EFFECT OF DIETARY FERMENTED AND AUTOCLAVED CASTOR SEED MEAL ON GROWTH , HAEMATOLOGY AND CARCASS COMPOSITION OF *Clariasgariepinus* FINGELINGS.

BY

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FAQ/13/0996

DEPARTMENT OF FISHERIES AND AQUACULTURE, FEDERAL UNIVERSITY OYE EKITI.

FEBRUARY 2019.

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A PROJECT IN THE DEPARTMENT OF FISHERIES AND AQUACULTURE,

[illegible] ACULTY OF AGRICULTURE IN PARTIAL FULFILLMENT OF THE REQUIREMENT [illegible] R THE AWARD OF DEGREE OF BACHELOR OF FISHERIES AND AQUACULTURE [illegible] FISHERIES AND AQUACULTURE).

FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE.

FEBRUARY 2019.

DECLARATION

I hereby declare that this project titled "EFFECT OF DIETARY FERMENTED AND AUTOCLAVED CASTOR SEED MEAL ON THE GROWTH, HAEMATOLOGY AND CARCASS COMPOSITION OF AFRICAN CATFISH (*Clarias gariepinus*) FINGERLINGS" was carried out by me in the Department of Fisheries and Aquaculture, Federal University Oye-Ekiti, Ekiti State under the supervision of Dr. T.O. Babalola.

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SALAMI, MICHAEL DIMEJI

FAQ/13/0996

22/03/2019

.....

DATE

CERTIFICATION

This is to certify that this project titled "EFFECT OF DIETARY FERMENTED AND AUTOCLAVED CASTOR SEED MEAL ON THE GROWTH, HAEMATOLOGY AND CARCASS COMPOSITION OF AFRICAN CATFISH (*Clarias gariepinus*) FINGERLINGS" by Salami, Michael Dimeji meets the regulations governing the award of the Degree of Bachelor of Fisheries and Aquaculture of Federal University Oye-Ekiti, Ekiti State and is approved for its contribution to knowledge and literary presentation. The above declaration is confirmed by

....

Dr. T.O. BABALOLA **DATE**

PROJECT SUPERVISOR

....

Dr. J.B. OLASUNKUNMI **DATE**

HEAD OF DEPARTMENT

DEDICATION

This project is dedicated to GOD, the CREATOR OF FISHES who gave human beings the Wisdom to multiply fishes, and also to my ever-supporting parents, Mr. and Mrs. Salami.

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ABSTRACT

This study investigated the effects of dietary fermented and autoclaved castor seed meal on growth performance, haematology and carcass composition of *Clarias gariepinus* fingerlings. 150 *Clarias gariepinus* fingerlings were randomly distributed into 5 groups of ten fish per tank each to five treatments. The experimental design is a complete randomize design in which there were three replicates for each treatment. The experimental diets which were designated 0%, 5%, 10%, 15%, and 20%, respectively were isonitrogenous (42% Crude Protein content) and isocaloric (2858.38Kcal/kg). The results showed that the best diet with fermented and autoclaved castor seed meal was T3 (10%), inclusion as it gave the best WG (13.83g), SGR (1.31), RGR (1.34), and the lowest FCR (1.39) when compared with T2 (5%), T4 (15%) and T5 (20%). The lowest growth and feed utilization parameters were observed in diet T5 (20%). The carcass composition showed that Crude protein, and moisture of fish fed the control were significantly higher ($p<0.05$) than the fish fed the CSM5, CSM 10, CSM15 and CSM20 and while for the Fat content, ASH and nitrogen free extract of the fish fed CSM20 was significantly higher than CSM15, CSM10, CSM5 and the treatment respectively. The weight gain of fish also decreased with increase in castor seed meal replacement level above 10%. The use of castor seed meal at 10% inclusion level was the best because there was no significant difference between control diets T1 and (10%) T3 ($P>0.05$). The results of the haematological parameters showed that the packed cell volume (PCV) and haemoglobin (Hb), Red Blood Cell, Neutrophils values were significantly ($P<0.05$) higher in control compared to CSM5, CSM10, CSM15, and CSM20, but the parked cell volume, haemoglobin, Red blood cell, and Neutrophils were similar ($p>0.05$) for CSM15 and CSM20 and Control and CSM10, while CSM5 showed no significant different from the others. Mean corpuscular haemoglobin concentration of the fish shows similar effect ($p>0.05$) for Control, CSM5, CSM10, CSM15 and CSM20. White blood cell was similar ($p>0.05$) in control and CSM5 but were significantly ($P<0.05$) different in CSM 10, CSM15 and CSM20. Hence in conclusion 10% is the best replacement level for soybean meal with no deleterious effect on the growth of *C. gariepinus* fingerlings. The use of castor seed meal at 10% replacement level for soybean is recommended in the diet of *C. gariepinus* fingerlings.

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CHAPTER ONE

1.1 INTRODUCTION

Protein is an essential nutrient which is vital for growth of animal/fish. Presently fish feed alone account for between 60 and 75% of the total cost of fish production (Babalola, 2010). Therefore, conventional protein feedstuff such as groundnut cake, fishmeal and soybean which is quite expensive can be replace by underutilized plant protein like castor seed meal in the diet of fish, because they are readily available and relatively cheap. Castor seed meal can be used to replace plant protein such as groundnut cake, soybean meal in the compounded diet of aquafeed (Cai *et al.* 2005).

Castor seeds are obtained from castor plant - *Ricinus communis* – a crop from the Euphorbiacea family which is widely cultivated, in the temperate tropical and subtropical countries. (Wikipedia 2008). Unprocessed castor seed meal contains about 35 to 40% crude protein content (Ani, 2012). The meal left after oil extraction has protein content of 39%, crude fiber 18%, mineral matter 7.5%, soluble carbohydrate 3%, and 40% ether extract (Devendra, 1988). Compared with other plant protein castor seed meal is high in essential amino acid and minerals. Amongu and his colleague (2008) gave the report that castor seed meal when compared with soybean meal contain 2.8% methionine, 9.36 arginine and 5.02 alanine to 1.60%, 8.10% and 4.30% respectively for methionine, arginine and alanine in soybean meal. The mineral of castor seed meal includes phosphorus 6.40, calcium 32.21, magnesium 48.09% and potassium 23.17%.

Even though, castor seed meal has a high percent of protein, the anti-nutritional factors present in the seed include ricin, ricinine and allergen (Darby *et al.*, 2001, Olsnes 2004). Detoxified castor seed meal has been used in diet fed poultry and fish such as *Oreochromis niloticus* and Carp

(Balogun *et al.*, 2005). The improved growth performance of *O. Niloticus* and Carp fed detoxified castor seed meal shows that herbivores had great response to the utilization of detoxified castor seed meal. However, similar response is yet to be obtained in catfish. Mustapha *et al* (2015) revealed that feeding of detoxified castor seed meal by boiling in water for 30 minutes and fermenting for 3 days to broilers appeared to lower the anti-nutritional factors in the meal and improved daily feed intake, daily body weight gain and feed conversion ratio more than those fed on the meal boiled in water for 30 minutes, soaked in water for 72hours and soaked in water for 72 hours followed by boiling for 20 minutes.

The aim of this study was to determine the effects of substituting soyabean meal with fermented and autoclived castor seed meal in the diets of *Clarias gariepinus* fingerlings on growth performance, haematology and carcass composition.

1.2 STATEMENT OF PROBLEM

Protein source constitutes 64 – 67% of the total cost of fish feeds therefore recent researches in aquaculture are focused on reducing its cost (Prendergast et al., 1994). Conventional feedstuffs are expensive and in highly competitive use with human and other livestock. To ameliorate this problem, there is need to source for non-conventional but efficient protein sources of feed stuff for fish.

1.3 JUSTIFICATION

Castor seed has a crude protein content ranging from 21 - 48 % depending on the extent of decortication and oil extraction process (Adedeji *et al.*, 2006). The cake contained about 32 - 48% crude protein depending on levels of decortications and deoiling (Rama Rao, 2004). Castor seed cake has high crude protein which is like that obtained from most conventional protein feed resource such as soybean meal and peanut meal (Akande *et al.*, 2012). However, the seed and the cake are not fully utilized as feed ingredient in livestock ration because of its toxicity due to the presence of ricin, ricinine and thermostable castor allergens (Ani and Okoric, 2009). With proper processing, the anti-nutritional factors present in the feedstuff can be eliminated and that will render the feed suitable for animal feeding.

1.4 Aims and Objectives

Aim

The general aim is to determine the suitability in term of growth performances of fermented and autoclaved castor seed meal for replacing soybean in the feed of *Clarias gariepinus*. This aim was achieved through the following objectives,

Objectives

The specific objectives are:

I. To determine the replacement level of soybean with castor seed meal in the diet that produced the best growth in *Clarias gariepinus* fingerlings.

II. To determine the haematology and carcass composition of the fish fed with castor seed meal at different inclusion level.

1.5 Research Hypotheses

The research hypotheses include:

Hy: There is significant difference in the levels of growth performance of *Clarias gariepinus* fed different inclusion level of castor seed meal

Ho: There is no significant difference in the growth and feed utilization by *Clarias gariepinus* fed diets containing different levels of substituted Fermented and autoclaved castor seed meal.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Fish Feed Development in Africa

Fish feed is considered as one of the pivotal challenges facing the development of aquaculture in Africa. Hetch (2000) observed that in spite of a lot of researches on inexpensive feed ingredients, not much has been contributed to aquaculture development in Africa. He suggested that more research on how best plant protein can be used as fish feed should be carried out. Fish feed technology is one of the least developed sectors of aquaculture particularly in the developing countries (F.A.O, 2003). The need for such researches was reiterated by Jamu and Ayinla (2003) since feed accounts for at least 60% of the total cost of fish production in Africa. However, opportunities exist in Nigeria to reduce feed cost with the availability of local feed ingredients of plants such as castor seed and animal origins rich in crude protein (MSME, 2005). The purpose of fish culture is to produce fish with fast growth and economic viability. These artificial feeds are well compounded mixture of feed stuffs in marsh or pelleted form.

2.1.1 Plant Protein Sources as Alternatives to Soybean in Fish Feed.

Plant protein sources are known as the cheaper feed sources for fish as well as other livestock. Investigations have been carried out to replace animal protein (fish meal) by plant protein in the diet of fish. El-dahhar *et al.* (1993) reported that the replacement of animal protein by plant protein reduced the growth performance of fish in linear relationship to the rate of replacement. He attributed this to the deficiency of some amino acids in the plant protein and suggested that the deficient amino acids should be supplemented in order to improve the growth of the fish. FAO

(1997) suggested the need to seek for other sources of protein to replace fishmeal in fish feed. Plant protein supplements, cereal grains and grain by-products are widely used in feeds for aquaculture species. Global availability and relatively low cost compared to ingredients of animal origin are their most obvious positive attributes. The inclusion of non-conventional plant fed stuffs (NCPF) in fish diets largely depends on the availability, nutrient level, processing technique, fish species and cultural farming pattern (Gabriel *et al.*, 2007).

