NUTRIENT UTILISATION AND GROWTH PERFORMANCE OF *CLARIAS GARIEPINUS* FED DIFFERENTLY PROCESSED *MUCUNA UTILIS* MEALS AS A REPLACEMENT FOR SOYBEAN-BASED DIET.

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A THESIS IN THE DEPARTMENT OF WILDLIFE AND FISHERIES MANAGEMENT SUBMITTED TO THE FACULTY OF AGRICULTURE AND FORESTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF IBADAN, NIGERIA.

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ABSTRACT

High cost of feed and competition between fish and other livestock's feed industries necessitate research into low cost, non-conventional feedstuffs for profitable fish farming. The feed potential of *Mucuna utilis* in this direction has not been documented. The feed potentials of *Mucuna utilis* and its effects on growth and biochemical parameters of the African catfish, *Clarias gariepinus* were therefore investigated.

Proximate, mineral composition and level of L-DOPA (3, 4-dihydroxylphenyalanine) in Raw *Mucuna* Meal (RMM), Cooked *Mucuna* Meal (CMM) and Toasted *Mucuna* Meal (TMM) were determined. These were used to substitute soybean meal at 0%, 10%, 20% and 30% inclusion levels in 40% crude protein isocaloric and isonitrogenous diets. *Clarias gariepinus* (mean weight 6.60 ± 1.09g) were randomly allotted to the 12 treatments in a 4 by 3 factorial experiment. The experiment was replicated thrice and the fish were fed twice daily at 5% body weight. The feeding trial lasted for 84 days during which growth and nutrient utilization parameters such as Mean Weight Gain (MWG), Specific Growth Rate (SGR), and Food Conversion Ratio (FCR) were measured. Packed Cell Volume (PCV), White Blood Cell (WBC), plasma glucose, plasma protein and albumin were determined. Also, histo-pathological evaluations of fish fed MSM supplemented diets were carried out. Data were analyzed using descriptive statistics and ANOVA.

Crude protein (29.2%), fat (0.7%), fibre (9.6%), Potassium (1.4%), Phosphorus (0.1%) and Iron (132.1mg/kg) were highest in RMM while CMM had the least values. The level of L-DOPA was highest (6.9%) in RMM and lowest in TMM (5.2%). All the fish responded positively to experimental diets with increase in growth ranging from 12.69 ± 2.10g in 30% RMM to 18.48 ± 3.9g in 10% CMM. The MWG (20.98 ± 5.19g), SGR (11.9%), and FCR (2.81

± 0.29) recorded in fish fed control diet (0% inclusion level) were higher but not significantly different from values obtained in fish fed diets 10% RMM and 10% TMM. Inclusion of Mucuna Seed Meal (MSM) in the diet beyond 20% in all treatments resulted in lower weight gain. Carcass protein increased in all except in fish fed 20% RMM. The PCV increased progressively from 27.0% in the control diet to 37.7% recorded in 30% inclusion level of CMM. Fish in 20% RMM had the highest WBC (8.20 X 10^3 ± 0.59mm^3) value while the lowest value (1.01 X 10^3 ± 1.29mm^3) was obtained in 20% inclusion level of TMM. There were no significant differences in the plasma glucose in all the treatments compared to the control value of 39.33 ± 9.24mg/l. Feeding MSM-based diets at 30% inclusion level in all the processed forms resulted in severe vacuolation of the hepatocytes of the liver and spongiosis of the white matter of the cerebellum of the brain.

Mucuna seed meals have good potential as feed ingredient in the diet of C*larias gariepinus*. Substitution of *Mucuna* seed meal as a replacement for soybean meal beyond 20% may lead to serious nutritional and health hazards to the fish.

Key words: Feedstuff, Mucuna seed meal, Soybean, *Clarias gariepinus*.

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This work is dedicated to the LORD JESUS CHRIST by whose GRACE I am redeemed from the power sin and the devil and to all those who have truly been saved by GRACE.

CERTIFICATION

I hereby certify that this research work was carried out by Mr. Jabez Bunmi OLASUNKANMI in the Department of Wildlife and Fisheries Management, University of Ibadan, Ibadan.

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CHAPTER ONE

INTRODUCTION

1.1 Population Growth and Food Crisis

Recent advances in technological development have enhanced rapid and self- sustaining economic growth in the developing countries. This has led to reduction in death rates without a corresponding reduction in birth rates, high population growth and pressure on available natural resources. Africa has about three quarters of a billion people (Bongaarts, 2002), its population has nearly doubled in the last 25 years and is expected to increase by at least 50 percent in the next 25 years. This growth in population has been and will be one of the principal causes of rising demand for food, water and other natural resources (Bongaarts, 2002).

In Nigeria, high population has resulted in increase pressure on available staple food sources and shortage of fertile land with adverse consequences on food security (Ezeagu, 1999). This has resulted in high incidence of hunger and malnutrition a situation in which children and women especially pregnant and lactating women are most vulnerable. There is much concern internationally that in the next decade population growth will outrun the world's capacity to produce food because of limited land, water and energy (Pinstrup-Anderson, 1994). Some researchers *(*Ezeagu *et al.*, 2002a, Ezeagu *et al.*, 2002b, Ukachukwu *et al.*, 2002*)* however believe that the rising demand can be satisfied by improved technology through a coordinated increase in both crop and animal production and fuller utilization of marginal lands and hitherto underutilized resources. One of such resources is fish which has a great role to play in enhancing food security.

1.2 Importance of fish as source of quality food

Data from various agricultural organizations in the world has shown that although fish is often excluded from projections of future food supply, even though it is an important source of food and it offers livelihood to large number of people especially from the developing world. Delgado *et al,(*2003) observed that fish is an important source of protein especially in the developing world. Fin fish provides around 16 percent of the animal protein consumed around the world. Moreover, fish accounts for about 20 percent of animal protein in low – income food deficit countries and 13 percent in the industrialized countries (Anderson, 2009).

In Nigeria, fish alone contributes, between 20 and 25 percent per caput animal protein intake (Omitoyin, 2007). FAO (1997) indicated that fish plays a major role in human nutrition by supplying one – third of the total animal protein intake in Asia. According to FAO (1992), fish is the cheapest protein source in the diet of the rural poor in the developing countries. FAO (2002) and Choo and Williams (2003) opined that, fish form an important component of the rapid growth of the animal products in developing countries over the past two decades. Some scholars (Bernacsek 1987, Lovell 1989, Hatch and Tai, 1997) believed that the dietary supply from fish is richer than that of beef, chicken and pork. Fish and shellfish are rich in polyunsaturated fatty acids (PUFA) and are essential for rapid brain development in humans as well as maintaining good health conditions (Inst. of Aqua., 2003). Fisheries occupies a very significant position in the primary sector providing employment for over a million people and contributing about 50% of the animal protein intake of the population, particularly the resource poor (Miller 2008, Ayinla 2009).

1.3 Fish Demand and Supply Potentials

The major sources of fish to the world are the oceans, lagoons, lakes and rivers. Supplementary fish production comes from aquaculture particularly fish farming. Aquaculture's contribution to

world food supply is gaining increasing importance, for instance, it contributed 16 percent of total world fin fish and shellfish landings in 1993 (Tacon, 1996), 31 per cent in 1997 (Delgado *et al 2003*), 43 per cent in 2006 (FAO 2008) and 46 per cent in 2008 (FAO, 2010). Global production of aquatic food totalled approximately 93.2 million metric tonnes (mmt) in 1997, out of which, capture fisheries supplied 64.5 mmt and aquaculture 28.6 mmt (Delgado *et al 2003*). Capture fisheries and aquaculture supplied the world with about 110 million tonnes of food fish in 2006 providing an apparent per capita supply of 16.7 kg (live weight equivalent), which is among the highest on record. Fish production in 2008 was about 142 million tonnes providing an estimated apparent per capita supply of about 17 kg (live weight equivalent) (FAO, 2010).

The current fish demand in Nigeria is estimated at about 2.1 million metric tonnes (mmt). The per capita fish consumption for the year 2007 was estimated at 8.90 kg The total domestic fish production (artisanal, industrial and aquaculture) accounts for about 615.507mmt, fish imports make up to about 740 mmt bringing the total fish supply to approximately 1.4mmt leaving a shortfall of about 650,000 mmt (Ayinla, 2009). The deficit can be supplied if the aquaculture sector is developed since the estimated potential production from the sector (aquaculture) exceeds the total demand. Aquaculture production is currently low due to a number of constraints which include, among others, high cost of fish feed, lack of capital, poor quality fish seed, unstable and defective government fisheries policies, poaching and poor marketing structure (Olasunkanmi 2001; Olasunkanmi *et al.*, 2010).

1.4 Statement of the Problem

Despite the huge potentials of fish farming, its contribution to total domestic fish production is low due to some problems. One of such problems is that of high cost of production input especially cost of fish feed. Experts have estimated that the cost of feed can account for as

much as 70 per cent of total cost of production in intensive fish farming (Omitoyin, 2007). Livestock and fish production also compete for the same feed ingredients leading to high cost of these conventional protein and energy sources. With the already existing food gap and expected population increases, the already heavy competition with farm animals for the available staples is almost certain to increase. The solution to the problem must be sought through a combination of all available sources. Balogun (1982) observed that there are resources in the tropical and sub-tropical world which must be scrutinized or processed for increased protein supply for human consumption and feed for the growing livestock and fish industries.

1.5 Justification for the Research

Increased fish production can be achieved by providing balanced and low cost fish rations. The keen competition for the available resources, especially soybean, by the feed millers and direct human consumption coupled with limited resources for production has imposed serious constraint on overall fish and livestock production in the country. The most logical way therefore to increase supply of feed ingredients would be to make more proteins available for human consumption and to search for and develop production of unconventional proteins sources for fish and livestock feed. This could both reduce the cost of fish and livestock production and release for human use protein feed that would otherwise have been consumed by fish and livestock. There is therefore an urgent need to source for alternative sources of feed ingredients in order to ameliorate the situation.

Mucuna is an important cover crop in many parts of the world (Ani, 2008). The foliage is frequently fed to grazing animals and the beans are sometimes eaten by humans and animals (Buckles, 1995; Camara *et al*., 2003; Muinga *et al.*, 2003). It is reported to contain sufficient protein to be used as substitute protein source in the food and feed industry and was

recommended as a potential food resource (Ezeagu 1997, Del Carmen 2000, Bressani 2002, Ukachukwu 2002, Iyayi and Taiwo 2003,). In Nigeria, *Mucuna* is cultivated in southern and middle belt states (Onweluzo and Eilitta, 2003). However, there is a low sustained interest in *Mucuna* cultivation due to its low utilization as food and feed, and subsequently the lack of market for the beans (Eilitta and Carskey, 2003). This is because the seed is known to contain a number of anti-nutritional factors (Ezeagu 1999, Carew *et al* 2001, Flores 2001) which limited its use. There is therefore the need to explore ways by which this plant could be put into better use. This calls for a multi-disciplinary research approach, thus, the need for this work.

1.6 Research Objectives

The general objective of this work therefore is to explore the feed potential of *Mucuna* seed in the nutrition of the African catfish, *Clarias gariepinus.*

The specific objectives are:

i. To assess the effect of processing on the nutritive value of *Mucuna* seed.

ii. To determine the effect of processed *Mucuna* seed meal diet on the growth of *Clarias gariepinus*.

iii. To determine the effect of processed *Mucuna* based diets on the haematological and histo-pathological parameters of *C. gariepinus*

CHAPTER TWO

LITERATURE REVIEW

2.1 Legumes as Food and Feed

The Leguminosae is one of the five largest families of flowering plants and is second only to the Gramineae in economic importance. It includes a number of familiar food products which are important sources of food for animals. The family Leguminosae has a potential of supplying increased vegetable protein that the world will need in future. In developing countries especially, cultivation of legumes is the best and quickest way to augment the production of plant proteins. Leguminous plants are found throughout the world, but the greatest variety grows in the tropics and subtropics. Of the thousands of known legume species, less than 20 are used extensively today. Those in common use include peanuts, soybeans, peas, lentils, pigeon peas, chick peas, mung peas, kidney beans, cowpeas, Lucerne, sweet clover, (*Melilitus* species), other clover (*Trifolium* species) and vetches (Turner 1959, NAS 1979).

Grain legumes are important and economical sources of protein, energy and other nutrients in the diets of most of the developing communities worldwide (Apata, 1990). They are the bedrock of national poultry and livestock industries. They play a central role in providing a better nutrition to a vast majority of people in the tropics where the insufficiency of proteins of animal origin. Legume seeds contain as high as 20 to 50 percent protein which in general runs well above twice the level found in cereal grains and significantly more than the levels in conventional root crops. The protein is high in lysine, a factor of much nutritional importance when legumes are combined with cereal proteins that have lower level of this amino acid. Grain legumes such as common beans, lentils and kidney beans represent the main supplementary

protein source in cereal and starchy food based diets of the large population in developing countries (Bressani, 2002). Furthermore, grain legumes contain high level of dietary fibre and phyto-chemicals (phyto-nutrients) which may be closely associated with health promotion (Ezeagu, 1997).

The utilization of grain legumes is limited by two main constraints: technological and nutritional (Egounlaty 1994). In general, the processing of grain legumes is energy- and time-consuming.- as compared to cereal, root and tuber processing. In Africa, the processing techniques and the long cooking time of grain legumes are very important and liable to affect their utilization (Doughty and Oraca-Tetteh, 1966). A hasty and defective cooking makes legumes indigestible thereby reducing their consumption. The cooking time of grain legumes varies not only among varieties and even the same cultivars but also depend on the moisture, the storage time and the cooking conditions such as the nature of cooking water, the treatments applied to grains before cooking and on the fire-kettle unit (Molina *et al.,* 1975)

Legumes have been an important crop ever since man started domesticating plants and have been part of our cultural heritage (Crepso, 1991). The reasons for the cultivation of pulses are many: (1) grain legumes have a low water content and impervious seed coat features valuable during transportation and storage. (2) They can easily be cultivated and mature rapidly. (3) They have an important place in the crop rotation. (4) The production per acre is higher for pulses than for cereals. (5) The roots of many legumes contain nitrogen fixing bacteria capable of using free atmospheric nitrogen to produce nitrates and nitrites which can be readily used by plants, thus augmenting their supply of nitrogenous materials. In addition to the major cultivated edible legumes soybean, peanuts, groundnut, peas and beans, there are more than 50 minor tropical legumes that have received little scientific attention (mainly because research efforts

concentrated on more conventional species (NAS, 1975). The knowledge acquired on grain legumes production and processing and the need for nutrients by the ever greater number of people indicate the need to increase the number of resources such as Mucuna and other beans to feed men.

