SUBTITUTION OF *Glycine max* (SOYBEAN) WITH *Mucuna utilis* (VELVET BEAN) IN THE DIET OF *Clarias gariepinus* (CATFISH)

BY

OLANIKANJU, OLAJUMOKE MARY

FAQ/13/1469

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF FISHERIES AND AQUACULTURE IN FACULTY OF AGRICULTURE, FEDERAL UNIVERSITY OYE-EKITI, NIGERIA.

IN PARTIAL FUILFILMENT FOR THE AWARD OF BACHELOR OF FISHERIES AND AQUACULTURE.

DECLARATION

I declare that the project titled "Subtitution of *Glycine max* (Soybean) with*Mucunautilis* (Velvet bean) in the diet of *Clarias gariepinus* (Catfish)" has been carried out by me in the Department of Fisheries and Aquaculture under the supervision of Dr. J. B Olasunkanmi.

Olanikanju Olajumoke Mary

22-03-2019

(Signature and Date)

CERTIFICATION

This is to certify that **OLANIKANJU, OLAJUMOKE MARY** with matriculation number FAQ/13/1469 carried out this research work titled "Subtitution of *Glycine max* (Soybean) with *Mucuna utilis* (Velvet bean) in the diet of *Clarias gariepinus* (Catfish)" and has satisfied the regulations governing the award of the degree of Bachelor of Fisheries and Aquaculture of the Federal University, Oye-Ekiti, Ekiti State during the 2017/2018 session under the supervision of Dr. J.B. Olasunkanmi

PROJECT SUPERVISOR DATE

HEAD OF DEPARTMENT DATE

DEDICATION

I dedicate this project to God Almighty, the beginning and the end, my creator, my strong pillar, my source of wisdom, knowledge and understanding. He has been my strength and provider throughout this project.

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My utmost appreciation goes to Almighty God for the gift of life, protection, provision and guidance for making it possible for me to complete this project successfully, without his order nothing is possible.

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ABSTRACT

An eight-week feeding trial was conducted to determine the potential of substituting ***Mucuna utilis***(Velvet bean) for Soybean meal in the diets of *Clarias gariepinus* (African catfish).***Mucuna*** *utilis* was substituted for Soybean Meal at 0% (T1); 25% (T2); 50% (T3); 75% (T4); and 100% (T5). The diets were isonitrogenous (40%) and fed twice daily to triplicate groups of *Clarias gariepinus* (African catfish) fingerling with initial weight of 7.14 in T1, 6.82 in T2, 7.01 in T3, 6.73 in T4 and 6.76 in T5 at 5% body weight.

The growth and nutritional responses were evaluated by analyzing the data using One-way ANOVA. The result showed that final weight, weight gain, daily weight gain, and specific growth rate, were highest ($p>0.05$) in T1 and lowest in T5, a similar pattern of growth was observed in length measurements. The lowest Feed Conversion Ratio FCR was recorded in T2 and the highest in T5. Similarities ($p>0.05$), was recorded in the survival of fish fed the experimental diet.

Red Blood Cell (RBC), Packed Cell Volume (PCV) and Haemoglobin (Hb) values were significantly higher ($p>0.05$) in control and the lowest in T5. White Blood Cell (WBC) was significantly higher in T5 and the lowest in control. Highest Neutrophil was observed in T4 and the lowest in T3. Lymphocyte was significantly higher in T3 and the lowest value was recorded in T4. Mean corpuscular haemoglobin concentration (MCHC) of fish fed the experimental diet shows no significant difference ($p>0.0$) in all the fish fed the experimental diet. The Highest Mean corpuscular haemoglobin (MCH) was recorded in control and lowest in T4.The Highest mean corpuscular volume observed in T5 and the lowest in T4.

In conclusion Soybean meal can be substituted with *Mucuna utilis* (Velvet bean) up to 25% in the diet of *Clarias gariepinus* fingerlings without adverse effects on the blood, growth and nutrient utilization.

TABLE OF CONTENTS

Contents **Page**

LIST OF TABLES

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

Feed production and quality is one of the major factors influencing the success of fish **production** in aquaculture (F.A.O, 2011). The high cost and scarcity of feed stuffs due to highly competitive use of the conventional feed stuff particularly the protein sources such as soybean meal, groundnut cake and fish meal etc. are factors militating against commercial fish and animal production in developing countries like Nigeria (Gabriel *et al*, 2007). These factors have led to increase in the price of feed, its reduction in supply and have inflicted high cost of production on the farmers and invariably affected the price of the fish, which use to be the cheap source of protein. Diets usually represent the most important aspect of fish farm operation in aquaculture, the selection of feed ingredients in diet acts as a major role in ensuring the ultimate nutritional and economic success of farmed fish (Woke *et al.* 2012).

In Nigeria, Aquaculture covers an estimated 60,000 hectares and produces some 25,000 to 30,000 metric tons (mt) of fish per year (Ebeniro and Orji, 2012). The exponential growth of the aquaculture sector during the past two decades is a result of the progressive intensification of production systems and use of quality feeds, which meet the nutritional requirements of cultured fish (FAO, 2006). Stimulated by higher global demand for fish, world fisheries and aquaculture production reached 157million tons in 2012 and is projected to reach about 172 million tonnes in 2021 with most of the growth coming from aquaculture (FAO, 2013). The target to increase aquaculture production must be supported by a corresponding increase in the production of diets for the cultured aquatic animals (Rahman et al., 2013).

Catfish, aside tilapia is the major fish cultured in Nigeria because it is found all over the country, acceptable, resistant to harsh environmental conditions, commands good price, tasty and easily transported for days during marketing with estimates which puts the current production **output of** *Clarias gariepinus* in the Nigeria at over 253,898 metric tonnes per year. Most **recent** investment in fish production in the country has been in catfish farming due to the fact that **live** catfish alone attracts premium price in Nigeria, with a high Return On-Investment (ROI) **ranging** between 30-40% in some successful enterprise (Adelakun *et al*, 2015).

Formulated feeds are complete feeds supplying the needed nutrients for optimal growth and health. The main issue in formulating feed is to meet the protein and essential amino acids (EAAs) requirements of the species. Protein deficiency is the commonest form of malnutrition in the developing countries, particularly in regions where diets are based mainly on roots, stems and tubers crops (Tuleun 2009) because protein is the most expensive and important components of fish feed. One major source protein is soybean. Soybean is however expensive, dwindling in supply and faced competition from many users (George *et al* 2010). Although non-conventional plant proteins have their limitations on usage as fish feeds, they have potential as dietary protein and energy sources (Balogun *et al.*, 2008; Naylor *et al.*, 2009).

The intensive use of soybean meal in poultry and fish feeds has led to increasing price of soybean meal with its unavailability (Soltan, 2005) and the high cost of soybean has necessitated increased research into alternative protein sources for fish. One of such alternative feed ingredients is *Mucuna utilis* (Tuleun *et al.* 2009; Ezeagu *et al.*, 2002). *Mucuna utilis* is popularly known as velvet seed and is a widely available leguminous crop that thrives well in tropical, subtropical and temperate regions (Ukachukwu and Obioha 1997), *Mucuna utilis* is an underutilized tropical legume and has a nutritional quality comparable to soya bean and other conventional legumes as it contains similar proportion of protein, lipids, minerals and other nutrients (Woke *et al.* 2012), the proximate composition, mineral assay and amino acid profile of velvet bean (*Mucuna utilis*) makes it a high potential as a other known plant protein feedstuff (Tuleun *et al.* 2009). The raw beans, though high in crude protein (Olasunkanmi ,2010), energy (Del Carmen *et al.,* 1999) and having a good amino acid profile (Siddhuraju *et al.*, 1996), contains anti-nutritional factors like 3, 4 dihydroxyphenylalanine (L-DOPA), trypsin inhibitors, tannin and alkaloids. However, appropriate processing of the beans using methods such as soaking in water, dehulling, and extrusion and cooking, have proved successful in ameliorating the adverse effects on poultry (Del Carmen *et al.*, 2002)

1.1. STATEMENT OF PROBLEM

Soybean has been a traditional protein source in formulated feeds, but competition between human beings, livestock and fish for this commodity has led to its rising cost and its accompanying scarcity is making it increasingly uneconomical to feed it to livestock as well as fish. To solve this problem, there is a need for increased research into alternative protein sources for livestock and fish.

1.2. JUSTIFICATION

In aquaculture, diets generally represent the largest single cost item of most fish farm operation. It follows that the selection of feed ingredients use within diets will play a major role in dictating the ultimate nutritional and economic success of farmed fish (Ebeniro and Orji, 2012). Soybean is widely used protein of plant origin, but the present high cost of soybean and its scarcity due to its demand in other animals and human feed deterrent to fish feed production and intensive aquaculture.

There is greater emphasis on the use of alternative protein feedstuff in fish nutrition due to the high costs of the conventional protein sources such as fish meal and soya bean which are used in other animal feeds (Fagbenro *et al.*, 2003; Tacon *et al.*, 2009). *Mucuna utilis,* an underutilized tropical legume, has a nutritional quality comparable to Soybean and other conventional legumes as it contain similar proportion of protein, Lipids, minerals and other nutrient, it has a crude protein range from (24-35.4%) (Ukachukwu, 2006), crude lipid 6-7% , total dietary fibre 8-10% , ash 3%, and carbohydrate of 50-60% and it is sparsely used for animal or human consumption due to its large amount of anti-nutritional factors making it readily available for fish feed (Woke *et al.* 2012; Ifesan *et al.* 2012). High cost of soybean and its scarcity due to its demand necessitated the need to obtain an alternative to soybean in order to sustain aquaculture in developing countries.

1.3. OBJECTIVES

The general objective is to explore the potentials of *Mucuna utilis* (velvet bean meal) in the nutrition of *Clarias gariepinus* (Catfish). Specific objectives are:

i. To investigate the growth performance of the fish fed on *Mucuna utilis* (velvet bean meal).

ii. To investigate the effect of *Mucuna utilis* (velvet bean meal) on haematological parameters of the fish.

CHAPTER TWO

LITERATURE REVIEW

2.1. History and Origin of *Mucuna utilis* (Velvet Bean)

Genus *Mucuna* belongs to the family Leguminosae, it consists of 100 species of climbing vines and shrubs and the genus name was derived from the word "Mucuna" (Umberto, 2000), *Mucuna* is a tropical legume which is widely cultivated as cover crop and of which the seeds is relatively high in protein content, it is hardly consumed by man which makes it have little or no competitive demand of users, *Mucuna utilis*, commonly known as velvet bean is a highly productive (200 to 600 kg seed/ha) legume, native to South Asia and Malaysia, but is presently widely grown throughout the tropics (Szabo, 2003) while according to Ceballos *et al.* (2012) argued it out that velvet bean is native to China and India, but widely distributed in many tropical regions nowadays, which makes the plant a widely available seed unlike soybean seed which is not generally grown in all the tropics, supporting what Szabo (2003) said that it could found throughout the tropics (Fung *et al.* 2012; Vadivel and Janardhanan, 2000) reported that *Mucuna* utilis (velvet bean) is an under-utilized legume species which is commonly grown in continent such as Africa, Asia and some parts of America, also by Balogun and Olatidoye (2012) in their work that *Mucuna utilis* is widely spread in tropical and subtropical regions of world. However Taylor's (2003) said the plant is known to be indigenous to tropical regions, especially Africa, India, and the West Indies. When there is a contact with the seed pod hairs which is mostly used as medicinal purposes in India, it causes an itching sensation (Sathiyanarayanan and Arulmozhi 2007). Mucuna have been identified over 4500 years ago, as it was first used for medicinal purpose then (Kavitha and Thangamani 2014). *Mucuna utilis* is commonly known as velvet beans, but in other region of the earth it is called names as cowitch, cowhage, Alkushi, Nescafé, Bengal bean, Mauritius bean, itchy bean, *chiporro* and/or buffalo bean, depending on country (Kavitha and Thangamani 2014; Tropical Plant Database 2012) containing high amount of carbohydrate and rich source of macro- and micro – elements (Ebeniro and Orji 2012).