2.1.2 GENERAL DESCRIPTION OF CASTOR SEED PLANT

Castor plant belongs to the Euphorbiaceae or spurge family (Akande *et al.* 2012) and it is not a legume. It is the only member of the genus *Ricinus* and has no immediate relatives (IGS, 2009). Many different species have been described but all are thought to be different types of *Ricinus Communis L.* The name *Ricinus* is a Latin word for tick. The seed is so named because it has markings and a bump at the end which resembles certain ticks (Ombrello, 2011). Castor seed (*Ricinus communis*) can vary greatly in its growth habit and appearance. It is a fast growing, suckering shrub that can reach the size of a small tree, around 12m (39ft). The glossy leave was 15-45cm (5.9-17.7in) long, long-stalked, alternatate and palmate with five to twelve deep lobes with coarsely toothed segment. It has a large star shaped leaves which make a bold foliage plant (Reel1976). The leave has slightly serrated edged and prominent central vein. It has numerous flowers in long inbred senses, with make flower at the base and female flowers on the tips (Reel1976).

2.1.3 TAXONOMY

Botanical Classification

Kingdom: *Plantae*

Order: *Malpighiales*

Family: *Euphorbiaceae*

Sub-family: *Acalyphoideae*

Genus: *Ricinus*

Species: *Communis*

Binomial Name: *Ricinus communis*

(Wikipedia)

2.1.4 HISTORY AND ORIGIN OF CASTOR PLANT

The castor bean plant (*Ricinus communis*) has been cultivated for centuries for the oil produced by it seeds. The Egyptians burned castor oil in their lamps more than 4,000 years ago. Thought to be native to tropical Africa the plant is a member of the spurge family. Despite being a member of the family Euphorbiaceae a diverse and economically important family of the flowering plant. Castor bean plant is regarded as the one of the deadliest natural poison ever known to man according to Guinness book of records due to the presence of ricin. If the seed is swallowed without chewing and there is no damage to the seed coat, it will most likely pass harmlessly through the

digestive tract. However, if it chewed or broken and swallowed, the ricin toxin will be absorbed by intestines and will bring about abdominal pain, vomiting and diarrhea.

2.2.0 COMPOSITION AND NUTRITIVE VALVE OF CASTOR SEED MEAL

Castor seed meal contain protein, lipids, carbohydrates, vitamin and minerals. Proximate analysis of undehulled and dehulled full fat castor seed and castor seed cake as reported by Oyeniran (2015) showed that both contained high quantities of valuable nutrients (protein, nitrogen free extract, crude fibre and high total ash content) signifying the presence of high levels of macro and micro minerals. Castor seed are decorticated, deoiled and detoxify to obtain their optimum nutritional value for fish utilization, the fruit are decorticated by air-drying it, deoiled by mechanical pressing and detoxify by pressure cooking it in an autoclave, before it is being used as a feed ingredient in fish.

Castor seed cake is obtained after the oil is pressed out (extracted) from the seeds. The de-oiled residue of castor seeds (castor seed cake) that remained after extraction contains toxins and allergens. The toxins are water-soluble and not lipid soluble. So they remain in the cake and are not released during the pressing process. The cake contained about 32 - 48% crude protein depending on levels of decortications and deoiling (Rama Rao, 2003).

Castor cake has popularly found application as a fertilizer which is far below its potential. Despite the unhealthy toxins in the cake, if it can be successfully detoxified, it could become an important new generation feed resource. Castor seed cake has high crude protein which is like those of most conventional protein feed resources such as soybean meal and peanut meal (Akande *et al.*, 2012). No single mechanical method has been successfully used for the total extraction of castor oil

leaving the cake with varying level of oil. Mechanical pressing will only remove about 70% of the oil present.

An effective method to recover more than 99% of the oil present in castor seeds is mechanical in combination chemical (hexane) extraction (Marter, 1981). It has also been suggested that castor bean cake should be properly de-oiled before use as a livestock and fish inorder to avoid reduced nutrient utilization (Akande *et al.*, 2011). This observation may be related to the purgative effect of the oil which prevented absorption of the nutrients in the intestine.

2.2.1 Utilization and Nutrient Profile of Castor Seeds and Castor Seed Cake

Castor seed cake have been successfully used in livestock feeding after detoxification both in monogastric and ruminants. Cal *et al.*, (2005) reported that castor seed was used to replace fishmeal at 0, 40 and 100% with 40% inclusion in both, performing most. The potentials of castor seed (*Ricinus communis*) meal as feed ingredient for *Oreochromis niloticus* was determined by using boiled seeds to prepare five diets, the effect of the experiment diet on the weight gain, specific growth rate, feed conversion ratio protein efficiency ratio apparent net protein utilization digestibility and carcass composition were investigated, the best of these growth factor were obtained with feed formulation from *Ricinus communis* seed boiled for 50 and 60 minutes (Balogun *et al.*,2005). Proximate compositions of un-dehulled and dehulled full fat castor seeds and castor seed cake was determined and the protein content of castor seed varies from 21 - 48 % depending on the extent of dehulling (decortication) and oil extraction process (Adedeji *et al.*, 2006). Compared to un-dehulled full-fat castor seeds, un-dehulled castor seed cake contains higher values of dry organic matter, crude protein, fat, fibre, mineral matter, soluble carbohydrate except for the gross energy and crude fat contents. Dry matter content, crude fat, ash content and gross

energy were higher in dehulled full-fat castor seeds than those in dehulled castor seed cake while the reverse was the case with the crude protein, crude fibre and soluble carbohydrate.

Proximate analysis of un-dehulled and dehulled full fat castor seeds and castor seed cake showed that both contained high quantities of valuable nutrients (protein, nitrogen free extract, ether extract, crude fibre and high total ash content) signifying the presence of high levels of macro- and micro-minerals. The presence of high nutrients suggests that castor seeds and castor seed cake may serve as useful feedstuffs for livestock if properly processed (Lewis and Elvin, 1977; Gohl, 1981; Weiss, 1983; Okorie *et al.*, 1985). The amino acid composition of un-dehulled and dehulled castor seed cake compared with that of soybeans as a standard plant protein is shown in Table 2.1. Amino acid profile analysis of castor seed cake is similar to that of soybeans commonly used as a reference standard plant protein.

The amino acid profiles of both un-dehulled and dehulled castor seed cakes are closely related in values to that of soybeans. However, castor seed cake is found to be deficient in certain indispensable amino acids like iso-leucine, tryptophan, lysine and Methionine (Adedeji *et al.*, 2006; Annongu and Joseph, 2008). There is therefore a need to supplement the deficient amino acids in foods or diets containing treated castor seed cake for optimum utilization by the animals.

Table 2.1: Amino acid profile of undehulled and dehulled castor seed cake compared with soybean as a standard plant protein

Amino acid (g/16 gram of N)	Undehulled castor seed cake	dehulled castor seed cake	Soybean
Lysine	4.11	4.48	6.30
Histidine	1.38	1.88	2.70
Arginine	8.00	9.36	8.10
Aspartic acid	6.42	7.66	11.80
Threonine	3.00	3.88	3.90
Serine	2.13	3.02	5.20
Glutamic acid	13.19	15.64	17.90
Proline	2.76	3.08	5.90
Glycine	0.56	1.11	4.40
Alanine	3.96	5.02	4.30
Cystine	0.53	0.86	1.60
Valine	5.46	4.93	5.10
Methionine	2.06	2.86	1.60
Isoleucine	3.09	1.61	7.90
Leucine	5.09	8.56	5.10
Tryptophan	-	-	1.30

Adapted from Annongu and Joseph, 2008.

Annongu and Joseph, 2008 reported that the proximate analysis, mineral composition and amino acid profile analysis of castor seeds and castor seed cake have shown that the seeds and cake contain high levels of nutrients with potentials to meet the nutritional needs of farm animals if given proper treatments and supplementation with the deficient limiting amino acids. Currently, research into methods that will permit efficient processing and detoxification to enable full utilization of the castor seeds and castor seeds cake as alternatives to conventional feedstuffs is being give consideration (Ani, 2007; Oso *et al.*, 2011).

2.2.2 CASTOR OIL AND ITS COMPOSITION

Castor oil is a colourless to very pale-yellow liquid with a distinct taste and odor. It boiling point is 313°C (595°F) and its density is 961kg/m3. Castor oil is well known as a source of ricinoleic acid, a monounsaturated, 18 carbon fatty acid. Among fatty acids, ricinoleic acid is unusual in that it has a hydroxyl functional group on the 12th carbon. The functional group causes ricinoleic acid (and castor oil) to be more polar than most fats. The oil is obtained by mechanically pressing with hydraulic press which could only remove about 70% of the available oil in the seed. But further extraction with petroleum ether will remove the residual oil (Durowaiye, 2015). Also mechanically expelling in combination with press cake hexane extraction is an effective method to recover more than 99% of oil present in the seed (Kulkarni and kesharamurth, 2005). The seed could be crushed to increased surface area for better oil extraction and then preserved mechanically with hydraulic machine for an hour for better oil extraction. Includes expellers and hydraulic presses (40-41%), rotary mills 15% and screw presses 39%, village ghanis 35% and by boiling the seed in water 30% (Durowaiye 2015).

Castor oil is known to consist of up to 90% ricinoleic, 4% linoleic, 3% oleic, 1% stearic and less than 1% linolenic fatty acids, castor oil is valuable due to the high content of ricinoleic acid (RA) which is used in a variety of applications in the chemical industry.

The hydroxyl functionality of RA makes the castor oil a natural polyol providing oxidative stability to the oil and a relatively high shelf life compared to other oils by preventing peroxide formation (Dunford, 2012). The presence of the hydroxyl group in RA and RA derivative provide a functional group location for performing a variety of chemical reaction including halogenation, dehydration, alkylation and esterification. Major castor oil producing countries include india, china and brazil, even though castor oil account for only 0.15% of the world production of vegetable oils, worldwide consumption of this commodity has increased more than 50% during the past 25years, rising from approximately 400,000tons in 1985 to 610,000tons in 2010 (Severino *et al.*,2012). Castor oil has been an impetus to a country like Nigeria. Ukachukwu *et al.* (2011) reported that cross river state of Nigeria, has gone into a project of producing castor oil seed for it oil.

2.2.3 Castor Seeds: Physical Characteristics

It is also referred to as Castor beans or Castor bean meal. Physical characteristics of castor seeds varieties found in Nigeria are shown in table 2.1 (Akande *et al.*, 2012). The seeds of the castor plants that grow in Northern States of Nigeria have been classified into seven distinct varieties according to their sizes and colours (Van Rheenen, 1976).

Table 2.2: Physical characteristics of two main varieties of castor seeds in Nigeria.