Many of the legumes possess multiple uses as food, fodder and pharmaceuticals. Some legume seeds are known to possess anti-cancerous compounds that retard or arrest the cancer growth (Sridhar and Bhat, 2007). A wide variety of legumes have been assessed either as alternatives to fish meal or soybean, most of which are of limited relevance in human nutrition (Alegbeleye, 2005). Of all plant protein sources, soybean *(Glycine max*) has received the most attention as dietary plant protein sources in livestock feed. Soybean has high protein and fat content and contains a good mix of essential amino acid. However, soybean contains anti-nutritive factors which have to be deactivated before feeding to monogastric animals like fish, poultry and pigs. Heat processing or extrusion is a common processing method. Other processing methods involve de-oiling or oil extraction through solvent or mechanical means. Extrusion produces full fat soybean meal, a product that has received the considerable attention. However the extensive use of soybean in livestock feeds is limited by decline in productivity and the various uses it is put to in human nutrition especially in infant formula (FAO, 2001).

2.1.1 Origin and History of Soybean

Soybean (*Glycine* spp) originated from China and was domesticated there (Nagata 1960, Hymowitz 1970, Probst and Judd, 1973, Ma and Zhang, 1983). Long before the dawn of civilization, primitive men of China subsisted on wild soybeans (Beaten, 1991). The present *Glycine max* is the derivative of *Glycine ussuriensis*, as this wild species is still found in China, Korea, Manchina, and some part of Japan (Morse, 1950). However, Hymowitz (1970) was of the

opinion that historical and geographical evidence points to the eastern half of North China as the area where Soybean was first domesticated around the eleventh century BC. According to Beaten (1991), the first mention of Soybean in US literature was in 1804. It was initially used exclusively as a forage crop, but then become increasingly important as an oil-seed crop. Demand for oil during the Second World War stimulated the crushing of Soybean for oil and meal.

Soybean was introduced to Nigeria as early as 1908 although its establishment as a crop can be attributed to the introduction and testing of varieties by the Institute for Agricultural Research (IAR), Zaria, beginning from 1930. The variety which was introduced from Malaysia in 1937 became virtually the sole variety in the Nigeria's soybean growing regions and has remained so up to the present time (Nyiakura, 1980). Before the civil war (1967- 1970), all the soybeans produced in the country was exported, however, increased production in recent years has been consumed internally. Soybean is used primarily as food and feed ingredient for livestock and fish.

Soybean meal is a principal protein source in the diet of poultry and swine all over the world. It is a by-product of agricultural products which must have been processed before use in the preparation of livestock feeds (Sotolu, 2008). Soybean meal is prepared by grinding the flakes after solvent extraction has been used to remove the oil from de-hulled soybeans. De-hulled, solvent-extracted soybean meal contains 48% protein and is the predominant protein source used in catfish feeds. It has the best amino acid profile of all common plant protein sources and is highly palatable and digestible to catfish. Anti-nutritional factors are destroyed or reduced to insignificant levels with heat that is applied during the extraction process. Levels of soybean meal up to 50% have been used in commercial catfish feeds without detrimental effect.

Full-fat soybean meal is prepared by grinding heated soybeans that have not undergone the oil extraction process. The meal contains 39% protein and 18% fat. It is rarely used in catfish feeds because of its high fat content. A limited amount can be used in catfish feeds as long as the total fat level in the finished feed does not exceed about 6%. Soybean meal contains variety of toxic substances including trypsin inhibitors haemagglutinins, Saponins and anti-vitamin A (Herman, 1970, Faris and Singh, 1990; Grimaud, 1988; Liener 1994b, D'Mello 2000, Francis *et al*., 2001). The heat labile toxic substances in raw soybeans meal are capable of depressing animal performance but are destroyed by adequate processing. Nevertheless, either under-heating or overheating of soyabean meal can be deleterious to the nutritional value of soybean and so should be avoided. The negative effect of soybean overheating has been attributed to the reduction in the available lysine or that the amino group of lysine in it becomes irreversibly bound to carbonyl group provided by reducing sugars (Grant *et al.,* 1991) while D'Mello (2000) reported that the negative effect was due to the denaturing of the proteolytic enzymes.

Decrease in chick growth was recorded when chicks were fed soya bean meal based diet processed by heating and it was stated further that solubility values of soya bean meal decreases with increase in heating time and that when protein solubility drops below 74%, nutritive value begins to be impaired (Liener, 1989).

2.1.2 Taxonomic Status of *Mucuna*

The genus *Mucuna* belongs to the family Fabaceae (leguminosae) and includes up to 150 species of annual and perennial legumes of pantropical distribution (Allen and Allen 1981, Eilitta *et al* 2002, Bachmann 2006). It is thought to originate from China, Malaysia or India. The most commonly cultivated species are vigorously growing, twining annual legumes. Several of the vinyl species are rated highly as soil renovators, cover crops, green manures and forages. The

barbed hairs or trichomes on the pods of several Mucuna species cause an intensive stinging irritation and itching. The substance responsible for the stinging and itching can be viewed as a member of the histamine-liberator groups similar to that in bee and snake venom. However, this itching property can be destroyed by boiling (Allen and Allen, 1981). Many common names are used for *Mucuna* (e.g. velvet bean, pica-pica, Bengal bean, Nescafe, Ojo de Venado, pois mascate, Kara benguk, Atmagupta (Kay,1979, Eillita *et al* 2002), Cow itch (cowhage),a corruption of Kwatch, a Hindi word meaning bad rubbing (Shelly and Arthur, 1955).

According to Eilitta *et al.* (2002), *Mucuna* is adapted to a wide range of environmental conditions contributing to its popularity as a green manure/cover crop. It grows best in warm humid conditions and at altitudes below 1600m.It is adapted to a wide variety of soils, typically nodules well and throughout its range has been relatively free from disease and pests. *Mucuna* can produce substantial biomass. Maasdorp *et al* (2002) reported a peak biomass of about 4 ton dry matter at a spacing of 11x 30cm^2. Smart (1990) reported that Mucuna is capable of producing prodigious yields of pods, seeds and forage. Gilbert (2002) reported that various authors working with Mucuna in Malawi recorded different yields.5700kgha^{-1} biomass,1770kgha^{-1} seed (Kuwenda and Gilbert 1998), 2130kgha^{-1} seed (Gilbert, 1986) 3200kgha^{-1} of Mucuna leaf litter fall (Gilbert, 1998). He claimed that Mucuna has consistently showed superior agronomic performance compared to other annual legumes in large scale on farm trials. *Mucuna pruriens* var. *utilis* is a strong growing pasture and fodder crop found growing in many places. The pods are glabrous ad pressed velvet or black woolly hairs. The pods are about 9.5-10cm long. The seeds are white or black with longest dimension 1.2-1.9cm, shorter 1-1.3cm and thickness 4.5-6.5mm, the aril sometimes separating easily .The flowers are dark purple or blue 3-3.5cm long in elongated racemes, often 2-3 together. The calyx tube is about 5-6

mm long. The leaves are thinly pressed pubescent with silvery straight hair below and glabrous above.

The roots are fleshy, usually well modulated and produced near the soil surface. The stems bear numerous, alternate, trifoliate leaves on short hairy fleshly petioles with large ovate leaflets they are glabrous and leaflets are about 10cm long x 8cm wide. The inflorescence is auxiliary and the flowers usually 5-30 are showy and purple, red or greenish yellow in colour. Pods are 9-14 cm long, hard, curved, slightly ridged and covered with soft black, white or grey hairs which give them a velvety appearance. The pods contain 4-6 seeds, globular, approximately 1.2x1.2cm, often a molted brown or black colour, sometimes with a pale-grey background; a few cultivars produce pure grey, white or black seed. The funicular hilum is about 4mm long and is surrounded by a distinctive white aril. The seed coat is hard, thick and glossy. St-Laurent *et al* (2002) quoting Prain (1897) classified *Mucuna* into two sub-genera: *Mucuna* (Adams) and Stizolobium. The subgenus *Mucuna* has among other characteristics, large discoid seeds with a long hilum over about three-quarters of the seed circumference and trifoliate first leaves, similar to the mature plant's leaves.

Sub- genus Stizolobium possesses small reniform seeds with a short hilum and entire first leaves. Sub-genus *Mucuna* is also divided into two sections as suggested by Weight and Walker-Arnott (1834). *Mucuna* species is an important cover crop (or green manure crop) in many parts of the world, especially among subsistence farmers (Carew *et al.* 2002). Buckles (1995) opined that its notable resistance to insects, weeds and drought as well as its nitrogen-fixing properties makes it ideal for use in low input agriculture. Ezeagu *et al* (2003) identified 12 varieties of *Mucuna* in Nigeria (Table 2.1) and reported that there is relatively large variability in the plant. However the authors could not tell whether such variability is at the species, sub-species or

variety level. Sridhar and Bhat (2007 reported that) *Mucuna* grown in Taiwan consists of three species and one variety. In Malaysia, *M. bracteata* is frequently planted in large plantations and small holdings of oil palm and rubber as cover crops along with some of the other legumes.

Table 1: Physical Characteristics of Seeds of 12 *Mucuna* Accessions from Nigeria.

Characteristics	M. utilis	M. cochinensis	M. veracruz	M. Georgia	M. rajada	M. Ghana	M. preta	M. jaspeada
Colour	Black	White	White, Black, Mottled	Black	White	Black	Black	White
Seed shape	Flat/Varied	Flat/Short helium	Ecliptic/short helium	Aried	Round	Rounnd/short helium	Varied.	Ecliptic/sho helium
Kernel Dimension (mm)								
Length[a]	13.80±1.64	14.71±2.04	14.16±1.71	13.90±1.13	10.10±0.59	13.81±1.96	14.36±0.74	14.93±1.72
Breadth[a]	10.50±0.56	10.37±0.91	10.71±0.93	10.53±0.75	8.77±0.75	10.16±0.89	10.67±0.46	10.86± 0.78
Depth (thickness)[a]	6.47±0.59	6.23±0.66	7.18±0.48	6.98±0.36	7.49±0.34	7.32±0.37	6.54±0.59	7.03±0.41
Hull content (%)[a]	14.98±0.33	10.88±0.06	12.56±0.32	14.22±1.18	11.71±0.24	10.34±0.26	13.89±0.49	11.13±0.38
1000 Seed weight (g)[b]	609.05±39.45	802.12±32.20	693.26±50	784.20±24.60	573.18±31.2	783.32±3369	867.16±59.15	925.63±23
Bulk density (g/cm^3)[c]	0.82	0.73	0.94	0.86	0.97	0.93	0.85	0.71
True density (g/cm^3)[c]	2.26	1.23	1.46	1.31	1.92	1.41	1.42	2.37
Grain hardness(kg)*[d]	96.46±16.79	105.18±32.35	143.90±24.64	82.94±10.60	65.00±8.49	119.98±16.66	101.60±27.35	115.88±6.8
WHC g H_2Og^{-1} **	0.72	0.82	1.56	0.69	0.91	0.76	1.19	0.67

*100mm min^{-1} speed; **WHC Water Holding Capacity; a= mean±SD of ten independent determinations; b= mean±SD of five independent determinations; c= mean of two independent determinations; d= mean±SD of five independent determinations.

Source: Ezeagu *et al* (2003)

2.1.3 *Mucuna* as Food and Feed

Mucuna beans have been used more as a cover crop than as a food source for humans. This may explain in part why culinary practices associated with *Mucuna* are still poorly developed, why acceptability of the beans is limited and why chemical and nutritional information is yet forthcoming in comparison to that available for other edible grain legume crops of economic significance. However, Bunch (2002) reported the use of *Mucuna* as flour and coffee in Honduras. Diallo *et al* (2002) reported that a number of recipes were made from *Mucuna*. *Mucuna* is also known to have a lot of potential as an alternative protein source for livestock (Flores *et al* 2002).

Various authors have reported that it contains sufficient crude protein (ranging from 18-35) to be used as substitute protein source (Janarhanan and Lakshamann1985, Ezeagu, 1997, Iyayi and Egharevba 1998, Ahenkora *et al* 1999, Bressani, 2002, Ukachukwu 2002, Iyayi and Taiwo 2003, Del Carmen, 2000). Ezeagu *et al* (2003) reported that nutrient content of Mucuna is comparable to those in commonly consumed legumes and that the plant has potential for exploitation as food and feed. Report by Bressani (2002) showed that people in different countries of Asia and Africa have been consuming *Mucuna* regardless of their knowledge of its possible deleterious effects. Kay (1979) reported that Mucuna is regarded as a famine food in parts of Asia and Africa where it is eaten. Dako and Hill (1977) and Iyayi and Egharevba (1998) reported that seeds of *Mucuna* constitute source of food for tribes and ethnic groups of Asia and Africa. seed is also known to have similar amino acids profile as others legumes seed such as soybeans (Iyayi *et al* 2005, Sridhar and Bhat 2007). Table 2.2 shows the approximate values of different amino acids in both soybean and *M. pruriens* as reported by Sridhar and Bhat (2007). Adebooye and Philips (2006) opined that *Mucuna* can be a good source of essential amino acids for man and animals

consumption. Bressani (2002) reported lysine, threonine, valine, isoleucine, leucine and phenylalanine in *M. utilis* is more than the recommended standard by Food and Agriculture Organization (FAO) and World Health Organization of the United Nations.