2.2. Description of Velvet bean plant

Mucuna utilis is a twinning annual crop but sometimes biannual with the stem reaching15m - 18m in length (Kavitha and Thangamani 2014), Mucuna is a perennial plant species with vigorous unidentified growth pattern, which can produce vines of 3 m–18 m in length and have a life cycle length from about 120 days–330 days (Pugalenthi *et al.* 2005). The plant is almost completely covered with fuzzy hairs when young, but losses its hairs as it grows older (Sahaji, 2011) which is due to wind action. Pugalenthi *et al.* (2005) further reported that the leaves are trifoliate with lateral leaflets which are 7-15cm long conspicuously asymmetrical arranged and 5–12 cm wide, and the flowers are white or purple, arranged in hanging racemes of 32 cm long, while the pods which are S-shaped, 5cm–15cm long with 4-6 seeds (having black coat and oblong) in each, 5 cm–6 cm thick and densely covered with persistent pale-brown or grey trichomes that cause irritating blisters if they come in contact with skin. The pods are having different colours (black, maroon, creamy, white, grey, beige, brown, and mottled) surrounded by a prominent, cream-coloured aril which causes the irritation on the skin (Musonda 2012).

2.3. Composition and Nutritional composition *Mucuna utilis*

Legumes are viable food crops which provide proteins, minerals, energy which are required by man and livestock, however, *Mucuna utilis* is a legume with a low human preference for food. but has a high potential as an energy and protein source in livestock feed.(Siddhuraju et al., 1996; Iyayi and Taiwo, 2003).Like other beans, *Mucuna* contains a high level of protein, vitamins and minerals making it an attractive and important source of plant protein for feeding animál (Iyayi *et al.* 2006), this was backed up in the report of Probat (2011) asserting that, as a food source, Mucuna contains high levels of essential daily protein, carbohydrates, lipids, fibres and minerals required by livestock. however, Woke *et al.* (2012) reported that though *Mucuna utillis* has a high nutrient present in it, it was still reported to be an underutilized tropical legume, with its nutritional quality comparable to that of Soybean and other conventional legumes as it contain similar proportion of protein, Lipids, minerals and other nutrients, likewise in the report of Ebeniro and Orji (2012). In Mugendi *et al.* (2014) research work, it also stated that the proximate and mineral content of the velvet bean compares favorably with edible legumes, claiming that the *Mucuna utilis* is rich in protein and micronutrients. While Kalidass and Mahapatra (2014) referenced that the nutritional value of this wild legume can supply significant amount of energy, vitamins and minerals in addition to protein (24-31%). While from other research works the seed was examined to have on dry matter proteins (25.4% to 35%) in the grain, foliage 11–23% , starch (31.2% to 39.5%), desirable amino acids, fatty acids and mineral composition with good nutritional properties (Siddhuraju and Becker, 2003; Pugalenthi et al., 2005; Vadivel and Janardhanan, 2005), also in the work of Mugendi and Njagi (2010) dehulled mucuna seeds contained higher crude protein, crude fat and ash content, but lower crude fibre content than whole bean, implying that the seed coat comprised mainly of fibre, while protein fat and ash are concentrated in the cotyledon. According to research (Iyayi *et al.* 2005; Vijayakumari *et al,* 2002 and Adebowaale *et al.* 2005) the seed is found to contain in dry matter per kg, about 220-350g crude protein, 4600-5400 kcal metabolizable energy (ME) and about 480 g carbohydrate, while The crude protein content of the dried leave ranges from 21 – 32 % on dry matter basis (Ujowundu et al., 2010).

2.3.1. Protein, Amino Acid and Mineral composition availability of velvet seed meal

Based on research work on the utilization of *Mucuna utilis* (velvet bean) as an inclusion in the diet of ruminant and non-ruminant animals, it has be observed to yield a positive outcome on the growth performance, due to the composition of protein and amino acid present in the feed stuff being able to meet the required dietary protein requirement of the livestock.

According to Tuleum *et al.* (2008) the seed of the velvet bean has nutritional potential enough to be classified as a source of protein (23%–35%), also in report of Ifesan *et al.* (2017) who reported velvet beans crude protein content to be around 28.38%-32.55% after been processed, which was similar to the report of Pugalenthi *et al.*, (2005) which they considered as a viable source of dietary proteins having protein as high as between 23–35% in addition to being digestible (Digestible foliage of 60–65%, grain of >95% and husks 78%), and metabolisable energy of about 1 kcal/g for raw seeds and 3.2 kcal/g for processed seeds. However, according to Musonda (2012) there is difference experienced in the crude protein content of the seed meal due to the variety of the seed which could be identify with the coat colour of the seed, with speckled having 24.95%, green 23.7%, cream velvet beans was found to be 28.1%,and black 22.5%. while In the earlier research conducted by Tuleun *et al.* (2001), the crude protein is 24% in speckled and black types, with the gross energy of 10-11 MJ/kg (Pugalenthi *et al.*, 2005; Siddhuraju *et al.*, 2000). Tuleun *et al.* (2008) further reported in its later report that Mucuna seed meal high contents of nitrogen and good essential amino acid levels, suggesting that it could serve as a source of essential nutrients to livestock on the basis that adequate information on the chemical composition of the seed is a prerequisite for its effective utilization in animal nutrition.

This claim was also supported by Vadivel *et al.,* (2007) that they contain desirable amino acids, fatty acids and have a good mineral composition, having high potassium (K), magnesium (Mg), calcium (Ca) and Iron (Fe), while Ifesan *et al.* (2017) reported the percentage concentration of the mineral in its work after the meal was processed by fermentation as magnesium having 189.00 mg/100g, sodium 3.22 mg/100g, iron 3.62 mg/100g, calcium 179.33 mg/100g, and highest in potassium 471.33 mg/100g, also high in lysine contents in grain while according to Balogun and Olatidoye (2012) mineral concentration of velvet seed meal includes Calcium 148.88±0.2, phosphorus 377.12±0.2, magnesium 23.66±0.3, iron 3.44±0.2 and manganese 5.28±0.1and from the research work cited by Ifesan *et al.* (2017) Both raw, cooked and fermented velvet beans were found to be rich in essential amino acids, most especially, lysine which makes it a useful supplement being a cereal grains which are generally low in lysine (Iyayi and Taiwo, 2003; Ogungbenle *et al.*, 2005), Aspartame and glutamate were also earlier reported to be predominant in fermented velvet beans (Siddhuraju *et al.*, 2000) and this is in consistent to previous reports, while according to Mugendi and Njagi (2010) all processing methods aid in increasing the concentration of essential amino acids (EAAs) in mucuna beans. While Carew and Gernat (2006) reported that mucuna seed has amino acids profile which is similar to that of the standard protein soybean and other legumes

2.3.2. Oil composition

Bachman (2014) reported that unsaturated oil present in the seeds has been found to be 47.2% linoleic acid, 14.2% oleic acid, 3.8% linolenic and 0.5% palmitoleic acid, the saturated fatty acids as palmitic 19.5%, stearic 12.6% and arachidic 2.2%. While Musoda (2012) reported that oil present in the seed to be highly unsaturated with 47.2% linoleic acid, 14.2%t oleic acid, 3.8% Linolenic acid and 0.5%palmitoleic acid while the saturated fatty acid s are Palmitic 19.5%, Stearic 12.6% and Arachidic 2.2% which was accurately similar to the report of Bachman (2014),also similar to the report of Balogun and Olatidoye (2012) who reported that the fatty acid profiles of the seeds revealed that the seed lipids contained higher concentration of palmitic acid and linoleic acid with Linoleic acid being the dominating fatty acid, followed by palmitic acid and oleic acid.

2.4. Utilization of Velvet seed meal

According to research work that visited for this study there is only but an handful of known published work of its utilization in the diet of fish which one of them is on tilapia and carp which was an herbivore which was done by Woke *et al.* (2012); Kakwi and Audu (2016) and they reported that about 20% of soybean meal could be replace in the diet of Herbivorous fish which will result in no adverse effect on the fish, also on the research work of Ani (2008) its inclusion level in the diet of other bird is also reported to be 20% of soybean meal can be replaced with Mucuna meal with no adverse effect on the birds. Another report by Olaboro *et al.*, (1991) indicated its utilization after been heat-treated by autoclaving in the diet of chick, being replace with soybean meal, and an improvement in growth parameters identified, other research works have been purely on poultry, rabbit, its medicinal utilization, and agricultural utilization as manure (Dossa *et al.,* 1998; Nyirenda *et al.,* 2003; Iyayi and Taiwo 2003; Iyayi *et al.,*2006)While on the contrary Vadivel *et al.* (2010) reported its inclusion in the diet of broiler bird, with up to 40% inclusion yielding better growth performance such as in feed intake, body weight gain, feed conversion ratio and protein efficiency ratio in both the starter and finisher phases, in that case, if appropriate treatment measures is employed by the use of soaking and boiling, probably up to that amount can be include in the diet of fish. Aside being utilized as feed ingredient, it was reported that virtually all part of *Mucuna utilis* is useful for purpose of manure and medicinal purposes, that is the seed, foliage and stem (Ujowundu *et al.* 2010).

2.5. Anti-nutritional Factors of *Mucuna utilis*

Inspite of its nutritional advantages and its utility pros, *Mucuna utilis* have been researched on to contain some anti-nutritional factors such as tannins, lectins, phytic acid, cyanogens, trypsin inhibitors and 3-4 dihydroxyl-L-phenylalanine (L-Dopa) which is the prominent among the anti-nutritional factors Olasunkanmi (2011). According to Carew *et al.,* (2003) L- Dopa, a potentially neurotoxic agent occurs in large amounts in *Mucuna.* In percentage concentration, Pugalenthi *et al.* (2006) reported that the seeds of the velvet bean contain tannins 3.1%–4.9%, L-dopa 4.2%–6.8%, lectins 0.31%–0.71%, protease inhibitors (trypsin and chymotrypsin), phytic acid, flatulence factors (oligosaccharides), saponins (1.15%–1.31%), hydrogen cyanide (58 mg/kg) and alkaloids.

2.5.1. Trypsin inhibitors

Trypsin inhibitors belong to the group of proteinase inhibitors that include polypeptides or proteins that inhibit trypsin activity. Tannins exhibit weak interactions with trypsin, and thus also inhibit trypsin activity.

2.5.2. Cyanogenic glycosides

Cyanogenic glycosides are plant toxins that through hydrolysis liberate hydrogen cyanide and the toxic effects of the free cyanide are well documented to affect spectrum of organisms since their mode of action is inhibition of the cytochromes of the electron transport system, which is known to cause chronic and acute effect on the system of the animal that feed on the seed without it been treated (Lampariello *et al.,* 2012 Laurena *et al.*, 1994).

2.5.3.3-4 dihydroxyl-L-phenylalanine (L-Dopa)

Mucuna utilis beans was found to have L-dopa (5%), along with minor amounts of methylated and non-methylated tetrahydroisoquinolines (0.25%) (Sidhuraju *et al.*, 2001; Misra and Wagner, 2004). However, in addition to L-dopa, 5-indole compounds, two of which were identified as tryptamine and 5-hydroxytryptamine, were also reported in velvet seed extracts (Tripathi and Updhyay, 2001).