Parameter	Large seeded variety	Small seeded variety
Whole fruit (g)	1.29 ±0.12	0.238±0.01
Whole seed (g)	0.68 ±0.1	0.116±0.02
Husk (g)	0.604±0.01	0.122±0.02
Testa (g)	0.168±0.002	0.036±0.003
Kernel (g)	0.515±0.01	0.08±0.01
Percentage seed	52.7±2.15	48.7±2.12
Percentage kernel	75.7±2.55	69.0±2.53
Husk : seed ratio	0.89±0.07	1.05±0.08
Coat : kernel ratio	0.33±0.01	0.45±0.01
Oil : cake ratio	0.33±0.01	0.37±0.01
Seed width (cm)	1.30±0.17	0.45±0.03
Seed thickness (cm)	0.74±0.09	0.40±0.05
Seed height (cm)	1.75±0.09	0.85±0.03

Adapted from Akande *et al.* 2012.

2.2.4 USES OF CASTOR OIL

Castor seed is the source of castor oil, which has a wide variety of uses. The seed contain between 40% and 60% oil that is rich in triglycerides mainly ricinolein. Castor bean oil is widely used for its lubricating properties and medicinal purposes in industry. It is also used for manufacturing soaps, lubricants, hydraulic and brake fluids paints, dyes, coatings, inks, cold resistant plastics, waxes and polishes, nylon, pharmaceutical and perfumes. Ricinoleic acid in castor oil inhibits the growth of many viruses, bacteria, yeast and moulds. Topical application of castor oil exert remarkable analgesic and anti-inflammatory effects due to the presence of ricinoleic acid (Akande *et al.*, 2011).

2.3.1 ANTI NUTRITIONAL FACTORS IN CASTOR SEED

The anti-nutritional factor present in Castor seed meal include ricin, ricinine and allergen, but the most poisonous of the toxin is ricin.

2.3.2 Ricin

Ricin, a lectin (a carbohydrate-binding protein) produced in the seed of the castor seed plant. Chemically it is a glycoprotein composed of two polypeptide chains - A and B, linked by a disulfide bond. A-chain of ricin is the toxin portion of the protein. The A-chain functions by depurinating the 28S subunit of the infected cells ribosomes. Whereas the B-chain of ricin is responsible of getting the A-chain into the target cell. The B-chain consist of a galactose binding region capable of binding a target cell membrane and initiating cell entry without the B-chain functionality, the A-chain cannot invade the cell. However, once it is in the cell, the A-chain can disrupt up to 1500 ribosomes per minute, this unique action makes ricin highly toxic. It prevents

cells from assembling various amino acids into proteins according to the various to the message it receives from messenger RNA in a process conducted by the cell ribosomes. Ricin is resistant, but not impervious, to digestion by peptidases.

2.3.3 RICININE

Ricinine belongs to the family of Alkyl Aryl Ethers. These are organic compounds containing the alkyl aryl ether functional group with formula R-O-R', where R is an alkyl group and R' is an aryl group. Ricinine is one of the toxic components in castor seed. It is a water-soluble alkaloid concentrated mostly in seed coat. It is also present in leaves and steams. It is slightly bitter to taste and is irritant to the throat (EFSA, 2008). It is only mild toxic to human being. Alkaloid ricinine a powerful haemagglutinin but weak cytotoxin. Munroe (2006) revealed that *Ricinus communis* agglutinin (RCA), does not penetrate the intestinal wall and not affect red blood cell but causes haemolysis and agglutination of red blood cells through injection intravenously.

2.3.4 ALLERGEN

An allergen is any substance (antigen), most often eaten or inhaled, that is recognized by the immune system and causes an allergic reaction. FAO 2012 reported that castor bean allergen-1 (CBIA) is the principal allergen of the castor bean. It is virtually non-toxic, does not cause death though may cause allergic reactions.

2.4.0. Processing of Castor Seeds and Castor Seed Cake by Heat Treatment

Since the discovery of the beneficial effects of heat treatment on the nutritional value of raw legume grains, the challenge of Animal Nutritionists has been to determine the appropriate level of heat treatment for optimum utilization. The most common way by which legumes are processed is by subjecting them to heat though this depends on the variety or type of grains. It has been

established that heat treatment exerts beneficial effects by destroying the anti-nutritional factors inherent in legume grains (Balogun *et al*, 2001). Although it reduces the availability of some amino acids, heat treatment is the most widely used processing method (Dhuradhar and Cheng, 1990). Heat treatment causes denaturation of proteinaceous inhibitors and it is a good method of decreasing the activity of lectins, trypsin and chemotrypsin inhibitors (Hanovar *et al.*, 1962; Kadam *et al.*, 1987; Van der Poel *et al.*, 1990; Estevez *et al.*, 1991). The effects of heat treatment depend on temperature and time duration of processing (Van der Poel *et al.*, 1990; Ichihara *et al.*, 1994; Quin *et al.*, 1996; Kaankuka *et al.*, 2000). Excessive heat treatment can result in reduction in protein solubility and it may destroy certain amino acids (Almas and Bender, 1980; Metebe, 1989; Dhurandhar and Chang, 1990; Van Barneveld *et al.*, 1993). It is therefore expedient to find the appropriate condition of heating which can maximize the destruction of anti-nutritional factor and minimize damage to the feed protein. Investigation on the effects of thermal treatment on certain anti-nutritional compounds in the seeds it shows that autoclaving seems to be the most efficient method of improving in-vitro protein digestibility and eliminating the anti-nutrients such as phytohaemaglutinin, hydrogen cyanide and oligosaccharide, inherent in the seeds (Ekpenyong and Borcher, 1980; Vijayakumari *et al.*, 1995). Another method of heat treatment that have been shown to inactivate anti-nutritional factor include steam heating at various pressure (Smith and Circle, 1978). Several reports have shown that processing of legume by cooking and roasting reduced their anti-nutritional factor to a threshold level (Faris and Singh, 1990; Kaankuka *et al.*,1996; Iyayi and Egbarevbe, 1998). According to Ogundipe (1980), boiling in water and toasting (commonly referred to as dry frying or roasting) are the most commonly used methods in developing countries.

Cooking can destroy some level of anti-nutritional factors (Jambunathan and Singh, 1980). Various researchers have boiled or cooked castor seeds for variable length of time (Okorie *et al.*,1985; Ani and Okorie, 2002; Aniand Okorie, 2005). Balogun *et al.*, 2001 reported that 30 minutes cooking time for soybean is adequate for its inclusion in diet of weaning and growing pigs. Bawa *et al.* (2003b) indicated that cooking lablab seeds at 100°C for 45 minutes significantly decreased trypsin inhibitory activity (TIA), phytic acid, tannin and cyanide levels.

To ensure standardization of cooking time, the ratio of water to seed should be 3 : 1 and as soon as the legume grain are poured into the boiling water, the specified time of cooking can then be recorded (Balogun *et al.*, 2001). A lot of nutrients are lost in water during the boiling process (Ogundipe, 1980; Bawa *et al.*, 2003a). Advantages of boiling the castor seeds include uniform cooking, low cost of processing and ease of adoption by both literate and the illiterate farmers. Processing of legumes by roasting reduced their anti-nutritional factor of legume grains to a threshold level. Udedibe (1995) and Olupona *et al.* (1998) reported that heat treatment alone was not sufficient to significantly destroy the anti-nutritional factor to a threshold level especially glucosides. Toasting involves dry frying of castor oil seed in an open pan with constant stirring to prevent charring (Akanji *et al.*, 2003).

The advantage of toasting as a processing method includes reduction of nutrient loss through leaching as in the case of boiling and the fact that it does not need to be dried in the sun, making it the best method of processing beans in the wet season. However, it has been reported by Abeke (1997) that charring may occur which may adversely affect the nutrients in the seeds.

2.4.1. Processing of Castor Seeds and Castor Seed Cake by Fermentation

Fermentation usually involve pouring water (slightly warm or even boiled) on the seeds and then covered in an air-tight container and then allowing it to stand for a few days in the water. This will allow natural fermentation to take place. After duration of fermentation, the water is drained off and the material sun dried. The warm water helps to weaken the outer, tougher seed coat and softens it for adequate and quick fermentation to take place. Ologhobo *et al.* (1993) stated that the length of time depends on the size of the grain legume. Sometimes, enzymes and other substrates are added to the water to quicken the fermentation process. The principle of this processing method is that certain microbes and enzymes act to destroy certain anti-nutritional factors in grain legume seeds. Some of the enzymes involved in the fermentation process include phytases and trypsinases. Faris and Singh (1990) reported that the nutritive value of legume based fermented foods were higher than their raw component. Leiner (1981) reported the destruction of trypsin inhibitor during fermentation. Fermentation has certain advantages which include ease of operation, low cost, and it can be adopted by both peasant and large scale farmers (Oso *et al.*, 2011).

2.4.2. Processing of Castor Seeds and Castor Seed Cake by Soaking

This involves placing the grains into a known quantity of water and allowing it to stay for certain duration of time. Soaking differs from fermentation in that microbial activities did not take place. To avoid microbial activities (fermentation), the water should be replaced as many times as possible within 24 hours. Nwokolo and Orji (1985) also showed that soaking in water and cooking can also improve nutritional status of legume grains. Ologhoho (1989) reports that soaking in water and germination are the most effective method of reducing phytic and oxalic acid in soybeans.

2.5.0 Carcass Composition of African Catfish (*Clarias gariepinus*)

Proper nutrition is one of the key factors that influence the ability of the fish to attain genetic potential, growth, reproduction and longevity. Knowledge of the carcass composition of the fish is a good measure for assessing the growth and quality of protein and its utilization, whether the diet promoted growth or simply accumulated only fat. Studies have been carried out on the carcass composition of some indigenous fishes. Abdullahi *et al.* (2001) reported that *C. gariepinus* has the following nutrients: moisture 75.00%, ash 10.50%, carbohydrate 10.20%, crude lipid 27.10 and crude protein 52.20%. The amino acid composition (g/100g) was also reported as lysine 10.13 histidine 2.84, arginine 4.40, aspartic acid 8.30, threonine 2.89, serine 2.07, glutamic acid 14.85, praline 2.54, glycine 8.86, alanine 4.90, cystine 2.62, valine 3.01, methionine 1.97, isoleucine 5.16 leucine 5.64, trysine 1.10, phnylamine 2.00. The mineral content (mg/100g) was reported as calcium 3910, potassium 633, iron 53, magnesium 31, sodium 261, copper 2, phosphorus 16 and zinc 19. These indices were either higher or close to the reference values of the FAO/WHO (1991). Minerals are important in the metabolic and physiological activities and subsequent growth and development of any organism. Eyo (2003) reported that all feedstuff namely maize, corn, palm kernel cake, blood meal, soya beans meal and fish meal contain a fair amount of calcium, potassium, sodium, phosphorus, magnesium and manganese. Carcass composition of *C. gariepinus* and *O. niloticus* fed Castor seed as feed ingredient boiled for 0, 20, 35, 50 and 65 minutes showed the highest protein content in fish feed seeds boiled for 65 and 35 minutes respectively (Agboola, 2004). Similarly, carcass nutrient consumption as reported by Balogun *et al.* (2004) for *O. niloticus* fingerlings fed boiled Delonix regia were significantly high. The proximate and mineral composition in some selected freshwater fishes in Nigeria were studied. The result obtained for C. gariepinus was crude protein 44.28%; crude fibre, 0.10%; ether extract,

7.80%; Ash, 4.00%; moisture, 6.60% and dry matter, 93.40%. The findings also noted that the variation in the calcium and phosphorus content was due to an increase in the proportion of bone to flesh as the fish grew (Fawole *et al.*,2007).