2.1.4 Anti-nutritional Factors in *Mucuna.*

The use of plant-derived materials such as legume seeds, different types of oil seed cake leaf meals, leaf protein concentrates and root tuber meals as fish feed ingredients is limited by the presence of a wide variety of anti-nutritional substances (Francis *et al.*,2001). *Mucuna* seed is not an exception. It is known to contain several anti-nutritional factors such as 3,4-dihydroxylpenylalanine (L-DOPA), total free phenols, tannins, anti-vitamins, protease inhibitors, phytic acid, flatulence factors, saponins and hydrogen cyanide (Vadivel and Janadharnan 2000, Del-Carmen 2002). Other toxic compounds known to be present in Mucuna include nicotine, physositignine and serotonin (in Mucuna pod hairs) (Duke, 1981; Preakash and Misra, 1987), bufotenine, choline, N, N-dimethyltryptamine, two unidentified 5-oxy-indole-3-3alkylamines, an unidentified B-carboline (Ghosal *et al*., 1971), bufotenine (reduces cholinesterase enzyme).

Table 2: Amino acid composition of Soybean and *Mucuna* bean (mg/100g protein).

Amino acid	Soybean	*Pruriens*
Glutamic acid	16.90	17.23
Asparitic acid	11.30	8.16
Serine	5.67	4.10
/Threonine	3.76	3.64
Proline	4.86	ND
Alanine	4.23	2.81
Glycine	4.01	5.12
Valine	4.59	5.57
Cystine	1.70	0.84
Methionine	1.22	1.28
Isoleucine	4.62	4.12
Leucine	7.72	7.85
Tyrosine	1.24	4.76
Phenlalanine	4.84	3.85
Tryptophan	3.39	1.35
Lysine	6.08	6.60
Hisitidine	2.50	3.14
Arginine	7.13	7.16

Source: Sridhar and Bhat (2007)

mucunain (treats parasitic intestinal worms; pesticide), serotonin (reduces cholinesterase enzyme and intestinal gas; relaxes muscles; clotting agent) (Bauer, 1996).

2.1.4.1 L-DOPA

L-DOPA is a potentially neurologic agent found in relatively large amounts in velvet beans. Although written record of the effect of L-DOPA on fish is scanty or unavailable, it is known to have negative effects on the growth of swine and poultry (Carew *et al 2002, Flores et al* 2002, Iyayi and Taiwo 2003). L-DOPA is a non -protein amino acid and precursor to the neurotransmitter dopamine used in the treatment of Parkinson's disease. This compound confers the plant competitive advantages through allelopathy and disease resistance, however, it is also known to induce gastro-intestinal problems and neurological effects when consumed by humans or animals (Carew *et al 2002).* L-DOPA is converted to dopamine on ingestion; thus its ingestion resulted in significantly elevated levels of systemic dopamine. Most side effects are directly from dopamine's activity as a neurotransmitter involved in the regulation of the heart, vascular system, digestive tract, and excretory system, rather than from its well/known effect on receptors in the brain (Szabo and Tebbett, 2002). It has been recognized that the presence of L-DOPA in *Mucuna* is a major impediment to consider it as food or feed (Sridhar and Bhat, 2007). According to Iyayi and Taiwo (2003), processing can reduce the anti-nutrients. Various methods have been used to process *Mucuna* seeds with the primary aim of reducing L-DOPA content (Flores *et al* 2002, Del-Carmen 2002) with varying level of success. Most of the processing methods employed involved application of heat to eliminate or reduce the level of toxic and inhibitory substances; however detectable levels of some anti-nutrients will remain even after thermal treatment. Eilitta *et al* (2002) reported that variation in L-DOPA is dependent on the genetic make-up as well as geographic location. However, Capo-chichi *et al (*2003) worked on

36 accessions of *M. utilis* of Africa, America and India and inferred that L-DOPA is influenced mainly by genotype vs. accessions rather than genotype vs. environment.

2.1.4.2 Phenols.

Another anti-nutrient in *Mucuna* is free phenol compounds. These compounds have the ability to decrease digestibility by complexing with dietary proteins. They are also known to lower the activity of several digestive enzymes (Sridhar and Bhat 2007). Phenols also complex with iron and prevents its absorption (Brune *et al.* 1989, Hurrel *et al* 1999), reduce the absorption of nutrients like vitamin B_{12} and cause damage to mucosa of digestive tract (Liener, 1994a). Tannins belong to the family of high molecular weight phenols known to strongly bind the proteins and decrease the in vitro digestibility (Sathe and Salunkhe, 1984). Decortications of *Mucuna* seeds before using for formulation will be effective to reduce the concentration of tannins and other phenols since they are present in the seed coat than cotyledon (Sridhar and Bhat 2007).

2.1.4.3 Protease inhibitors

Protease inhibitors are compounds which suppress the proteolytic activity of digestive enzymes and they are found throughout the plant kingdom particularly among legumes. The best known of this group of antimetabolites are the trypsin inhibitors which inhibit the activity of the digestive enzyme, trypsin, secreted by the pancreas. Its presence cause considerable decrease in the digestibility of dietary protein due to the formation of irreversible trypsin and trypsin inhibitor complexes. Various levels of trypsin and chymotrypsin inhibitor have been reported in *Mucuna* harvested from various regions of the world (Ravindran and Ravindran 1988, Udedibie and Carlini 1998, Ezeagu *et al 2003*, Carew *et al* 2002, Del-Carmen *et a*l (2002). Egounlety (1994) reported that the activity of trypsin inhibitor is affected by some processes such as heat

treatment, soaking, germination , fermentation and de-hulling and that the extent to which it is destroyed in legumes is a function of temperature, duration of heating , particle size and moisture conditions. These factors are controlled closely in the commercial processing of soybean –oil meal in order to obtain a product with maximum nutritive value.

2.1.4.4 Lectins (Phyto-haemaglutinins)

Lectins are glycoproteins of 60,000-100,000 molecular weights that are known for their ability to agglutinate (clump) erythrocytes in vitro (Ezeagu, 1997, Cornell, 2008)). The terms phytohemagglutinins, phytagglutinins, and lectins are used interchangeably. Lectins-containing plants have been found in many botanical groups including mono- and dicotyledons, molds and lichens, but most frequently they have been detected in leguminoseae and euphorbiaceae. They may exist in various tissues of the same plant and have different cellular localizations and molecular properties (Cornell, 2008). They are found in most types of beans, including *Mucuna*.

Reduced growth, diarrhoea, and interference with nutrient absorption are caused by this class of toxicants (Cornell University, 2008). Large amounts of lectins can damage the heart, kidneys and liver, lower blood clotting ability, destroy the lining of the intestines, and inhibit cell division. Cooking neutralizes lectins to some extent, and digestive juices further destroy them. Raw black beans contain enough lectins to kill rats in one week.

Not much is known about the functions of lectins in the organism they are found. However, there is evidence that lectins may be involved in the recognition between cells or cells and various carbohydrate- containing molecules (Cornell University, 2008). This suggests that they may be involved in the regulating physiological functions. They seem to play an important role in the defence mechanisms of plants against the attack of microorganisms, pests, and insects. Fungal infection or wounding of the plant seems to increase lectins. In legumes, the role of

lectins in the recognition of nitrogen-fixing bacteria Rhizobium genus, which have sugar-containing substances, has received a special attention.

According to Cornell University (2008), pathological lesions occur in animals injected with kidney beans extracts. Various tissues suffer from parenchymatous, fatty degeneration, and oedema. In the liver local necrosis and fatty changes can be observed. Haemorrhages are observed in the stomach, the intestinal wall, and other organs. Distinctions of capillary vessels may be present in the Kidney and myocardial with numerous thrombi. Morphological changes in rats fed navy beans include: increased weight of kidney and heart, pancreatic atrophy, and fatty metamorphosis of the liver. Such changes may be attributed to the low availability of essential amino acids and low food intake of the animals consuming the raw bean diet. For example, rats fed raw kidney beans develop multiple histological lesions. Also, lectins from red kidney beans are found to induce small intestinal epithelial growth, crypt cell hyperplasia and DNA synthesis. Small amounts of isolated black bean agglutinin showed low food absorption and nitrogen retention rate.

2.1.4.5 Flatulence factors

Large-scale consumption of seeds of most of the legumes results in flatus. Oligosaccharides are known to be the main compounds responsible for flatus (Reddy and Salunkhe 1980). Such oligosaccharides cannot be hydrolyzed or absorbed in monogastric animals as they lack of a-1,6 galactosidase activity in the small intestine. Thus, microorganisms in the large intestine utilize these oligosaccharides and result in generation of flatus gases (e.g. *Entamoeba histolytica*, *E. hartmanni*). In *M. pruriens*,

verbascose has been considered as the main oligosaccharide responsible for flatus (Vijayakumari *et al* 1996).

2.1.4.6 Melanin

Melanin in seeds is responsible for negative health effects (Dollery, 1999; Hegedus 2001). It has been predicted that melanin may be present in *Mucuna* seeds even after processing. For instance, cooking or soaking in water with sodium bicarbonate resulted in darkening, which is presumed to be due to conversion of L-DOPA into melanin (Nyirenda *et al*.,2003). Hence, future studies may be directed towards alkaline additives in minimizing and understanding the conversion of L-DOPA into melanin.

2.1.4.7 Phytic acid

Phytic acid is otherwise known as inositol hexaphosphate (Hans and Wifred, 1988) or myo-inositol hexaphosphate (Hendricks, 2002). It is widely present in cereal grain, legumes and processed food products (Reddy and Salunkhe, 1982). The ability of phytic acid to complex with metals or protein is well-known (Alegbeleye, 2005) and is one of the main nutritional concerns associated with phytates (Rackis and Anderson, 1977). Phytic acid and its derivatives interacts with essential dietary minerals (especially calcium, magnesium, iron, zinc and molybdenum), proteins and vitamins thus making them unavailable or partially available for absorption in the intestinal tract (O'Dell, 1969, Davies and Martindale, 1982). Phytic acid is not heat labile, but is lowered by cooking. Phytic acid (as phytate) can be extracted by dilute hydrochloric or trichloroacetic acid and by soaking in warm water (Hans and Alfred, 1988). These methods are not relevant to products meant for fish feeding for reasons that include cost and possibility of residues (Alegbeleye, 2005).

2.2 Biological Characteristics of *Clarias gariepinus*

Froese and Pauly (2009) reported that *C. gariepinus* which is generally considered to be one of the most important tropical catfish species for aquaculture has an almost Pan-African distribution**.** It is widely distributed throughout Africa. It inhabits tropical swamps, lakes and rivers, some of which are subject to seasonal drying. It has a scaleless slimy skin, which is darkly pigmented in the dorsal and lateral parts of the body. The fish turns lighter in colour when exposed to light. During stress they will show a mosaic-like pattern of dark and light spots (Viveen *et al*. 1985).

With a wide mouth, the African catfish has the ability to feed on a variety of food items, ranging from minute zooplankton to fish. It is able to suck benthos from the bottom, to tear pieces of cadavers with the small teeth on its jaw and to swallow prey such as fish whole. The mouth circumference of this gape-limited predator, which is about one-quarter of its total length, determines the maximum size of its prey. Around the mouth eight barbells can be distinguished (nasal, maxillary, outer mandibular, and inner mandibular). The fish can move the maxillary barbells independently of its mouth. Close to the nasal barbells two olfactory organs are located. Catfish recognizes its prey mainly by touch and smell. This is of relevance during feeding at night and in muddy waters, visibility being of less importance (Holden and Reed 1972, Viveen *et al*. 1985, De Graaf and Jansen, 1996)).

The median fins of *Clarias gariepinus* consist of a dorsal, a caudal and an anal fin while the paired fins consist of pectoral and ventral fins. The pectoral fins have developed strong spines which have a locomotory and protective function. The fish is capable of migrating over land by sculling with its tail as its elbows along on its spines. The sharp spines are not poisonous (Viveen *et al* 1985).

The gills and the arborescent organs are distributed over five brachial arches. These can be observed by cutting away the operculum. The accessory breathing organs enable Clarias not only to live in stagnant pools but to travel over damp ground (Holden and Reed 1972, de Moor and Bruton1988). It is able to survive out of water for some hours depending on the humidity of the environment. Because it can tolerate low oxygen levels in the water it is very suitable for fish culture. Furthermore t is able to withstand adverse environmental conditions and habitat instability. The fish is highly fecund. Spawning usually takes place after rain with rising water levels. Hatching, although temperature dependent, occurs approximately 18-24 hours after fertilization at 24- 28 ^{0}C. Growth under natural conditions and particularly under controlled aquaculture conditions is fast. The species is an opportunistic omnivore capable of switching feeding modes depending on prey available. It is hardly and does not succumb to disease (De Graaf and Jansen 1996).

Clarias gariepinus is one of the most commonly cultured species in Nigeria (NSPFS, 2004).Its suitability for culture covers almost every part of Africa. In Nigeria, *C. gariepinus* contributes significantly to fish production through aquaculture production the yield from *C. gariepinus* cuts across almost all ecological zones in the country. This may be linked to its hardy nature and ability to grow and breed under a wide range of culture conditions (Ajani 2005, Olasunkanmi 2008). A bottom feeder which occasionally feeds at the surface forages at night on a wide variety of prey (Burgess, 1989). It feeds on plankton, invertebrates and fish but also takes young birds, rotten flesh and plants.

2.3 Nutritional Characteristics of Fish

Fish are poiklothermic animals, and their metabolic rate is determined by the water temperature. Thus, fish are divided into coldwater and warm-water fish, according to the

temperature optimum for their growth. The metabolic rate differs from species to species, even at the same water temperature. Since fish live in water, they can maintain their body position against gravity with the aid of their buoyancy, and require little energy to do so. Therefore, their energy expenditure for maintenance is considerably lower than that of homoeothermic terrestrial animals.

In nature fish normally consume an array of food organisms, such array of organisms appear to guarantee an adequate representation of the complex series of amino acids, vitamins, energy source, minerals and other nutrients essential for normal growth. These nutrients may come from natural aquatic organisms or from prepared feeds. In culturing fish in captivity nothing is more important than well-balanced diets and adequate feeding. If there is no utilizable feed intake by the fish, then there will be no growth and death eventually results. An undernourished or malnourished fish is never able to maintain its health and be productive, regardless of the quality of its environment. According to Cho *et al* (1985) the production of nutritionally balanced diets for fish requires research, quality control and biological evaluation.

