EFFECT OF AUTOCLAVED CASTOR SEED MEAL ON GROWTH PERFORMANCE, BODY COMPOSITION AND HAEMATOLOGY OF Clarias gariepinus FINGELINGS.

BY

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FAQ/12/0462

DEPARTMENT OF FISHERIES AND AQUACULTURE, FEDERAL UNIVERSITY

OYE EKITI.

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A PROJECT IN THE DEPARTMENT OF FISHERIES AND AQUACULTURE, FACULTY OF AGRICULTURE IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF BACHELOR OF FISHERIES AND AQUACULTURE (B. FISHERIES AND AQUACULTURE).

FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE.

DECLARATION

I, ADEDAPO, AKINOLA MICHAEL hereby declare that this project titled "EFFECT OF AUTOCLAVED CASTOR SEED MEAL ON GROWTH PERFORMANCE, BODY COMPOSITION AND HAEMATOLOGY OF *Clarias gariepinus* FINGELINGS" has been performed by me in the Department of Fisheries and Aquaculture under the supervision of Dr. T.O. Babalola. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree at any University.

…….. …….

Adedapo, Akinola Michael DATE

FAQ/12/0462

CERTIFICATION

This is to certify that the experiment reported here was conducted by:

Mr. ADEDAPO, AKINOLA MICHAEL

The report has been read and approved having met the requirement of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University Oye-Ekiti, for the award of Bachelor of Fisheries and Aquaculture.

……. ……..

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

PROJECT SUPERVISOR

……. …….

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

HEAD OF DEPARTMENT

DEDICATION

I dedicate this project to all sufficient, unquestionable, awesome and gracious living God almighty. My father Mr Adeniyi Emmanuel, the Adedapo and the family of Mr. and Mrs. Omotoso and to my Late mother Mrs. Adeniyi Abimbola Iyabo

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ABSTRACT

Eight weeks trial was conducted to study the effect of dietary inclusion of autoclaved castor seed meal on growth performance, body composition and haematology of Clarias gariepinus fingerlings. 150 Clarias gariepinus fingerlings were divided randomly into 5 groups of ten fish/tank each to five treatments. The experimental design is a complete randomize design in which there were three replicate for each of the treatment. The experimental diet which were designated 0%, 10%,15%, 20%, and 25%, respectively were isonitrogenous (40% C.P) and isocaloric (2881 Kcal/kg). Fishmeal, soybean meal, and groundnut cake was used as control (diet 1) protein source. The result shows significant difference ($p<0.05$) among the treatments in respect to growth rate, feed intake, feed conversion ratio, and specific growth rate. The result of the growth performance indicates that the final body weight, total weight gain, daily weight gain, standard growth rate, protein efficiency ratio, were significantly ($p<0.05$) higher in the control group (CTRL) compared to CSM10, CSM15, CSM20, and CSM25. The total feed intake and the daily feed intake were significantly ($p<0.05$) higher in control compared to all other treatments, while the feed conversion ratio was significantly lower ($p<0.05$) in control than the remaining diet. Crude protein, and moisture of fish fed the control were significantly higher ($p<0.05$) than the fish fed the CSM10, CSM15, CSM20 and CSM 25, while for the Fat content, ASH and nitrogen free extract of the fish fed CSM25 was significantly higher than CSM20, CSM15, CSM10 and control respectively. The results of the haematological parameters showed that the packed cell volume (PCV) and haemoglobin (Hb), Red Blood Cell, Mean Corpuscular Volume, Mean corpuscular haemoglobin, Mean corpuscular Haemoglobin concentration, Neutrophils values were significantly ($P<0.05$) higher in control compared to CSM10, CSM15, CSM20, and CSM25, but the parked cell volume, Red blood cell, and mean corpuscular volume were similar ($p>0.05$) for CSM15 and CSM20,

CSM20 and CSM25 and CSM15 and CSM20 respectively, mean corpuscular haemoglobin concentration of the fish shows similar effect ($p>0.05$) for CSM10, CSM15, CSM20 and CSM25 but was however significantly different ($p<0.05$) from the control, while in white blood cell and lymphocyte there were significant difference ($p<0.05$) in the CSM25 and the lowest in control respectively. Hence the inclusion of 10% castor seed meal may give a satisfactory performance if the seed is well treated and dehulled properly.

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CHAPTER ONE

1.1 INTRODUCTION

One of the challenges facing aquaculture industry today is the high cost of conventional feedstuff used in compounding aquafeeds. The conventional protein sources like soybean meal, groundnut cake, fish meal and the conventional energy supplement such as maize are not only expensive but have high competitive use by humans and other industrial users. The average price of the ingredients commonly used in fish feed jumped between 20% and 92% from June 2007 to June 2008 (FAO, 2010). The protein intake of Nigerians has been on the decline as a result of the ever increasing population and high cost of animal protein. In Nigeria, the average daily animal protein intake per adult is still far less than the 65g per adult per day recommended by FAO (Adewumi *et al*., 2010). Protein is one of the most important nutrients in aquafeed as component of the feedstuff for aquatic animals. It affects not only the growth of fish but also feed costs (Yunyun *et al*. 2015). Fish growth and production improves with the levels of protein in a diet but this can affect the cost of production economically if it is excessive and also an excess of protein in the diet can adversely affect the level of ammonia in the culture system, but suboptimum level of dietary protein results in poor growth and survival of fish.

Feed represents a major cost in aquaculture production. Considering the increasing prices of fishmeal and soybean meal, which are typically the main protein sources in fish diets, there is need to source for alternative feed stuffs to replace the ingredients that are expensive, scarce and highly competitive with humans. Studies on alternative feedstuffs have allowed considerable reduction in production costs (Albuquerque *et al*., 2014) as the partial or total replacement of expensive ingredients contributes for the economic viability of animal production (Santos *et al*., 2013).

Castor seed meal is one of the underutilized plant protein that can be used as ingredient in animal diet. Castor plant (*Racinus communis*) is a crop from the Euphorbiaceae family, it is indigenous to East Africa (Ethiopia) (wikipedia 2008). This plant in Nigeria grows widely as annual or perennial shrub that could attain the height up to 10 m high in most part of Nigeria (Ombrello, 2007). Unprocessed castor seed meal have a crude protein content of 35 to 40% (Ani, 2012) but detoxified castor seed meal (DCM) contained 41 % crude protein (CP) (Abdalla *et al*. 2008).

In spite of the quality of the seed meal, the anti-nutritional factor present in the seed has reduced the use of the seed in animal feeds. The potent antinutritional factors present in the seed include ricin, ricinine, and allergen (Darby *et al*., 2001, Olsnes 2004). These anti-nutritional factors can be deactivated through physical and chemical treatments like soaking, steaming boiling autoclaving, lye, fermentation, and treatment with ammonia (Anandan *et al*., 2012; Benge, Martinez-Herrera *et al*., 2006; Nsa and Ukachukwu, 2007; Ani, 2012)

Detoxified castor seed meal have been used in diet fed poultry, and fish such as *Oreochromis niloticus* and carp Balogun *et al.* (2005), Cal .X *et al.* (2005), with improvement on the growth performance of the fish fed detoxified castor seed meal performing more better than the control without detoxified castor seed meal, this report indicated that herbivores had great response to the utilization of detoxified castor seed meal with 40% in inclusion of the boiled detoxified castor seed meal performing most.

Castor seed meal can be used to replace plant protein such as groundnut cake, soybean meal and partially animal protein (fish meal) in the compounded diet of aquafeed (Cal *et al.* 2005).

Compared with other plant protein castor seed meal is high in essential amino acid and minerals. Annongu and his colleague (2008) gave the report that castor seed meal when compared with soybean meal contain 2.86% 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 include phosphorus 6.40, calcium 32.21, magnesium 48.09% and potassium 23.17%.

The aim of this study is to evaluate the effects of substituting autoclaved castor seed meal in the diets of *Clarias gariepinus* fingerlings on growth performance, body composition and haematology of the fish and to reduce the high dependency and competition on the conventional feedstuff, if successful, may reduce the cost of feed production and subsequently that of animal production in general and may increase the production of the livestock sector of Nigerian Agriculture (Annongu and Joseph, 2008).

1.1 Objective

To determine the effect of growth performance on *Clarias gariepinus* fingerlings fed Autoclaved castor seed meal

To determine the effect of body composition on *Clarias gariepinus* fingerlings fed Autoclave castor seed meal

To the determine the effect of Haematology on *Clarias gariepinus* fed fingerlings Autoclaved Castor seed meal

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and origin of castor plant

Castor seeds have been found in Egyptian tombs dating back to 4000 BC and the oil gotten from the seed was used in wick lamp for lighting (Durowaiye 2015). Castor plant is native to the Ethiopian region of tropical East Africa (Akande *et al*. 2012). The plant is called by various names in different languages (Ombrello, 2007). Castor plant belongs to the Euphorbiaceae or spurge family (Akande *et al*. 2012). It is the only member of the genus *Ricinus* and has no immediate relatives (IGS, 2009). 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, 2007). Castor plant grows as perennial in the tropics and as an annual crop in the temperate climate. The common name "castor oil plant" is probably derived from its use as a replacement for castoreum, a perfume-base made from the dried perineal glands of the beaver (castor in Latin). Its other common name is palm of Christ (or Palma Christi) which is derived from castor oil's reputed ability to heal wounds and cure ailments (Durowaiye 2015)

2.1.1 Description of Castor oil Plant

Castor oil plant has been bred to adapt to mechanized cultivar and can be cultivated in dry areas with a height between 160-120m and (Rehm and Espig, 1991). It has large star shaped leaves which make a bold foliage plant (Reed, 1976). The leaves have slightly serrated edged and prominent central veins. It has numerous flowers in long inbred senses, with male flower at the base and female flowers on the tips (Reed, 1976).