2.5.1 Haematological Parameters in Fish

2.5.2 Hemoglobin (Hb).

Hemoglobin molecules are responsible for binding and releasing oxygen on to the red blood cells. Blood is a complex mixture of suspended cellular components (erythrocytes, leukocytes and thrombocytes) and dissolved substances (electrolytes, proteins, carbohydrates, amino acids, etc.).

A high level of hemoglobin shows high red blood cell count. Low levels of Hb indicate anaemia, iron deficiency and bleeding. Normal range from 9 to 16g/dl (Dawn, 2010). Haematological parameters reflect the condition of fish more quickly than other commonly measured parameters and since they respond quickly to changes in environmental conditions, they have been widely used for the description of healthy fish, for monitoring stress responses and for predicting systematic relationships and the physiological adaptations of animals (Atamanalp and Yanik, 2003).

Blood cell responses are important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases (Golovina, 1996; Luskova, 1997). White blood cells protect the body against infection. The major types of white blood cells include basophils, neutrophils, eosinophils, B Cells, T Cells, Band Cells and monocytes. Haemoglobin is a protein that is carried by red cells. It picks up oxygen in the lungs and delivers it to the peripheral tissues

to maintain the viability of cells. Haemoglobin is made from two similar proteins alpha and beta) that "stick together".

Both proteins must be present for the haemoglobin to pick up and release oxygen normally. Neutrophil are primary white blood cells responsible for fighting infection. High neutrophil count indicates infection while low neutrophil indicates sepsis (Dawn, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 TEST INGREDIENT

5kg of castor seed for this study were locally soureed within Ikole-Ekiti, Ekiti State, Nigeria. The seeds were dehulled manually by carefully cracking them between two stones. Decorticated seeds were obtained by separating the hulls from the cotyledons. The oil in the seeds was obtained using Manual hydraulic oil extractor. The residue (cake) after oil extraction was soaked in water to allow natural fermentation at a ratio of 1:4 under an air tight environment for 5 days. The water were drained off at end of the fermentation process, seeds was washed several times with water to remove the sour taste and fermenting odour. The washed seeds were sun dried to a moisture content of 10-12%, as described by Ani and Okorie (2006) and Ani (2012). Then the milled seed cake were autoclaved at 120°C for 30minute to further remove any anti-nutritional factor present. The milled samples were package and used to compound the experimental diets.

3.1.1 EXPERIMENTAL SITE

The research was conducted in the wet laboratory, Department of Fisheries and Aquaculture, Federal University Oye-Ekiti. fifteen (15) rectangular aquaria tanks were used, all of the same size, and it were subjected to feeding trial which last for 8 weeks. Analysis were done on sample collected base on the growth performance and at the end of the experiment fish careass was analysed for proximate composition and the haematological analysis was carried out in the analytical laboratory of the General Hospital Ikole-Ekiti.

3.1.2 EXPERIMENTAL DESIGN

150 Catfish fingerlings (*Clarias gariepinus*) were purchased from Afe Babalola fish farm in Ekiti State. Prior to the commencement of the experiment, fish were acclimatized to the experimental condition for two weeks. Fifteen (15) rectangular aquaria tanks were used, all of the same size. The fish were randomly distributed into five treatment. Each treatment have three replicate in each of the plastic aquarium. 10 *Clarias gariepinus* fingerlings were stock into each of the 15 aquarium tank using complete randomize design (CRD).

3.1.3 Experimental Procedure

The experimental fish were batch weighed at the beginning of the experiment. Fish were offered 5% of their body weight per day, sub divided into two equal feeds at 08:00, and 17:00h daily. The mean total length and standard length of the fish were determined weekly while the record of fish mortality in each treatment was recorded daily. Faecal matter was collected once a day at about 8:00 am before feeding commenced during the latter part of the experiment. Faeces collection was performed by siphoning materials from the bottom of tank. One fish from each of the treatments was sacrificed and dried at end of the experiment for carcass and gross chemical analysis.

3.2 DIET FORMULATION

Five isonitrogenous (42% protein) and isocaloric (2858.38kcal/kg ME) ration were formulated as shown in the table below1:0, diet 0 was the control, while diet 1,2,3 and 4 will have portion of soybean replaced by castor seed meal at different inclusion level of 5%, 10% 15% and 20% respectively.

Table 3.2: Composition of the Experimental diets (g/kg)

	Diets				
	0% CSM	5% CSM	10% CSM	15% CSM	20% CSM
Ingredients (g/kg)					
MAIZE	10.97	9.00	5.97	4.20	4.50
WHEATOFFAL	5.00	6.87	8.00	10.77	9.27
FISH MEAL	28.00	28.00	28.00	28.00	28.00
GNC	14.00	14.10	15.00	15.00	16.20
SOYBEAN MEAL	33.00	28.00	23.00	18.00	13.00
CSM	0.00	5.00	10.00	15.00	20.00
SALT	0.50	0.50	0.50	0.50	0.50
METHIONINE	1.00	1.00	1.00	1.00	1.00
VIT.PREMIX	2.00	2.00	2.00	2.00	2.00
BINDER	2.00	2.00	2.00	2.00	2.00
VIT.C	0.03	0.03	0.03	0.03	0.03
VEG.OIL	3.00	3.00	3.00	3.00	3.00
CHROMIC OXIDE	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100

Vit. A: 4000000iu, Vit. D3 800000iu, Tocopherols; 4000 iu, Vit k3; 800mg, folacin; 200mg,vit.B11.8mg, Vit.B2,5mg, thiamine; 600mg, riboflavin 1800mg, niacin 6000mg, calcium pantothenic 2000mg, pyridoxin 600mg, cyanocolabamin 4mg, biotin 3mg, magnesium 30000mg, zinc 20000mg, iron 8000mg, copper 2000mg, iodine 480mg cobalt 80mg;selenium 40mg, Choline chloride 80000mg, manganese.30000mg, BHT 26000mg, anticaking agent 6000

3.3 Chemical and nutritional analyses

Proximate analysis of both the fish carcasses and feed were analysed for moisture content, crude protein, crude lipid, and ash content. Proximate composition of feed and fish was determined using association of official analytical chemistry (AOAC, 2003) method, sample of experimental diets were taken to the central laboratory for proximate analysis using the method described by AOAC, crude protein by the kjedahl method, ether extract by subjecting the samples to petroleum ether extraction at 60 – 100° using the soxlet extraction apparatus, dry matter by oven drying the samples at 105° C over a 6 hours period. Crude fiber by boiling the sample under flux in weak sulphuric acid (0.255N NaOH) FOR Hour. The residues which consist of cellulose, lignin and mineral matter were dried and weighted. The ash content was determined by igniting a weighted sample in a muffle 30 furnace at 600° C, the nitrogen free extract (NFE) was obtained by the difference after the percentage of the fractions were subtracted from 100%.

3.4 Analysis of Growth Performance and Feed Utilization of Clarias Fed Test Diets

a) Growth Performance of Clarias

i) Weight gain (WG)

WG = Final weight of fish – Initial weight of fish

ii) Average Weight gain $= \frac{\text{Weight gain}}{\text{Experimental Period (in days).}}$

iii) Relative growth rate (RGR)

$$\textbf{RWG (\%)} = \frac{WF_{(g)} \times 100}{W_I}$$

Where

WF = Final average weight of fish

W_I = Initial average weight at the beginning of the experiment

iv) Specific Growth Rate (SGR) %

$$SGR\ (\%/day) = \frac{\text{Log}_e WF - \text{Log}_e W_I}{\text{Time (days)}} \times 100$$

b. Feed Utilization Analysis

i) Feed conversion ratio (FCR)

$$FCR = \frac{\text{Dry feed consumed (g)}}{\text{Gain in weight (g)}}$$

ii) Protein efficiency ratio (PER)

$$PER = \frac{\text{Gain in wet weight (g)}}{\text{Protein fed (g)}}$$

3.5 Haematological Assessment

Haematological examination of the fish was carried out at the end of the experiment in order to investigate the possible effect(s) of the feeds on the fish. Haematological parameters such as Packed Cell Volume (PCV), Haemoglobin, Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC). Blood samples were collected from fish according to the method described by Morgan and Iwama (1997), Ajani (2005) and Omitoyin (2006). The samples were analysed at the State General Hospital Ikole Ekiti Ekiti State.

3.6 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 calculated from RBC, Hb and PCV (Hamening 1992).

Using

MCV (Fl)= PCV/RBC ($10^{6}\mu l^{-1}$)

MCH (pg)= [Hb(gdl^{-1}) X10] /RBC ($106\ \mu l^{-1}$)

And MCHC (gl^{-1}) = [Hb(gdl^{-1})X 10]/PCV

3.7 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)

3.8.0 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).

3.8.1 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.

3.8.2 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, 200mg; 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 Statistical analysis

The data were subjected to analysis of variance (ANOVA) and if significant ($p<0.05$) difference were found, Duncan's multiple range test (Duncan, 1955) was used to rank the group using Statistical Analysis System (SAS). The data presented as mean ± S.E.M. of three replicate group. The statistical model used was a one-way analysis of variance.

CHAPTER FOUR

4.0 Results

4.1.1 Data on the average water quality, DO and temperature magnitude which values were identical on all the treatment groups in the course of the experiment.

4.3 Proximate composition of experimental diet

Proximate of experimental diet is presented in Table_4.1. Moisture content differ significantly ($p<0.05$) in all experiment with the highest recorded in control diet and lowest in CSM20. Fat content where similar ($p>0.05$) in CSM5 and CSM10 respectively however differ significantly ($p<0.05$) from the Control, CSM15 and CSM20 groups. Crude protein was significantly different ($p<0.05$) in all in all the experimental diet. Fiber content in the experimental diet CSM5 and CSM10 are not significantly different from each other but differ significantly ($p<0.05$) from other diets. Ash content in the control diet is similar to that of CSM5 and CSM20 differ significantly ($p<0.05$) from other diets. NFE differ significantly ($p<0.05$) in all experiment with the highest recorded in CSM20 and the lowest in control diet.