2.3.1 Nutrient Requirement of Fish

In culturing fish in captivity, nothing is more important than well balanced diets and adequate feeding. If there is no utilizable feed intake by the fish, then there will be no growth and death eventually results. An undernourished fish is never able to maintain its health and be productive, regardless of the quality of its environment (Cho *et al.*1985).The production of nutritionally balanced diets for fish requires research, quality control and biological evaluation. Diets can negatively influence the well-being of a fish by inducing nutrient deficiencies, imbalance, or toxicoses or by introducing infective agents into the fish. A well-balanced diet, however, not only results in higher production but also provides the nutrients necessary to hasten

recovery from diseases or aid the fish in overcoming the effect of environmental stress. Malnutrition on the other hand, impairs fish productivity and results in deterioration of health until recognizable diseases ensue.

Feeding represents the single most expensive production costs in intensive aquaculture. Therefore the development of formulated feeds which satisfy requirements of the fish is considered to be one of the major tasks in aquaculture. Much research should therefore be directed towards the development of least-cost feeds to rear the fish as cost effective as possible (Omitoyin, 2007).

The dietary requirements of fish during different stages of their life cycle are determined by the functional morphology and the ontogenetic development of the gut.

2.3.1.1 Energy Requirement

Fish, like all other animals require energy to sustain life. This energy is derived from that stored in the chemical bonds of the feed they eat and it is released when these bonds are broken by oxidative reactions (Smith, 2002). Fats, carbohydrates and proteins can all be used by the fish as a source of energy.

Energy is the most important component of the diet because feed intake in animals that are fed *ad libitum* is largely regulated by dietary energy concentration. Thus, feeding standards for many animals are based on energy needs. In fish that are not fed *ad libitum*, feed intake is more of a function of feed allowance than of the dietary energy concentration except when the fish are fed to satiety (Robinson *et al.*, 2001). Although feed intake in fish may not be strictly regulated by dietary energy concentration, balance of dietary energy in relation to dietary nutrient content is important when formulating catfish feeds. If dietary energy is excessively high, food intake may decline resulting in a reduced intake of essential nutrients. An excessively

high dietary energy to other nutrients ratio may lead to an undesirable level of visceral or tissue fat that may reduce dressed yield and shorten shelf life of frozen products.

One of the notable differences in the nutrition of fish as compared with other livestock concerns energy requirements. Less energy is required for protein synthesis in fish. Maintenance energy requirements are lower for fish than for warm-blooded animals because fish do not have to maintain a constant body temperature and they expend less energy to maintain their spatial position.

2.3.1.2 Protein requirement

Protein is required by all animals for body maintenance and growth. However, the protein needed for these functions varies with the species and culture environment Chuapoehuk and Pothisoong (1997). The protein requirements of most animals are the sum of the requirements for individual amino acids and the requirements for nonessential nitrogen (Cho *et al.*1985). Generally, fish require a higher level of dietary protein than terrestrial farmed vertebrates. In fish, dietary protein requirements are size dependent – smaller fish require higher levels of protein for maximal growth than larger fish (Kaushik and Seiliez, 2010). According to Craig and Helfrich (2009) protein requirements are usually lower for herbivorous fish (plant eating) and omnivorous fish (plant-animal eaters) than they are for carnivorous (flesh-eating) fish, and are higher for fish reared in high density (recirculating aquaculture) than low density (pond aquaculture) systems. Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Protein requirements also vary with rearing environment, water temperature and water quality, as well as the genetic composition and feeding rates of the fish. Protein is used for fish growth if adequate levels of fats and

carbohydrates are present in the diet. If not, protein may be used for energy and life support rather than growth.

Protein comprises about 70% of the dry weight of fish muscle. A continual supply of protein is needed throughout life for maintenance and growth (Robinson *et al.* (2001). A reduction or cessation of growth with weight loss is a consequence of inadequate dietary protein, with the withdrawal of protein from less vital tissue to maintain the functioning of those more important (Wilson, 1989).

2.3.1.3 Lipid Requirement

Dietary lipids serve both as sources of essential fatty acids (EFA) and energy. In addition they also act as carriers of fat-soluble vitamins. The requirements of EFA by fish differ from species to species. Lipids play an important role as an energy source in fish diets, especially for carnivorous fish in which the availability of dietary carbohydrates for energy is low. Dietary lipids provide the essential fatty acids necessary for normal growth and development (Fletcher, 1997). Fish cannot synthesize either linolenic [18:3(n - 3)[or linoleic acid [18:2(n - 6)] and hence one or both of these fatty acids must be provided by the diet, depending on the essential fatty acids requirements (NRC, 1993). The polyunsaturated fatty acid (PUFA) from fish tissues are predominantly of the n-3 series, of which, quantitatively, the main components are eicosapentenoic acid [20: 5(n – 3)] eicosahexaenoic acid [22:6 (n – 3)]. The diet must supply the PUFA themselves or their metabolic precursors.

2.3.1.4 Vitamins Requirement

Vitamins are complex organic substances, usually of comparatively small molecular size. They are distributed in feedstuffs in small quantities and form a distinct entity from other major and minor food components. In natural waters the diet of post larval and early juveniles of

African catfish consists mainly of zooplankton and other invertebrates, whereas the adult fish have a wide ranging natural diet. Vitamins are needed for normal growth, maintenance and reproduction of animals. According to Cho *et al.* (1985) the absence of vitamins from the diet leads to specific deficiency disease in mammals and birds. In fish, many of the vitamin deficiency are non specific (Table 3).

The requirement of fish for a particular vitamin depends upon the environmental conditions, the type of feed and the rate of growth (Andrews *et al.* 1973, NRC 1993). According to Edwin (1984) and Wilson (1984) the requirement for some vitamins is affected by the amino acid composition of the diet. Halver (2002) reported that vitamins A and D are stored in fish's body fat and their prolong absence from the diet does not produce any noticeable change in growth because an deficit in these vitamins in the food is supplemented at the expense of stored reserves. However, Edwin (1984) that fish feed should be formulated to contain vitamins in excess of the minimum requirements because they are easily destroyed during manufacture and decompose during storage. Other reasons for over-fortification of vitamins in fish feed are that anti-metabolites may reduce the activities of some vitamins in feed ingredients and the content and bioavailability of vitamins in feed ingredients vary considerably.

Table 3: Nutritional deficiency symptoms in finfish

Code	Symptom	Possible nutrient deficiencies
01	Anaemia	Folic acid, inositol, niacin, pyridoxine, riboflavin, rancid fat, vitamins B_{12}, , and K
02	Anorexia (poor appetite)	Biotin, folic acid, inositol, niacin, pyridoxine, riboflavin, thiamine, Vitamins A, B_{12} andC
03	Ascites	Vitamins A, C and E
04	Ataxia	Pyridoxine, pantothenic acid, riboflavin
05	Gill Atrophy	Pantothenic acid
06	Muscle Atrophy	Biotin, thiamine
07	Renal calcinosis,	Magnesium
08	Cartilage abnormality	Vitamin C, tryptophan
09	Cattaract	Methionine, riboflavin, thiamine, zinc
10	Ceroid liver	Rancid fat, vitamin E
11	Cloudy lens	Methionine, riboflavin, zinc
12	Clubbed gills	Pantothenic acid
13	Slow Clotting blood	Vitamin K
14	Dark skin colouration	Biotin, pyridoxine, folic acid, riboflavin
15	Convulsions	Biotin, pyridoxine, thiamine
16	Discolouration of skin	Fatty acds thiaminie
17	Bone Deformation	Phosphorus
18	Lens Deformation	Vitamin A
19	Degeneration of gills	Biotin
20	Dermatitis	Pantothenic acid
21	Diathesis, exudative	Selenium
22	Low Disease resistance	Protein, Vitamin C
23	Distended stomach	Inositol

24	Dystrophy, muscular	Selenium, Vitamin E
25	Oedema	Niacin, pyridoxine, vitamins A and E
26	Epicarditis	Vitamin E
27	Equilibrium loss	Pyridoxine, thiamine
28	Erosion of fins	Fatty acids
29	Exophthalmos	Pyridoxine, vitamins A, C, and E
30	Exudated gills	Panthothenic acid
31	Fatty liver	Biotin, choline, fatty acids, inositol, vitamin E
32	Poor Feed efficiency,	Biotin, calcium, choline, energy, fat, folic acid, inositol, niacin, protein, riboflavin
33	Erythrocytes fragility	Biotin, vitamin E
34	Fin fragility	Folic acid
35	Erythrocytes Fragmentation	Biotin, vitamins B_{12} and E
36	Rapid gasping	Pyridoxine
37	Goitre	Iodine
38	Poor Growth	Biotin, calcium, Choline, energy, fat, folic acid, inositol, niacin, Panthotenic acid, protein, pyridoxine, riboflavin, thiamine, vitamins A, $B_{12,}$ C, D, and E
39	Reduced Haematocrit	Iron, vitamins C and E
40	Low haemoglobin	Iron, vitamins B_{12}, and C
41	Hemorrhagic eye	Riboflavin, vitamin A
42	Hemorrhagic gill	Vitamin C
43	Hemorrhagic kidney	Choline, Vitamins A and C
44	Hemorrhagic liver	Vitamin C
45	Hemorrhagic skin	Niacin, panthotetic acid, riboflavin, vitamins A and C
46	Irritability	Fatty acids, pyridoxine, thiamine
47	Colon lesion	Biotin, niacin

48	Eye lesion	Methionine, riboflavin, vitamins A and C, Zinc
49	Skin lesion	Biotin, inositol, niacin, panthotenic acid
50	Lethargy	Folic acid, niacin, panthotenic acid, thiamine, vitamin C
51	Lipoid liver	Fatty acids, rancid fat
52	Lordosis	Vitamin C
53	Cardiac myopathy	Essential fatty acids
54	Necrosis (liver)	Panthotenic acid
55	Nerve disorder	Pyridoxine, thiamine
56	Pale liver (glycogen)	High digestible carbohydrate, biotin
57	Photophobia	Niacin, riboflavin
58	Pinhead	Starvation
59	Pigmentation (iris)	Riboflavin
60	Prostration	Panthothennic acid, vitamin C
61	Rapid rigor mortis	Pyridoxine
62	Scoliosis	Phosphorus, tryptophan, vitamins C and D
63	Shock syndrome	Essential fatty acids
64	Slime, blue	Biotin, Pyridoxine
65	Spasm, muscle	Niacin
66	Swimming, erratic	Pyridoxine, Panthotenic acid
67	White muscle tetany	Niacin, vitamin D
68	Vascularisation (cornea)	Riboflavin

Source: Cho *et al.* (1985)

2.3.1 Nutritional Characteristics of *Clarias gariepinus*

In natural waters the diet of post larval and early juveniles of African catfish consists mainly of zooplankton and other invertebrates, whereas the adults have a wide ranging natural diet (Wikipedia, 2009). The dietary requirements of fish during different stages of their life cycle are determined by the functional morphology and the ontogenetic development of the gut. The dietary requirements during the larval and early juvenile stages as well as during the grow-out phase must be satisfied in order to maintain growth rates and conditions. Research has shown that the nutritional requirements of the early juveniles up to an age of approximately six weeks differ from those of older fish (de Moor and Bruton, 1988). Thereafter there do not seem to be any further changes in nutritional requirements. The most important nutrients are proteins and lipids. Unlike many other vertebrate animals, fish generally rely more on lipids than on carbohydrates for their energy requirement and are generally not able to utilize carbohydrate efficiently. However, there are indications that omnivorous fish such as African catfish have the ability to utilize carbohydrates as indicated by the presence of amylase.

2.3.2 Nutritional Requirements of Larval and Early Juveniles

Due to the high densities at which catfish are reared, it is essential that a reliable source of high quality larval feed, which satisfies all the nutritional requirements, is always and readily available. Live organisms particularly rotifers, cladocerams and *Artemia nauplii* have been used in large scale rearing of African catfish (Hogendoorn 1980; Hogendoorn and Vismans, 1980; Viveen *et al.,* 1985). However the collection of live food from pond is cumbersome and only available on a seasonal basis and the cultivation of Artemia is expensive particularly for hatcheries in developing countries in Africa. Therefore artificial dry-feed for larvae based primarily on a single cell protein (SCP) Torula yeast (*Candida utilis)* (Litchfield, 1983) and fish

meal have been formulated. However, live-food is essential only for the first few days after the start of exogenous feeding (Burgess, 1989).

2.4 Protein Quality Evaluation

Protein quality is the term used to denote the efficiency with which a protein is utilized for growth and foe maintenance. Chemical analysis and animal feeding experiments have been the methods for learning about proteins (Ezeagu, 1997). Other terms that are sometimes used almost interchangeably with protein quality are Biological Value (BV) and Net Protein Utilization (NPU).

2.4.1 Biological Value (BV)

The biological value of a protein is defined as the percentage of absorbed nitrogen retained. It is generally accepted that the proportion or the balance of amino acids in dietary protein is a major determinant of the efficiency with which it is utilized (Harper and Kunita, 1959)). Incomplete digestion and absorption will adversely affect protein utilization just as will a poor balance of amino acids. The BV of food protein, therefore, depends upon the number and kind of amino acid present in the molecule. The nearer the food protein approaches the body protein in amino acid make up, the higher will be the BV. Animal proteins generally have higher BVs than plant proteins.

2.4.2 Net Protein Utilization (NPU)

According to Ezeagu (1997), the usefulness of a protein to an animal will depend upon its digestibility as well as its BV. The product of these two values is the proportion of the nitrogen intake which is retained, and is termed the net protein utilization (NPU).

2.4.3 Growth Rate Criterion

In method of protein evaluation based on fish growth, test protein sources are fed to juvenile fish under standardized conditions for period of time. Growth alone or growth rate and feed intake may be measured and in some cases the carcasses are analyzed for nitrogen content. Depending on the procedure employed, Protein Efficiency Ratio (PER), Net Protein retention (NPR), Protein Retention Efficiency (PRE), Net Protein Utilization (NPU) may be calculated. Some workers (Jansen, 1962) however, believed that it is better to assess proteins in terms of growth requirements. Growth alone has the advantage of simplicity and is used as a screening method (Jansen *et al.*, 1962).