2.2.1 Composition and nutritive value of castor seed meal

Castor seed meal contains proteins, lipids, carbohydrates, vitamins, and minerals. The protein content is the principle nutrient of interest, accounting for between 21% -48% of total crude protein content depending on the level of deoiling and decortication (Adeniji *et al*., 2007). Proximate compositions of un-dehulled and dehulled full fat castor seeds and castor seed cake are Compared to un-dehulled full-fat castor seeds, dehulled castor seed cake contains higher values of dry organic matter, crude protein, 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 un-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 as reported by Oyeniran (2015) 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.

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. The utilization of castor seed meal as fish ingredient is recent and had been used on two fish only, but have been used on both monogastric and ruminant animal

2.2.2 Protein, amino acid and mineral composition of castor seed meal

Castor seed meal contains from 21 to 48% protein depending on the level of decortication and deoiling, the amino acid profile compared to that of the conventional feedstuff such as soybean

meal, groundnut cake meets the amino acid demand of fish (Adeniji *et al*. 2007, Annongu and Joseph 2008), It has an ideal amino acid profile with moderately high Cystine, methionine, and isoleucine. The amino acid profiles of both un-dehulled and dehulled castor seed cakes are closely related in values to that of soybeans (Oyeniran 2015), Macro- and micro-minerals of un-dehulled and dehulled full-fat castor seeds and castor seed cake shows that dehulled castor cake contains higher values of both macro- and micro-minerals than un-dehulled full-fat castor seeds. Dehulled castor seed cake contains higher values of phosphorus, magnesium, sodium and potassium than the dehulled full-fat castor seeds, while the opposite was recorded calcium. The macro- and micro-mineral compositions of un-dehulled and dehulled castor seed and castor seed cake revealed that they contain sufficient amounts of these nutrients to meet the nutritional requirements of animals if the anti-nutrients in the castor seeds that could inhibit utilization or availability of the minerals are removed by adequate treatment of the castor seeds or the cake. 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 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; Ani and Okorie 2009). Toxic substances such as ricin, ricinine, hydrocyanide, allergens and other alkaloids have however limited the use of castor seeds for human and animals. Onwuliri and Anekwe, (2001) Annongu and Joseph, (2008) reported the mineral composition and amino acid profile analysis of castor seed cake as shown in the Table 2.1 and 2.2.The cake contain high levels of nutrients with potentials to meet the nutritional needs of fishes 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).

Table 2.1: **Amino acid profile of dehulled castor seed cake compared with soybeans as standard plant protein**

Amino acid (g/16 gram of N)	[a]dehulled castor seed cake	[b]Dehulled castor seed cake	Soybeans
Lysine	7.3± 0.12	4.48	6.30
Histidine	2.2 ± 0.06	1.88	2.70
Arginine	7.4 ± 1.14	9.36	8.10
Aspartic acid	12.4 ± 2.0	7.66	11.80
Threonine	2.3 ± 0.08	3.88	3.90
Serine	4.1 ± 0.2	3.02	5.20
Glutamic acid	13.6± 2.6	15.64	17.90
Proline	9.7 ± 0.50	3.08	5.90
Glycine	5.7 ± 0.46	1.11	4.40
Alanine	3.8 ± 1.2	5.02	4.30
Cystine	0.53	0.86	1.60
Valine	3.1 ± 0.10	4.93	5.10
Methionine	1.9± 0.11	2.86	1.60
Iso-leucine	3.8± 0.05	1.61	7.90
Leucine	5.1± 0.16	8.56	5.10
Tryptophan	-	-	1.30

Source: **a**:**Onwuliri and Anekwe , 2001**., **b**: **Annongu and Joseph, 2008**.

Table 2.2: **Macro- and micro-mineral composition of full-fat castor seed meal and castor seeds cake**

Macro element (mg/100g)	Un-dehulled		dehulled	
	Ful-fat castor seed	Castor seed cake	Ful-fat castor seed	castor seed cake
Phosphorus	3.4	5.6	3.3	6.4
Calcium	19.37	27.22	30.34	32.21
Magnesium	49.95	61.47	57.82	48.59
Potassium	21.89	23.61	19.62	23.17
Sodium	74.32	105.29	73.45	56.87
Microminerals, ppm				
Copper	0.88	5.33	0.88	0.44
Iron	51	72	26	49
Manganese	1.62	8.08	0.81	0.81
Cobalt	1.64	1.64	-	-
Lead	-	-	-	-

Source: **a**:**Onwuliri and Anekwe , 2001**., **b**: **Annongu and Joseph, 2008**.

2.2.3 Oil composition

The oil content of the seed varies from 35 to 55 percent of the weight of the seeds, depending on the variety of the seed (Durowaiye, 2015), Akande *et al.* (2012) reported that the large seed of castor yield of 39.4% oil while the small seed yield 37.2%, showing that the size of the seed cause variation among in the level of oil present in the seed, the oil of castor seed have a great aroma that could stimulate feeding, The oil is obtained by mechanical 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, mechanical 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 increase surface area for better oil extraction and then pressed mechanically with hydraulic machine for an hour for better oil extraction. Oil from the seed can be produced locally by boiling the seed meal in water and separating the oil which is used for medicinal purposes in the rural areas (Kulkarni and Kesharamurth, 2005). The average yield of oil by different methods of extraction includes expellers and hydraulic presses (40-41%), rotatory mills 15% and screw presses (39%), village gbanis (35%) and by boiling the seed meal in water (30%) (Durowaiye, 2015). The oil is graded as first grade oil which is pale yellow to colour less and is prized for medicinal purposes, while the poorer grade is used for industrial purposes. Castor oil has long been used commercially as a highly renewable resource for the chemical industry. Major castor oil producing countries include Brazil, China, and India, Even though castor oil accounts for only 0.15% of the world production of vegetable oils, worldwide consumption of this commodity has increased more than 50% during the past 25 years, rising from approximately 400,000 tons in 1985 to 610,000 tons in 2010 (Severino *et al*. 2012). Castor oil has been an impetus to a country like Nigeria such that Benue

state government, received over two billion naira for castor oil seed production imitative in 2008 (Durowaiye 2015). Ukachukwu *et al*. (2011) reported that cross river state of Nigeria has gone into a project of producing castor oil seed for its oil. Castor oil is mostly ricinoleic acid with small amount of dihydroystearic, linoleic, stearic and eicosanonic acids (Ombrello, 2007), the unique structure of castor oil offers interesting properties, making it appropriate for various industrial applications. 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 derivatives provides a functional group location for performing a variety of chemical reactions including halogenation, dehydration, alkylation, and esterification. As a result, this unique functionality allows the castor oil to be used in industrial applications such as paints, coatings, inks, and lubricants (Dunford, 2012). The chemical responsible for these flavours and aroma are obtained from ricinoleic acid and account for about 90% of the total triglyceride fatty acids of castor oil (Cadha, 2006). Castor oil is used in manufacturing of soaps, inks, plastics, brake fluids and certain insecticidal oil. It is also useful in dyeing and finishing of textile products and preservation of leather (Cadha, 2006; Ogunniyi, 2006). 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 Utilization of castor seed cake in animal nutrition**.**

Castor seed cakes have been successfully used in livestock feeding after detoxification both in monogastric and ruminants. Poultry appear to be more tolerant to castor toxins than other mammals. Up to 40% of the cake has been used in chick ration, boiled castor bean meal supplemented with Beta-xylanase was fed to pullet chicks at 15% levels of inclusion and the weight gain were comparable to the control but higher levels led to reduced weight gain (Babalola *et al*., 2006, Ani and Okorie, 2009). The potentials of castor seed (*Ricinus cummunis*) meal as feed ingredient for *Oreochrornis niloticus* was determined by using boiled seeds to prepare five diets, The effects of the experimental diets 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 factors were obtained with feed formulated from *R. cummunis* seeds boiled for 50 and 65 minutes (Balogun *et al.*, 2005). Cal *et al*., (2005) reported that castor seed was use to replace fishmeal at 0, 40 and 100% with 40% inclusion in both performing most

2.4 Factors Affecting the Nutritional Quality of Castor Seeds and Castor Seed Cake

The use of castor seeds and castor seed cake as feed ingredient in poultry rations is limited by the presence of phytotoxins, primarily the ricin and ricinine (Babalola *et al.* 2006) another problem associated with the meal is the high level of fiber 300g/kg if the hull is not removed before oil extraction (Apata *et al*., 1999). There are also the presence of extremely potent allergens in the seed meal (Babalola *et al*., 2006). The anti-nutritional factors hindered the use of both castor seeds and castor seed meals as sources of protein.