2.6. Detoxification of velvet beans

According to research, the most common and effective method for detoxifying L-DOPA in velvet beans is with the use of Heat treatment method as they are heat labile, the various method employed in the processing method includes the processing by roasting at 120° C for 30 min, autoclaving for 30 min, boiling and other methods aside heat treatment includes grilling for 1 hour after the seed has being pre-soaked for 24 hours, water-soaking for 48 hours and boiling for 30 minutes, or soaking the cracked seeds for 24 h in 4% $Ca(OH)_2$ (Siddhuraju *et al.*, 1996; Dossa *et al.*, 1998; Del Carmen *et al.*, 1999; Ukachukwu and Szabo, 2003; Nyirenda *et al.* 2003; Cook *et al.*, 2005; Pugalenthi *et al.*, 2005).Also Feedipedia (2013) states that the anti-nutritional factors can be reduced efficiently by a wide range of treatment processes such as boiling in water, autoclaving and ensiling. However, according to Ebeniro and Orji (2012), they reported that about 12% boiled Mucuna processed seed meal can be added to the diet of animal with no side effect. While Mugendi and Njagi (2010) treated the velvet seed meal by Extraction at high pH (9.0) which reduced L-Dopa by 99.65% to 0.02%, but reported that the extract and the bean sample turned blackish, implying chemical conversion of L-Dopa to melanin, rather than actual extraction into water, while Fermentation and germination reduces L-Dopa levels by 73% and 22%, respectively, also processing of the seed meal by autoclaving, at low pH of 3.2, alkaline fermentation, moderate temperature of 60°C reduced trypsin inhibitor present in the seed completely while germination and fermentation method increase the crude protein to 32.9% and 37.6%, respectively. Ifesan *et al.* (2017) in their research study reported that the protein content in the sample increase in relative to the cooking time (28.38%-32.55%) also, which has been referenced by nutritionist to detoxify fully all the anti-nutritional factor (Mubarak, 2005).However, there could be leaching of nutrient when processing by germination, cooking and fermentation, which means, the crude protein content could be more than that. However, aside detoxification by toasting, all other detoxification methods have been able to reduce the level of anti-nutrition present in the velvet seed meal except by dry heat-treatment method (toasting) as the its detoxification requires moisture (Ramachandran *et al.* (2005)

Table 1: The proximate composition of *Mucuna utilis* (Velvet bean)

Parameters (%)	**Processed Mucuna seed meal (MSM)**
Crude protein	27.12
Lipid	0.52
Crude fiber	9.21
Ash	3.00
Moisture	10.80
Nitrogen Free Extract	49.35

Table 2: Amino acid profile of *Mucuna utilis* (Velvet bean)

Amino acid	(%)
Aspartic acid	14.28
Threonine	3.86
Serine	4.53
Glutamic acid	13.28
Proline	3.64
Glycine	5.49
Alanine	4.28
Cysteine	4.52
Valine	4.47
Methionine	0.69
Isoleucine	7.24
Leucine	6.14
Tyrosine	3.94
Phenylalanine	4.58
Lysine	5.72
Histidine	3.13
Arginine	7.41
Tryptophan	0.81

(Source: Olatidoye; 2012)

Table 3: Mineral composition of *Mucuna utilis* (Velvet bean)

Mineral	(%)
Calcium (%)	5.25
Phosphorus (%)	0.02
Magnesium (%)	1.63
Potassium (%)	0.13
Sodium (%)	1.17
Zinc (ppm)	0.12
Manganese (ppm)	0.0

Source: (Okafor; 2015)

Catfish production in Nigeria

Fish constitutes about 41% of the total animal protein intake by the average Nigerian, which however leads to great demand of fish in the country to meet protein dietary requirement of the citizen Issa *et al.* (2013). Catfish is the major fish cultured in Nigeria because it is found all over the Country, north, south, east and west of the country, acceptably eaten by most tribes, with high resistant harsh environmental conditions, resistance to diseases, commands good price, it provides cheap and quality protein, create employment opportunity,, as well as constitute an important element in the social stability and progress of the people in Nigeria and can be kept alive for days during marketing, also easily spawned under proper conditions which gives the farmer control over the production process estimates put the current production output of *Clarias gariepinus* in the Country at over 253,898 metric tonnes per year, with its production actively supported by Federal Government of Nigeria through the Department of Fisheries (the competent authority) through training, policy formulation, implementation, signatories to relevant international conventions, and sustainable through infrastructure development, awareness as one of the profitable means of Fish Production that is environmentally friendly (Anetekhai 2013, Adelakun*et al.* 2015; Rana, 2007)) and catfish production in the country is rated as being up to 90% out of the total percentage of the number of fish cultured in the country. All stated report from previous study on the species indicates that it can help in attaining some goals of millennium development goals, which includes alleviation of poverty and also increase food security in the country (FDF 2005; Adelakun *et al.* 2015).

2.7.1. Protein requirement in *Clarias gariepinus* (African catfish)

Better food utilization is not only the result of increased protein level, but also of improved protein quality (Pantazis 2014), protein is a basic nutrient in the feed of fish because it provides amino acids and nitrogen for body protein and non-protein nitrogen substances biosynthesis and can be used as a source of energy and synthesis of glucose (National Research Council [NRC], 2011), protein is the most important nutrient in the diet of fish because of the fact that the growth of the fish depends on the protein supplied from the diet given to the fish and when too high, it will lead to high cost of production and eutrophication, but when low would result in poor growth, for this reason the protein requirement of the fish must be known and be met. In Abdel-Tawwab & Ahmad (2009) words dietary protein is an important aspect in achieving efficient fish production, which should be met to accommodate fish requirements due to age, size and reproduction. According to research work, digestibility experiments indicated that adult *C. gariepinus* accept purified diets comprised mainly (48-50%) of purified proteins with high digestibility values for most nutrients (Pantazis and Neofitou 2004)

It is really difficult to set a level of protein that is best, as over the years there have been debate over the best percentage level required for catfish as situation of to which factor to which the fish are under varies as these factors affects the dietary protein requirement of the fish and this includes the size of the fish, water quality parameters type of feed fed, feed allowance, fish size, amount of non-protein energy in the diet, protein quality, natural food available ,age of the fish and management practices, as well as the genetic composition and feeding rates of fish Woke *et al.*, (2012), and Fernandes *et al.* (2016), according to Robinson *et al.* (2006) Fry diets used in the hatchery should contain 45 to 50 percent protein, while according to NRC (1993) catfish at fry state requires protein level of 40% and above, but fingerlings requires 40% and while adult requires protein level of about 35% NRC (1993).

2.8. Haematology

Haematological parameters are tested to provide important information in assessment studies, by providing an indication as to the general physiology and health status of the organism under investigation (Ayoola *et al.,* 2014) which was similar to what Satheeskumar *et al.,* (2011) that said haematological and biochemical changes in blood are important indicators used in monitoring physiological and pathological changes in fish, and toxic effect of the feed or the environmental effluent which can affect the health and cause stressor on the fish. While Bahmani *et al.*, (2001) reported that blood parameters analyses have proven to be a valuable tool to analyze the health status of farmed and uncultured fish as these indices provide reliable information on possible exposure to mutagens, metabolic disorders, deficiencies and chronic stress status before clinical symptoms appears. According to Ranzani-Paiva *et al.* (2000) haematological characteristics of most fish are studied with the aim of establishing any deviation from the normal value as this may indicate a disturbance in the physiological process. Blood samples can regularly be obtained from test organisms, which will enable the use of non-destructive approach in effecting assessment of the fish condition (Akinrotimi *et al.,* 2010), since fish are so intimately associated with the aqueous environment, the blood will reveal measurable physiological changes in the fish more rapidly than any physiological assessment parameters (Ezeri *et al.,* 2004), according to the report of Ani (2008). The values of PCV and Hb range fall between normal range when he replace soybean meal with velvet seed meal by 20%, which indicated that 20% of processed velvet seed meal can be used to replace soybean meal. However, the composition of blood can be changed by dietary treatment, malnutrition and disease condition (Feist *et al.*, 2000) reason why haematology analysis is very essential to ascertain the effect of the seed meal on the health of the fish fed experimental feed sample.

2.9: Taxonomy Classification *Clarias gariepinus*

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Clariidae

Genus: *Clarias*

Species: *C.gariepinus*

(Source; Gruntar and Fink 2004)

CHAPTER THREE

MATERIALS AND METHODS

3.1. Experimental Site

The research was conducted in the wet Laboratory, Department of Fisheries and Aquaculture, Federal University Oye- Ekiti, Ikole Campus, Ekiti State, Nigeria. The haematological analysis was carried out in the laboratory of the Ekiti State Hospitals' Management Board Specialist Hospital, Ikole Ekiti.

3.2. Collection and processing of *Mucuna utilis*

Five kilograms of *Mucuna utilis* was procured from Institute of Agriculture Ahmadu Bello University (A.B.U) Zaria. The seeds were soaked in water for 48hours, dehulled by hand, and then cooked in already boiling water one hour using fire-wood at 400°C, the seeds were then washed, dried to constant weight before and milled. The resulting meal was sieved to pass 2mm mesh.

3.3. Diet Formulation

Feedstuffs was prepared and formulated into feeds containing 40% crude protein. Five isonitrogenous feeds were formulated to contain MSM at varying levels: Diet 1 (MSM 0%) which also serves as control, Diet 2 (MSM 25%), Diet 3 (MSM 50%), Diet 4 (MSM 75%) and Diet 5 (MSM 100%) (Table 4).The feed ingredients for each of the diets mixed thoroughly and water was added then stirred to form consistent dough which was passed through a 2mm die pelleting machine. The pellets produced were sundried to constant weight stored in an air tight polythene bag and kept in a cool dry place until used.

Table 4: Percentage Composition of Experimental Diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize	9.315	9.315	9.315	9.315	9.315
Wheat offal	9.315	9.315	9.315	9.315	9.315
Soybean	23.28	17.46	11.64	5.82	0
Mucuna seed	0	5.82	11.64	17.46	23.28
Groundnut cake	23.28	23.28	23.28	23.28	23.28
Fish Meal	23.28	23.28	23.28	23.28	23.28
Methionine	1.00	1.00	1.00	1.00	1.00
Binder	2.00	2.00	2.00	2.00	2.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00
Vitamin C	0.03	0.03	0.03	0.03	0.03
Common salt	0.5	0.5	0.5	0.5	0.5
Chromic oxide	1.00	1.00	1.00	1.00	1.00
Palm Oil	5.00	5.00	5.00	5.00	5.00
Total	100	100	100	100	100

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

3.4. Experimental design and feeding trials

One hundred and fifty *Clarias gariepinus* fingerlings were purchased from a reputable farm in Ado-Ekiti. The fish were acclimatized for fourteen days, during which they were fed. At the end of the acclimatization period, fish were randomly selected and stocked into 15 rectangular aquaria at 10 fish per aquaria and were randomly assigned to each diet groups for eight weeks during which they were fed 5% of their body at 08:00 and 16:00 hours. Fish were weighed every two weeks. Data obtained was subjected to statistical analysis at the end of the experiment.

3.5. Chemical analyses

Feeds were analyzed for proximate analysis using Association of Official Analytical Chemists (A.O.A.C, 2000) method.

3.6. Growth performance and nutrient utilization of *Clarias gariepinus*

The growth performance parameters such as weight gain, relative growth rate and specific growth rate and the nutrient utilization parameters include daily feed intake, Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Net Protein Utilization (NPU) were evaluated using:

(i) Weight gain (WG)

WG = Final weight of fish – Initial weight of fish

(ii) Relative growth rate (RGR)

$$RWG\ (\%) = \frac{WF\ (g) \times 100}{W1}$$

where;

WF = Final average weight of fish

W_1 = Initial average weight at the beginning of the experiment

(iii)Specific Growth Rate (SGR) %

$$SGR\ (\%/day) = \frac{\log \text{ of } W1 - \log \text{ of } W0 \times 100}{T1 - T0}$$

(iv)Percentage Survival

$$\text{Survival Rate} = \frac{\text{Initial number of fish stocked} - \text{Mortality} \times 100}{\text{Initial number of fish stocked}}$$

(v) Feed conversion ratio (FCR)

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

(vi)Feed Efficiency Ratio (FER)

$$FER = \frac{\text{Weight gain} \times 100}{\text{Feed Intake}}$$

(vii) Protein efficiency ratio (PER)

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

(viii) Feed Intake

Feed Intake = Percentage body weight x weekly total weight x weight of fish

3.7 Water quality assessment

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.