2.5 Haematological Parameters in Fish

In its broadest interpretation haematology is the study of blood. Blood is a complex mixture of suspended cellular components (erythrocytes, leukocytes and thrombocytes) and dissolved substances (electrolytes, proteins, carbohydrates, amino acids, etc.). Clinical haematology is concerned primarily with the cellular components of blood. Blood analysis is valuable as a means of evaluating physiological conditions of cultured fishes and diagnosing a disease as well as determining the effect of diet and other environmental factors (Badari and Said 1991; Swbodova *et al*, 1991).

According to Omoregie (1998), haematological parameters are important due to their relationship with energy (plasma glucose level), respiration (erythrocyte, haematocrit and haemoglobin level) and defence mechanisms (leucocyte level). Changes in haematological parameters are quick responses to environmental or physiological alterations. They are easy to measure and can provide an integrated measure of the physiological status of organisms. Haematological tests and analysis of serum constituents are useful in the detection of stress and

metabolic disturbances and disease in fish (Aldrin *et al.*, 1982; Morgan and Iwama 1997). In haematological analyses measurement of blood indices and the morphological composition of the blood is important. To investigate the fish blood factors and their changes, the normal rate of these factors must be initially measured in healthy fish. Haematological determination can provide substantial diagnostic information once reference values are established under standardized conditions. Haematological studies in animal research and in human diseases are well accepted and considered to be routine procedure in diagnoses (Ranzani-Paiva *et al.*, 2001; Jamalzadeh *et al*., 2008). Haematological parameters reflect the condition of fish more quickly than other commonly measured parameters and since they respond quickly to changes in environmental conditions, they have been widely used for the description of healthy fish, for monitoring stress responses and for predicting systematic relationships and the physiological adaptations of animals (Atamanalp and Yanik, 2003).

Changes in blood cell morphology and in the cellular compositions of blood are correlated with an animal health. Changes in blood haemoglobin, red blood cell numbers or haematocrit and packed cell volume values following acute stress may indicate that haemo-dilution or haemo-concentration has occurred due to impaired osmo-regulation. Increased red blood cell numbers resulting from splenic contraction or erythrocyte swelling through the actions of epirephrine to facilitate gas transfer (Morgan and Iwama, 1997). Decreases may indicate anaemia or a reduction in circulating red blood cell numbers resulting from infectious diseases indicating reduced fish activities Grant *et al.*, 1991, Adeyemo, 2005).

The count of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation (Adeyemo, 2007). Studies have shown that when the water quality

is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986). Blood cell responses are important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases (Golovina, 1996; Luskova, 1997).White blood cells protect the body against infection. The major types of white blood cells include basophils, neutrophils, eosinophils, B Cells, T Cells, Band Cells and monocytes. Haemoglobin is a protein that is carried by red cells. It picks up oxygen in the lungs and delivers it to the peripheral tissues to maintain the viability of cells. Haemoglobin is made from two similar proteins alpha and beta) that "stick together". Both proteins must be present for the haemoglobin to pick up and release oxygen normally.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Collection and Preparation of *Mucuna* seed meals

Fifty kilograms of raw fresh *Mucuna* seeds (*M. pruriens* var. *utilis)* was obtained from International Livestock Research Institute (ILRI) located in International Institute of Tropical Agriculture [IITA], Ibadan, Nigeria. These samples of Mucuna seeds were then prepared into meals as follows:

(i) **Raw Mucuna Meal (RMM)**

Two Kilograms of fresh Mucuna seeds were de-hulled by crushing it in rotating blades of a Popular^{R} Model three-blade Blender three to five times per batch. The hull was separated from the de-hulled seeds by winnowing. Seeds were then milled in a grinding machine. The resulting flour then was made to pass through 1mm sieve and used as Raw *Mucuna* Meal (RMM)

(ii) **Cooked Mucuna Meal [CMM]**

Two Kilograms of fresh Mucuna seeds were cooked in boiling water for 30 minutes using a Bunsen burner. The seeds were quickly removed from the water and fresh cold water was allowed to run over it to cool it down speedily. The seeds were de-hulled with hands and sun – dried for three days as described by Nchang (2007). The dried seeds were then milled into meal with a grinding machine.

(iii) Toasted Mucuna Meal [TMM]

Raw whole seeds were de-hulled as described in section 3.1(i). The seeds were then toasted for 30 minutes at 130^{0}C in an Astell Hearson^{R} Oven. The oven has been allowed to stabilize at this temperature for 15 minutes before the seeds were put into it. Toasted seeds were allowed to cool down to room temperature and then milled into meal with a grinding machine.

3.2 Proximate Analysis of samples.

Samples of the processed meals were analysed for proximate and mineral compositions using the procedures described by A.O.A.C (1990) for crude protein, crude fat, crude fibre, nitrogen free extract, moisture and ash.

3.3 Method of L-DOPA Determination

L-DOPA content of processed seeds was determined by the method described by Arnon (1937) as modified by Ezeagu (2003).

Determination of L-DOPA Content

Reagents: 0.5 HCl, Nitrite Molybdate reagent (made by dissolving 10g of sodium molybdate in 100ml distilled water) , 1N NaOH and Stock Standard L-DOPA solution (dissolve 50mg in 500ml of distilled water, add 2ml 0.1N HCl and make up to 1L (prepared for each batch daily).

Procedure

The samples were milled to pass through 1mm mesh. 0.1g of was put in 50ml centrifuge tubes with 20ml 0.5N HCl for 2 hours at room temperature (25 -30^{0}C) with manual agitation at 20 minutes interval. Then centrifuge at 3000mg for 20 minutes.

1ml of aliquots of extract to 10ml with distilled water was used for the analysis as follows:

1. 1ml of diluted extract was placed in a test tube
2. 1ml of 0.5ml HCl was added
3. 1ml of nitrite molybdate reagent was added (a yellow colour developed)
4. 1ml of 1N NaOH was added (a red colour developed)
5. The reagent mixture was made up to 5ml with distilled water and absorbence at 510mm was read against reagent blank.

6. Levels of L-DOPA was calculated from a standard curve prepared from absorbence of 1ml aliquot of standard L-DOPA solutions containing 0.05 mg/ml.

3.4 Experimental diet: Preparation and inclusion.

Twelve isonitrogenous diets containing 40% crude protein with four diets for each processed *Mucuna* seed meal (RMM, CMM and TMM) in which soybean meal (SBM) diet was replaced were formulated and prepared and these were incorporated at 0, 10 20 and 30% inclusion level respectively. The diet having 100% Soybean Meal (SBM) served as the control while others had SBM replaced with MSM at 10%. 20% and 30% respectively (Tables 4, 5 and 6). This replacement was done for all the differently processed MSM. Each of the feed ingredients was properly ground into fine powder, mixed thoroughly and pelletized using a locally fabricated pelletizing machine with a die hole of 2mm. The feeds were than sun-dried and packed separately in air tight polythene bags and stored in a cool dry place.

Table 4: Ingredient Composition of Experimental Diet (RMM)

Ingredient	D1 (0%)	D2 (10%)	D3 (20%)	D4 (30%)
Fish Meal	29.41	29.41	29.41	29.41
Soya bean	45.54	40.91	36.36	31.82
Mucuna	-	6.84	13.68	20.52
Starch	21.34	19.04	16.75	14.44
Bone Meal	2.50	2.50	2.50	2.50
[1]Fish Premix	0.50	0.50	0.50	0.50
Vitamin C	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
Vegetable oil	0.45	0.45	0.45	0.45
Calculated				
Crude Protein	40.00	40.00	40.00	40.00
Calculated	3097.9	3256.3	3154.6	3162.6
gross energy				

[1]contains/kg: vitamins. A:. 4 $x10^7$ i.u, D3: $6x10^6$ i.u, K_3: 800mg; Tocophenols ,$2x10^5$ i.u , Folacin 100mg, Thiame 200mg, Riboflavin 6000mg, Niacin $4x10^5$,Calcium panththenate 10000mg, Pyridoxine 3000mg,Cyanocobalamin 12mg, Biotin 80mg, Mn 6000mg, Zn 8000mg, Fe 8000mg,Cu 800mg, Choline chloride $8x10^5$mg, iodine 1`0^3mg,Co $4x10^3$mg, Se $2x10^3$mg BHT 2x10mg

Table 5: Ingredient Composition of Experimental Diet (CMM)

Ingredient	D1 (0%)	D5 (10%)	D6 (20%)	D7 (30%)
Fish Meal	29.41	29.41	29.41	29.41
Soya bean	45.45	40.91	36.36	31.82
Mucuna	-	7.01	14.82	21.03
Starch	21.34	18.87	16.41	13.94
Bone Meal	2.50	2.50	2.50	2.50
[1]Fish Premix	0.50	0.50	0.50	0.50
Vitamin C	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
Vegetable oil	0.45	0.45	0.45	0.45
Calculated Crude Protein	40.00	40.00	40.00	40.00
Calculated gross energy	3097.9	3245.7	3151.6	3542.3

[1]contains/kg: vitamins. A:. 4 $x10^7$ i.u, D3: $6x10^6$ i.u, K_3: 800mg; Tocophenols ,$2x10^5$ i.u , Folacin 100mg, Thiame 200mg, Riboflavin 6000mg, Niacin $4x10^5$,Calcium panththenate 10000mg, Pyridoxine 3000mg,Cyanocobalamin 12mg, Biotin 80mg, Mn 6000mg, Zn 8000mg, Fe 8000mg,Cu 800mg, Choline chloride $8x10^5$mg, iodine 10^3mg,Co $4x10^3$mg, Se $2x10^3$mg BHT 2x10mg

Table 6: Ingredient Composition of Experimental Diet (TMM)

Ingredient	D1 (10%)	D8 (D10%)	D9 (20%)	D10 (30%)
Fish Meal	29.41	29.41	29.41	29.41
Soybean	45.45	40.91	36.36	31.82
Mucuna	-	7.01	14.82	20.53
Starch	21.34	19.04	16.75	14.44
Bone Meal	2.50	2.50	2.50	2.50
*Fish Premix	0.50	0.50	0.50	0.50
Vitamin C	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
Vegetable oil	0.45	0.45	0.45	0.45
Calculated Crude Protein	40.00	40.00	40.00	40.00
Calculated gross energy	3097.90	2985.97	3014.8	3128.9

*contains/kg: vitamins. A:. 4 $x10^7$ i.u, D3: $6x10^6$ i.u, K_3: 800mg; Tocophenols ,$2x10^5$ i.u , Folacin 100mg, Thiame 200mg, Riboflavin 6000mg, Niacin $4x10^5$,Calcium panththenate 10000mg, Pyridoxine 3000mg,Cyanocobalamin 12mg, Biotin 80mg, Mn 6000mg, Zn 8000mg, Fe 8000mg,Cu 800mg, Choline chloride $8x10^5$mg, iodine 10^3mg,Co $4x10^3$mg, Se $2x10^3$mg BHT 2x10mg

3.5 Experimental Set up and Procedure

This experiment was carried out in Bunmbola Farms Ltd., Osogbo, Osun State, Nigeria. The fish were reared using circular 38-litre capacity plastic tank which was filled with 18-litre of water. Six hundred *C. gariepinus* fingerlings (average weight 6.60g±1.09) were obtained from Olly Bee Farms, Iyaana –Offa, Oyo State, Nigeria and brought to the experimental site in a polythene oxygenated bag and they were acclimatized for fourteen days. They were fed with commercial diet (Caps feed^{R}) during the period of acclimatization.

One hundred and twenty *C. gariepinus* juveniles (average weight 6.60g±1.09) were randomly distributed into twelve aquaria at the stocking rate of 10 fish per aquarium. This was replicated thrice for each of treatment. The experimental fish were batch weighed at the beginning of the experiment with a digital balance and subsequently at two week interval throughout the experimental period. Fish were fed twice daily (800h and 1600h) at 5% of their body weight. The quantity of feed was adjusted bi-weekly when fish were weighed. The experiment lasted for twelve weeks. Prior to the end of the experiment, two experimental fish were sacrificed from each tank and were oven dried for proximate analysis. Similar analysis was carried out at the beginning of the experiment. Water quality parameters were monitored throughout the period of the experiment.

3.6 Growth Performance and Nutrients Utilization Parameters

3.6.1 Growth performance parameters

Fish response to treatments was measured using mean weight gain, average daily growth, specific growth rate, percentage weight gain, survival rate, as described by Hardy (2002).

3.6.1.1 Specific Growth Rate (SGR) = $\frac{\log_e W_1 - \log_e W_0}{T_1 - T_0} \times 100$

Where:

SGR= Specific growth rate

W_1 and W_0 = final and initial weights of fish respectively

T_1 and T_0 = final and initial time of the experiment respectively

Log_e = Natural log to base e

3.6.1.2 Average Daily Growth (ADG) = $\frac{\text{Mean weight gain}}{\text{Experimental Period (in days).}}$

3.6.1.3 Mean Weight Gain = Final weight (g) – Initial weight (g)

3.6.1.4 % weight gain = $\frac{\text{Mean weight gain}}{\text{Initial Mean weight}} \times 100$

3.6.1.5 Survival Rate (SR) = $\frac{\text{Initial number of fish stocked – Mortality}}{\text{Initial number of fish stocked}} \times 100$

3.6.1.6 Feed Conversion Ratio (FCR) = $\frac{\text{Total Food Consumed (g)}}{\text{Weight gained (g)}}$

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

3.6.2 Nutrients utilization parameters

Nutrient utilization was measured using food conversion ratio, protein efficiency ratio and productive protein value as described by Hardy (2002) as follows:

3.6.2.1 Protein Efficiency Ratio (PER) = $\frac{\text{Mean weight gain (g)}}{\text{Crude Protein intake (g)}}$

3.6.2.2 Productive Protein Value (PPV) = $\frac{\text{Increment in body protein}}{\text{Protein consumed}}$

3.6.2.3 Apparent Net Protein Utilization (APP.NPU) = $\frac{N_b - Na}{N_i} \times 100$

Where

N_b – body nitrogen at the end of experiment

N_a - body nitrogen at the beginning of experiment

N_i- amount of nitrogen ingested

3.6.2.4 Apparent Protein Digestibility (APD)

$$= 1 - \frac{\%\ Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in feaces}} \times \frac{\%\text{protein in feaces}}{\%\text{protein in feed}} \times 100$$

As described by Oliva-Teles and Gonçalves (2001).