2.4.1 ANTI NUTRITIONAL FACTORS

Castor seed meal have been shown to contain a number of anti-nutritional, which include ricin, ricinine and allergens, but the most dangerous of the toxin is ricin which varies depending on the variety of castor seed, large size seed contains 3.7 Hu mg/m/ while small size variety contains 3.1 Hu mg/ml/ (Durowaiye 2015)

2.4.1.1 RICIN

Ricin component of the castor seeds remain a serious impediment limiting the application of castor seed meal in animal diet (EFSA, 2008). Ricin is the most notorious deadly poison found in abundance in the seed and in smaller amounts in the remaining plant parts. Ricin is a highly toxic naturally occurring protein found in castor plant although the lethal dose in adults is considered to be four to eight seeds, reports of actual poisoning are relatively rare (Horton and Maurice, 1989). According to FAO 2012, Ricin is a toxic glycoprotein (Toxalbumin – a carbohydrate binding protein) found in castor beans. Chemically it is a glycoprotein composed of two polypeptide chains – A and B, linked by a disulfide bond. Ricin functions by disrupting the protein synthesis mechanism of the cell. The A-chain of ricin is the toxic portion of the protein. The A-chain functions by depurinating the 28S subunit of the infected cell's ribosomes. The B-chain of ricin is responsible for getting the A-chain into the target cell. The B-chain consists of a galactose-binding region capable of binding a target cell's membrane and initiating cell entry. Without the B-chain's functionality, the A-chain cannot enter 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, with an LD50 (lethal dose for 50% of a population) of 3–5μg/kg when inhaled and 1–20 mg/kg when ingested.

2.4.1.2 RICININE

Ricinine is one of the toxic components in castor seed, it is a water soluble alkaloid concentrated mostly in seed coat. It also present in leaves and stems. It is slightly bitter to taste and is irritant to the throat (EFSA, 2008). It is only mild toxic to human being. Alkaloid ricinine or ricines communis agglutinin (RCA) which a powerful haemagglutinin but weak cytotoxin (Durowaiye 2015). Munroe (2006) revealed that 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.

2.4.1.3 ALLERGEN

FAO 2012 reported that Castor bean allergen-1 (CB1A) is the principal allergen of the castor bean. It is virtually non-toxic, does not cause death though may cause allergic reactions. It is a polysaccharididic protein factor. CB1A is among one of the most heat-stable proteins found in normal heating conditions.

2.4.2 Processing Of Castor Seed Meal

The toxins in the seeds are water-soluble and not lipid soluble. So the toxins remain in the castor seed cake and are not released during oil extraction process. The various methods of processing legume seeds for incorporation into poultry feeds can be used for processing castor seeds (Oyeniran 2015). These processing methods include heat treatment which include cooking, roasting and autoclaving, and treatment without heating include soaking, fermentation, lye treatment as well as their combination. The adoption of any of the processing methods is influenced by cost, location, environmental factors, choice and facilities available at the farmer's disposal

(Bawa, *et al*., 2003a). These processing methods have effects on proximate compositions and levels of destruction of anti-nutrients of the grain legume (Najime 2003, Bawa *et al*., 2003b and Abeke *et al*., 2003).

2.4.2.1 Autoclaving

This method of processing is most preferred due to the fact that the nutrient are not leached out through the water as in the case of soaking etc. and also the microbes and enzyme that cause deterioration can be adequately deactivated. The cake obtained after oil extraction are to be pressure cooked using autoclaving machine, a method described by Purushothan *et al*., (1986). Barnes *et al* (2009) showed that the ricin in castor seed meal was denatured when subjected to hot pressured and boiling for 60 min. similar results was also obtained by Balogun *et al.* (2005).
Castor seed meal detoxified by autoclaving affect the crude protein with increase in crude protein with reduce in fat content Durowaiye (2015)

2.4.2.2 Lye Treatment

This method of detoxifying castor seed meal was reported by Akande *et al.* 2011, to prove efficient on detoxifying the cake more than fermentation and boiling, the techniques affect the chemical profile of castor seed meal such as the energy value compared to that of untreated castor seed meal, but most nutrient were lost as the techniques involve washing with fluid, according to the results in his work, the method removed about 55% of ricin in the castor seed meal Akande *et al*.2012

2.4.2.3 Fermentation

Effect of fermentation on castor seed meal had a decree on fiber content of product and may influence positively the bioavailability of other nutrient Akande *et al* 2012 with varying degree of anti-nutrient factor left in it

2.4.2.4 Processing of Castor Seeds and Castor Seed Cake by boiling

According to Akande *et al.* (2012), least detoxification was attained with boiling treatment as it affect the lectins which is heat labile and agglutination which is observed in boiling treatment method but tannin and oxalate appear not to have been affected by treatment with over 50% of phytate removed. This processing method had a short fall on the energy value compared to untreated castor seed meal, but loss of nutrient was recorded, since the treatment involve washing with fluid, particularly soluble carbohydrate, however there were residue of ricin on a varying degree, depending on the boiling time Akande *et al*. (2012)

2.5.1 Protein and amino acid Requirement of Catfish

Protein is the major constituent of fish diet. Knowledge of the protein requirement of fish is essential for the formulation of a well balance artificial diet for an economical fish feeding. Fish meal, soybean meal, fish hydroxylate, skim milk powder, legumes, and wheat gluten are excellent sources of protein. Additionally, the building blocks of proteins (free amino acids) such as lysine and methionine are commercially available to supplement the diet, unlike domesticated farm animals, many fish species currently being cultured have high dietary protein requirement, majorly in a high density fish culturing medium as they expand a lot of energy to get feed (30%-45%), which vary for the catfish with each particular life stage. For example, fish will require less protein

at lower temperature and pH and higher protein content at higher temperature and pH levels. Also fish at a fry state will require a protein level of 40% and above, fingerlings will require 40% and adult will require the protein of about 35% NRC (1993), gave the following amount of amino acid requirement for warm water catfish. Lysine 1.43%, leucine 0.98%, arginine 1.2%; methionine 0.64%, valine 0.84%, histidine 0.42%, tryptophan 0.14%, isoleucine 0.72%, phenylalanine 1.40% and threonine 0.65% .

2.5.2 Energy requirement of *Clarias gariepinus* fingerlings

Energy is one of the most important parts of the diet as this affect the utilization of other nutrient when too high or low. Feeding standards for many animals are based on energy needs. Feed intake for catfish may be more a function of how much feed the catfish are allowed to have rather than energy concentration in the feed. Although catfish feed intake may not be strictly regulated by dietary energy, balance of dietary energy is important when formulating catfish feeds. This is true mainly because a lack of non-protein energy in the diet will result in the more expensive protein's being used for energy. Also, if dietary energy is too high, catfish may not eat as much, resulting in less intake of essential nutrients (Babalola *et al.* 2006). Too high of a dietary energy/protein ratio may lead to higher body fat, which may reduce dressed yield and shorten shelf life of frozen products.

We don't know the absolute energy requirements for catfish. Estimates have been made by measuring weight gain or protein gain of catfish fed diets with a known amount of energy.

Energy requirements reported for catfish, which have generally been expressed as a ratio of digestible energy (DE) to crude protein (DE/P), range from 7.4 to 12 kilocalorie/ gram (kcal/g).

Based on current knowledge, a DE/P ratio of 8.5 to 9.5 kcal/g is adequate for use in commercial catfish feeds. Increasing the DE/P ratios of catfish diets above this range may increase fat deposition and reduce processed yield, and if the energy value is too low the fish will grow slowly. Carbohydrates and lipids (fats and oils) are the major energy sources for catfish.

2.5.3 Energy and Protein of *Clarias gariepinus* fingerlings

Dietary nutrients are essential for the construction of living tissues. They also are a source of stored energy for fish digestion, absorption, growth, reproduction and the other life processes. The nutritional value of a dietary ingredient is in part dependent on its ability to supply energy. Physiological fuel values are used to calculate and balance available energy values in prepared diets. They typically average 4, 4, and 9 kcal/g for protein, carbohydrate and lipid, respectively.

To create an optimum diet, the ratio of protein to energy must be determined separately for each fish species.

Excess energy relative to protein content in the diet may result in high lipid deposition. Because fish feed to meet their energy requirements, diets with excessive energy levels may result in decreased feed intake and reduced weight gain. Similarly, a diet with inadequate energy content can result in reduced weight gain because the fish cannot eat enough feed to satisfy their energy requirements for growth. Properly formulated prepared feeds have a well-balanced energy to protein ratio.

2.6 Haematology of *Clarias gariepinus*

Several factors affect haematological values in fish these include sex, age, size, environmental and physiological conditions. Fish cultures are especially at risk to the adverse effects of stress.