3.8 Heamatological Assessment

3.8.1. Sample Collection

At the end of the feeding trial, fish blood samples were collected from the caudal peduncle using heparinized plastic and syringe (2 mL). Blood samples were preserved in di-sodium salt of Ethylene Diamine Tetra-Acetic Acid (EDTA) bottles as anticoagulant for analysis as described by Joshi *et al.* (2002). After the collection, the blood samples were taken to the analytical laboratory of Ekiti State Hospitals' Management Board Specialist Hospital, Ikole Ekiti., where the haematological analysis was carried out. A total number of five fishes were randomly selected from each treatment and blood samples were collected following the procedures by Wedemeyer and Yasutake (1977). The following haematological parameters were analyzed;

3.8.2. Determination of Red blood cells counts

Standard haemocytometer was used in the counting of the red blood cells according to method of Blaxhall and Daisley. The blood samples for the red blood cell count was diluted with Hayem's fluid, comprising of 1g NaCl, 5g Na_2SO_4, 0.5g $HgCl_2$ and 200ml diluted water. A haemocytometer placed on a compound microscope was used to estimate the erythrocyte number. The number of cells counted was multiplied by a diluting factor 200 and volume factor (VF) of 50 as described by Svobodova *et al.* (1991).

3.8.3. Determination of White blood cells counts

White blood cells were determined using the method described by Blaxhall and Daisley, 1973.

3.8.4. Packed cell volume

Blood were collected into microhaematrocrit haprinised tube which was sealed with critaseal at one end. The sampled tubes were then centrifuged for 5 minutes at 12000rpm using Hawksley microhaematocrit centrifuge. The haematocrit values were read on a microhaematocrit reader. A mean of two readings was recorded as percentage for the fish haematocrit

3.8.5. Determination of haemoglobin concentration

The cyanmet-haemoglobin method was used as described by Larsen and Snieszko (1961), 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.

3.8.6. Determination of Mean Corpuscular Hemoglobin (MCH)

Mean Corpuscular Haemoglobin· Concentration, express the concentration of haemoglobin in unit volume of erythrocyte, Mean Corpuscular Hemoglobin (MCH) was calculated using the formula described by Dacie and Lewis (1977)

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

3.8.7. Determination of Mean Corpuscular Volume (MVC)

The Mean Corpuscular Volume (MCV) was determined as described as described by Dacie and lewis (1977)

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

3.8.8. Determination of Mean Corpuscular Haemoglobin Concentration (MCHC)

The Mean Corpuscular Haemoglobin Concentration (MCHC) was calculated using the formula described by Dacie and lewis (1977).

$$MCHC\ (g/dL) = \frac{Hb\ (gb1^{-1})}{PCV\ (\%)}$$

3.9. Statistical Analysis

Data collected from the experiment were subjected to one-way Analysis Of Variance (ANOVA) test using Statistical Package for Social Scientists (SPSS) version 20. Duncan's Multiple Range test was used to separate the means in cases of significant differences. Statistical significance effect on the parameters to be measured was set at $p \leq 0.05$.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1RESULTS

4.1.1: Proximate composition of experimental diet

Proximate of experimental diet is presented on Table 5. Crude protein range from 39.98% in T1 to 39.81% in T5, and Crude lipid ranges from 7.94% in T4 and 8.88% in T3. T5 has the highest percentage of Crude fibre (6.45%) and the lowest was observed in T1 (2.31%). Ash ranges from 10.40% in T2 to 11. 28% in T1, moisture content also ranges from 4.38% in T1 to 8.54% in T3

4.1.2: Effect of Mucuna Seed Meal (MSM) based diet on growth performance of *Clarias gariepinus* fingerlings

Growth performance of the fish fed the experimental diets is presented in Table 6. Mean Weight gained in T1 and T2 were significantly different from the other treatments. Highest mean weight gain was recorded in T1 (8.25 ± 0.34) and lowest in T5 (3.92 ± 0.64). Average daily weight gain recorded were similar ($p>0.05$) in T3, T4 and T5 but were significantly different ($p<0.05$) from T1and T2. Similar protein efficiency ratio ($p>0.05$) was observed in T3, T4 and T5, but significantly different from T1 and T2, with highest protein efficiency ratio recorded in T1 (0.21 ±0.01) and lowest in T5 (0.10 ± 0.02). Feed conversion ratio of fish fed for this study recorded that there were similarities ($p>0.05$) in T1, T2, T3 and T4 but was significantly different ($p<0.05$) from T5. The lowest FCR in T2 (0.96 ± 0.04) and highest in T5 (1.54±0.26). Feed efficiency ratio of *Clarias gariepinus* fed experimental diet also recorded similarities ($p>0.05$) in, T5, T4 and T3, likewise T1 and T2 respectively, highest FER in T2 (103.87 ± 2.83) and lowest in T5 (69.74 ±14.28). There was significant difference ($p<0.05$) in the specific growth rate of all the experimental treatment but similarities ($p>0.05$) was recorded in T3 and T4, with highest in T1 (0.28 ± 0.01) and lowest in 100%MSM (0.10 ± 0.00). Relative growth rate of the fish fed experimental diet was recorded to be similar ($p>0.05$) in T3, T4 and T5 likewise in T1 and T2. Finally, similarities ($p>0.05$), was recorded in the survival rate of fish fed the experimental diet.

4.1.3: Carcass composition of *Clarias gariepinus* fingerlings fed experimental diets

Table 7 shows the proximate composition of *Clarias gariepinus* fed experimental diet, crude protein of *Clarias gariepinus* fed the experimental diet was observed to be similar ($p>0.05$) in T2 andT4, but significantly different from T1, T3 and T5 with highest crude protein observed in the flesh of fish in T1 (56.65±0.03) and lowest in T5 (51.52 ± 0.01). Crude lipid of *Clarias gariepinus* in T3 and T5 are similar ($p>0.05$), but significantly different ($p<0.05$) from crude lipid in T1, T2 and T4, with highest crude lipid observed in T3 (8.23 ±0.13) and lowest in T1 (5.97 ± 0.02). Crude fiber of fish fed the experimental diet was observed to be significant different ($p<0.05$) in all the experimental treatment, with highest fiber recorded in T3 (16.59 ±32) and lowest in T1 (9.82 ± 0.01). The ash content of fish fed the experimental diet similar ($P>0.05$) in T1 and T5 but was however significantly different ($P<0.05$) in T2, T3 and T4, with highest in T5 (17.61 ±0.27) and lowest in T3 (9.98 ± 0.03). Moisture of fish fed experimental diet were however significantly different ($p<0.05$) in all experimental fish with highest recorded in T1 (10.35 ± 0.00) and lowest in fish fed T4 (7.39 ± 0.14).

4.1.4. Haematological analysis of *Clarias gariepinus* fingerlings fed Mucuna seed meal

Hematological parameters of *Clarias gariepinus* fingerlings fed experimental diet is presented in Table 8. White Blood Cell (WBC) was similar ($p>0.05$) in T1 and T2 but were significantly different ($p<0.05$) in T3, T4, and T5, with the highest in T5 (13.63 ± 0.97) and lowest in T1 (2.93 ±0.37). Neutrophil observed showed that there were similarities ($p>0.05$) in T1, T2 and T5 respectively but were however significantly different ($p<0.05$) from neutrophil of T3 and T4. Lymphocyte of *Clarias gariepinus* fed the experimental diet shows no significant difference ($p>0.05$) in T1, T2 and T3 and T5, but were however significantly different ($p<0.05$) from recorded result in T4, with the highest (76.33 ± 0.88) in T3 and lowest (64 ± 0.58) in T4. Packed cell volume (PCV) was similar ($p>0.05$) in result of the analysis of the blood samples in T2 and T3, likewise in T4 and T5, but were significantly different from T1. Red blood cell of *Clarias gariepinus* fingerlings fed mucuna seed meal-based diet were similar ($p>0.05$) T2, T3, T4 and T1, but were however significantly different ($p<0.05$) from T5, with the highest recorded RBC in T1 (2.67 ± 0.15) and lowest in T5 (1.73 ± 0.12). There was no significant difference ($p>0.05$) in the haemoglobin concentration in T1, T2, T3 and, T4 but were significantly different ($p<0.05$) from observed haemoglobin concentration in T5. Mean corpuscular haemoglobin concentration (MCHC) shows no significant difference ($p>0.05$) in all the fish. Mean corpuscular haemoglobin (MCH) of fish fed the experimental diet were similar ($p>0.05$) for T2, T3 and T4 likewise for T1 and T5, with highest MCH recorded in T1 (5.27 ± 0.52) and lowest in T4 (4.14 ± 0.46). Highest mean corpuscular volume was observed in T5 (158.6 ± 13N.75) and lowest in fish fed T4 (124.21 ± 0.93)

4.1.5: Water quality parameters of the experimental units.

Water quality parameters of the experimental units are presented on Table 9. There was no significant difference ($p>0$) in water temperature and p^H in all treatments. However significant differences were observed in dissolved oxygen values in T1, T4 and T5.

Table 5: The proximate composition of the experimental feed.

PARAMETER	CTRL	T2	T3	T4	T5
Crude Protein	39.98	39.90	39.95	39.93	39.81
Crude lipid	8.75	8.04	8.88	7.94	8.09
Crudė fiber	2.31	3.18	3.35	4.21	6.45
Ash	11.28	10.40	10.43	10.68	11.15
Moisture	4.38	6.08	8.54	6.41	5.72
NFE	33.29	32.39	28.86	30.83	28.77

Table 6: Effect of Mucuna seed meal (MSM) based diet on growth performance of *Clarias gariepinus* fingerlings

PARAMETERS	T1	T2	T3	T4	T5
Initial weight (g)	7.14 ± 0.02^{a}	6.82 ± 0.18^{a}	7.01 ± 0.12^{a}	6.73 ± 0.32^{a}	6.76 ± 0.13^{a}
Final weight (g)	15.39 ± 0.36^{c}	13.50 ± 0.14^{b}	11.65 ± 0.56^{a}	11.30 ± 0.53^{a}	10.67 ± 0.51^{a}
Mean Weight gain (g)	8.25 ± 0.34^{c}	6.68 ± 0.50^{b}	4.63 ± 0.44^{a}	4.57 ± 0.26^{a}	3.92 ± 0.64^{a}
Average daily weight gain	0.15 ± 0.01^{c}	0.12 ± 0.00^{b}	0.09 ± 0.01^{a}	0.08 ± 0.00^{a}	0.07 ± 0.01^{a}
Protein efficiency ratio	0.21 ± 0.01^{c}	0.17 ± 0.00^{b}	0.12 ± 0.01^{a}	0.12 ± 0.02^{a}	0.10 ± 0.02^{a}
Feed intake (g)	8.17 ± 0.76^{c}	6.44 ± 0.16^{b}	5.39 ± 0.28^{a}	6.05 ± 0.39^{ab}	5.7 ± 0.23^{ab}
Feed conversion ratio	1.00 ± 0.04^{a}	0.96 ± 0.04^{a}	1.18 ± 0.08^{ab}	1.32 ± 0.07^{ab}	1.54 ± 0.26^{b}
Feed efficiency ratio	101.04 ± 4.33^{b}	103.87 ± 2.83^{b}	85.7 ± 5.28^{ab}	75.91 ± 3.76^{a}	69.74 ± 14.28^{a}
Specific growth rate	0.28 ± 0.01^{d}	0.23 ± 0.00^{c}	0.15 ± 0.01^{b}	0.15 ± 0.02^{b}	0.10 ± 0.00^{a}
Relative growth rate	215.58 ± 4.65^{b}	198.17 ± 3.23^{b}	165.9 ± 5.41^{a}	168.02 ± 2.88^{a}	158.33 ± 10.79^{a}
Survival rate (%)	100 ± 0.00^{a}	90 ± 0.00^{a}	93.33 ± 3.33^{a}	90 ± 5.78^{a}	93.33 ± 3.33^{a}