3.6.2.4.1 Procedure for APD Determination

Fish were starved for two days to empty their stomachs at the beginning of the experiment. The formulated diets were adjusted to accommodate 1% chromic oxide (Table 3.4) which also served as a marker as described by Smith (1989). Fish were fed as described earlier. Remnant feeds were removed from the system one hour after feeding in order to prevent possible contamination of feacal materials with uneaten feed. Feacal materials were siphoned from the tank and packed separately according to treatment. These were oven dried at 105^{o}C for 24 hours. Samples of experimental feed and feaces were analysed for protein and chromic oxide by the wet acid digestion method described by Furukawa and Tusukhara (1966).

3.7 Fish Carcass Analysis

The proximate analysis of fish was carried out at the beginning and end of the experiment in order to investigate possible changes that might occur in the experimental fish using the methods described by AOAC (2006).

3.8 Methods of Water Quality Assessment

Water quality parameters were monitored throughout the period of the experiment. Temperature was measured using mercury-in glass Celsius scale thermometer while pH,

dissolved oxygen, ammonia and nitrite was measured using the procedure described by APHA (2000).

3.9 Haematological Assessment

Haematological examination of the fish was carried out at the beginning and the end of the experiment in order to investigate the possible effect(s) of the feeds on the fish. Haematological parameters such as Packed Cell Volume (PCV), Haemoglobin, Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) were determined while plasma biochemistry parameters such as Total protein (TP), Albumin, Globulin, Lymphocytes and glucose were measured. Blood samples were collected from fish according to the method described by Morgan and Iwama (1997), Ajani (2005) and Omitoyin (2006). The samples were analysed at the haematology laboratory of Ladoke Akintola University of Technology Teaching Hospital, Osogbo haematological and plasma biochemistry analysis.

3.9.1 Blood collection and preservation

A 2ml plastic syringe needle was inserted at the ventral midline just posterior to the anal fin of the fish at angle 45^0 until it penetrated the caudal vessel lying between adjacent haemal arches. This was accomplished by inserting the needle until it stopped at the backbone. Blood was then drawn slowly into the syringe and preserved. The blood for haematological analysis was preserved in labeled Ethylene diminitetra acetate (EDTA) bottles while that for biochemical analysis was preserved in bottles containing lithium heparin anticoagulant. The use of plastic syringe is a necessary precaution with fish blood because contact with glass result in decreased coagulation time. The plasma obtained by centrifugation from the lithium heparinised samples was stored at 20^{0C} until analysed.

3.9.2 Packed Cell Volume (PCV)

Sample bottles containing EDTA were filled with blood from experimental fish and were immediately sealed with plasticine. The ample bottle were set on a tray and centrifuged for 5 minutes in a micro haematocrit centrifuge (SP6-500 UV Spectrophotometer) at 12,000G. PCV was then read by the haematocrit reader described.

3.9.3 Haemoglobin (Hb) Concenttration

About 0.02ml of well-mixed blood was added to 4ml of Drabkins solution (Potassium ferricyanide , 200mg potassium cyanide, 50mg potassium di-hydrogen phosphate). The entire mixture was then made up to 1 litre with distilled water and pH adjusted to neutral (pH =7.0). The mixture was allowed to stay for about 5 minutes and the Hb was then read by comparing with a cyanomethahaemoglobin standard with a yellow- green filter at 625nm photometrically.

3.9.4 Red Blood Cell (RBC) Counts

Red blood cell was determined by diluting the blood with Dacies fluid (99ml of 3% aqueous solution of sodium citrate and 1ml of 40% formaldehyde) at a ratio 1:200. The diluted blood was introduced into a Neubauer counting chamber and red blood cells counted under the light microscope (Svoboda *et al. 2001;* Alegbeleye 2005; Adesina 2008; Sotolu 2008).

3.9.5 White Blood Cell (WBC) Counts

White blood cell count was determined by diluting the blood sample with 3% aqueous solution of acetic acid at a ratio 1:200 and gentian violet was added. This was also counted under the light microscope.

3.9.6 Mean Corpuscular Volume (MCV)

This represents the mean volume of a single red cell in a blood sample and it is determined by:

$$\text{MCV (fl)} = \frac{\text{Volume of red cell in ml per 100ml blood}}{\text{Number of red cells per 100ml blood.}} \times 100$$

3.9.7 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC was derived from the relationship between the haemoglobin concentration and the haematocrit expressed in pictograms (10^{-12}). Thus,

$$\text{MCHC (g/cl)} = \frac{\text{Haemoglobin content}}{\text{Haematocrit}} \times 100$$

3.9.8 Mean Corpuscular Haemoglobin (MCH)

$$\text{MCH} = \frac{\text{Haemoglobin}}{\text{Erythrocyte count (RBC)}}$$

3.10.1 Histo-pathological Examination of Organs and Tissues

Fish organs and tissues such as the brain, liver, gills and kidney from each experimental unit were removed and examined for possible changes due to the treatment effects on the fish. One fish was sacrificed from each replicate (i.e. three fish per treatment) and selected organs and tissues promptly removed, fixed in Boulin's solution and preserved in 10% formalin before taken to the laboratory for gross lesions and other clinical signs as described by Rodrigues *et al* (2001) and Udoh (2005).

3.11 Statistical Analysis

Data collected from the experiment were subjected to one-way analysis of variance test (ANOVA) using Statistical Package for Social Scientists (SPSS) version 15. Least Square Difference (LSD) was used to separate the means in cases of significant difference.

CHAPTER FOUR

4.0 RESULTS

4.1 EXPRIMENT 1: PROCESSING OF MUCUNA SEEDS INTO MEALS (MSM).

4.1.1 Proximate Composition of MSM

Table 7 shows the proximate composition of processed MSM. The crude protein value ranged from 27.5% (TMM) to 29.2% (RMM). The CP values for RMM and TMM were not significantly different, these were however significantly different from CMM value. The values of other components in MSM were not significantly different from each other. The highest values of crude fat (0.7%), crude fibre (9.6%), K (1.4%), P (0.07%) and Fe (132.1mg) were obtained in RMM while TMM had the least values of 0.69%, 0.64%, 1.18%, 0.071% and 128.56 mg/kg respectively. However, the highest value of Zn (15.38mg/kg) was recorded in TMM while the least value (12.76mg/kg) was recorded in CMM.

4.1.2: Effect of Processing on L-DOPA of MSM.

Processing of Mucuna in this study resulted to reduction in L-DOPA content of MSM (Table 8). Cooking reduced L-DOPA by 17.5% of the original content while toasting reduced the quantity by 25.4%. Other phyto-chemicals presented in Table 8 are as reported by Ezeagu *et al.*, (2003).

Table 7: Proximate Composition of Differently Processed Mucuna Seed Meal (MSM)

Parameters	Raw (RMM)	Cooked (CMM)	Toasted (TMM)	SEM
Crude Protein (%)	29.2	28.5	27.5	0.175
Ether Extract (%)	0.74	0.69	0.64	0.009
Crude Fibre (%)	9.63	9.46	9.06	0.045
Ash (%)	3.28	3.3	3.25	0.124
Moisture (%)	10.04	10.9	9.84	0.438
Mg (%)	0.21	0.3	0.19	0.054
K (%)	1.43	1.4	1.18	0.032
P (%)	0.076	0.073	0.071	0.002
Na (%)	0.19	0.23	0.16	0.031
Fe (mg/kg)	132.14	129.53	128.56	0.245
Mn (mg/kg)	24.87	25.14	21.67	0.249
Zn (mg/kg)	13.11	12.76	15.38	0.191

Table 8: Phyto-chemicals Present in MSM

Phyto-chemical	Raw		Cooked	Toasted
L-DOPA ($g100g^{-1}$)	6.82[a]	6.9[b]	5.7[b]	5.2[b]
Tyrpsin inhibitors (TUI/mg)	30.81[a]	ND[b]	ND[b]	ND[b]
Phytate ($g100g^{-1}$)	0.85[a]	ND[b]	ND[b]	ND[b]
Total oxalate ($g100g^{-1}$)	1.35[a]	ND[b]	ND[b]	ND[b]
Soluble oxalate ($mg100g^{-1}$)	1.12[a]	ND[b]	ND[b]	ND[b]
Cyanide ($mg100g^{-1}$)	0.12[a]	ND[b]	ND[b]	ND[b]
Nitrate-N($mg100g^{-1}$)	1.80[a]	ND[b]	ND[b]	ND[b]
Tannin($g100g^{-1}$)	1.62[a]	ND[b]	ND[b]	ND[b]

Legend:

[a,b]Source: [a]Ezeagu *et al*. (2003)

[b] Current study

ND: Not detected

4.2 EXPRIMENT 2: USE OF RAW MUCUNA MEAL (RMM) AS A SUBSTITUTE FOR SOYBEAN IN *C. gariepinus*

4.2.1 Proximate Composition of the Experimental Diets

The proximate composition of the experimental diets is presented in Table 9. The crude protein (CP) value for all treatments is approximately 40%. Ether extract values ranged from 12.0% diet containing 20% RMM to 8.3% in diet containing 30% RMM. Highest value of crude fibre (2.9) was recorded in diet containing 10 % followed by 2.6 in diet containing 0% RMM, 2.2 in diet containing 30% RMM and lowest (1.9) in diet containing 20% RMM. Ash was least (9.0) in diet containing 20%, followed by 10.9 in diet containing 10 % RMM, 11.9 in diet containing 20% RMM and highest (12.4) in diet containing 30% RMM. The moisture content in control diet (4.6) was least, followed by 5.4 in diet containing 20%, 5.5 in diet containing 30% RMM and highest (6.5) in diet containing 10% RMM.

4.2.2 Growth Response and nutrient Utilization of *C. gariepinus* fed RMM Based Diets.

The growth response and nutrient utilization of *C. gariepinus* fed RMM based diets is presented in Table 10. The mean weight gain decreased as inclusion level increased. Diet containing 0% RMM recorded the highest mean weight gain (20.98±5.19g), this was closely followed by diet containing 10% RMM

Table 9: Proximate Composition (%DM) of RMM Experimental feed

Proximate Composition	Diet 1 (0% RMM)	Diet 2 (10% RMM)	Diet 3 (20% RMM)	Diet 4 (30%RMM)
Crude Protein (%)	39.99	40.09	39.89	39.96
Ether Extract (%)	8.67	9.00	12.00	8.33
Crude Fibre (CF) (%)	2.55	2.93	1.89	2.18
Ash (%)	11.88	10.90	9.00	12.38
Moisture (%)	4.63	6.47	5.42	5.50

Table 10: Growth Response and Nutrient Utilization of *C. gariepinus* fed RMM based Diets.

Parameters	Diet 1 (0% RMM)	Diet 2 (10% RMM)	Diet 3 (20% RMM)	Diet 4 (30%RMM)	SEM
Initial Mean weight (MW)(g)	6.6	6.6	6.6	6.6	-
Final Mean weight (MW) (g)	27.58	24.64	24.38	15.16	0.94
Mean weight gain (MWG) (g)	20.98^{a}	18.04^{b}	17.78^{b}	8.56^{c}	0.94
% Weight gain	317.87^{a}	273.33^{b}	269.39^{b}	129.70^{a}	-
Specific weight gain (SGR)	11.91^{c}	10.89^{b}	10.71^{b}	6.89^{a}	0.02
Feed conversion Ratio (FCR)	2.81^{a}	3.06^{a}	3.15^{a}	5.18^{b}	0.03
Feed conversion efficiency (FCE)	35.59^{a}	32.74^{a}	31.76^{a}	19.31^{b}	-
Percentage survival (% SR)	86^{a}	76^{b}	86^{a}	80^{a}	-
Protein efficiency ratio (PER)	0.5^{a}	0.45^{a}	0.45^{a}	0.21^{b}	0.02
Apparent net protein utilization	15.95^{a}	17.7^{a}	17.0^{a}	33.38^{b}	-
Protein productive value (PPV)	0.16^{a}	0.18^{a}	0.28^{a}	0.33^{a}	-
Nitrogen metabolism (Nm) (X 10)	788.12^{a}	720.33^{b}	714.34^{b}	501.74^{a}	-
Average daily growth (ADG)	0.23^{a}	0.21^{a}	0.20^{a}	0.15^{a}	-
Feed consumed/ fish (g)	58.95^{a}	55.10^{b}	55.99^{b}	44.33^{c}	0.2

Means with the same superscripts along the same row are not significantly different ($p>0.05$).

(18.04±3.18g), diet containing 20% RMM (17.78±1.94g) and the least value was obtained in diet containing 30% RMM (8.56±2.10g).

The percentage weight gains (%WG) were significantly different ($p < 0.05$) in fish fed diets containing 0% (317.9%), 10% RMM (273.3%) and 30% RMM (129.7%). The specific growth rate (SGR) values were also significantly different ($p<0.05$) with the exception of diet containing 10% RMM and 20% RMM. However, diet containing 30% recorded the least SGR value (6.89) while control diet recorded the highest (11.91).

The best FCR value (2.81) was obtained in fish fed control diet and this was significantly different ($p<0.05$) from what was obtained in fish fed diet containing 30% (5.18). FCR values in other treatments (diet containing 10% and diet containing 20%) were inferior to that of control diet but not significantly different ($p>0.05$) from it. FCE values ranged between 19.31in fish fed diet containing 30% to 35.59 in fish fed control diet. FCE value in fish fed diet containing 30% was significantly lower ($p<0.05$) than that of other treatments.