Table 4.1 Data on water quality, DO and temperature in plastic tanks used for the experimental fish

Replicates	Water pH	Water temperature (°C)	Dissolved Oxygen (D.O)
T1R1	6.5	28.5	4.50
T1R2	6.7	28.7	4.10
T1R3	6.5	28.5	4.45
T2R1	6.7	28.7	4.20
T2R3	6.6	28.3	4.30
T2R3	6.7	28.6	4.40
T3R1	6.5	28.2	4.80
T3R2	6.6	28.2	4.20
T3R3	6.5	29.0	4.65
T4R1	6.2	28.5	4.50
T4R2	6.3	28.6	4.70
T4R3	6.2	28.5	4.50
T5R1	6.9	28.9	4.58
T5R2	6.8	28.8	4.50
T5R3	7.0	29.2	4.60

Table 4.2: Proximate composition of fermented and autoclaved castor seed meal

Parameters (%)	processed castor seed meal
Moisture	13.8
Fat	23
Crude Protein	42.5
Ash	8.0
NFE	12.7

Table 4.3: Proximate composition of experimental diet

Parameter (%)	CTRL	CSM5	CSM10	CSM15	CSM20
Moisture	8.25±0.03^{e}	7.99±0.003^{c}	7.90±0.01^{b}	8.19±0.01^{d}	7.61±0.01^{a}
Fat	17.45±0.04^{a}	18.09±0.01^{b}	18.69±0.01^{b}	19.64±0.03^{c}	20.23±0.01^{d}
Crude Protein	43.39±0.01^{e}	42.71±0.01^{b}	42.94±0.003^{d}	42.76±0.01^{c}	42.35±0.03^{a}
Fiber	2.5±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	22.89±0.05^{e}	21.78±0.02^{d}	20.85±0.01^{c}	19.44±0.05^{b}	19.12±0.06^{a}

Mean ± S.E with different super script are significantly different from each other ($P<0.05$).

4.4 Effect of castor seed meal based diet on growth performance of *Clarias gariepinus* Fingerlings fed for 8 weeks

Growth performance of the fish (*Clarias gariepinus*) fingerlings fed castor seed meal was Presented in table 4.2, initial weight was similar ($p>0.05$) in all the experimental treatment final weight was however showed there was significant different between Control and CSM20, and no significantly different in CSM5 and the other treatment. The initial length showed that the Control, CSM5, CSM10 differ significantly from CSM15 and CSM20. Final length showed there is no significant different in Control, CSM5, CSM10 and CSM15 however there was significant different between Control and CSM20.

The feed intake of the fish was significantly different ($p<0.05$) in all treatment. Weight gained showed there is no significant different between Control and CSM10 and there is no significant different in CSM5, CSM10 and CSM15, while there is no significant different between CSM15 and CSM20. However, there is a significant different in Control and CSM20.

Average weight gained also showed there is no significant different between Control and CSM10 and there is no significant different in CSM5, CSM10 and CSM15, while there is no significant different between CSM15 and CSM20. However, there is a significant different in Control and CSM20. Feed conversion ratio were similar ($p>0.05$) in CSM5, and CSM10, but were significantly different ($p<0.05$) from control CSM15, and CSM20 with highest (1.33 ± 0.02) in CSM20 while lowest (1.03 ± 0.01) in Control.

Relative growth rate was similar ($p>0.05$) for CSM5 and CSM15 and however significantly different ($p<0.05$) from Control, CSM10 and CSM20. Specific growth rate was similar ($p>0.05$) for CSM5 and CSM15 and however significantly different ($p<0.05$) from Control, CSM10 and CSM20. Percentage mortality was similar ($p>0.05$) in all the experimental treatment.

4.5: Carcass composition of fish (*Clarias gariepinus* fingerlings) fed experimental diet after 8weeks

Carcass composition of fish fed experimental diet is presented on table 4.3, moisture content in CSM15 and CSM20 are not significantly different from each other whereas they are significantly different in control, CSM5 and CSM10. Fat content of fish fed experimental diet was significantly different ($p<0.05$) with the highest (1.31 ± 0.02) recorded on CSM20 and the lowest recorded on fish fed the control diet (0.65 ± 0.02). The crude protein of fish fed the experimental diet were significantly different ($p<0.05$) with the highest in Control (21.35 ± 0.04) and the lowest in control (15.04 ± 0.02). Fibre content of fish fed experimental diet was significantly different ($p<0.05$) with the highest (0.86 ± 0.01) recorded on CSM20 and the lowest recorded on fish fed the control diet (0.45 ± 0.22). The ash content of fish fed the experimental diet in CSM15 and CSM20 are not significantly different from each other while other differ significantly ($p<0.05$) with the highest recorded in CSM20 and the lowest recorded in the control. NFE of fish fed experimental diet were however significantly different ($p<0.05$) with the highest recorded in CSM20 and the lowest in control of fish fed experimental diet.

Table 4.4: Growth performance of fish (*Clarias gariepinus*) fingerlings fed castor seed meal for 8 weeks

Parameter	CTRL	CSM5	CSM10	CSM15	CSM20
Initial weight (g)	5.97±0.21^{a}	6.52±0.29^{a}	6.48±0.02^{a}	5.98±0.41^{a}	5.84±0.04^{a}
Final weight (g)	11.96±0.97^{a}	9.87±0.29^{abc}	10.613±0.90^{ab}	8.79±1.07^{bc}	7.24±0.71^{c}
Initial length (g)	7.04±0.01^{b}	7.31±0.09^{b}	7.27±0.16^{b}	8.18±0.20^{a}	8.37±0.27^{a}
Final length (g)	11.19±0.30^{a}	10.4±0.40^{ab}	10.80±0.22^{ab}	9.91±0.88^{ab}	9.40±0.21^{b}
Feed intake (g)	10.42±0.01^{e}	8.18±0.04^{c}	9.5±0.29^{d}	7.13±0.18^{b}	5.84±0.45^{a}
Weight Gained (g)	5.997±0.78^{a}	3.33±0.01^{bc}	4.13±0.92^{ab}	2.81±0.73^{bc}	1.40±0.66^{c}
Average Weight Gain (g)	0.107±0.01^{a}	0.06±0.00^{bc}	0.07±0.02^{ab}	0.05±0.01^{bc}	0.02±0.012^{c}
Feed Conversion Ratio	1.03±0.01^{d}	1.11±0.003^{c}	1.13±0.012^{c}	1.23±0.01^{b}	1.33±0.02^{a}
Relative Growth rate	199.89±10.16^{a}	151.27±2.37^{bc}	163.81±14.46^{b}	146.15±10.74^{bc}	123.81±11.13^{c}
Specific Growth Rate	1.47±0.09^{a}	0.76±0.03^{bc}	0.87±1.55^{b}	0.67±0.14^{bc}	0.37±0.16^{c}
Mortality (%)	36.67±0.33^{a}	30.00±1.00^{a}	30.00±0.58^{a}	33.33±0.33^{a}	26.67±0.67^{a}

Mean ± S.E with different super script are significantly different from each other ($P<0.05$).

Table 4.5: Carcass composition of fish fed experimental diet after the 8 weeks

Parameter (%)	CTRL	CSM5	CSM10	CSM15	CSM20
Moisture	73.49±0.02^{a}	73.11±0.06^{b}	72.84±0.04^{c}	72.07±0.04^{d}	71.96±0.02^{d}
Fat	0.65±0.02^{e}	0.85±0.02^{d}	0.92±0.01^{c}	1.11±0.02^{b}	1.31±0.02^{a}
Crude Protein	21.35±0.04^{a}	19.42±0.01^{b}	19.13±0.06^{c}	17.09±0.02^{d}	15.04±0.02^{e}
Fiber	0.45±0.22^{e}	0.53±0.01^{d}	0.62±0.01^{c}	0.72±0.01^{b}	0.86±0.01^{a}
Ash	3.57±0.02^{d}	3.86±0.02^{c}	4.02±0.04^{b}	5.01±0.01^{a}	5.04±0.01^{a}
NFE	0.49±0.02^{e}	2.22±0.05^{d}	2.47±0.05^{c}	4.00±0.03^{b}	5.78±0.03^{a}

Mean ± S.E with different super script are significantly different from each other ($P<0.05$).

4.6 Haematological analysis of fish (*Clarias gariepinus*) fingerlings fed castor seed meal

Haematogical parameter of fish fed experimental diet is presented on table 4.4. packed cell volume however showed that there are no significant different in Control and CSM5 and no significantly different CSM5 and CSM10, while CSM5, CSM15 and CSM20 are significantly differ from that in Control and CSM10. Haemoglobin observed showed that there are no significant different in Control and CSM5 and no significantly differentCSM5 and CSM10, while CSM5, CSM15 and CSM20 are significantly differ from that in Control and CSM10.

Red blood cell of *Clarias gariepinus* fingerling fed castor seed meal there are no significant different in Control and CSM5 and no significantly differentCSM5 and CSM10, while CSM5, CSM15 and CSM20 are significantly differ from that in Control and CSM10.

Mean cell volume of the fish shows similarity ($p>0.05$) in Control, CSM5 and CSM10 respectively whereas there is no significant different also in CSM15 and CSM20 but differ significantly from Control, CSM5 and CSM10 respectively. Mean corpuscular haemoglobin of the fish as recorded, there were similarity ($p>0.05$) in control, CSM5 and CSM10 respectively whereas there is no significant different also in CSM15 and CSM20 but differ significantly from Control, CSM5 and CSM10 respectively. Mean corpuscular haemoglobin concentration of the fish shows similar effect ($p>0.05$) in all treatment, they are not significantly different from each other.

For Neutrophils in CSM5 and CSM15 they are not significantly different from each other while other differ significantly ($p<0.05$) with the highest recorded in control and the lowest recorded in CSM15.

While in lymphocyte there were no significant different ($p>0.05$) between CSM5 and CSM10 but however differ significantly ($p<0.05$) in control, CSM15 and CSM20respectively with the highest recorded in CSM10 and the lowest recorded in Control.

Table 4.6: Heamatology analysis of the fish (*Clarias gariepinus*) fingerlings fed processed castor seed meal

Parameter (%)	CTRL	CSM5	CSM10	CSM15	CSM20
Packed cell volume (%)	44.00±1.15^{a}	39.67±1.45^{ab}	41.33±4.63^{a}	32.67±1.45^{b}	32.00±1.15^{b}
Haemoglobin (g/dL)	14.67±0.38^{a}	13.22±0.48^{ab}	13.78±1.54^{a}	10.89±0.48^{b}	10.67±0.38^{b}
Red blood cell (x 10^{12}/L)	3.40±0.12^{a}	2.97±0.15^{ab}	3.13±0.45^{a}	2.27±0.15^{b}	2.20±0.12^{b}
Mean corpuscular volume (fL)	129.48±1.00^{b}	133.87±1.69^{b}	133.46±5.27^{b}	144.49±2.93^{a}	145.71±2.41^{a}
Mean corpuscular haemoglobin (Pg)	4.32±0.03^{b}	4.46±0.06^{b}	4.45±0.18^{b}	4.82±0.10^{a}	4.86±0.08^{a}
Mean corpuscular haemoglobin concentration (g/dL)	33.33±0.00^{a}	33.33±0.00^{a}	33.33±0.00^{a}	33.33±0.00^{a}	33.33±0.00^{a}
White blood cell (x10^{3}/L)	5100.0±2.08^{d}	6300.0±2.08^{d}	8316.7±3.09^{c}	13346.7±1.18^{b}	17133.3±2.33^{a}
Neutrophils (%)	49.00±2.08^{a}	26.00±3.06^{c}	17.67±1.45^{d}	25.67±2.33^{c}	40.00±2.31^{b}
Lymphocytes (%)	51.00±2.08^{d}	74.00±3.06^{b}	82.33±1.45^{a}	74.33±2.33^{b}	62.00±1.15^{c}

Mean ± S.E with different super script are significantly different from each other ($P<0.05$).