Blood chemistry and hematological measurements can provide valuable physiological indices that may offer critical feedback on different stressors (Binukumari *et al*., 2011)

Assessment of nutrient utilization and biological values of feeds and feedstuffs may seem inconclusive without adequate consideration of their implications on the physiological and health status of the animal (Anyanwu *et al*., 2011), Bhaskar and Rao (1990) reported Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) values of 132.8-308.4fl, 20.90- 47.20pg and 10.90–38.10% respectively in their studies in relation to stocking and feeding conditions. In 2007 Ochang *et al.* reported similar result but with the MCHC little bit higher, while Babalola *et al.* 2006 reported red blood cell, Haemoglobin, Packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration white blood cell values of 6.13, 4.30, 13.85, 22.59, 7.02, 31.01, 12.82 when fed boiled castor seed meal at 150g kg^{-1}

2.6.1 Hemoglobin (Hb).

Hemoglobin molecules are responsible for binding and releasing oxygen on to the red blood cells. 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).

2.6.2 White Blood Cell Differential

It is the analysis of different types of white blood cells. The distribution of these types of cells shows underlying cause of disease in animal. Basophils are common in parasitic infection. The normal values of basophil ranges from 0-0.2%) high level of basophil indicates possible parasitic infection. Low level indicates absence of parasites. Eosinophils fight against allergies and

parasites. High eosinophil count indicates an allergy or parasite causing illness. Lymphocytes are white blood cells which develop anti-bodies to prevent against future attacks. High lymphocytes may be due to infection by virus. Neutrophil are primary white blood cells responsible for fighting infection. High neutrophil count indicates infection while low neutrophil indicate sepsis (Dawn, 2010)

CHAPTER THREE

3.0 MATERIALS AND METHODS

20 kg of castor seed was gathered in Ekiti and preparation of the seed to castor seed meal done by the following procedure, the wet laboratory of the Department of Fisheries and Aquaculture Federal University Oye-Ekiti was used for the feeding trial and the proximate analysis done in the central laboratory of the Federal University Oye-Ekiti, and the haematological analysis was carried out in the analytical laboratory of the Federal Medical Center Ido-Ekiti

3.1 DEHULLING OF THE SEED

3.1.1 Castor seed meal Processing

The castor seed undergo various processing in the course of its preparation for extraction. The unit operations involved are:

- Cleaning: The castor beans had some foreign materials and dirt which was separated by hand picking.
- Drying: The cleaned beans were sun dried in the open, until the seed dehiscent and release the seed. The beans were further dried in the sun for few hours to allow for easy breaking of the seed without mashing the coat with the cotyledon
- Breaking of coat: this is done by manual breaking of the coat covering the cotyledon between two stone
- Winnowing: The separation of the coat from the nibs (cotyledon) was carried out using tray to blow away the cover in order to achieve very high yield by separating the coat away from the cotyledon.

❖ Grinding (particle size reduction): Mortar and pestle were used to crush the beans into a paste (cake) in order to increase the surface area and allow the easy release castor fat for extraction.

3.1.2 Oil extraction process

The castor seed meal that had been grounded was pretreated with heat to assist in oil extraction and to improve the nutritional value in the oven for 30 minutes at 105^0 C after which I put in a muffling cloth to allow for removal of oil while the cake is left in the muffling cloth. Manual hand oil press was used for this process provided by my supervisor.

3.1.3 Preparation of castor cake for feeding trials/detoxification of the cake.

The cake obtained after oil extraction was pressure cooked using autoclaving machine using the method described by Purushothan *et al*., (1986). The autoclaving machine lid was lifted open and the water level was gauged with distil water. A perforated iron bucket was placed inside the autoclaving machine slightly above the water. Another iron bucket was lined with foil paper and was filled with castor seed cake to a level and placed inside the perforated iron bucket. The autoclaving machine was locked properly and connected with source of electricity. The timing of the autoclaving machine started when steam was rushing out properly and collected inside a container For 1 hour in steam at 125 ^{O}C

3.2 Diet formulation

Five isonitrogenous (40% protein) and isocaloric (2881.75 kcall/kg ME) ration was formulated as shown in the Table 3.1 below 1:0 diet 1 was the control, , diet 2,3,4 and 5 have portion of castor seed meal at 10%, 15%, 20% and 25% respectively.

Table 3.1: Diet formulation table for each of the treatment

Ingredient	0% CSM	10% CSM	15% CSM	20% CSM	25% CSM
MAIZE	7.64	7	7.03	6.03	6
WHEAT OFFAL	10	8.4	7	7	6
FISHMEAL	30	30	30	30	30
GROUNDNUT CAKE	10.95	10.98	10.45	8.95	5.95
SOYBEAN MEAL	32	24.1	21	18.5	17.53
CASTOR SEED MEAL (CSM)	0	10	15	20	25
METHIONINE	1	1	1	1	1
VIT. MIN. PREMIX	2	2	2	2	2
SALT	0.5	0.5	0.5	0.5	0.5
BINDER	2	2	2	2	2
VIT. C	0.015	0.015	0.015	0.015	0.015
VEGETABLE OIL	4	4	4	4	4
CHROMIX OXIDE	0.005	0.005	0.005	0.005	0.005
TOTAL	100	100	100	100	100

Vit. A; 4000000iu, Vit. D_3 800000iu, Tocopherois; 4000 iu, Vit k_3; 800mg, folacin; 200mg, vit.B11.8mg, Vit.B2,5mg, thiamine; 600mg, ribofavin 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 Fish, and sample collection

150 *Clarias gariepinus* (fingelings) was gotten from Afe Babalola Fish Farm (ABUAD) fish farm Ekiti state. The fishes were acclimatized in a randomly selected 15 aquaria tank for one week before the commencement of the feeding trial, during which period fishes were placed on commercial catfish diets (vital feed). After which it was subjected to the feeding trials as stated above, and analysis was done on the sample collected (data) base on growth performance, body composition, and on the haematology of the fish.

3.4 Experimental design

Catfish fingerlings (*Clarias gariepinus*) that were purchased were randomly assigned to the dietary treatment. Each treatment had three replicate in each of the plastic aquarium, ten *Clarias gariepinus* fingerlings were stocked in each of the five dietary treatments compared, with three tanks per treatment as replicates and ten in each of the replicate using complete randomize design (CRD). Feed was suspended for 24 hours before the feeding trial to increase appetite and reception of new diet (Madu and Akilo, 2001)

3.5 Experimental Procedure

One hundred fifty *Clarias gariepinus* fingerlings were purchased from a reputable farm (Afe Babalola farms, Ado-Ekiti, Ekiti-State) and used during the experiment. The fish were acclimatized for one week, during the period of acclimatization the fish were fed at 5% body weight twice daily with a formulated diet of 40% crude protein. At the end of the acclimatization period, the fish were randomly selected and stocked into 15 rectangular aquaria of 25 liters holding 10 fishes. The trial lasted 8 weeks and was conducted in fifteen plastic tanks, the tank was properly

placed in the wet laboratory of Department of Fisheries and Aquaculture of Federal University Oye-Ekiti, and properly monitored to ensure good water quality state. The photoperiod was natural and all tanks had similar lighting condition

3.5.1 Feeding trial

Three tanks were randomly assigned to each diet groups in eight weeks experiment, at the start of the experiment, 10 fish were batch weighted and stocked into each tank. During the feeding period, fish were fed the experimental diet of 5% body weight twice a day at 09:00 and 16:00 respectively, 0ne hour later the uneaten feed was siphoned.

3.5.2 Experimental conditions

The water quality parameters were monitored on a weekly bases for temperature using clinical thermometer; dissolved oxygen using a dissolved oxygen meter, Hydrogen iron concentration (pH) were measured using a pH meter in the laboratory at room temperature

3.6 Chemical analyses

Chemical Analysis of both fish carcasses and feed were analyzed for proximate composition: moisture, ash, crude protein, crude fat, and carbohydrate according to the methods Proximate composition of feed and fish was determined using Association of Official Analytical Chemistry (A.O.A.C, 2003) method, Samples of experimental diets were taken to the central Laboratory for proximate analysis using the methods described by A.O.A.C. Crude protein by the Kjedahl procedure, ether extract by subjecting the samples to petroleum ether extraction at 60-100^{o} C using the soxhlet extraction apparatus. Dry matter by oven drying the samples at 105^{o}C over a 6- hour

period. Crude fiber by boiling the samples under flux in weak sulphuric acid (0.255N H2S04), then in a weak sodium hydroxide (0.312N NaOH) for 1 hour. The residues which consist of cellulose, lignin and mineral matter were dried and weighed. The ash content was determined by igniting a weighed sample in a Muffle 30 furnace at 600^{o}C. The nitrogen free extract (NFE) was obtained by the difference after the percentages of the other fractions were subtracted from 100%.