The protein efficiency ratio (PER) value was highest in fish fed diet containing 0% RMM (0.50) and lowest in diet containing 30% (0.21). The protein productive values (PPV) were 0.33, 0.28, 0.18 and 0.16 for diets containing 30%, 20%, 10% and 0% respectively. APP.NPU value was significantly higher in fish fed diet containing 30% (33.38) compared to other treatments, the lowest value (15.95) was however obtained in fish fed control diet. Nitrogen metabolism (Nm) values ranged from 501.74 in fish fed diet containing 30% to 788.12 in the fish fed diet containing 0% RMM. Nm value in control was significantly higher than the other treatments. Average daily growth (ADG) was highest (0.23) diet containing 0% RMM but not significantly different ($p>0.05$) from other treatments.

Fish fed diet containing 30% RMM recorded least feed intake (44.33g/fish), diet containing 10% RMM recorded 55.10g/fish, diet containing 20% RMM (55.99g/fish) and highest value (58.95g/fish) was recorded in diet containing 0% RMM. The growth curves of fish in response to the test diets over the experimental period are shown in Figure 1. Fish showed similar growth pattern for the first four weeks after which fish on control diet exhibited better growth until week eight. However, fish growth in diet containing 20% RMM was not inferior to that of the control from week eight to week twelve.

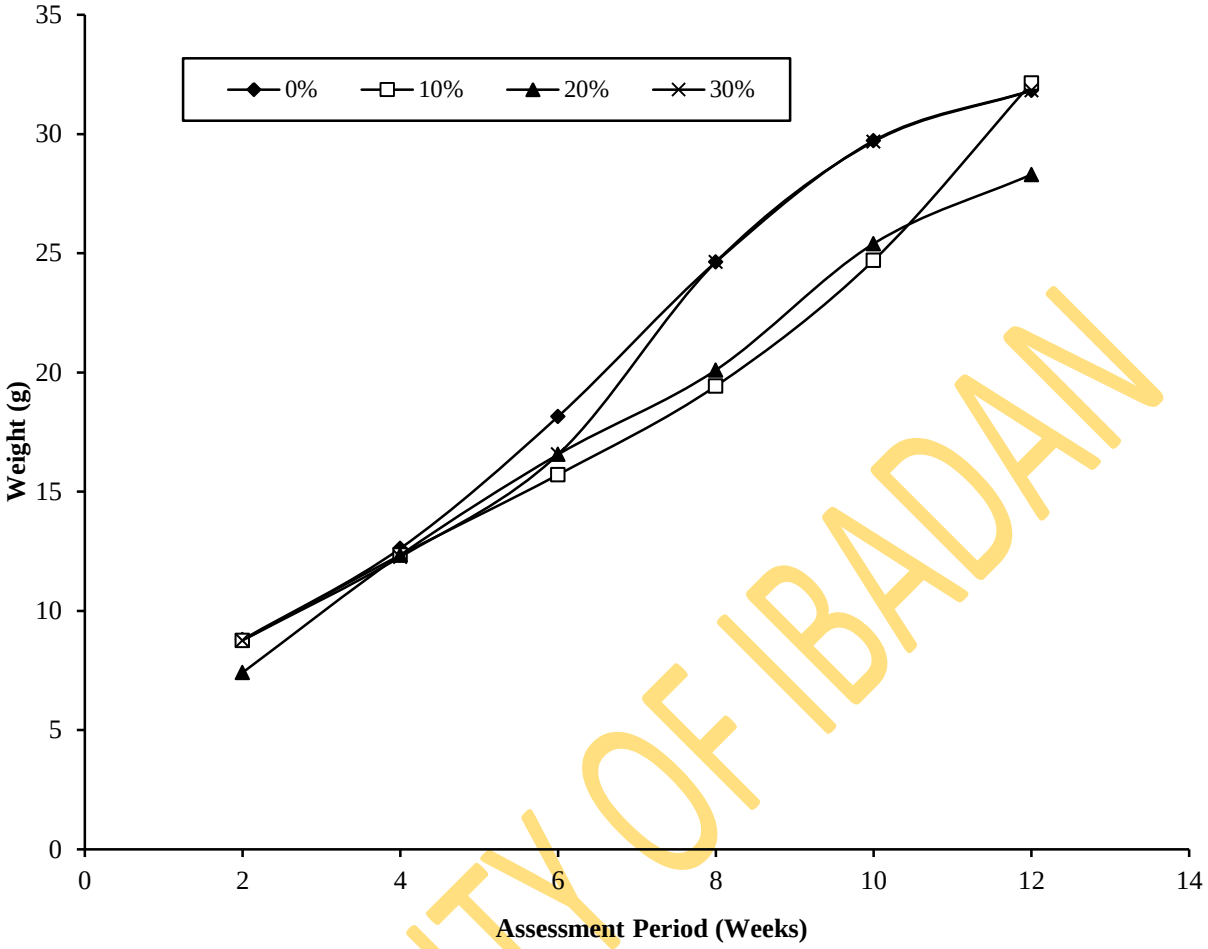

Figure 1 : Weight gain of *Clarias gariepinus* fed varying levels of raw mucuna seed meal.

4.2.3 Carcass Composition of the *C. gariepinus* fed RMM Based Diets

Carcass composition of *C. gariepinus* fed RMM based diets is presented in Table 11. Crude protein value was highest (65.97%) in fish fed diet containing 30% RMM, followed by those fed diet containing 20% RMM (59.70), diet containing 0% RMM (59.00) and least in fish fed diet containing 20% RMM. Ether extract however, decreased from the initial value of 22.05 to 5.73 in fish fed diet containing 0% RM, 11.76 in fish fed diet containing 10% RMM, 9.62 in diet containing 20% RMM and 8.33 in diet containing 30% RMM. Although experimental fish did not contain fibre at the beginning of the study, carcass fibre was recorded at the end of the trial. Fish fed diet containing 20% RMM recorded the highest value of 18.92 while the least value (8.92) was recorded in fish fed diet containing 10% RMM. Ash content increased above initial value (10.86) in diet containing 0% RMM (21.20) and diet containing 30% RMM (21.00) but decreased in diets containing 10 and 20% RMM (7.25 and 3.63 respectively). Moisture content in all treatments decreased when compared to what was obtained at the beginning of the study. However, the lowest moisture content recorded was 6.67 in fish fed diet containing 20% RMM followed by 8.50 in fish fed diet containing 30% RMM, 9.36 in fish fed diet containing 20% and 10.84 in fish fed the control diet containing 0% RMM.

Table 11: Carcass Composition of *Clarias gariepinus* fed RMM Based Diets.

Parameters	Initial	Diet 1 (0% RMM)	Diet 2 (10% RMM)	Diet 3 (20% RMM)	Diet 4 (30%RMM)
Crude Protein (%)	52.62	59.00	59.70	59.42	65.97
Ether Extract (%)	22.05	5.73	11.76	9.62	8.33
Crude Fibre (%)	-	10.04	8.92	18.92	11.98
Ash (%)	10.86	21.20	7.25	3.63	21.00
Moisture (%)	19.96	10.84	9.36	6.67	8.50

4.2.4 Haematological Assessment of fish fed RMM Based Diets

PCV values in all treatments were higher than the initial value (Table 12). However, the highest PCV (30.33%) was recorded in fish fed diet containing 30% RMM while the lowest was (26.33%) was obtained in fish fed diet containing 10% RMM. WBC values in fish fed diet containing 0% RMM (1.83±2.32 x 10^3) and those of diet containing 20% RMM (2.03±5.92 x10^3) were lower than the initial value (2.9 x 10^3) while the values recorded in fish diets containing 10 (7.13±0.53 x 10^3) and 30% RMM (8.00±3.61 x 10^3) were significantly higher ($p<0.05$) than the other treatments. RBC values increased above the initial value in all treatments. However the diet containing 0% RMM recorded the highest RBC value (3.53±0.15 x 10^{12}/L) and the lowest (2.37±0.11 x $10^{12/}$L) was recorded in diet containing 20% RMM. Haemoglobin (Hb) values ranged from 7.7±1.30 in diet containing 20% RMM to 10.00±0.72 in diet containing 30% RMM. Hb values were not significantly different from each other. Initial MCHC value was 37 this dropped to 32.06±1.74 in diet containing 20% RMM, 32.06±2.06 in diet containing 10% RMM, 33.00±3.02 in diet containing 30% RMM and 34.46±3.45 in diet containing 0% RMM.

Table 12: Haematological and Serum Assessment of fish fed RMM Based Diets

Parameter	Initial	Diet 1 (0% RMM)	Diet 2 (10% RMM)	Diet 3 (20% RMM)	Diet 4 (30%RMM)	SEM
PCV (%)	23.00^{c}	27.00^{a}	26.33^{a}	26.89^{a}	30.33^{b}	1.50
WBC(x10^{3}mm)	2.90^{c}	1.83^{c}	7.13^{a}	5.63^{b}	8.00^{a}	0.31
RBC(x10^{12}/L)	1.70^{b}	3.53	2.80^{a}	2.37^{a}	3.17^{a}	0.14
Hb (g/L)	3.80^{b}	9.03^{a}	7.90 ^{a}	7.70^{a}	10.00^{a}	0.46
MCHC (g/L)	37.00^{b}	33.46^{a}	32.06^{a}	32.06^{a}	33.00^{a}	0.45
MCH (pg)	23.00^{b}	25.60^{b}	28.37^{a}	28.37^{a}	32.40^{a}	2.35
MCV (fl)	70.00^{c}	76.47^{c}	88.76^{b}	88.77^{b}	98.03^{a}	2.23
Lymphocytes (%)	93.00^{b}	75.00 ^{a}	90.3^{b}	100.00^{b}	100.00^{b}	2.99
Neutrophil (%)	7.00^{b}	18.33^{a}	14.5^{a}	0.00^{c}	0.00^{c}	2.86

Means with the same superscripts along the same row are not significantly different (p>0.05)

There was no significant difference between initial MCH value and that of the diet containing 0% RMM. However, MCH in fish fed diet containing 0% RMM differs significantly from the other treatments. MCV values ranged between 76.47±3.55fl in fish fed diet containing 0% RMM to 98.03±16.17fl in diet containing 30% RMM. MCV values in diets containing 10% and 20% RMM (88.76±17.79fl and 88.77±11.09fl respectively) are not significantly different from each other but are significantly different from that of diet containing 30% (98.03±16.17fl) and 0% RMM. Initial lymphocytes value was 93. This increased in all treatments except the control. The highest value obtained was 100 in diets containing 20 and 30% RMM followed by 90.33 and 75 in diet containing 10 and 0% RMM respectively. Initial neutrophil value was 7. Neutrophil in diet containing 0 and 10% RMM were 18.33 and 14.59 respectively. Neutrophil in diets containing 20 and 30% RMM was zero.

4.2.5 Plasma Biochemistry of *C. gariepinus* fed RMM based Diets

Plasma biochemistry of *C. gariepinus* fed RMM based diets is presented in Table 13. The blood glucose in fish at the commencement of the feeding trial was 38g/L. There was no significant difference ($p>0.05$) in blood glucose in fish fed diets containing 0, 10 and 20% RMM. However, blood glucose in fish fed diet containing 30% RMM was significantly different ($p<0.05$) from that of other treatments. Blood cholesterol in diets containing 0 and 30% RMM were not significantly different ($p>0.05$) but these were significantly different from that of diets containing 10 and 20% RMM.

Albumin in fish fed diet containing 20% RMM was significantly different from those of other treatments. Globulin in diet containing 0 (110.00±45.56), 10 (77.66±22.50), 20 (33.00±19.09) and 30% RMM (53.33±5.77) were significantly different ($p<0.05$) from each other and the initial value (12.00) obtained at the beginning of the study. Globulin-albumin ratio

in all treatments was significantly higher than the initial value. Total protein was significantly lower at the beginning than at the end of the study in all treatments and the total protein in fish fed diets containing 10, 20 and 30% RMM were significantly lower ($p<0.05$) than that of diet containing 0%RMM.

Table 13: Plasma Biochemistry of *C. gariepinus* fed RMM based Diets

Parameter	Initial	Diet 1 (0% RMM)	Diet 2 (10% RMM)	Diet 3 (20% RMM)	Diet 4 (30%RMM)	SEM
Glucose (g/L)	38[a]	39.3[a]	39.00[a]	39.67[a]	32.67[b]	4.22
Total Protein (g/L)	38[d]	129[a]	94.00[b]	40.67[d]	69.33[c]	13.37
Cholesterol(mol)	ND	2.2[a]	0.90[b]	0.9[b]	2.17[a]	0.30
Globulin (g/L)	12[e]	110[a]	77.66[b]	33.00[d]	53.33[c]	14.03
Albumin (g/L)	26[a]	18.67[b]	16.00[b]	7.67[c]	15.67[b]	1.96
Glo-Alb. Ratio	0.46[c]	5.77[a]	4.81[a]	3.02[b]	3.98[b]	-

Means with the same superscripts along the same row are not significantly different ($p>0.05$).

4.2.6 Histological Changes in Organs of *C. gariepinus* fed RMM Based Diets.

Histological changes observed in *C. gariepinus* fed RMM based diets are presented in Table 14. There was no histological lesion observed in the brain of fish fed the diet containing 0% RMM (Plate 1). The brain of fish fed diets containing 10, 20 and 30% RMM showed marked spongiosis of the white matter (Plate 2). Only fish fed diet containing 0% RMM showed moderate spongiosis of the white matter (Plate 3). The gill of *C. gariepinus* fed diet containing 0% RMM did not show any lesion (Plate 4). The gill of *C. gariepinus* fed diet containing 20% RMM suffered mild clumping of the secondary lamella (Plate 5) while those fed diet containing 10% RMM suffered marked congestion as well as oedema and severe oedema (Plates 6 and 7). Fish fed diet diet containing 10% RMM had mild diffuse vacuolation of hepatocytes of the liver (Plates 8 and 9) while those fed diets containing 20% and containing 30% RMM suffered moderate diffuse and marked diffuse vacuolation of hepatocytes respectively (Plates 10 and 11). There was no lesion observed in kidney of *C. gariepinus fed* diet containing 30% RMM (Plate 12) but the kidney in fish fed diets containing 10% and 20% RMM suffered congestion (Plate 13).

