.5691	.5947	.6097	.6194

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	1.6733	3	5
A			
A	1.4700	3	3
A			
A	1.3067	3	1
A			
A	1.2933	3	4
A			
A	1.1333	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for RGR

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1479.262

Number of Means	2	3	4	5
Critical Range	69.97	73.12	74.97	76.16

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	255.89	3	5
A			
A	218.84	3	3
A			
A	210.48	3	1
A			
A	210.36	3	4
A			
A	189.47	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for _Mortality

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	86.66667

Number of Means	2	3	4	5
Critical Range	16.94	17.70	18.15	18.43

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	40.000	3	4
	A			
B	A	23.333	3	3
B				
B		16.667	3	5
B				
B		6.667	3	2
B				
B		6.667	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for INITIAL_LENGTH

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.246967

Number of Means	2	3	4	5
Critical Range	.9041	.9448	.9687	.9840

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	9.1300	3	4
	A			
	A	9.0333	3	3
	A			
B	A	8.7333	3	2
B	A			
B	A	8.6233	3	1
B				
B		7.8967	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for FINAL_LENGTH

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.98802

Number of Means	2	3	4	5
Critical Range	1.808	1.890	1.938	1.968

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	12.1100	3	1
	A			
B	A	11.6200	3	4
B	A			
B	A	11.5167	3	3

B				
B	C	10.0967	3	5
	C			
	C	9.4833	3	2

The ANOVA Procedure

Duncan's Multiple Range Test for WBC

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	594968.3

Number of Means	2	3	4	5
Critical Range	1403	1466	1504	1527

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	10368.3	3	5
	B	5266.7	3	4
	B			
C	B	4133.3	3	3
C				
C		3233.3	3	2
C				
C		2833.3	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for NEU

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	94.6

Number of Means	2	3	4	5
Critical Range	17.69	18.49	18.96	19.26

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	32.333	3	3
A			
A	30.667	3	4
A			
A	28.333	3	2
A			
A	23.000	3	5
A			
A	18.000	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for LYM

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	94.6

Number of Means	2	3	4	5
Critical Range	17.69	18.49	18.96	19.26

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	82.000	3	1
A			
A	77.000	3	5
A			
A	71.667	3	2
A			
A	69.333	3	4
A			
A	67.667	3	3

The ANOVA Procedure

Duncan's Multiple Range Test for PCV___

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	8.266667

Number of Means	2	3	4	5
Critical Range	5.231	5.466	5.605	5.693

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	44.667	3	1
A			
A	41.667	3	2
B	34.000	3	3
B			
B	33.333	3	4
C	27.333	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for RBC__10___

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.09

Number of Means	2	3	4	5
Critical Range	.5458	.5703	.5848	.5940

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	3.4667	3	1
A			
A	3.1667	3	2
B	2.4000	3	3
B			
B	2.3000	3	4
C	1.7333	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for HB

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.919933

Number of Means	2	3	4	5
Critical Range	1.745	1.823	1.870	1.899

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	14.8867	3	1
A			
A	13.8900	3	2
B	11.3333	3	3
B			
B	11.1100	3	4
C	9.1100	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for MCH

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.047293

Number of Means	2	3	4	5
Critical Range	.3956	.4134	.4239	.4306

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	5.2800	3	5
	A			
B	A	4.8867	3	4
B				
B	C	4.7300	3	3

	C			
D	C	4.3900	3	2
D				
D		4.2967	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for MCV

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	42.60809

Number of Means	2	3	4	5
Critical Range	11.88	12.41	12.72	12.93

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TSM
	A	158.287	3	5
	A			
B	A	146.663	3	4
B				
B	C	141.860	3	3
	C			
D	C	131.713	3	2
D				
D		128.883	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for MCHC

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.000075

Number of Means	2	3	4	5
Critical Range	.01571	.01642	.01684	.01710

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TSM
A	33.335859	3	2
A			
A	33.333538	3	3
A			
A	33.329749	3	4
A			
A	33.328752	3	5
A			
A	33.328334	3	1