3.7 Growth and nutritional analysis

The parameters measured were according to Halver (1989) Maynand *et al*., as explained below

Mortality: this measure the death rate in the fishes as expressed in

i. % mortality $= \frac{\text{No of dead fish}}{\text{No of fishes stocked}} \text{ X } \frac{100}{1}$

ii. Specific growth rate (SGR): this is expressed as

$$\frac{\ln \text{MFW (mean final weight)} - \ln \text{MIW(mean initial weight}}{\text{time in days}} \text{ X100}$$

iii. Feed conversion ratio (FCR) $= \frac{\text{weight of feed fed(dry gram weight)}}{\text{weight gain of fish (wet gram weight)}}$

iv. Protein efficiency ratio (PER) is expressed as $\frac{\text{weight gain of fish}}{\text{protein feed}}$

Where protein feed = feed intake X percentage protein

Determination of digestibility by

Amount of chromic oxide $= \frac{1}{4} \left(\frac{y-0.032}{0.2089} \right)$

Percentage of chromic oxide $= \frac{X}{Y} \text{ X } 100$

Percentage of apparent digestible nutrient = $100 - 100 \left(\frac{\% \text{ of Cr2 O3 in feacal}}{\% \text{ of Cr2O3 in feed}} \text{ X } \frac{\% \text{ nutrient in feed}}{\% \text{ nutrient in feacal}} \right)$

3.8 Haematology

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

Using

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

MCH (pg)= [Hb(gdl^{-1}) X10] /RBC ($10^{6}\ \mu l^{-1}$)

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

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 microhaematocrit centrifuge at 12,000 rpm. The PCV was read by the use of haematocrit reader (Kelly, 1979)

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).

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.

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

3.9 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 SPSS version 16.0 (SPSS 2008). The data presented as mean ± S.E.M. of three replicate group.

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

$Yij= \mu + Bi + Eij$

Yij = the jth observation of the ith. treatment

μ= the overall estimate of population mean

Bi= the effect of the ith treatment

Eij = the random error.

CHAPTER FOUR

4.0 Results

4.1 Proximate composition of experimental diet

Proximate of experimental diet is presented on table 4.1. Moisture content was similar ($p>0.05$) in CSM10 and CSM15 as well as CSM20 and CSM25 respectively. They however differ significantly ($p<0.05$) from the moisture content in the control. Fat content differed significantly ($p<0.05$) in all the experimental diet with the highest recorded in CSM 25 and lowest in the control. Crude protein were similar ($p>0.05$) in all the experimental diet with the highest recorded in CSM 15, while the lowest in CSM25 and the control respectively. Fiber content in the experimental diet differ significantly ($p<0.05$) with the highest recorded in CSM25 and the lowest recorded in the control. The control diet, CSM10 and CSM15 had similar ($p>0.05$) ash content but however significantly differ ($p<0.05$) from ash content of CSM20and CSM25 respectively. NFE where similar ($p>0.05$) in CSM15 and CSM20 respectively, but however differ significantly ($p<0.05$) from NFE recorded in control, CSM10 and CSM25

Table 4.1: Proximate composition of experimental diet

Parameters (%)	**CTRL**	**CSM10**	**CSM15**	**CSM20**	**CSM25**
Moisture	8.30 ± 0.10^{b}	7.90 ± 0.10^{ab}	8.20 ± 0.20^{ab}	7.60 ± 0.00^{a}	7.60 ± 0.00^{a}
Fat	17.52 ± 0.10^{a}	18.68 ± 0.02^{b}	19.61 ± 0.04^{c}	20.24 ± 0.07^{d}	21.25 ± 0.08^{e}
Crude Protein	40.06 ± 0.31^{a}	40.23 ± 0.44^{a}	40.45 ± 0.48^{a}	40.28 ± 0.44^{a}	40.06 ± 0.22^{a}
Fiber	2.06 ± 0.06^{d}	3.10 ± 0.03^{c}	3.42 ± 0.06^{b}	4.16 ± 0.27^{b}	4.66 ± 0.13^{a}
Ash	5.50 ± 0.10^{a}	5.70 ± 0.10^{a}	5.80 ± 0.20^{a}	5.70 ± 0.10^{ab}	6.20 ± 0.00^{b}
NFE	26.56 ± 0.13^{d}	24.34 ± 0.43^{c}	22.53 ± 0.50^{b}	22.03 ± 0.41^{b}	20.23 ± 0.37^{a}

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

4.2: **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 with the highest (2.54 ± 0.00) in CSM25 and lowest (2.50 ± 0.01) in CSM10, final weight was significantly different ($p<0.05$) in fish fed castor seed meal with highest recorded (8.37 ± 0.18) in the control and the lowest recorded (3.62 ± 0.02) in CSM25. The feed intake of the fish were similar ($p>0.05$) in CSM20 and CSM25, however significantly different ($p<0.05$) as observed in the control CSM10, and CSM15. Weight gained was significantly different ($p<0.05$) in all treatment with highest (7.96 ± 0.10) in the control and the lowest (2.79 ± 0.03) in CSM25. Daily weight gained was similar ($p>0.05$) in CSM20 and CSM25, but significantly different ($p<0.05$) in the control, CSM10 and CSM15. Protein intake were similar ($p>0.05$) in CSM20, and CSM25, however were significantly different ($p<0.05$) from control CSM10, and CSM15 with highest (3.37 ± 0.07) in control while lowest (2.15 ± 0.01) in CSM15. Feed conversion ratio were similar ($p>0.05$) for CSM10 and CSM15, and significantly different ($p<0.05$) from control, CSM20, and CSM25 with highest (1.30 ± 0.01) in CSM25 and lowest (1.05 ± 0.01) in control. Protein efficiency ratio were similar ($p>0.05$) for CSM20 and CSM25 and also for CSM10 and CSM15 respectively, however significantly different ($p<0.05$) from form the control. Standard growth rate were significantly different ($p<0.05$) with the highest recorded (2.33 ± 0.01) in control and lowest (1.65 ± 0.01) in CSM25 respectively, however significantly different ($p<0.05$) from standard growth rate in the CSM15. Percentage mortality were similar ($p>0.05$) for CSM15, CSM20 and CSM25. However were significantly different ($p<0.05$) from control and CSM10.

Table 4.2: Growth performance of fish (Clarias gariepinus) fingerlings fed castor seed meal for 8 weeks

PARAMETERS	CTRL	CSM10	CSM15	CSM20	CSM25
Initial Weight (g)	2.53 ± 0.01^{a}	2.50 ± 0.01^{a}	2.51 ± 0.01^{a}	2.52 ± 0.02^{a}	2.54 ± 0.00^{a}
Final Weight (g)	8.37 ± 0.18^{e}	6.57 ± 0.04^{d}	5.31 ± 0.03^{c}	3.80 ± 0.03^{b}	3.62 ± 0.02^{a}
Feed Intake (g)	10.49 ± 0.09^{d}	8.40 ± 0.03^{c}	7.21 ± 0.03^{b}	5.53 ± 0.06^{a}	5.33 ± 0.03^{a}
Weight Gained (g)	7.96 ± 0.10^{e}	5.89 ± 0.02^{d}	4.70 ± 0.02^{c}	3.02 ± 0.05^{b}	2.79 ± 0.03^{a}
Daily Weight Gain (g)	0.14 ± 0.00^{d}	0.11 ± 0.00^{c}	0.08 ± 0.00^{b}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}
Protein Intake	3.37 ± 0.07^{d}	2.64 ± 0.02^{c}	2.15 ± 0.01^{b}	1.53 ± 0.01^{a}	1.45 ± 0.01^{a}
Feed Conversion Ratio	1.05 ± 0.01^{a}	1.11 ± 0.00^{b}	1.13 ± 0.00^{b}	1.26 ± 0.02^{c}	1.30 ± 0.01^{c}
Protein Efficiency ratio	0.38 ± 0.01^{c}	0.36 ± 0.00^{b}	0.36 ± 0.00^{b}	0.32 ± 0.01^{a}	0.31 ± 0.00^{a}
Specific Growth Rate	2.33 ± 0.01^{e}	2.11 ± 0.01^{d}	1.96 ± 0.00^{c}	1.70 ± 0.01^{b}	1.65 ± 0.01^{a}
Mortality rate (%)	50 ± 5.77^{a}	70 ± 5.77^{b}	63.33 ± 6.67^{ab}	63.33 ± 3.33^{ab}	53.33 ± 6.67^{ab}

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

4.3: Proximate composition of fish (Clarias gariepinus fingerlings) fed experimental diet after 8 weeks

Proximate composition of fish fed experimental diet is presented on table 4.3, moisture content was significantly different ($p<0.05$) with highest recorded in the control (75.66 ± 0.01) and the lowest recorded in CSM25 (72.97 ± 0.01). Similar trend was also recorded in the crude protein, the crude protein content of the fish fed experimental diet was significantly different ($p<0.05$) with the highest recorded in the control (19.29 ± 0.01) and the lowest recorded in the fish fed the experimental diet having CSM25 (14.15 ± 0.01), Fat content of fish fed experimental diet was significantly different ($p<0.05$) with the highest (1.52 ± 0.20) recorded on CSM25 and the lowest recorded on fish fed the control diet (0.86 ± 0.03).

The ash content of fish fed the experimental diet were significantly different ($p<0.05$) with the highest in CSM15 (5.09 ± 0.01) and the lowest in control (3.60 ± 0.08).

NFE of fish fed experimental diet were however significantly different ($p<0.05$) with the highest recorded in CSM25 and the lowest in control of fish fed experimental diet.