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

Table 7: Carcass composition of fish fed experimental diets

Parameter	**T1**	**T2**	**T3**	**T4**	**T5**
Crude protein (%)	56.65 ± 0.03^{d}	55.38 ± 0.10^{b}	55.67 ± 0.24^{c}	54.7 ± 0.20^{b}	51.52 ± 0.01^{a}
Crude Lipid (%)	5.97 ± 0.02^{a}	7.24 ± 0.02^{b}	8.23 ± 0.13^{d}	7.70 ± 0.20^{c}	7.99 ± 0.13^{d}
Crude fiber (%)	9.82 ± 0.01^{a}	13.06 ± 0.04^{b}	16.59 ± 0.32^{c}	15.68 ± 0.11^{d}	15.04 ± 0.14^{e}
Ash (%)	17.21 ± 0.02^{d}	16.04 ± 0.07^{c}	9.98 ± 0.03^{a}	14.53 ± 0.11^{b}	17.61 ± 0.27^{d}
Moisture (%)	10.35 ± 0.00^{e}	8.28 ± 0.02^{c}	9.52 ± 1.13^{d}	7.39 ± 0.14^{a}	7.84 ± 0.16^{b}

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

Table 8: **Haematology parameters of *Clarias gariepinus* fed Mucuna seed meal.**

PARAMETERS	CTRL	T2	T3	T4	T5
White blood cell ($\times 10^3$mm)	2.93 ± 0.37^a	4.00 ± 0.12^{ab}	5.00 ± 0.12^b	7.00 ± 0.25^c	13.63 ± 0.97^d
Neutrophil (%)	27.67 ± 1.45^b	28.00 ± 1.15^b	23.67 ± 0.88^a	36.00 ± 0.58^c	29.00 ± 1.53^b
Lymphocyte (%)	72.33 ± 1.45^b	72.00 ± 1.15^b	76.33 ± 0.88^c	64.00 ± 0.58^a	73.33 ± 0.88^{bc}
Packed cell volume (%)	41.67 ± 1.76^c	34.67 ± 1.45^b	35.00 ± 1.45^b	30.00 ± 0.58^a	27.33 ± 1.20^a
Red blood cell($\times 10^{12}$/L)	2.67 ± 0.15^b	2.60 ± 0.06^b	2.50 ± 0.06^b	2.47 ± 0.23^b	1.73 ± 0.12^a
Haemoglobin conc.(g/L)	13.89 ± 0.58^c	11.57 ± 0.48^b	11.67 ± 0.19^b	10.00 ± 0.19^a	9.11 ± 0.40^a
Mean corpuscular haemoglobin concentration (g/L)	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
Mean corpuscular haemoglobin (pg)	5.27 ± 0.52^b	4.45 ± 0.17^{ab}	4.67 ± 0.03^{ab}	4.14 ± 0.46^a	5.28 ± 0.14^b
Mean corpuscular volume (fl)	157.94 ± 15.61^c	133.37 ± 15.61^{ab}	$140.04 \pm 5,21^{ab}$	124.21 ± 0.93^a	158.6 ± 13.75^c

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

Table 9: Water quality parameters of the experimental units.

TRT	T1	T2	T3	T4	T5
Temperature (°C)	28.60 ± 0.20^{a}	28.90 ± 0.03^{ab}	28.80 ± 0.33^{ab}	29.00 ± 0.11^{b}	29.00 ± 0.05^{b}
Dissolve Oxygen (mg/L)	4.50 ± 0.06^{c}	4.37 ± 0.03^{bc}	4.46 ± 0.03^{bc}	4.17 ± 0.12^{a}	4.23 ± 0.09^{ab}
p^{H}	6.30 ± 0.03^{a}	6.47 ± 0.09^{a}	$6.6 \pm 0.15.00^{a}$	6.57 ± 0.15^{a}	6.30 ± 0.06^{a}

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

4.2: Discussion

The proximate composition of MSM in this study revealed that Mucuna is rich in crude protein crude fiber, Nitrogen free extract and Lipid this result is like that of Tuleun *etal.* (2009), Ujowundu *et al.* (2010), Olasunkanmi, (2011), Balogun and Olatidoye (2012) and Sese *et al* 2014). This might be responsible for the relatively good performance of fish placed on MSM-based diets.

The various treatment, protein in control and 25%MSM were effectively utilized by *Clarias gariepinus* fingerlings, but less efficient at higher inclusion level, which may be as a result of none or low anti-nutritional factors present, as the same pattern was observed in the report of Kakwi and Audu (2016) likewise in the report of Alegbeleye *et al.* (2005).

The inclusion of Mucuna seed meal were similar to that obtained in the control, as it did not affect the carcass protein and proximate composition, as there were no significant differences in the utilization of the test diets which conform to report of Kakwi and Audu (2016) that *Mucuna at* 25% inclusion can be used to replace soybean meal, as this also conform to reports of Eyo (2001), Hossain *et al.* (2001), Fagbenro *et al.* (2003), Hasan *et al.* (2006), Davies and Gouveia (2008) observed non-significant differences in the carcass protein contents of diets containing boiled full-fat soybean or defatted soybean meal fed to Nile tilapia and Common carp.

Even though the fish grows well on MSM based diet, the high values of White Blood Cells and neutrophil may indicate that feeding Mucuna Seed Meal to fish introduces some stressors into the body of fish.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.0: Conclusion

The results of this study on the effect of Mucuna seed meal on the growth performance, body composition and haematology of *Clarias gariepinus* fingerlings revealed that 25% inclusion of Mucuna seed meal can be included in the diet of *Clarias gariepinus* fingerlings.

5.1. Recommendations

Based on the results of this study, it is recommended that:

- Soybean meal can be replaced with MSM for up to 25% in the diet of *Clarias gariepinus.*
- More research should be conducted on processing methods that will improve the utilization of Mucuna seed as a feed ingredient for fish.

REFERENCES

Adebowale K.O, Lawal O.S, (2003). Functional properties and retrogradation behavior of native and chemically modified starch of Mucuna bean (*Mucuna pruriens*). *J. Sci. Food Agric.,* 83: 1541-1546.

Adelakun, K.M., Amali R.P., Ogundiwin, D.I., and Bakare, K. (2015), Assessment of catfish farming in Osogbo and its environs, PeerJ PrePrints, 10(1): 1-14

Agharkar S.P (1991). Medicinal plants of Bombay presidency. Scientific Publication, Jodhpur, India. pp. 1–2.

Akinmutimi, A. (2006). Effect of quantitative substitution of cooked *Mucuna utilis* seed meal for soya bean meal in broiler finisher diet. *International Journal of Poultry, Science* 5(5): 447-481.

AOAC (Association of Official Analytical Chemist) (2005*). Official Methods of Analysis of AOAC* International (18th edition). W. Horwitz and G. W. Latimer (Eds.). Arlington, Virginia. APHA, AWWA, WCPF (American Public Health Association, American Water Works Association and Water Control Pollution Federation) (1985). Standard Methods for Examination of Water, 15th ed. Washington D.C. USA: APHA, 1976Pp

Anetekhai, M.A., (2017), Catfish aquaculture industry Assessment in Nigeria, *Inter-African Bureau for Animal Resources, African Union,* 10(1): 1-97 https://www.researchgate.net/publication/312473322

Ani, A.O. (2008), The Feeding Value Of Processed Velvet Bean (*Mucuna Pruriens*) For **Pullet** Chicks, *Agro-Science Journal of Tropical Agriculture, Food, Environment and Extension* 7(2): pp 149-155

Anibeze, C.I.P and Eze, A (2000). Growth response of two African Catfishes (Osteichthys: Claridae) in Homestead concrete ponds. *Journal of Aquatic Sciences*, 15:55-58

Association of Official Analytical Chemists (A.O.A.C,2000). Official Methods of Analysis, 14th edition. Williams (ed) Arlington V.A.p102.

Atanda J.O. (eds) (2009). Conference Proceedings of Fisheries Society of Nigeria, Owerri, Nigeria. pp. 40-41

Balogun, I.O., Olatidoye, O.P. (2012). Chemical composition and nutritional evaluation of Velvet Bean seads (*Mucuna utilis*) foor domestic consumption and Industrial utilization in Nigeria

Bachmann, T. 2014. *Mucuna pruriens* (L.) D.C. http://www.fao.org/ag/agp/AGPC/doc/Gbase/DATA/PF000416.HTML Date of access: 10 December 2018.

Bressani R (2002). Factors influencing nutritive value in food grain legumes: Mucuna compared to other grain legumes. In: Food and Feed from Mucuna: Current Uses and the Way Forward (Editors, Flores BM, Eilittä M, Myhrman R, Carew LB and Carsky RJ), Workshop, CIDICCO, CIEPCA and World Hunger Research Center, Tegucigalpa, Honduras (April 26-29, 2000), pp. 164-188.

Carew, L. B., Hardy, D., Ger lat, A.G.and Zakrzewska, E. I. (2003). Heating raw velvet beans (*Mucuna pruriens*) reverses so ne anti nutritional effects on organ growth, blood chemistry and organ histology in growing chickens. *J. Tropical and Subtropical Agroecosystems* 1 (2-3):267- 275.

De Silva S.S, Anderson T.A, (1995). Fish Nutrition in Aquaculture. Chapman and Hall, Melbourne.

Dossa, CS, Mensah, GA, Dossa, AD, Adoun, C. (1998. Influence of various physicochemical treatments of *Mucuna pruriens* seeds on the nutrient chemical composition. *Tropiculture.* 16-17 (3) 141.

Ebeniro, l.a. and Orji, r.c.a. (2012), Growth Response of *Heterobranchus longifilis* Fingerlings To *Mucuna pruriens* Seed Meal Based Diets, *Journal of Agriculture and Social Research (JASR)* 12 (2), 2012: 67-73

El-Sayed AFM, S Teshima (1992). Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia *(Oreochromis niloticus*)

Fagbenro, O.A., Akande, T.T. and Fapounda, O.O. (2003). Use of Roselle (*Hibiscus Sabdariffa)* seed meal as a soybean meal replacer in practical diets for fingerlings of African Catfish (*Clarias gariepinus*), *Proceeding of the third international conference on African fish and Fisheries,* Cotonou, Benin, 10-14 November.

FAO, (2012): FAOSTAT, Statistics Division, Food and Agriculture Organization of the united Nations, http://www.fao.org/corp/statistic/en/ (accessed 12. 06. 2017).

FAO. (2011). Cultured Aquatic Species Information programme. *Cyprinus carpio* (Linnaeus, 1758). Fisheries and Aquaculture Department.

Federal Department of Fisheries (FDF) (2008) Fisheries Statistics of Nigeria. Federal Department of Fisheries Area 11, Garki, Abuja, FCT, Nigeria. Fourth Edition.

Fung, S.Y. Tan, H.N., Sim, S., and Aguiyi .J.C. (2012), Effect of *Mucuna pruriens* seed extract pretreatment on The responses of spontaneously beating rat atria and aortic Ring to Naja sputatrix (Javan Spitting Cobra) Venom, Hindawi Publishing Corporation, 12(486390) pp. 1-6.

Gabriel, U.U. Akinrotimi, O.A, Bekibele, D.O, Onunkwo, D.N and Anyanwu, P.E. (2007) Locally produced fish feed: potential for aquaculture development in sub-Saharan Africa.