4.7: Discussion

The growth performance in fish's fed fermented and autoclaved castor seed meal diets can slightly measure up to the standard obtained for fish fed the control diet. This finding agrees with the reports of Ani and Okorie (2009) and Ani (2007) who stated that processed castor bean meal can be included in the diets at 10 and 15 % level with DL-Methionine and L-Lysine supplementations respectively.

The numerical increase in the value of feed conversion ratio across the treatments fed diet containing castor seed meal may be due to the presence of fiber which may have reduce the utilization of the nutrient and some may have bypassed without being utilized which was in contrast with the report of Durowaye (2015). Mulky and Gandhi (1994) low palatability could result in low consumption and utilization.

This may be attributed to poor palatability due to the presence of residual ricin which increased in the diets as the dietary level of Castor seed meal increased above 10% inclusion. Ani (2007) made similar observations.

It is well established that poor palatability will cause depressed feed intake leading to decreased final weight and weight gain and consequently resulting in poor feed conversion ratio. The resultant effect of poor diet palatability is inadequate intake of nutrients required to sustain rapid growth and development (Esonu *et al.*, 2002).

The carcass analysis showed that all diets there were slight increase in the fat content of the fish fed castor seed meal and this may be due to the residue of lipid in the castor seed meal. The low crude fibre obtained in all the fish carcass sample could also imply that the available fibre was well digested by the fish. and also there were increase in the nitrogen free extract and ash which may be due to the fiber content in the feed, slightly in contrast to report of Adebayo *et al* (2016), there

were reduce in the whole body crude protein but increase in fiber, Nitrogen free extract of the fish and Ash content, this is due to varying inclusion and the presence fiber in the diet composition which was in contrast with the report of Cal *et al.* (2005).

The pH range of 6.10 – 7.0, Temperature range of 28.1 – 29.2 (^{0}C) and Dissolved Oxygen (D.O) range of 4.2 – 4.86mgl-1 recorded during this study suggest that they are within acceptable range for growth and maintenance of *Clarias gariepinus*. The pH range of 6 – 8.5, Temperature range of 24- 30 (^{0}C) and D.O. of 4 – 8.0mg-1has been reported (Balogun *et al.*, 2004). Since all the physico-chemical parameters of the culture water were within acceptable ranges, the fish growth and feed utilization may therefore be affected by other intrinsic and extrinsic factors.

Reduction in the haematological value with the inadequate nutrient utilization in the fish fed Fermented and autoclaved castor seed meal as the level of inclusion increase in the red blood cell count, packed cell volume, haemoglobin, neutrophils and increase level of mean cell volume, mean cell haemoglobin and white blood cell this trend may have been due to due to the possible residue of ricin or allergen on the blood variables, which is in contrast with what was reported by Akande *et al* (2013) when differently processed castor bean cake was fed to broiler chickens.

CHAPTER FIVE

5.1. CONCLUSION

The results of this study on effect of dietary inclusion of fermented and autoclaved castor seed meal on the growth performance, haematology and carcass composition of *Clarias gariepinus* fingerlings revealed that, castor seed, if to be used as an alternative feedstuff for animals, has to be processed to remove or eliminate the toxic factors, ricin, ricinine and allergens before good results can be obtained. The best performance is observed when the seed is fermented and autoclaved and included at 10% level in diet of African catfish.

5.2. RECOMMENDATIONS

Given the nutritional potential of the castor seed meal, I will recommend that:

More castor seed should be planted to improve the production and availability in the market and more research work should be carried out on the seed to know it full potential.

REFERENCES

Adebayo, I. A., Fapohunda, O. O., Ajibade A. O. (2016). Evaluation of Nutritional Quality of *Clarias gariepinus* from Selected Fish Farms in Nigeria. *American Journal of Food Science and Nutrition Research.* Vol. 3, No. 4, 56-62.

Adedeji, J. A., Apata, D. F., Aderinola, O. A., Rafiu T. A. and Amao, S. R. (2006). Performance and haematological/serum characteristics of rabbits fed boiled castor seed cake based diet. *World Journal of Zoology*, 1 (2): 91-93.

Aiyeloja, A. A., George, F. A., Obasa, S. O. and Sanni, L. O. (2011). Effect of post slaughter time intervals on the quality of the African catfish, *Clarias gariepinus* (Burchell, 1822). *Journal of Food Technology* 6 (9): 790 – 797.

Ajani, F. (2005) Hormonal and Heamatological Responses of Adult and Broodstock *Clarias gariepinus* to Amonia and Nitrite Toxicity under Different Culture Environments. *PhD Thesis in University of Ibadan.* 180p.

Akande T. O., Odunsi A. A., Rafiu T. A., Olaniyi C. O. and Binuomote R. T. (2013). Growth and serological assessment of broiler chickens fed differently processed castor *(Ricinus communis* Linn.) kernel cake based diets. African Journal of Agricultural Research Vol. 8(41), pp. 5161-5165.

Akande, T. O., Odunsi, A. A. Olabode O. S., Ojediran T. K. (2012). Physical and Nutrient Characterization of Raw and Processed Castor (*Ricinus communis* L.) Seeds in Nigeria *World Journal of Agricultural Sciences* 8 (1): 89-95

Akande, T. O., Odunsi, A. A. and Adedeji, O. S. (2011). Toxicity and nutritive assessment of castor *(Ricinus communis)* and processed cake in rat diet. *Asian Journal of Animal Science, 5(5):330-339.*

Ani, A.O. (2012). Nutritional Evaluation of Castor oil bean (Ricinus communis L) Meal. LambertnAcademic Publishers, Saarbrucken, Deutschland (Germany). 172pp.

Ani, A. O. (2007). Effects of graded levels of dehulled and cooked castor oil bean (*Ricinus communis* L) meal and supplementary L-lysine on performance of broiler finishers. *Journal of Agriculture, Food, Environment and Extension,* 6(1): 89-97.

Ani, A.O. and Okorie, A.U. (2009). Performance of broiler finishers fed graded levels of processed castor oil bean (*Ricinus communes L*) meal supplemented with DLMethionine. *Journal of Animal Production,* 36(1): 61-73.

Ani, A. O and Okorie, A .U (2006).The efficacy of two stage cooking as a method of detoxifying castor oil Bean(Ricinus communis.L.) meal for livestock feeding. Journal of Sustainable Agriculture and the Environment, 8(1):14-22

Annongu, A.A, Joseph, J. K. (2008). Proximate Analysis of Castor Seeds and Cake. J. Appl. Sci. Environ. Manage. 12(1) 39 – 41.

AOAC (Association of official Analytical chemists). (2003). Official methods of analysis 13th AOAC, Gaithersburg, Maryland, USA

Babalola, T.O.O. 2010. *Utilization of dietary animal fats and vegetable oils by African catfish (Heterobranchus longifilis) fingerlings*. Ph.D. Thesis University of Ilorin, Nigeria.

Balogun, J. K. S., Abdullahi, A. S., Auta, J. and Ogunlade, O. P. (2004). Feed Conversion, Protein Efficiency, Digestibility and Growth Performance of Oreochromis niloticus fed Delinix regia seed meal. Proceedings of the National Conference of Fisheries Society of Nigeria (FISON), Lagos, Nigeria. Pp. 838 -842.

Balogun ,T. F., Kaankuka, F. G. and Bawa. G. S. (2001). Effect of boiling full fat soybeans on its aminoacid profile and on its performance on pigs. *Proceedings of the 26th Annual Nigerian Society of Animal Production (NSAP).* 26(1): 45-51

Balogun, J.K., Auta, J., Abdullahi, S.A. and Agboola, 0.E., (2005): Potentials of castor seed meal (Ricinus communis- L.) as feed ingredient for *Oreochromis niloticus* Journal of Fish Biology 18: 1-12.

Bawa, G.S; Tegbe T.S.B; Ogundipe, S.O; Dafwang, I.I and Abu, E. A (2003). The Effect of duration of cooking Laglab seeds on the level of some anti-nutritional factors. Pros.28th Ann. Conf. Nigerian Society of Animal Production Ibadan, Nigeria. 28.pp 2013-214.

Cai. X., Luo .L., Xue. M., Wu .X. and Zhan . W., (2005). Growth performance, body composition and phosphorus availability of juvenile grass carp (*Ctenopharyngodon idellus*) as affected by diet processing and replacement of fishmeal by detoxified castor bean meal. Aquaculture Nutrition 2005 11; 293–299

Darby, S.M, Miller, M.L and Allen, R.O. (2001): Forensic determination of ricin and alkaloid marker ricinine from castor bean for Forensic Science Extracts. *Scientific Journal.* 46:1033-1042.

Dunford N.T. Food and Industrial Bioproducts and Bioprocessing. John Wiley & Sons; 2012.

Durowaiye. G.G. (2015): effect of autoclaved castor seed cake in maize and sorghum based diets with enzyme supplementation on performance of Japanese Quail. Faculty of Agriculture, Ahmadu Bello university pg. 1-85.

Davendra, C. (1988). *Non-traditional feed resources in Asia and Pacific* (2nd ed.). Thailand: FAO Regional Office for Asia and pacific Bangkok.

Edwards P., Kamal M. and Lwee K. (1985). Incorporation of Composted and dried Water Hyacinth in pelleted feed for Tilapia (*Oreochromis niloticus* Peters). *Aquaculture and Fisheries Management* 1: 233 – 248.

EFSA (2008) Ricin (from *Ricinus cummunis*) as undesirable substance in Animal Feed: Scientific Opinion of the Panel on contaminants in the food chain. European Food Safety Authority. The EFSA Journal. 726, 1 - 38.

Esonu, B.O., Iheukwumere, F.I., Emenalom, O.O. Uchegbu, M.C. and Etuk, E.B. (2002). Performance, nutrient utilization and organ characteristics of broiler finishers fed microdesmis puberula leaf meal. *Livestock Research for Rural Development*. Retrieved October 20, 2011, from http://www.cipav.org.co/lrrd/lrrd14/6/eson146.html.

FAO, (2012): FAOSTAT, Statistics Division, Food and Agriculture Organization of the United Nations, http://www.fao.org/corp/statistic/en/ (accessed 12. 09. 2018).