Table 4.3; Proximate composition of fish fed experimental diet after the 8 weeks

Parameters (%)	**CTRL**	**CSM10**	**CSM15**	**CSM20**	**CSM25**
Moisture	75.66 ± 0.01^{e}	74.98 ± 0.01^{d}	74.04 ± 0.00^{c}	73.97 ± 0.01^{b}	72.97 ± 0.01^{a}
Fat	0.86 ± 0.03^{a}	0.93 ± 0.10^{b}	1.05 ± 0.11^{c}	1.31 ± 0.37^{d}	1.52 ± 0.20^{e}
Crude Protein	19.29 ± 0.01^{e}	17.05 ± 0.01^{d}	16.15 ± 0.01^{c}	15.02 ± 0.77^{b}	14.15 ± 0.01^{a}
Ash	3.60 ± 0.08^{a}	4.04 ± 0.12^{b}	5.09 ± 0.01^{e}	4.99 ± 0.68^{c}	5.03 ± 0.03^{d}
NFE	0.62 ± 0.62^{a}	1.00 ± 0.82^{c}	0.88 ± 0.31^{b}	1.71 ± 0.29^{d}	2.68 ± 0.44^{e}

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

4.4: 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 was similar ($p>0.05$) in CSM15 and CSM20, but however significantly different ($p<0.05$) in control, CSM10 and CSM25 with the highest in control while the lowest is in CSM25, haemoglobin observed showed that there are significant difference ($p<0.05$) in all the treatment with the highest (4.17 ± 0.03) in control while the lowest (1.70 ± 0.06) in CSM25. Red blood cell of *Clarias gariepinus* fingerling fed castor seed meal was similar ($p>0.05$) for CSM20 and CSM25, but significantly different ($p<0.05$) from control, CSM10 and CSM15 with the highest recorded in the control (1.03 ± 0.03) and the lowest in CSM15 (0.73 ± 0.03) mean cell volume of the fish shows similarity ($p>0.05$) in treatment CSM15 and CSM20 but were significantly different ($p<0.05$) from control, CSM10 and CSM25. Mean corpuscular haemoglobin of the fish as recorded, there were similarity ($p>0.05$) in CSM10 and CSM15, but however were significantly different ($p<0.05$) from control CSM20 and CSM25. mean corpuscular haemoglobin concentration of the fish shows similar effect ($p>0.05$) for CSM10, CSM15, CSM20 and CSM25 but was however significantly different ($p<0.05$) from the control, Neutrophils shows significant difference ($p<0.05$) with the highest (35.00 ± 0.58) in control and the lowest (24.67 ± 0.67) in CSM25, while in lymphocyte there were significant difference ($p<0.05$) recorded with the highest (74.67 ± 0.67) in the CSM25 and the lowest (65.00 ± 0.58) in control

Table 4.4: Heamatology analysis of the fish (*Clarias gariepinus*) fingerlings fed autoclaved castor seed meal

PARAMETERS	CTRL	CSM10	CSM15	CSM20	CSM25
Packed cell volume (%)	11.00 ± 0.58^{d}	8.67 ± 0.33^{c}	7.33 ± 0.33^{b}	6.33 ± 0.33^{b}	3.67 ± 0.33^{a}
Haemoglobin (g/dL)	4.17 ± 0.03^{e}	2.90 ± 0.06^{d}	2.70 ± 0.06^{c}	2.53 ± 0.03^{b}	1.70 ± 0.06^{a}
Red blood cell (x 10^{12}/ L)	1.03 ± 0.03^{d}	0.83 ± 0.03^{c}	0.73 ± 0.03^{b}	0.46 ± 0.03^{a}	0.40 ± 0.03^{a}
Mean corpuscular volume (fL)	156.67 ± 18.33^{c}	115.60 ± 2.26^{b}	103.33 ± 3.33^{ab}	92.80 ± 3.73^{ab}	80.23 ± 3.73^{a}
Mean corpuscular haemoglobin (Pg)	59.17 ± 4.64^{c}	43.17 ± 0.92^{b}	40.20 ± 0.99^{b}	36.2 ± 0.67^{ab}	32.60 ± 0.38^{a}
Mean corpuscular haemoglobin concentration (g/dL)	49.73 ± 5.27^{b}	40.90 ± 0.85^{a}	37.47 ± 0.37^{a}	35.23 ± 0.23^{a}	33.13 ± 0.44^{a}

White blood cell ($x10^9$/L)	10.77 ± 0.15^a	12.43 ± 0.18^b	13.00 ± 0.06^c	13.40 ± 0.12^d	15.00 ± 0.06^e
Neutrophils (%)	35.00 ± 0.58^e	32.33 ± 0.67^d	29.67 ± 0.67^c	27.33 ± 0.33^b	24.67 ± 0.67^a
Lymphocytes (%)	65.00 ± 0.58^a	67.67 ± 0.67^b	70.33 ± 0.67^c	72.67 ± 0.33^d	74.67 ± 0.67^e

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

CHAPTER FIVE

5.1: Discussion

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, 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 general decrease in body weight gain, daily weight gain, standard growth rate, and low feed intake of fish (*Clarias gariepinus*) fingerlings fed castor seed meal based diet may be due to lower absorption of dietary nitrogen, fiber and presence of residual ricin in the diet as, the higher the level of inclusion the lower her weight gain, this agreed with report of Balogun *et al.* et al. (2005) and Durowaiye (2015), reduced weight gained also in contrast with the report of Ani and Okorie (2009) who reported that when treated castor seed was added to birds feed,. Mortality rate increased with reduce feed intake, this could be attributed to gradual reduction in the activity of digestive enzyme inhibitiors (trypsin and chemotrypsin) which affect the utilization of amino acids in any diet (Jobling, 1981), this could also be attributed to low palatability as reported by Cal *et al. (*2005) and also the uncontrolled changed in water quality.

The numerical increase in the value of feed conversion ratio and protein efficiency 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 and (1994) low

palatability could result in low consumption and utilization (NRC, 1981, Pompa, 1982) with the report of Balogun *et al* (2005)

Reduction in the haematological value with the inadequate nutrient utilization in the fish fed autoclaved castor seed meal as the level of inclusion increase in the red blood cell count, packed cell volume, haemoglobin, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, white blood cell, lymphocyte and increase level of neutrophils, this trend may have been due to increase fiber level or due to the possible residue of ricin or allergen on the blood variables, which is in contrast with what was reported by Babalola et al (2006) when boiled castor seed meal was fed to pullet chick

5.2 CONCLUSION

The result of this study on effect of dietary inclusion of castor seed meal on the growth performance, body composition and haematology of *Claris gariepinus* fingerlings revealed that 10% inclusion of castor seed meal can be included in the diet of castor seed meal

5.3. RECOMMENDATIONS

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

More castor seed should be planted to improve the production and availability in the market

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APPENDIXES

Appendix 1: Growth performance of *Clarias gariepinus* fingerlings fed Autoclaved castor seed meal

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MIW	CTRL	3	2.5300	.01732	.01000	2.4870	2.5730	2.51	2.54
	CSM10	3	2.5033	.02082	.01202	2.4516	2.5550	2.48	2.52
	CSM15	3	2.5133	.02517	.01453	2.4508	2.5758	2.49	2.54
	CSM20	3	2.5167	.03215	.01856	2.4368	2.5965	2.48	2.54
	CSM25	3	2.5367	.00577	.00333	2.5223	2.5510	2.53	2.54
	Total	15	2.5200	.02236	.00577	2.5076	2.5324	2.48	2.54
AFI	CTRL	3	8.3667	.30860	.17817	7.6001	9.1333	8.15	8.72
	CSM10	3	6.5667	.07234	.04177	6.3870	6.7464	6.52	6.65
	CSM15	3	5.3133	.05508	.03180	5.1765	5.4501	5.25	5.35
	CSM20	3	3.8000	.04583	.02646	3.6862	3.9138	3.76	3.85
	CSM25	3	3.6200	.03000	.01732	3.5455	3.6945	3.59	3.65
	Total	15	5.5333	1.84470	.47630	4.5118	6.5549	3.59	8.72
MFW	CTRL	3	10.4867	.14844	.08570	10.1179	10.8554	10.36	10.65
	CSM10	3	8.3967	.05508	.03180	8.2599	8.5335	8.34	8.45
	CSM15	3	7.2100	.05292	.03055	7.0786	7.3414	7.15	7.25
	CSM20	3	5.5333	.10408	.06009	5.2748	5.7919	5.45	5.65
	CSM25	3	5.3267	.05859	.03383	5.1811	5.4722	5.26	5.37
	Total	15	7.3907	1.98392	.51225	6.2920	8.4893	5.26	10.65
BWG	CTRL	3	7.9567	.16503	.09528	7.5467	8.3666	7.82	8.14
	CSM10	3	5.8933	.03512	.02028	5.8061	5.9806	5.86	5.93
	CSM15	3	4.6967	.04041	.02333	4.5963	4.7971	4.66	4.74