George F.O.A., O. J. Olaoye, O. P. Akande and R. R. Oghobase (2010). Determinants of Aquaculture Fish Seed Production and Development in Ogun State, Nigeria. *Journal of Sustainable Development in Africa, 12.*

Goldburg, R.J., Hua, K. and Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of National Academy of Science.* USA 106:15103-1511.

Gurumoorthi P, Pugalenthi M, Janardhanan K (2003). Nutritional potential of five accessions of a South Indian tribal pulse, *Mucunapruriens* var *utilis* II. Investigations on total free phenolics, tannins, trypsin and chymotrypsin inhibitors, phytohaemagglutinins, and *invitro* protein digestibility. *J. Trop. Subtrop. Agroecosysytems* 1: 153- 158.

Htun-Han, M. (1978). The reproductive biology of the dab Limanda Limanda (L.) in the North Sea: gonosomatic index, hepatosomatic index and condition factor.

Ifesan, B.O.T., Akintade, A.O., Babriel-Ajobiewe, R.A.O., (2017), Physicochemical and nutritional properties of *Mucuna pruriens and Parkia biglobosa* subjected to controlled fermentation, *International Food Research Journal* 24(5): 2177-2184.

Issa F.O., Abdulazeez M. O., Kezi D.M., Dare J.S., and Umar, R., (2014), Profitability analysis of small-scale catfish farming in Kaduna State, Nigeria, *Journal of Agricultural Extension and Rural Development,* 6(8), pp. 347-353

Iyayi, E. A. and Egharevba, J.I. (1998). Effect of germination and heat treatment on the nutritional composition of *Mucuna utilis. Nigerian Journal of Animal Production 25(1)*: 40-45.

Iyayi E.A, Taiwo V.O, (2003). The effect of diets incorporating Mucuna (*Mucuna pruriens*) seed meal on the performance of laying hens and broilers. Trop. Subtrop. Agroecosyst., 1: 239-246.

Kakwi, D.G., Audu, B.S., (2016) Effect of partial replacement of Soybean meal with *Mucuna pruriens* Meal in the diet of Common Carp, (*Cyrinus carpio* Linnaeus, 1785) Fingerlings *Nigerian Journal of Fisheries and Aquaculture* 4 (2):48 – 60

Kavitha C. and Thangamani C. (2014), Amazing bean *"Mucuna pruriens"*: A comprehensive review, *Journal of medic plants research*,8(2), pp138-143

Lampariello, R.L., Cortelazzo, .A., Guerranti. R., Sticozzi. C., Valacchi. G., (2010). The Magic Velvet Bean of Mucuna pruriens, *Journal of Traditional and Complementary Medicine,* 2(4): pp. 331-339

Laurena, A.C., Revilleza, M.J.R., Mendoza, E.M.T., (1994). Polyphenols, phytate, cyanogenic glycosides and trypsin inhibitor activity of several Philippine indigenous food legumes. *J. of Food Comp. and Analysis* 7, 194-202.

Misra, L., Wagner, H., (2004). Alkaloidal constituents of *Mucuna pruriens* seeds. Phytochemistry, 65, 2565-2567.

Mubarak, A.E. (2005). Nutritional composition and antinutritional factors of mung bean seeds *(Phaseolus aureus)* as affected by some home traditional processes. Food Chemistry 89: 489–495.

Mugendia, M.J., Njagi L. M., (2010), Effects of processing mucuna bean *(mucuna pruriens l.)* . On protein quality and antinutrients content, Conference on International Research on Food Security, Natural Resource Management and Rural Development, ETH Zurich, September 14 - 16, 2010

Musonda (2012), Nutritional variation among different varieties of velvet beans (*Mucuna pruriens*) when evaluated as potential protein supplements in broiler rations, pp 1-32.

Nyirenda, D, Musukwa, M. and Jonsson, L. O. (2003). The effects of different processing methods of velvet bean (*Mucuna pruriens*) on L-dopa content, proximate composition and broiler chicken performance. *Tropical and Subtropical Agroecosystems 1*: 253-270

Olabóro, G., Okot, M. W., Mugerwa, T. S and Latshaw, J. D. (1991). Growth depressing factors in velvet beans fed to broiler chickens. *East African Agricultural and Forestry Journal.* 57:103-110.

Olasunkami, J.B (2011). Nutrient utilization and growth performance of *Clarias gariepinus* fed differently processed *Mucuna utilis* meals as a replacement for soybean based diet. Pp 32-33

Siddhuraju .P., Becker .K., Richter N., (1996), Chemical composition and protein **quality of the** little-known legume, velvet bean (*Mucuna pruriens* (L.) DC). J. Agric. **Food** Chem., 44: 2636-2641.

Probat, E. 2011. What is so interesting about the velvet bean? http://bioweb.uwlax.edu/bio203/2011/probst_emil/facts.html Date of access: 27 December 2018.

Pugalenthi M, Vadivel V, Siddhuraju P (2005). Alternative Food/Feed Perspectives of an underutilized Legume *Mucuna pruriens* var. *Utilis* - A Review. Plant Foods for Human Nutrition 60: 201-218.

Rahman, R.A., Kian, A. Yong Seok, and Mustafa, S. (2013). Use of enriched live prey in promoting growth and maturation of Tiger Shrimp, (*Penaeus monodon). NAGA, World Fish Center Quarterly. 27(12)*:55-59.

Ramachandran, S., Bairagi, A. and Ray, A. K. (2005). Improvement of nutritive value of grass pea (*Lathyrus sativus*) seed meal in the formulated diets for rohu, *Labeo rohita* (Hamilton), fingerlings after fermentation with a fish gut bacterium. *Bio-resource Technology*, 96(13): 1465-1472.

Ravindran V, Ravindran G (1988). Nutritional and Antinutritional characteristics of Mucuna bean seeds. J. Sci. Food Agric., 46: 71-79.

Richter N, Siddhuraju P, Becker K (2003). Evaluation of nutritional quality of *Moringa* (*Moringa oleifera* Lam.) leaves as alternative protein source for tilapia (*Oreochromis niloticus* L.). Aquaculture. 217:599-611.

Sahaji P.S, (2011). Acute oral toxicity *of Mucuna pruriens* in albino mice. *Int. Res. J. Pharm.* 2(5):162-163.

Sathiyanarayanan, L., Arulmozhi, S., (2007). *Mucuna pruriens.* A comprehensive review. Pharmacognosy Rev., 1, 157-162

Siddhuraju, P. and Becker, K. (2003). Comparative nutritional evaluation of differently processed mucuna seeds (*Mucuna pruriens* (L.) on growth performance, feed utilization and body composition of Nile tilapia (*Oreochromis niloticus*). *Aquaculture Research*, 34(6): 256.

Siddhuraju P, Becker K, Makkar H.P.S, (2000). Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an underutilized tropical legume, *Mucuna pruriens* var. utilis. *J. Agric. Food Chem.*, 48: 6048-6060.

Tacon, A.G.J. (1995). Fishmeal replacers review of anti-nutritional factors within oilseeds and pulses-a limiting factor for the aquaculture green revolution paper presented at the feed ingredients Asia 95 conference 19-21 September 1995, Singapore International Convention and Exhibition Centire Singapore, Turret Group PLC (UK), Conference Proceedings, PP.23-48.

Tanzania's Trade with PTA Countries: A Special Emphasis on Non-Traditional Products by Flora Mndeme Musonda, Research Paper 31.

Taylor D.S., 2001, Physical variability and fluctuating asymmetry in heterozygous and homozygous populations of *Rivulus marmoratus*, Can. J. Zool., 79: 766–778 https://doi.org/10.1139/z01-038

Tuleun CD, Patrick J.P, Tiamiyu LO (2009). Evaluation of Raw and Boiled Velvet Bean (*Mucuna utilis*) as Feed Ingredient for Broiler Chickens. *Pakistan J. Nutr.*, 8(5): 601-606.

Tuleun C.D, Igba .F, (2008). Growth and carcass characteristics of broiler chickens fed water soaked and cooked velvet bean (*Mucuna utilis*) meal. *Afr. J. Biotechnol.* Vol. 7(15): 2676-2681.

Tripathi, Y.B., Updhyay, A.K., 2001. Antioxidant property of *Mucuna pruriens* Linn. *Curr. Sci.*, 80, 1377-1378.

Udensi .E.A, Onwuka .G.I, Okoli .E.G., (2004). Effect of processing on the levels of some antinational factors in *Mucuna utilis, Plant Production Resources Journal.* 8(1): 1-6.

Ukachukwu SA, Ezeagu IE, Tarowali G, Ikeorgu J.E.C. (2002). Utilization of *Mucuna* as food and feed in West Africa pp 189 – 217 in Food and Feed from *Mucuna, Cument* Uses and the way forward proceedings of an International workshop.

Ukachukwu SN, Obioha F.C, (1997). Chemical evaluation of *Mucuna cochinchinensis* as alternative feed stuff. *J. Appl. Chem Agric.Res.*4:43-48.

Umberto Q (2000). CRC World Dictionary of Plant Names. 3 M-Q. CRC Press. p. 1738.

Vadivel V, Janardhanan K (2001). Diversity in nutritional composition of wild jack bean (*Canavalia ensiformis* L. DC.) seeds located from south India. Food Chem., 74: 507-511.

Vadivel, V., Pugalenthi, M., 2008. Removal of antinutritional/toxic substances and improvement in the protein digestibility of velvet bean seeds during various processing methods. *J. of Food Sci. and Technol.*, 45, 242-246.

Wedemeyer G. A, Yasutake W.T. (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. United States Fish and Wildlife Service Technical. Pp 89.

Woke, G. N., Aleleye-Wokoma, I. P., Komi, G. W., and Bekibele, D. O. (2012), Effect of fermented and unfermented Mucuna bean seed, on growth performance of tilapia, *Global Journal of Pure and Applied Sciences* 1(9), 9-15.

APPENDICES

Appendix 1: Proximate composition of fish fed experimental diet

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
CRUDEP	CTRL	3	56.6500	.04359	.02517	56.5417	56.7583	56.60	56.68
	MSM(25)	3	55.3767	.17786	.10269	54.9348	55.8185	55.22	55.57
	MSM(50)	3	55.6733	.41968	.24230	54.6308	56.7159	55.20	56.00
	MSM(75)	3	54.7000	.34220	.19757	53.8499	55.5501	54.31	54.95
	MSM(100)	3	51.5167	.02082	.01202	51.4650	51.5684	51.50	51.54
	Total	15	54.7833	1.82393	.47094	53.7733	55.7934	51.50	56.68
LIPID	CTRL	3	5.9733	.02517	.01453	5.9108	6.0358	5.95	6.00
	MSM(25)	3	7.2433	.03055	.01764	7.1674	7.3192	7.21	7.27
	MSM(50)	3	8.2300	.22338	.12897	7.6751	8.7849	7.98	8.41
	MSM(75)	3	7.7033	.34790	.20086	6.8391	8.5676	7.45	8.10
	MSM(100)	3	7.9967	.22279	.12863	7.4432	8.5501	7.74	8.14
	Total	15	7.4293	.84608	.21846	6.9608	7.8979	5.95	8.41
CRUDEF	CTRL	3	9.8200	.02000	.01155	9.7703	9.8697	9.80	9.84
	MSM(25)	3	13.0600	.06083	.03512	12.9089	13.2111	12.99	13.10
	MSM(50)	3	16.5933	.55194	.31866	15.2222	17.9644	16.25	17.23
	MSM(75)	3	15.6767	.19502	.11260	15.1922	16.1611	15.54	15.90
	MSM(100)	3	15.0367	.24846	.14345	14.4195	15.6539	14.75	15.19
	Total	15	14.0373	2.50283	.64623	12.6513	15.4234	9.80	17.23
ASH	CTRL	3	17.2067	.04041	.02333	17.1063	17.3071	17.17	17.25
	MSM(25)	3	16.0433	.11590	.06692	15.7554	16.3313	15.91	16.12
	MSM(50)	3	9.9833	.04726	.02728	9.8659	10.1007	9.93	10.02
	MSM(75)	3	14.5300	.19000	.10970	14.0580	15.0020	14.34	14.72
	MSM(100)	3	17.6100	.46893	.27074	16.4451	18.7749	17.11	18.04
	Total	15	15.0747	2.86527	.73981	13.4879	16.6614	9.93	18.04
MOISTURE	CTRL	3	10.3533	.00577	.00333	10.3390	10.3677	10.35	10.36
	MSM(25)	3	8.2767	.03512	.02028	8.1894	8.3639	8.24	8.31
	MSM(50)	3	9.5200	.22338	.12897	8.9651	10.0749	9.27	9.70
	MSM(75)	3	7.3900	.25534	.14742	6.7557	8.0243	7.17	7.67
	MSM(100)	3	7.8400	.27875	.16093	7.1476	8.5324	7.53	8.07
	Total	15	8.6760	1.14959	.29682	8.0394	9.3126	7.17	10.36