Food and Agricultural Organisation (FAO) (1997). Review of the state of World Aquaculture. *FAO Fisheries Circular*. Rome. Pg.188-14

Gabriel, U. U. Akinrotimi, O. A. Bekibele, D. O., Onunkwo, D. N. and Anyanwu, P. E. (2007). Locally produced fish feed: potentials for aquaculture development in sub-saharan Africa. *African Journal of Agricultural Research*. 2(7):287 – 295.

Gohl, B. (1981). In: Tropical Feeds. Feed Information Summaries and Nutritive values. F. A. O., Rome. 1981. Pg. 32-76

Hetch, T. (2000). Consideration on Aquaculture. *Journal of World Aquaculture* 31:12–19.

Jamu, D. M. and Ayinla, O. A. (2003). Potential for the development of aquaculture in Africa. *NAGA* 26: 9 – 13.

Kelly, W.R., 1979. Veterinary Clinical Diagnosis. 4th Edn., Balliere Tindall. London. Pg.141-145

Kulkarni, S. H. and Kesharamurth, K. S. (2005). *Jatropha curcas* poisoning. *Journal of Pediatric*, 72:75-76

Leiner, I. E. (1981). Factors affecting the nutritional quality of soy products. *Journal of American Oil Chemical Society*, 58: 406-415.

Lewis, W H; Elvin, M. P. F. (1977). In: Medical Botany: Plants Affecting Man's Health. John Wiley and Sons Publishers. New York. Pg. 215-219

Metebe, K (1989). The effect of dry and moist heat on some chemical properties of winged beans seeds. *Tropical Grain Legume Bulletin*. 36: 32:33.

Morgan, J. D. and Iwama, G. K. (1997) Measurement of Stressed States in the Field: In Fish Stress and Health in Aquaculture (Iwama *et al.* eds.). Society for Experimental Biology Seminar Series 62: 247 – 268.

Mulky, M.J, and Gandhi, V.M., (1994). Detoxification of castor seed meal integration with all seed meal. *Journal of American Chemist's Society*, 68:616-617.

Mustapha, G.G., Igwebuike, J.U., Adamu, S. B., Kwari, I.D and Gashua, M.M. (2015) The effect of feeding raw castor seed *(Ricinus communis)* meal its replacement levels and processing on the productive performance of broilers. International Journal of Agriculture and Biosciences. 4(4), 161- 166.

M. S. M. E. (2005). Micro, Small and Medium Enterprise Report on Enterprise Level Survey of Catfish Farming Industry in Nigeria. *Consultancy Report submitted to Nigerian Investment Promotion Commission*, P. 86.

Olsnes, S. (2004). The history of ricin, abrin and related toxins. *Toxicon*. 44: 361-370.

Omitoyin, B.O. (2006) Haematological changes in the blood of *Clarias gariepinus* (Burchell 1822) juveniles fed poultry Litter. Livestock Research for Rural Development 18(11).

Oso, A. O., Olayemi, W. A., Bamgbose, A. M. and Fowoyo, O. F. (2011). Utilization of fermented castor oil seed (*Ricinus communis L*) meal in diets for cockerel chicks. *Archivos de Zootecnia*, 60:22-30.

Oyeniran. O. (2015) effects of feeding processed castor *(Ricinus communis)* seeds and cake mealson the performance characteristics of broiler chickens. world journal of agricultural sciences 8 (4): 29- 35.

Prendergast, A. F. D., Higgs, A., D. Beams, M., Dosaugh, B. S. and Deacon, G. (1994). Searching for substitute: Canola. *Northern Aquaculture* 10(3): 15 – 19.

Rama Rao, S.V., 2004. Vegetable protein supplements in poultry diets. Retrieved October, 20, 2011, from http://www.poultvet.com/index.php

Reed, C.F. (1976). Information Summaries on 1000 Economic Plants. Typescript submitted to the USDA.

Schalm, O.W., Jain .N.O., and Carrol .E.J., (1975). Veterinary Haemtology 3rd Edn., Lea and Febiger, Philadelphia, pp: 144-156

Severino .L.S., Auld .D.L., Baldanzi .M, (2012):. A review on the challenges for increased production of castor. *Agron J.* 104(4):853

Ukachukwu, S.N., Nsa, E.E., Iriobe, O.M., Odoemelam, V.U. and Ukweni, I.A. (2011). Acute toxicity of castor oil bean extract and Tolerance Level of Raw Castor Oil Bean *(Ricinus communes L)* by broiler. *Nigerian Journal of Animal Science*, 2011.13:113- 122.

Weiss, E A. (1983). Oil Seed Crops, Tropical Agriculture Series, 1st edition, Longmm, London.

Wikipedia, (2008). *http//en.Wikepedia. Org/wiki/castor-oil plant accessed 04/04/2017.*

The ANOVA Procedure

Class Level Information

Class	Levels	Values
CSM	5	1 2 3 4 5

Number of observations 15

The ANOVA Procedure

Dependent Variable: INITIAL_WEIGHT INITIAL WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.21553333	0.30388333	1.67	0.2334
Error	10	1.82386667	0.18238667		
Corrected Total	14	3.03940000			

R-Square	Coeff Var	Root MSE	INITIAL_WEIGHT Mean
0.399925	6.932914	0.427068	6.160000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	1.21553333	0.30388333	1.67	0.2334

The ANOVA Procedure

Dependent Variable: FINAL_WEIGHT FINAL WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	38.55490667	9.63872667	4.63	0.0226
Error	10	20.83966667	2.08396667		
Corrected Total	14	59.39457333			

R-Square	Coeff Var	Root MSE	FINAL_WEIGHT Mean
0.649132	14.89573	1.443595	9.691333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	38.55490667	9.63872667	4.63	0.0226

The ANOVA Procedure

Dependent Variable: INITIAL_LENGTH INITIAL LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4.24889333	1.06222333	12.23	0.0007
Error	10	0.86886667	0.08688667		
Corrected Total	14	5.11776000			

R-Square	Coeff Var	Root MSE	INITIAL_LENGTH Mean
0.830225	3.861219	0.294765	7.634000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	4.24889333	1.06222333	12.23	0.0007

The ANOVA Procedure

Dependent Variable: FINAL_LENGTH FINAL LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6.00904000	1.50226000	2.24	0.1376
Error	10	6.71473333	0.67147333		
Corrected Total	14	12.72377333			

R-Square	Coeff Var	Root MSE	FINAL_LENGTH Mean
0.472269	7.923879	0.819435	10.34133

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	6.00904000	1.50226000	2.24	0.1376

The ANOVA Procedure

Dependent Variable: WEIGHT_GAIN WEIGHT GAIN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	34.57642667	8.64410667	5.92	0.0104
Error	10	14.59993333	1.45999333		
Corrected Total	14	49.17636000			

R-Square	Coeff Var	Root MSE	WEIGHT_GAIN Mean
0.703111	34.19077	1.208302	3.534000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	34.57642667	8.64410667	5.92	0.0104

The ANOVA Procedure

Dependent Variable: AVERAGE_DAILY_WG AVERAGE DAILY WG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.01053333	0.00263333	4.70	0.0215
Error	10	0.00560000	0.00056000		
Corrected Total	14	0.01613333			

R-Square	Coeff Var	Root MSE	AVERAGE_DAILY_WG Mean
0.652893	37.36471	0.023664	0.063333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	0.01053333	0.00263333	4.70	0.0215

The ANOVA Procedure

Dependent Variable: FEED_CONSUMED FEED CONSUMED

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.32550667	3.58137667	0.89	0.5045
Error	10	40.24846667	4.02484667		

Corrected Total	14	54.57397333	

R-Square	Coeff Var	Root MSE	FEED_CONSUMED Mean
0.262497	14.88133	2.006202	13.48133

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	14.32550667	3.58137667	0.89	0.5045

The ANOVA Procedure

Dependent Variable: FCR FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.62422667	0.15605667	0.94	0.4813
Error	10	1.66606667	0.16660667		
Corrected Total	14	2.29029333			

R-Square	Coeff Var	Root MSE	FCR Mean
0.272553	28.00833	0.408175	1.457333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	0.62422667	0.15605667	0.94	0.4813

The ANOVA Procedure

Dependent Variable: FER FER

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1326.408493	331.602123	2.04	0.1644
Error	10	1625.639867	162.563987		
Corrected Total	14	2952.048360			

R-Square	Coeff Var	Root MSE	FER Mean
0.449318	47.67446	12.75006	26.74400

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	1326.408493	331.602123	2.04	0.1644

1

The ANOVA Procedure

Dependent Variable: SGR SGR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.31333333	0.82833333	2.37	0.1222
Error	10	3.49220000	0.34922000		
Corrected Total	14	6.80553333			

R-Square	Coeff Var	Root MSE	SGR Mean
0.486859	16.72495	0.590948	3.533333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	3.31333333	0.82833333	2.37	0.1222

1

The ANOVA Procedure

Dependent Variable: RGR RGR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	9414.17616	2353.54404	7.04	0.0058
Error	10	3342.86460	334.28646		
Corrected Total	14	12757.04076			

R-Square	Coeff Var	Root MSE	RGR Mean
0.737959	11.64658	18.28350	156.9860

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	9414.176160	2353.544040	7.04	0.0058

1

The ANOVA Procedure

Dependent Variable: _MORTALITY ORTALITY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	173.333333	43.333333	0.36	0.8308
Error	10	1200.000000	120.000000		
Corrected Total	14	1373.333333			

R-Square	Coeff Var	Root MSE	_MORTALITY Mean
0.126214	34.96101	10.95445	31.33333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	173.3333333	43.3333333	0.36	0.8308

1

The ANOVA Procedure

Dependent Variable: WBC WBC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	310247333.3	77561833.3	79.43	<.0001
Error	10	9765000.0	976500.0		
Corrected Total	14	320012333.3			

R-Square	Coeff Var	Root MSE	WBC Mean
0.969486	9.819611	988.1801	10063.33

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	310247333.3	77561833.3	79.43	<.0001

The ANOVA Procedure

Dependent Variable: NEU NEU

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1902.000000	475.500000	29.84	<.0001
Error	10	159.333333	15.933333		

Corrected Total	14	2061.333333

R-Square	Coeff Var	Root MSE	NEU Mean
0.922704	12.60524	3.991658	31.66667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	1902.000000	475.500000	29.84	<.0001

The ANOVA Procedure

Dependent Variable: LYM LYM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1811.600000	452.900000	33.47	<.0001
Error	10	135.333333	13.533333		
Corrected Total	14	1946.933333			

R-Square	Coeff Var	Root MSE	LYM Mean
0.930489	5.352233	3.678768	68.73333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	1811.600000	452.900000	33.47	<.0001

The ANOVA Procedure

Dependent Variable: PCV___ PCV()

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	342.9333333	85.7333333	5.04	0.0174
Error	10	170.0000000	17.0000000		
Corrected Total	14	512.9333333			