	CSM20	3	3.0167	.08083	.04667	2.8159	3.2175	2.97	3.11
	CSM25	3	2.7900	.05292	.03055	2.6586	2.9214	2.73	2.83
	Total	15	4.8707	1.98525	.51259	3.7713	5.9701	2.73	8.14
MR	CTRL	3	50.0000	10.00000	5.77350	25.1586	74.8414	40.00	60.00
	CSM10	3	70.0000	10.00000	5.77350	45.1586	94.8414	60.00	80.00
	CSM15	3	63.3333	11.54701	6.66667	34.6490	92.0177	50.00	70.00
	CSM20	3	63.3333	5.77350	3.33333	48.9912	77.6755	60.00	70.00
	CSM25	3	53.3333	11.54701	6.66667	24.6490	82.0177	40.00	60.00
	Total	15	60.0000	11.33893	2.92770	53.7207	66.2793	40.00	80.00
ADWG	CTRL	3	.1433	.00577	.00333	.1290	.1577	.14	.15
	CSM10	3	.1067	.00577	.00333	.0923	.1210	.10	.11
	CSM15	3	.0800	.00000	.00000	.0800	.0800	.08	.08
	CSM20	3	.0533	.00577	.00333	.0390	.0677	.05	.06
	CSM25	3	.0500	.00000	.00000	.0500	.0500	.05	.05
	Total	15	.0867	.03638	.00939	.0665	.1068	.05	.15
PI	CTRL	3	3.3567	.12503	.07219	3.0461	3.6673	3.27	3.50
	CSM10	3	2.6400	.02646	.01528	2.5743	2.7057	2.62	2.67
	CSM15	3	2.1533	.02082	.01202	2.1016	2.2050	2.13	2.17
	CSM20	3	1.5333	.01528	.00882	1.4954	1.5713	1.52	1.55
	CSM25	3	1.4500	.01000	.00577	1.4252	1.4748	1.44	1.46
	Total	15	2.2267	.73898	.19080	1.8174	2.6359	1.44	3.50
FCR	CTRL	3	1.0500	.02000	.01155	1.0003	1.0997	1.03	1.07
	CSM10	3	1.1133	.00577	.00333	1.0990	1.1277	1.11	1.12
	CSM15	3	1.1333	.00577	.00333	1.1190	1.1477	1.13	1.14
	CSM20	3	1.2633	.04041	.02333	1.1629	1.3637	1.22	1.30
	CSM25	3	1.3000	.02000	.01155	1.2503	1.3497	1.28	1.32
	Total	15	1.1720	.09951	.02569	1.1169	1.2271	1.03	1.32

PER	CTRL	3	.3800	.01000	.00577	.3552	.4048	.37	.39
	CSM10	3	.3600	.00000	.00000	.3600	.3600	.36	.36
	CSM15	3	.3600	.00000	.00000	.3600	.3600	.36	.36
	CSM20	3	.3200	.01000	.00577	.2952	.3448	.31	.33
	CSM25	3	.3133	.00577	.00333	.2990	.3277	.31	.32
	Total	15	.3467	.02717	.00701	.3316	.3617	.31	.39
SGR	CTRL	3	2.3333	.01528	.00882	2.2954	2.3713	2.32	2.35
	CSM10	3	2.1100	.01000	.00577	2.0852	2.1348	2.10	2.12
	CSM15	3	1.9567	.00577	.00333	1.9423	1.9710	1.95	1.96
	CSM20	3	1.6967	.02082	.01202	1.6450	1.7484	1.68	1.72
	CSM25	3	1.6533	.01155	.00667	1.6246	1.6820	1.64	1.66
	Total	15	1.9500	.26406	.06818	1.8038	2.0962	1.64	2.35

MIW

Duncan

		Subset for alpha = 0.05
TREATMENT	N	1
CSM10	3	2.5033
CSM15	3	2.5133
CSM20	3	2.5167
CTRL	3	2.5300
CSM25	3	2.5367
Sig.		.120

Means for groups in homogeneous subsets are displayed.

AFI

Duncan

TREATMENT	N	Subset for alpha = 0.05			
		1	2	3	4
CSM25	3	3.6200			
CSM20	3	3.8000			
CSM15	3		5.3133		
CSM10	3			6.5667	
CTRL	3				8.3667
Sig.		.162	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

MFW

Duncan

TREATMENT	N	Subset for alpha = 0.05				
		1	2	3	4	5
CSM25	3	5.3267				
CSM20	3		5.5333			
CSM15	3			7.2100		
CSM10	3				8.3967	
CTRL	3					10.4867
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

MR

Duncan

TREATMENT	N	Subset for alpha = 0.05	
		1	2
CTRL	3	50.0000	
CSM25	3	53.3333	53.3333
CSM15	3	63.3333	63.3333
CSM20	3	63.3333	63.3333
CSM10	3		70.0000
Sig.		.159	.086

Means for groups in homogeneous subsets are displayed.

ADWG

Duncan

TREATMENT	N	Subset for alpha = 0.05			
		1	2	3	4
CSM25	3	.0500			
CSM20	3	.0533			
CSM15	3		.0800		
CSM10	3			.1067	
CTRL	3				.1433
Sig.		.383	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

PI

Duncan

TREATMENT	N	Subset for alpha = 0.05			
		1	2	3	4
CSM25	3	1.4500			
CSM20	3	1.5333			
CSM15	3		2.1533		
CSM10	3			2.6400	
CTRL	3				3.3567
Sig.		.112	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

FCR

Duncan

TREATMENT	N	Subset for alpha = 0.05		
		1	2	3
CTRL	3	1.0500		
CSM10	3		1.1133	
CSM15	3		1.1333	
CSM20	3			1.2633
CSM25	3			1.3000
Sig.		1.000	.299	.072

Means for groups in homogeneous subsets are displayed.

PER

Duncan

TREATMENT	N	Subset for alpha = 0.05		
		1	2	3
CSM25	3	.3133		
CSM20	3	.3200		
CSM10	3		.3600	
CSM15	3		.3600	
CTRL	3			.3800
Sig.		.260	1.000	1.000

Means for groups in homogeneous subsets are displayed.

SGR

Duncan

TREATMENT	N	Subset for alpha = 0.05				
		1	2	3	4	5
CSM25	3	1.6533				
CSM20	3		1.6967			
CSM15	3			1.9567		
CSM10	3				2.1100	
CTRL	3					2.3333
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

APPENDIX 2

Body composition of *Clarias gariepinus* fingerlings fed Autoclaved castor seed meal

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Moisture	CTRL	3	75.6600	.01000	.00577	75.6352	75.6848	75.65	75.67
	CSM10	3	74.9800	.01000	.00577	74.9552	75.0048	74.97	74.99
	CSM15	3	74.0400	.00000	.00000	74.0400	74.0400	74.04	74.04
	CSM20	3	73.9700	.01000	.00577	73.9452	73.9948	73.96	73.98
	CSM25	3	72.9700	.01000	.00577	72.9452	72.9948	72.96	72.98
	Total	15	74.3240	.95485	.24654	73.7952	74.8528	72.96	75.67
CP	CTRL	3	19.2900	.01000	.00577	19.2652	19.3148	19.28	19.30
	CSM10	3	17.0500	.01000	.00577	17.0252	17.0748	17.04	17.06
	CSM15	3	16.1500	.01000	.00577	16.1252	16.1748	16.14	16.16
	CSM20	3	15.0200	.01000	.00577	14.9952	15.0448	15.01	15.03
	CSM25	3	14.1500	.01000	.00577	14.1252	14.1748	14.14	14.16
	Total	15	16.3320	1.83894	.47481	15.3136	17.3504	14.14	19.30
Fat	CTRL	3	.8600	.01000	.00577	.8352	.8848	.85	.87
	CSM10	3	.9300	.01000	.00577	.9052	.9548	.92	.94
	CSM15	3	1.0500	.01000	.00577	1.0252	1.0748	1.04	1.06
	CSM20	3	1.3100	.01000	.00577	1.2852	1.3348	1.30	1.32
	CSM25	3	1.5200	.01000	.00577	1.4952	1.5448	1.51	1.53
	Total	15	1.1340	.25525	.06591	.9926	1.2754	.85	1.53
Ash	CTRL	3	3.6000	.01000	.00577	3.5752	3.6248	3.59	3.61
	CSM10	3	4.0400	.01000	.00577	4.0152	4.0648	4.03	4.05
	CSM15	3	5.0900	.01000	.00577	5.0652	5.1148	5.08	5.10
	CSM20	3	4.9900	.01000	.00577	4.9652	5.0148	4.98	5.00
	CSM25	3	5.0367	.01528	.00882	4.9987	5.0746	5.02	5.05
	Total	15	4.5513	.63556	.16410	4.1994	4.9033	3.59	5.10

NFE	CTRL	3	.6200	.01000	.00577	.5952	.6448	.61	.63
	CSM10	3	1.0000	.01000	.00577	.9752	1.0248	.99	1.01
	CSM15	3	.8800	.01000	.00577	.8552	.9048	.87	.89
	CSM20	3	1.7100	.01000	.00577	1.6852	1.7348	1.70	1.72
	CSM25	3	2.6800	.01000	.00577	2.6552	2.7048	2.67	2.69
	Total	15	1.3780	.77060	.19897	.9513	1.8047	.61	2.69