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
CRUDEP	Between Groups	45.920	4	11.480	175.428	.000
	Within Groups	.654	10	.065		
	Total	46.574	14			
LIPID	Between Groups	9.578	4	2.394	53.896	.000
	Within Groups	.444	10	.044		
	Total	10.022	14			
CRUDEF	Between Groups	86.881	4	21.720	265.853	.000
	Within Groups	.817	10	.082		
	Total	87.698	14			
ASH	Between Groups	114.390	4	28.597	523.189	.000
	Within Groups	.547	10	.055		
	Total	114.937	14			
MOISTURE	Between Groups	18.114	4	4.528	116.673	.000
	Within Groups	.388	10	.039		
	Total	18.502	14			

CRUDEP

Duncan

TRT	N	Subset for alpha = 0.05			
		1	2	3	4
MSM(100)	3	51.5167			
MSM(75)	3		54.7000		
MSM(25)	3			55.3767	
MSM(50)	3			55.6733	
CTRL	3				56.6500
Sig.		1.000	1.000	.186	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

LIPID

Duncan

TRT	N	Subset for alpha = 0.05			
		1	2	3	4
CTRL	3	5.9733			
MSM(25)	3		7.2433		
MSM(75)	3			7.7033	
MSM(100)	3			7.9967	7.9967
MSM(50)	3				8.2300
Sig.		1.000	1.000	.119	.205

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

CRUDEF

Duncan

TRT	N	Subset for alpha = 0.05				
		1	2	3	4	5
CTRL	3	9.8200				
MSM(25)	3		13.0600			
MSM(100)	3			15.0367		
MSM(75)	3				15.6767	
MSM(50)	3					16.5933
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

MOISTURE

Duncan

TRT	N	Subset for alpha = 0.05				
		1	2	3	4	5
MSM(75)	3	7.3900				
MSM(100)	3		7.8400			
MSM(25)	3			8.2767		
MSM(50)	3				9.5200	
CTRL	3					10.3533
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ASH

Duncan

TRT	N	Subset for alpha = 0.05			
		1	2	3	4
MSM(50)	3	9.9833			
MSM(75)	3		14.5300		
MSM(25)	3			16.0433	
CTRL	3				17.2067
MSM(100)	3				17.6100
Sig.		1.000	1.000	1.000	.061

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 2: Effect of Mucuna seed meal (MSM) based diet on growth performance of *Clarias gariepinus* fingerlings.

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
INITIALWEIGHT	CTRL	3	7.1400	.02646	.01528	7.0743	7.2057	7.12	7.17
	MSM25	3	6.8167	.31086	.17947	6.0445	7.5889	6.52	7.14
	MSM50	3	7.0133	.20306	.11724	6.5089	7.5178	6.78	7.15
	MSM75	3	6.7300	.55381	.31974	5.3543	8.1057	6.10	7.14
	MSM100	3	6.7600	.22869	.13204	6.1919	7.3281	6.50	6.93
	Total	15	6.8920	.31303	.08082	6.7186	7.0654	6.10	7.17
FEEDINTAKE	CTRL	3	8.1700	.13000	.07506	7.8471	8.4929	8.02	8.25
	MSM25	3	6.4400	.28513	.16462	5.7317	7.1483	6.15	6.72
	MSM50	3	5.3900	.47634	.27502	4.2067	6.5733	5.11	5.94
	MSM75	3	6.0467	.67397	.38912	4.3724	7.7209	5.30	6.61
	MSM100	3	5.7000	.40150	.23180	4.7026	6.6974	5.28	6.08
	Total	15	6.3493	1.07407	.27732	5.7545	6.9441	5.11	8.25
FINALWEIGHT	CTRL	3	15.3933	.61582	.35554	13.8636	16.9231	14.76	15.99
	MSM25	3	13.4967	.23587	.13618	12.9107	14.0826	13.25	13.72
	MSM50	3	11.6467	.96692	.55825	9.2447	14.0486	10.62	12.54
	MSM75	3	11.3033	.92522	.53418	9.0050	13.6017	10.39	12.24
	MSM100	3	10.6767	.89030	.51401	8.4650	12.8883	10.02	11.69
	Total	15	12.5033	1.90085	.49080	11.4507	13.5560	10.02	15.99
WEIGHTGAIN	CTRL	3	8.2533	.59702	.34469	6.7703	9.7364	7.63	8.82
	MSM25	3	6.6800	.08660	.05000	6.4649	6.8951	6.58	6.73
	MSM50	3	4.6333	.77565	.44782	2.7065	6.5602	3.84	5.39
	MSM75	3	4.5733	.45654	.26359	3.4392	5.7075	4.29	5.10
	MSM100	3	3.9167	1.10821	.63983	1.1637	6.6696	3.17	5.19
	Total	15	5.6113	1.77056	.45716	4.6308	6.5918	3.17	8.82
ADG	CTRL	3	.1500	.01000	.00577	.1252	.1748	.14	.16
	MSM25	3	.1200	.00000	.00000	.1200	.1200	.12	.12
	MSM50	3	.0833	.01528	.00882	.0454	.1213	.07	.10
	MSM75	3	.0833	.00577	.00333	.0690	.0977	.08	.09
	MSM100	3	.0700	.01732	.01000	.0270	.1130	.06	.09
	Total	15	.1013	.03204	.00827	.0836	.1191	.06	.16
PER	CTRL	3	.2067	.01528	.00882	.1687	.2446	.19	.22
	MSM25	3	.1667	.00577	.00333	.1523	.1810	.16	.17
	MSM50	3	.1167	.01528	.00882	.0787	.1546	.10	.13
	MSM75	3	.1167	.01155	.00667	.0880	.1454	.11	.13
	MSM100	3	.0967	.02887	.01667	.0250	.1684	.08	.13
	Total	15	.1407	.04415	.01140	.1162	.1651	.08	.22

FCR	CTRL	3	.9967	.07371	.04256	.8136	1.1798	.94	1.08
	MSM25	3	.9633	.04726	.02728	.8459	1.0807	.91	1.00
	MSM50	3	1.1767	.13279	.07667	.8468	1.5065	1.10	1.33
	MSM75	3	1.3233	.11676	.06741	1.0333	1.6134	1.22	1.45
	MSM100	3	1.5400	.45044	.26006	.4210	2.6590	1.02	1.81
	Total	15	1.2000	.28906	.07464	1.0399	1.3601	.91	1.81
FER	CTRL	3	101.0433	7.49489	4.32718	82.4250	119.6617	92.60	106.91
	MSM25	3	103.8667	4.90787	2.83356	91.6748	116.0585	100.15	109.43
	MSM50	3	85.7000	9.13959	5.27674	62.9960	108.4040	75.15	91.21
	MSM75	3	75.9067	6.51111	3.75919	59.7322	92.0812	68.86	81.70
	MSM100	3	69.7433	24.73256	14.27935	8.3043	131.1824	55.17	98.30
	Total	15	87.2520	17.62213	4.55001	77.4932	97.0108	55.17	109.43
SGR	CTRL	3	.2800	.02000	.01155	.2303	.3297	.26	.30
	MSM25	3	.2300	.00000	.00000	.2300	.2300	.23	.23
	MSM50	3	.1500	.01732	.01000	.1070	.1930	.13	.16
	MSM75	3	.1500	.03000	.01732	.0755	.2245	.12	.18
	MSM100	3	.1033	.00577	.00333	.0890	.1177	.10	.11
	Total	15	.1827	.06745	.01742	.1453	.2200	.10	.30
RGR	CTRL	3	215.5767	8.05998	4.65343	195.5546	235.5988	207.01	223.01
	MSM25	3	198.1667	5.59129	3.22813	184.2771	212.0562	192.16	203.22
	MSM50	3	165.9000	9.37194	5.41089	142.6188	189.1812	156.64	175.38
	MSM75	3	168.0200	4.98410	2.87757	155.6388	180.4012	162.30	171.43
	MSM100	3	158.3267	18.68894	10.79006	111.9008	204.7526	146.21	179.85
	Total	15	181.1980	24.37534	6.29368	167.6994	194.6966	146.21	223.01
SURVIVALRATE	CTRL	3	100.0000	.00000	.00000	100.0000	100.0000	100.00	100.00
	MSM25	3	90.0000	.00000	.00000	90.0000	90.0000	90.00	90.00
	MSM50	3	93.3333	5.77350	3.33333	78.9912	107.6755	90.00	100.00
	MSM75	3	90.00 00	10.00000	5.77350	65.1586	114.8414	80.00	100.00
	MSM100	3	93.3333	5.77350	3.33333	78.9912	107.6755	90.00	100.00
	Total	15	93.3333	6.17213	1.59364	89.9153	96.7513	80.00	100.00

ANOVA

INITIALWEIGHT	Between Groups	.377	4	.094	.946	.477
	Within Groups	.995	10	.100		
	Total	1.372	14			
FEEDINTAKE	Between Groups	14.270	4	3.567	18.965	.000
	Within Groups	1.881	10	.188		
	Total	16.151	14			
FINALWEIGHT	Between Groups	44.548	4	11.137	18.448	.000
	Within Groups	6.037	10	.604		
	Total	50.585	14			
WEIGHTGAIN	Between Groups	39.084	4	9.771	20.338	.000
	Within Groups	4.804	10	.480		
	Total	43.888	14			
ADG	Between Groups	.013	4	.003	24.450	.000
	Within Groups	.001	10	.000		
	Total	.014	14			
PER	Between Groups	.024	4	.006	20.761	.000
	Within Groups	.003	10	.000		
	Total	.027	14			
FCR	Between Groups	.686	4	.172	3.547	.048
	Within Groups	.484	10	.048		
	Total	1.170	14			
FER	Between Groups	2711.780	4	677.945	4.144	.031
	Within Groups	1635.773	10	163.577		
	Total	4347.554	14			
SGR	Between Groups	.060	4	.015	46.245	.000
	Within Groups	.003	10	.000		
	Total	.064	14			
RGR	Between Groups	7201.844	4	1800.461	16.128	.000
	Within Groups	1116.354	10	111.635		
	Total	8318.198	14			
SURVIVALRATE	Between Groups	200.000	4	50.000	1.500	.274
	Within Groups	333.333	10	33.333		
	Total	533.333	14			

INITIALWEIGHT

Duncan

TRT	N	Subset for alpha = 0.05
		1
MSM75	3	6.7300
MSM100	3	6.7600
MSM25	3	6.8167
MSM50	3	7.0133
CTRL	3	7.1400
Sig.		.174

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

FEEDINTAKE

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
MSM50	3	5.3900		
MSM100	3	5.7000	5.7000	
MSM75	3	6.0467	6.0467	
MSM25	3		6.4400	
CTRL	3			8.1700
Sig.		.107	.074	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