R-Square	Coeff Var	Root MSE	PCV___ Mean
0.668573	10.86935	4.123106	37.93333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	342.9333333	85.7333333	5.04	0.0174

The ANOVA Procedure

Dependent Variable: RBC__10___ RBC(*10¹²)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.42933333	0.85733333	5.04	0.0174
Error	10	1.70000000	0.17000000		
Corrected Total	14	5.12933333			

R-Square	Coeff Var	Root MSE	RBC__10___ Mean
0.668573	14.76052	0.412311	2.793333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	3.42933333	0.85733333	5.04	0.0174

The ANOVA Procedure

Dependent Variable: HB HB

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	38.07676000	9.51919000	5.04	0.0174
Error	10	18.87340000	1.88734000		
Corrected Total	14	56.95016000			

R-Square	Coeff Var	Root MSE	HB Mean
0.668598	10.86527	1.373805	12.64400

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	38.07676000	9.51919000	5.04	0.0174

The ANOVA Procedure

Dependent Variable: MCHC MCHC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.3359901E-6	3.3399753E-7	0.73	0.5903
Error	10	4.5603897E-6	4.5603897E-7		
Corrected Total	14	5.8963798E-6			

R-Square	Coeff Var	Root MSE	MCHC Mean
0.226578	0.020260	0.000675	3.333242

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	1.3359901E-6	3.3399753E-7	0.73	0.5903

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The ANOVA Procedure

Dependent Variable: MCH MCH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.69722667	0.17430667	5.75	0.0115
Error	10	0.30326667	0.03032667		
Corrected Total	14	1.00049333			

R-Square	Coeff Var	Root MSE	MCH Mean
0.696883	3.801751	0.174146	4.580667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	0.69722667	0.17430667	5.75	0.0115

2

The ANOVA Procedure

Dependent Variable: MCV MCV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	630.0891733	157.5222933	5.71	0.0117
Error	10	276.0030667	27.6003067		

Corrected Total	14	906.0922400

R-Square	Coeff Var	Root MSE	MCV Mean
0.695392	3.823525	5.253599	137.4020

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	630.0891733	157.5222933	5.71	0.0117

The ANOVA Procedure

Dependent Variable: MOISTURE MOISTURE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.28086667	1.32021667	305.13	<.0001
Error	10	0.04326667	0.00432667		
Corrected Total	14	5.32413333			

R-Square	Coeff Var	Root MSE	MOISTURE Mean
0.991873	0.090486	0.065777	72.69333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	5.28086667	1.32021667	305.13	<.0001

The ANOVA Procedure

Dependent Variable: CP CP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	69.93764000	17.48441000	4803.41	<.0001
Error	10	0.03640000	0.00364000		
Corrected Total	14	69.97404000			

R-Square	Coeff Var	Root MSE	CP Mean
0.999480	0.327751	0.060332	18.40800

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	69.93764000	17.48441000	4803.41	<.0001

The ANOVA Procedure

Dependent Variable: FAT FAT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.75984000	0.18996000	207.99	<.0001
Error	10	0.00913333	0.00091333		
Corrected Total	14	0.76897333			

R-Square	Coeff Var	Root MSE	FAT Mean
0.988123	3.119897	0.030221	0.968667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	0.75984000	0.18996000	207.99	<.0001

The ANOVA Procedure

Dependent 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
CSM	4	0.31404000	0.07851000	206.61	<.0001

The ANOVA Procedure

Dependent 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
CSM	4	5.59910667	1.39977667	846.64	<.0001

The ANOVA Procedure

Dependent Variable: NFE NFE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	47.82840000	11.95710000	3113.83	<.0001
Error	10	0.03840000	0.00384000		
Corrected Total	14	47.86680000			

R-Square	Coeff Var	Root MSE	NFE Mean
0.999198	2.072499	0.061968	2.990000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CSM	4	47.82840000	11.95710000	3113.83	<.0001

The ANOVA Procedure

Duncan's Multiple Range Test for INITIAL_WEIGHT

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	0.182387

Number of Means	2	3	4	5
Critical Range	.7769	.8119	.8325	.8456

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	6.5200	3	2
A			
A	6.4867	3	3
A			
A	5.9833	3	4
A			
A	5.9667	3	1
A			
A	5.8433	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for FINAL_WEIGHT

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	2.083967

Number of Means	2	3	4	5
Critical Range	2.626	2.744	2.814	2.858

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	CSM
	A		11.963	3	1
	A				
B	A		10.613	3	3
B	A				
B	A	C	9.847	3	2
B		C			
B		C	8.793	3	4
		C			
		C	7.240	3	5

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	0.086887

Number of Means	2	3	4	5
Critical Range	.5363	.5604	.5746	.5837

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	8.3700	3	5
A			
A	8.1733	3	4
B	7.3067	3	2
B			
B	7.2767	3	3
B			
B	7.0433	3	1

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	0.671473

Number of Means	2	3	4	5
Critical Range	1.491	1.558	1.597	1.623

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	CSM
	A	11.1900	3	1
	A			
B	A	10.8033	3	3
B	A			
B	A	10.4000	3	2

B	A			
B	A	9.9100	3	4
B				
B		9.4033	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for WEIGHT_GAIN

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	1.459993

Number of Means	2	3	4	5
Critical Range	2.198	2.297	2.355	2.393

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	CSM
	A	5.9967	3	1
	A			
B	A	4.1300	3	3
B				
B	C	3.3300	3	2
B	C			
B	C	2.8100	3	4
	C			
	C	1.4033	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for AVERAGE_DAILY_WG

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	0.00056

Number of Means	2	3	4	5
Critical Range	.04305	.04499	.04613	.04686

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	CSM
	A	0.10667	3	1
	A			
B	A	0.07333	3	3
B				
B	C	0.06000	3	2
B	C			
B	C	0.05000	3	4
	C			
	C	0.02667	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for FEED_CONSUMED

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	4.024847

Number of Means	2	3	4	5
Critical Range	3.650	3.814	3.911	3.973

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	15.010	3	1
A			
A	13.813	3	4
A			
A	13.733	3	3
A			
A	12.553	3	2
A			
A	12.297	3	5

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	0.166607

Number of Means	2	3	4	5
Critical Range	.7426	.7760	.7957	.8082

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	1.7600	3	5
A			
A	1.6433	3	4
A			
A	1.3333	3	3
A			
A	1.2767	3	2
A			
A	1.2733	3	1

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	162.564

Number of Means	2	3	4	5
Critical Range	23.20	24.24	24.85	25.25

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	CSM
	A	40.19	3	1
	A			
B	A	32.82	3	3
B	A			
B	A	26.71	3	2
B	A			
B	A	21.15	3	4
B				
B		12.85	3	5

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	0.34922

Number of Means	2	3	4	5
Critical Range	1.075	1.123	1.152	1.170

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	4.1333	3	1
A			
A	3.8667	3	3
A			
A	3.7000	3	2
A			
A	3.0000	3	5
A			
A	2.9667	3	4

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	334.2865

Number of Means	2	3	4	5
Critical Range	33.26	34.76	35.64	36.20

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	199.89	3	1
B	163.81	3	3
B			
C B	151.27	3	2

C	B			
C	B	146.15	3	4
C				
C		123.81	3	5

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	120

Number of Means	2	3	4	5
Critical Range	19.93	20.83	21.35	21.69

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	36.667	3	1
A			
A	33.333	3	4
A			
A	30.000	3	3
A			
A	30.000	3	2
A			
A	26.667	3	5

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	976500

Number of Means	2	3	4	5
Critical Range	1798	1879	1926	1957

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	17133.3	3	5
B	13466.7	3	4
C	8316.7	3	3
D	6300.0	3	2
D			
D	5100.0	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	15.93333

Number of Means	2	3	4	5
Critical Range	7.262	7.589	7.781	7.904

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	49.000	3	1
B	40.000	3	5
C	26.000	3	2
C			
C	25.667	3	4
D	17.667	3	3

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	13.53333

Number of Means	2	3	4	5
Critical Range	6.693	6.994	7.171	7.284

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	82.333	3	3
B	74.333	3	4
B			
B	74.000	3	2
C	62.000	3	5
D	51.000	3	1

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	17

Number of Means	2	3	4	5
Critical Range	7.501	7.839	8.037	8.164

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	44.000	3	1
A			
A	41.333	3	3
A			
B A	39.667	3	2
B			
B	32.667	3	4
B			
B	32.000	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	0.17

Number of Means	2	3	4	5
Critical Range	.7501	.7839	.8037	.8164

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	CSM
	A	3.4000	3	1
	A			
	A	3.1333	3	3
	A			
B	A	2.9667	3	2
B				
B		2.2667	3	4
B				
B		2.2000	3	5

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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	1.88734

Number of Means	2	3	4	5
Critical Range	2.499	2.612	2.678	2.720

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	CSM
	A	14.667	3	1
	A			
	A	13.777	3	3
	A			
B	A	13.220	3	2

B			
B	10.890	3	4
B			
B	10.667	3	5

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	4.56E-7

Number of Means	2	3	4	5
Critical Range	.001229	.001284	.001316	.001337

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	3.3336508	3	4
A			
A	3.3333538	3	5
A			
A	3.3333443	3	1
A			
A	3.3331066	3	3
A			
A	3.3327553	3	2

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	0.030327

Number of Means	2	3	4	5
Critical Range	.3168	.3311	.3395	.3448

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	4.8567	3	5
A			
A	4.8167	3	4
B	4.4633	3	2
B			
B	4.4500	3	3
B			
B	4.3167	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	27.60031

Number of Means	2	3	4	5
Critical Range	9.56	9.99	10.24	10.40

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	145.707	3	5
A			
A	144.493	3	4
B	133.873	3	2
B			
B	133.457	3	3
B			
B	129.480	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for MOISTURE

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	0.004327

Number of Means	2	3	4	5
Critical Range	.1197	.1251	.1282	.1302

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	73.49000	3	1
B	73.11000	3	2
C	72.84000	3	3
D	72.06667	3	4
D			
D	71.96000	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for CP

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	0.00364

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	CSM
A	21.35333	3	1
B	19.42333	3	2
C	19.13333	3	3
D	17.09000	3	4
E	15.04000	3	5

The ANOVA Procedure

Duncan's Multiple Range Test for FAT

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	0.000913

Number of Means	2	3	4	5
Critical Range	.05498	.05745	.05891	.05984

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	1.31333	3	5
B	1.10667	3	4
C	0.91667	3	3
D	0.85333	3	2
E	0.65333	3	1

The ANOVA Procedure

Duncan's Multiple Range Test for FIBRE

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	0.00038

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	CSM
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

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The ANOVA Procedure

Duncan's Multiple Range Test for ASH

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	0.001653

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	CSM
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

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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	0.00384

Number of Means	2	3	4	5
Critical Range	.1127	.1178	.1208	.1227

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CSM
A	5.78000	3	5
B	4.00000	3	4
C	2.46667	3	3
D	2.21667	3	2
E	0.48667	3	1