Moisture

Duncan

		Subset for alpha = 0.05				
treatment	N	1	2	3	4	5
CSM25	3	72.9700				
CSM20	3		73.9700			
CSM15	3			74.0400		
CSM10	3				74.9800	
CTRL	3					75.6600
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

CP

Duncan

treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
CSM25	3	14.1500				
CSM20	3		15.0200			
CSM15	3			16.1500		
CSM10	3				17.0500	
CTRL	3					19.2900
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Fat

Duncan

treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
CTRL	3	.8600				
CSM10	3		.9300			
CSM15	3			1.0500		
CSM20	3				1.3100	
CSM25	3					1.5200
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Ash

Duncan

treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
CTRL	3	3.6000				
CSM10	3		4.0400			
CSM20	3			4.9900		
CSM25	3				5.0367	
CSM15	3					5.0900
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

NFE

Duncan

treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
CTRL	3	.6200				
CSM15	3		.8800			
CSM10	3			1.0000		
CSM20	3				1.7100	
CSM25	3					2.6800
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Appendix 3

Haematology of *Clarias gariepinus* fingerlings fed Autoclaved castor seed meal

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
PCV	CTRL	3	11.0000	1.00000	.57735	8.5159	13.4841	10.00	12.00
	CSM10	3	8.6667	.57735	.33333	7.2324	10.1009	8.00	9.00
	CSM15	3	7.3333	.57735	.33333	5.8991	8.7676	7.00	8.00
	CSM20	3	6.3333	.57735	.33333	4.8991	7.7676	6.00	7.00
	CSM25	3	3.6667	.57735	.33333	2.2324	5.1009	3.00	4.00
	Total	15	7.4000	2.58567	.66762	5.9681	8.8319	3.00	12.00
Hb	CTRL	3	4.1667	.05774	.03333	4.0232	4.3101	4.10	4.20
	CSM10	3	2.9000	.10000	.05774	2.6516	3.1484	2.80	3.00
	CSM15	3	2.7000	.10000	.05774	2.4516	2.9484	2.60	2.80
	CSM20	3	2.5333	.05774	.03333	2.3899	2.6768	2.50	2.60
	CSM25	3	1.7000	.10000	.05774	1.4516	1.9484	1.60	1.80
	Total	15	2.8000	.82722	.21359	2.3419	3.2581	1.60	4.20
RBC	CTRL	3	1.0333	.05774	.03333	.8899	1.1768	1.00	1.10
	CSM10	3	.8333	.05774	.03333	.6899	.9768	.80	.90
	CSM15	3	.7333	.05774	.03333	.5899	.8768	.70	.80
	CSM20	3	.4667	.05774	.03333	.3232	.6101	.40	.50
	CSM25	3	.4000	.00000	.00000	.4000	.4000	.40	.40
	Total	15	.6933	.24631	.06360	.5569	.8297	.40	1.10
MCV	CTRL	3	1.5667E2	31.75426	18.33333	77.7847	235.5486	120.00	175.00
	CSM10	3	1.1560E2	3.91535	2.26053	105.8737	125.3263	112.50	120.00
	CSM15	3	1.0333E2	5.77350	3.33333	88.9912	117.6755	100.00	110.00
	CSM20	3	92.8000	6.46297	3.73140	76.7451	108.8549	87.50	100.00
	CSM25	3	80.2333	5.35381	3.09103	66.9337	93.5329	75.00	85.70
	Total	15	1.0973E2	29.95255	7.73371	93.1395	126.3138	75.00	175.00

MCH	CTRL	3	59.1667	8.03638	4.63980	39.2032	79.1301	50.00	65.00
	CSM10	3	43.1667	1.60728	.92796	39.1740	47.1594	42.00	45.00
	CSM15	3	40.2000	1.70880	.98658	35.9551	44.4449	38.60	42.00
	CSM20	3	36.2000	1.15326	.66583	33.3352	39.0648	35.00	37.30
	CSM25	3	32.6000	.65574	.37859	30.9710	34.2290	32.00	33.30
	Total	15	42.2667	10.02323	2.58799	36.7160	47.8173	32.00	65.00
MCHC	CTRL	3	49.7333	9.13583	5.27457	27.0387	72.4280	42.50	60.00
	CSM10	3	40.9000	.85440	.49329	38.7776	43.0224	40.00	41.70
	CSM15	3	37.4667	.63509	.36667	35.8890	39.0443	37.10	38.20
	CSM20	3	35.2333	.40415	.23333	34.2294	36.2373	35.00	35.70
	CSM25	3	33.1333	.76376	.44096	31.2360	35.0306	32.30	33.80
	Total	15	39.2933	6.96270	1.79776	35.4375	43.1491	32.30	60.00
WBC	CTRL	3	10.7667	.25166	.14530	10.1415	11.3918	10.50	11.00
	CSM10	3	12.4333	.30551	.17638	11.6744	13.1922	12.10	12.70
	CSM15	3	13.0000	.10000	.05774	12.7516	13.2484	12.90	13.10
	CSM20	3	13.4000	.20000	.11547	12.9032	13.8968	13.20	13.60
	CSM25	3	15.0000	.10000	.05774	14.7516	15.2484	14.90	15.10
	Total	15	12.9200	1.43288	.36997	12.1265	13.7135	10.50	15.10
NEUTROPHIL	CTRL	3	35.0000	1.00000	.57735	32.5159	37.4841	34.00	36.00
	CSM10	3	32.3333	1.15470	.66667	29.4649	35.2018	31.00	33.00
	CSM15	3	29.6667	1.15470	.66667	26.7982	32.5351	29.00	31.00
	CSM20	3	27.3333	.57735	.33333	25.8991	28.7676	27.00	28.00
	CSM25	3	24.6667	1.15470	.66667	21.7982	27.5351	24.00	26.00
	Total	15	29.8000	3.85820	.99618	27.6634	31.9366	24.00	36.00
LYMPHOCYTE	CTRL	3	65.0000	1.00000	.57735	62.5159	67.4841	64.00	66.00
	CSM10	3	67.6667	1.15470	.66667	64.7982	70.5351	67.00	69.00
	CSM15	3	70.3333	1.15470	.66667	67.4649	73.2018	69.00	71.00
	CSM20	3	72.6667	.57735	.33333	71.2324	74.1009	72.00	73.00
	CSM25	3	74.6667	1.15470	.66667	71.7982	77.5351	74.00	76.00
	Total	15	70.0667	3.67359	.94852	68.0323	72.1010	64.00	76.00

PCV

Duncan

TREATMENT	N	Subset for alpha = 0.05			
		1	2	3	4
CSM25	3	3.6667			
CSM20	3		6.3333		
CSM15	3		7.3333		
CSM10	3			8.6667	
CTRL	3				11.0000
Sig.		1.000	.103	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Hb

Duncan

TREATMENT	N	Subset for alpha = 0.05				
		1	2	3	4	5
CSM25	3	1.7000				
CSM20	3		2.5333			
CSM15	3			2.7000		
CSM10	3				2.9000	
CTRL	3					4.1667
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

RBC

Duncan

TREATMENT	N	Subset for alpha = 0.05			
		1	2	3	4
CSM25	3	.4000			
CSM20	3	.4667			
CSM15	3		.7333		
CSM10	3			.8333	
CTRL	3				1.0333
Sig.		.145	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

MCV

Duncan

TREATMENT	N	Subset for alpha = 0.05		
		1	2	3
CSM25	3	80.2333		
CSM20	3	92.8000	92.8000	
CSM15	3	1.0333E2	1.0333E2	
CSM10	3		1.1560E2	
CTRL	3			1.5667E2
Sig.		.102	.106	1.000

Means for groups in homogeneous subsets are displayed.

MCH

Duncan

TREATMENT	N	Subset for alpha = 0.05		
		1	2	3
CSM25	3	32.6000		
CSM20	3	36.2000	36.2000	
CSM15	3		40.2000	
CSM10	3		43.1667	
CTRL	3			59.1667
Sig.		.272	.057	1.000

Means for groups in homogeneous subsets are displayed.

MCHC

Duncan

TREATMENT	N	Subset for alpha = 0.05	
		1	2
CSM25	3	33.1333	
CSM20	3	35.2333	
CSM15	3	37.4667	
CSM10	3	40.9000	
CTRL	3		49.7333
Sig.		.057	1.000

Means for groups in homogeneous subsets are displayed.

WBC

Duncan

TREATMENT	N	Subset for alpha = 0.05				
		1	2	3	4	5
CTRL	3	10.7667				
CSM10	3		12.4333			
CSM15	3			13.0000		
CSM20	3				13.4000	
CSM25	3					15.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

NEUTROPHIL

Duncan

TREATMENT	N	Subset for alpha = 0.05				
		1	2	3	4	5
CSM25	3	24.6667				
CSM20	3		27.3333			
CSM15	3			29.6667		
CSM10	3				32.3333	
CTRL	3					35.0000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

LYMPHOCYTE

Duncan

TREATMENT	N	Subset for alpha = 0.05				
		1	2	3	4	5
CTRL	3	65.0000				
CSM10	3		67.6667			
CSM15	3			70.3333		
CSM20	3				72.6667	
CSM25	3					74.6667
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.