WEIGHTGAIN

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
MSM100	3	3.9167		
MSM75	3	4.5733		
MSM50	3	4.6333		
MSM25	3		6.6800	
CTRL	3			8.2533
Sig.		.254	1.000	1.000

SURVIVALRATE

Duncan

TRT	N	Subset for alpha = 0.05
		1
MSM25	3	90.0000
MSM75	3	90.0000
MSM50	3	93.3333
MSM100	3	93.3333
CTRL	3	100.0000
Sig.		.080

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

FCR

Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
MSM25	3	.9633	
CTRL	3	.9967	
MSM50	3	1.1767	1.1767
MSM75	3	1.3233	1.3233
MSM100	3		1.5400
Sig.		.091	.082

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

FER

Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
MSM100	3	69.7433	
MSM75	3	75.9067	
MSM50	3	85.7000	85.7000
CTRL	3		101.0433
MSM25	3		103.8667
Sig.		.175	.127

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

SGR

Duncan

TRT	N	Subset for alpha = 0.05			
		1	2	3	4
MSM 100	3	.1033			
MSM 75	3		.1500		
MSM 50	3		.1500		
MSM 25	3			.2300	
CTRL	3				.2800
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

RGR
Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
MSM100	3	158.3267	
MSM50	3	165.9000	
MSM75	3	168.0200	
MSM25	3		198.1667
CTRL	3		215.5767
Sig.		.309	.071

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 3: Haematology analysis of *Clarias gariepinus* fingerlins fed *Mucuna utilis*

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
WBC	CTRL	3	2933.3333	642.91005	371.18429	1336.2562	4530.4104	2200.00	3400.00
	MSM(25)	3	4000.0000	200.00000	115.47005	3503.1725	4496.8275	3800.00	4200.00
	MSM(50)	3	5000.0000	200.00000	115.47005	4503.1725	5496.8275	4800.00	5200.00
	MSM(75)	3	7000.0000	435.88989	251.66115	5917.1895	8082.8105	6500.00	7300.00
	MSM(100)	3	13633.3333	1656.30110	956.26589	9518.8533	17747.8134	11900.00	15200.00
	Total	15	6513.3333	3999.26184	1032.60497	4298.6159	8728.0507	2200.00	15200.00
NEUT	CTRL	3	27.6667	2.51661	1.45297	21.4151	33.9183	25.00	30.00
	MSM(25)	3	28.0000	2.00000	1.15470	23.0317	32.9683	26.00	30.00
	MSM(50)	3	23.6667	1.52753	.88192	19.8721	27.4612	22.00	25.00
	MSM(75)	3	36.0000	1.00000	.57735	33.5159	38.4841	35.00	37.00
	MSM(100)	3	29.0000	2.64575	1.52753	22.4276	35.5724	27.00	32.00
	Total	15	28.8667	4.48596	1.15827	26.3824	31.3509	22.00	37.00
LYMP	CTRL	3	72.3333	2.51661	1.45297	66.0817	78.5849	70.00	75.00
	MSM(25)	3	72.0000	2.00000	1.15470	67.0317	76.9683	70.00	74.00
	MSM(50)	3	76.3333	1.52753	.88192	72.5388	80.1279	75.00	78.00
	MSM(75)	3	64.0000	1.00000	.57735	61.5159	66.4841	63.00	65.00
	MSM(100)	3	73.3333	1.52753	.88192	69.5388	77.1279	72.00	75.00
	Total	15	71.6000	4.50079	1.16210	69.1075	74.0925	63.00	78.00
PCV	CTRL	3	41.6667	3.05505	1.76383	34.0775	49.2558	39.00	45.00
	MSM(25)	3	34.6667	2.51661	1.45297	28.4151	40.9183	32.00	37.00
	MSM(50)	3	35.0000	1.00000	.57735	32.5159	37.4841	34.00	36.00
	MSM(75)	3	30.0000	1.00000	.57735	27.5159	32.4841	29.00	31.00
	MSM(100)	3	27.3333	2.08167	1.20185	22.1622	32.5045	25.00	29.00
	Total	15	33.7333	5.37809	1.38862	30.7550	36.7116	25.00	45.00
RBC	CTRL	3	2.6667	.25166	.14530	2.0415	3.2918	2.40	2.90
	MSM(25)	3	2.6000	.10000	.05774	2.3516	2.8484	2.50	2.70
	MSM(50)	3	2.5000	.10000	.05774	2.2516	2.7484	2.40	2.60
	MSM(75)	3	2.4667	.40415	.23333	1.4627	3.4706	2.10	2.90
	MSM(100)	3	1.7333	.20817	.12019	1.2162	2.2504	1.50	1.90
	Total	15	2.3933	.40438	.10441	2.1694	2.6173	1.50	2.90
HB	CTRL	3	13.8889	1.01835	.58794	11.3592	16.4186	13.00	15.00
	MSM(25)	3	11.5556	.83887	.48432	9.4717	13.6394	10.67	12.33
	MSM(50)	3	11.6667	.33333	.19245	10.8386	12.4947	11.33	12.00
	MSM(75)	3	10.0000	.33333	.19245	9.1720	10.8280	9.67	10.33
	MSM(100)	3	9.1111	.69389	.40062	7.3874	10.8348	8.33	9.67
	Total	15	11.2444	1.79270	.46287	10.2517	12.2372	8.33	15.00

MCHC	CTRL	3	33.3333	.00000	.00000	33.3333	33.3333	33.33	33.33
	MSM(25)	3	33.3333	.00000	.00000	33.3333	33.3333	33.33	33.33
	MSM(50)	3	33.3333	.00000	.00000	33.3333	33.3333	33.33	33.33
	MSM(75)	3	33.3333	.00000	.00000	33.3333	33.3333	33.33	33.33
	MSM(100)	3	33.3333	.00000	.00000	33.3333	33.3333	33.33	33.33
	Total	15	33.3333	.00000	.00000	33.3333	33.3333	33.33	33.33
MCH	CTRL	3	5.2648	.90096	.52017	3.0267	7.5029	4.48	6.25
	MSM(25)	3	4.4457	.30125	.17393	3.6974	5.1941	4.10	4.67
	MSM(50)	3	4.6681	.05343	.03085	4.5354	4.8008	4.62	4.72
	MSM(75)	3	4.1402	.79398	.45841	2.1679	6.1126	3.33	4.92
	MSM(100)	3	5.2762	.24683	.14251	4.6630	5.8893	5.09	5.56
	Total	15	4.7590	.66715	.17226	4.3895	5.1285	3.33	6.25
MCV	CTRL	3	157.9449	27.02870	15.60503	90.8019	225.0879	134.48	187.50
	MSM(25)	3	133.3713	9.03746	5.21778	110.9210	155.8216	123.08	140.00
	MSM(50)	3	140.0427	1.60299	.92549	136.0607	144.0248	138.46	141.67
	MSM(75)	3	124.2063	23.81944	13.75216	65.0356	183.3771	100.00	147.62
	MSM(100)	3	158.2846	7.40484	4.27519	139.8900	176.6792	152.63	166.67
	Total	15	142.7700	20.01441	5.16770	131.6864	153.8536	100.00	187.50

WBC

Duncan

TRT	N	Subset for alpha = 0.05			
		1	2	3	4
CTRL	3	2933.3333			
MSM(25)	3	4000.0000	4000.0000		
MSM(50)	3		5000.0000		
MSM(75)	3			7000.0000	
MSM(100)	3				13633.3333
Sig.		.146	.170	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

NEUT

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
MSM(50)	3	23.6667		
CTRL	3		27.6667	
MSM(25)	3		28.0000	

MSM(100)	3		29.0000	
MSM(75)	3			36.0000
Sig.		1.000	.461	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

LYMP

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
MSM(75)	3	64.0000		
MSM(25)	3		72.0000	
CTRL	3		72.3333	
MSM(100)	3		73.3333	73.3333
MSM(50)	3			76.3333
Sig.		1.000	.404	.067

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

PCV

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
MSM(100)	3	27.3333		
MSM(75)	3	30.0000		
MSM(25)	3		34.6667	
MSM(50)	3		35.0000	
CTRL	3			41.6667
Sig.		.151	.850	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

RBC

Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
MSM(100)	3	1.7333	
MSM(75)	3		2.4667

MSM(50)	3		2.5000
MSM(25)	3		2.6000
CTRL	3		2.6667
Sig.		1.000	.365

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

MCH

Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
MSM(75)	3	4.1402	
MSM(25)	3	4.4457	4.4457
MSM(50)	3	4.6681	4.6681
CTRL	3		5.2648
MSM(100)	3		5.2762
Sig.		.300	.124

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

MCV

Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
MSM(75)	3	124.2063	
MSM(25)	3	133.3713	133.3713
MSM(50)	3	140.0427	140.0427
CTRL	3		157.9449
MSM(100)	3		158.2846
Sig.		.300	.124

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 4: Water quality parameters of experimental unit.

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Temperature	CTRL	3	28.6000	.34641	.20000	27.7395	29.4605	28.20	28.80
	MSM25	3	28.8667	.05774	.03333	28.7232	29.0101	28.80	28.90
	MSM50	3	28.7667	.05774	.03333	28.6232	28.9101	28.70	28.80
	MSM75	3	29.0000	.20000	.11547	28.5032	29.4968	28.80	29.20
	MSM100	3	29.0000	.10000	.05774	28.7516	29.2484	28.90	29.10
	Total	15	28.8467	.22318	.05762	28.7231	28.9703	28.20	29.20
DissolvedO	CTRL	3	4.5000	.10000	.05774	4.2516	4.7484	4.40	4.60
	MSM25	3	4.3667	.05774	.03333	4.2232	4.5101	4.30	4.40
	MSM50	3	4.4567	.06028	.03480	4.3069	4.6064	4.40	4.52
	MSM75	3	4.1667	.20817	.12019	3.6496	4.6838	4.00	4.40
	MSM100	3	4.2333	.15275	.08819	3.8539	4.6128	4.10	4.40
	Total	15	4.3447	.17133	.04424	4.2498	4.4395	4.00	4.60
PH	CTRL	3	6.3333	.05774	.03333	6.1899	6.4768	6.30	6.40
	MSM25	3	6.4667	.15275	.08819	6.0872	6.8461	6.30	6.60
	MSM50	3	6.6000	.26458	.15275	5.9428	7.2572	6.40	6.90
	MSM75	3	6.5667	.25166	.14530	5.9415	7.1918	6.30	6.80
	MSM100	3	6.3000	.10000	.05774	6.0516	6.5484	6.20	6.40
	Total	15	6.4533	.19952	.05152	6.3428	6.5638	6.20	6.90

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Temperature	Between Groups	.344	4	.086	2.434	.116
	Within Groups	.353	10	.035		
	Total	.697	14			
DissolvedO	Between Groups	.244	4	.061	3.642	.044
	Within Groups	.167	10	.017		
	Total	.411	14			
PH	Between Groups	.217	4	.054	1.598	.249
	Within Groups	.340	10	.034		
	Total	.557	14			

Temperature

Duncan

TRT	N	Subset for alpha = 0.05	
		1	2
CTRL	3	28.6000	
MSM50	3	28.7667	28.7667
MSM25	3	28.8667	28.8667
MSM75	3		29.0000
MSM100	3		29.0000
Sig.		.128	.187

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

DissolvedO

Duncan

TRT	N	Subset for alpha = 0.05		
		1	2	3
MSM75	3	4.1667		
MSM100	3	4.2333	4.2333	
MSM25	3	4.3667	4.3667	4.3667
MSM50	3		4.4567	4.4567
CTRL	3			4.5000
Sig.		.100	.071	.256

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

PH

Duncan

TRT	N	Subset for alpha = 0.05
		1
MSM100	3	6.3000
CTRL	3	6.3333
MSM25	3	6.4667
MSM75	3	6.5667
MSM50	3	6.6000
Sig.		.097

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.