Table 14: Histological Changes in Organs of *C. gariepinus* fed RMM Based Diets

Organs/ Tissues	Histological signs	Diet 1 (0% RMM)	Diet 2 (10% RMM)	Diet 3 (20% RMM)	Diet 4 (30% RMM)
Brain	Moderate spongiosis of the white matter	-	+	-	-
	Marked spongiosis of the white matter	-	+	+	+
Gill	Mild clumping of the secondary lamella	-	-	+	-
	Marked congestion and oedema	-	+	-	-
	Severe oedema	-	+	-	-
Liver	Mild diffuse vacuolation of hepatocytes	-	+	-	-
	Moderate diffuse vacuolation of Hepatocytes	-	-	-	+
	Marked diffuse vacuolation of hepatocytes	-	-	+	-
	Marked widespread vacuolation hepatocytes	-	-	-	+
	Focal marked centrilobular vacuolation of hepatocytes	-	-	-	-
Kidney	Marked congestion	-	+	+	-

Legends: - No visible lesions + Observed lesion.

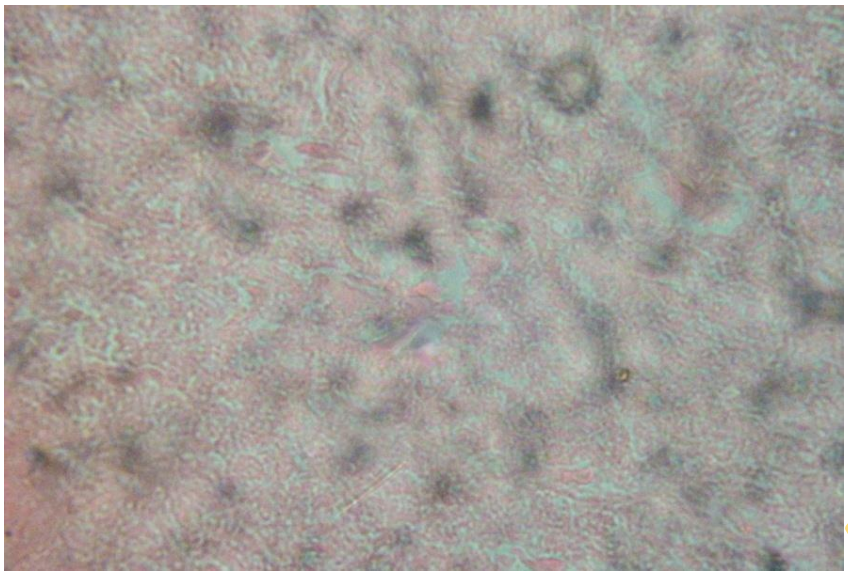

Plate 1: Photomicrograph of a section of brain of *C. gariepinus* fed 0% RMM showing normal cells

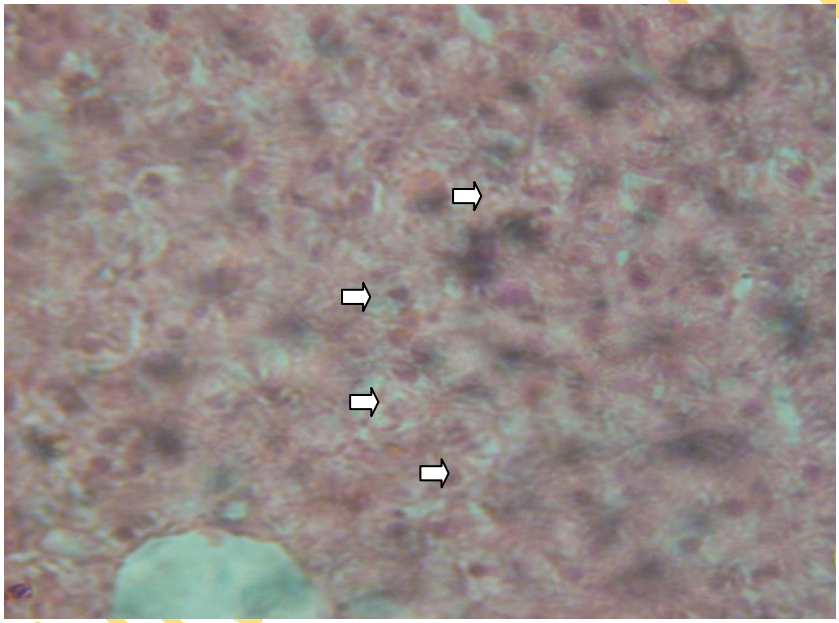

Plate 2: Photomicrograph of a section of brain of *C. gariepinus* fed 10, 20 and 30% RMM based diets showing marked spongiosis of the brain cell.

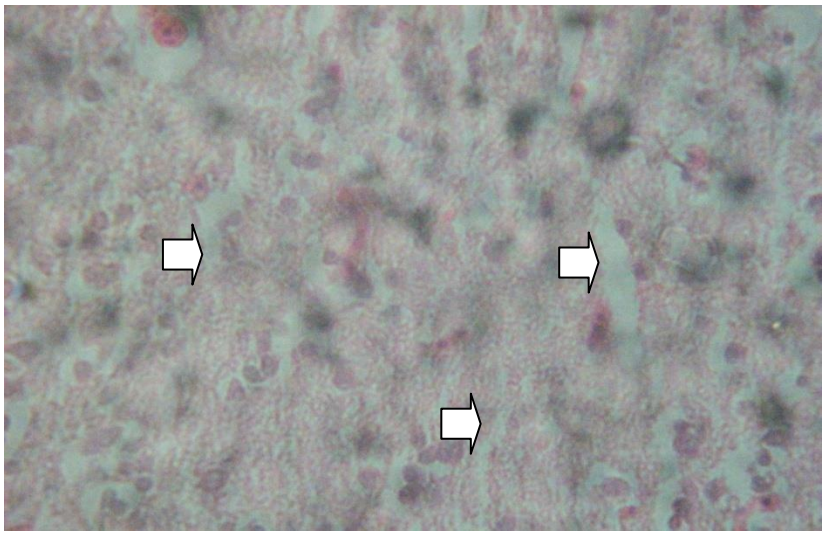

Plate 3: Photomicrograph of a section of brain of *C. gariepinus* fed 10% RMM based diets showing moderate spongiosis of the brain cells.

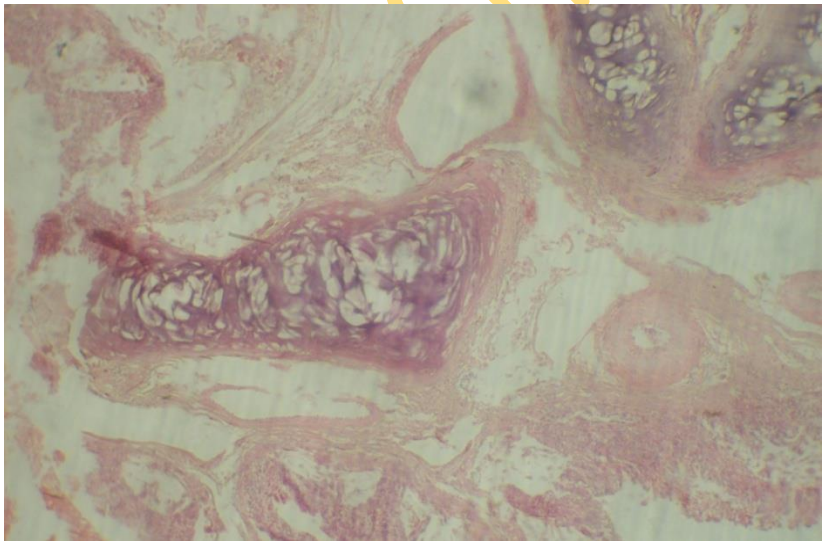

Plate 4: Photomicrograph of a section of gill of *C. gariepinus* fed 0% RMM showing normal cells

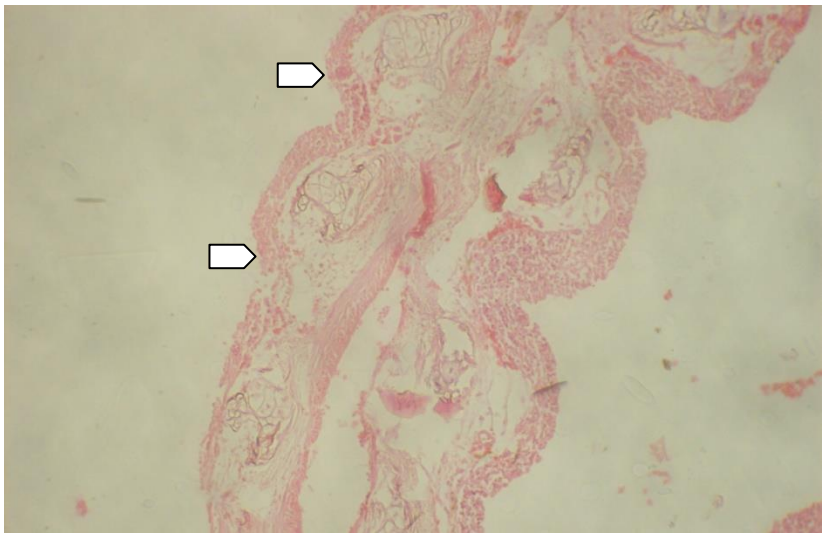

Plate 5: Photomicrograph of a section of gill of *C. gariepinus* fed 20% RMM showing mild clumping of the secondary lamella

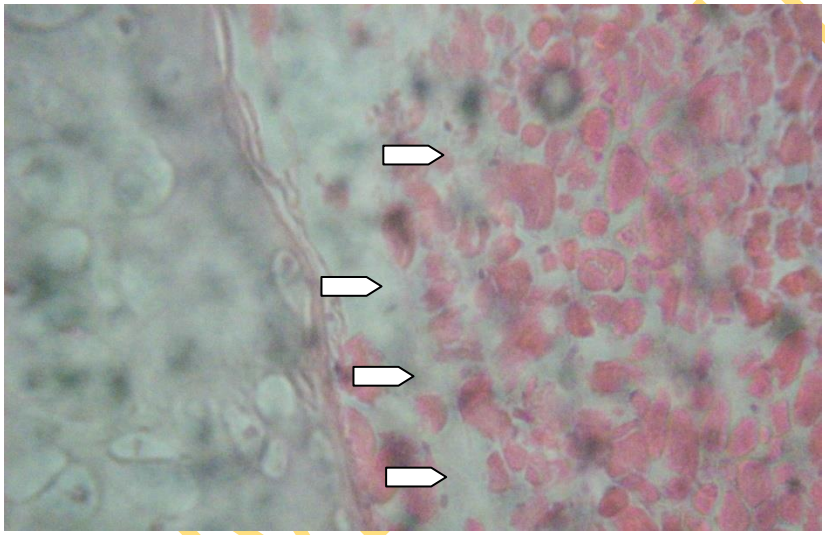

Plate 6: Photomicrograph of a section of gill of *C. gariepinus* fed 10% RMM showing severe oedema

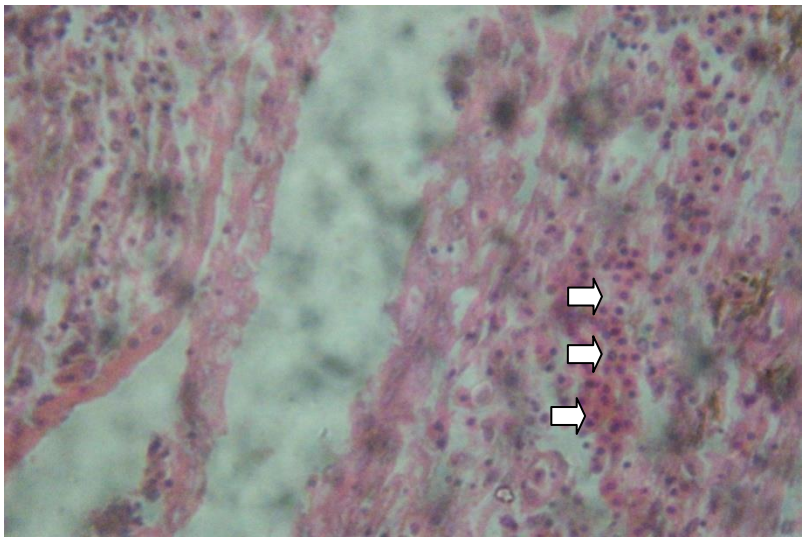

Plate 7: Photomicrograph of a section of gill of *C. gariepinus* fed 10% RMM showing marked congestion and oedema

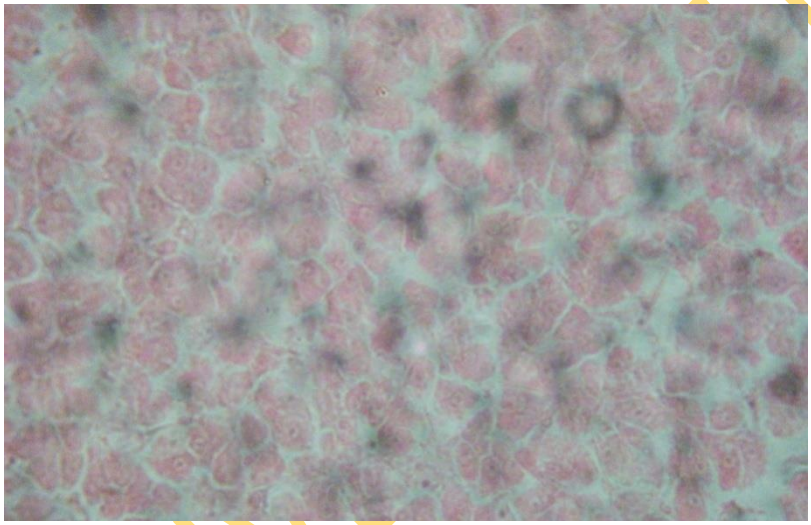

Plate 8: Photomicrograph of a section of liver of *C. gariepinus* fed 0% RMM showing normal cells

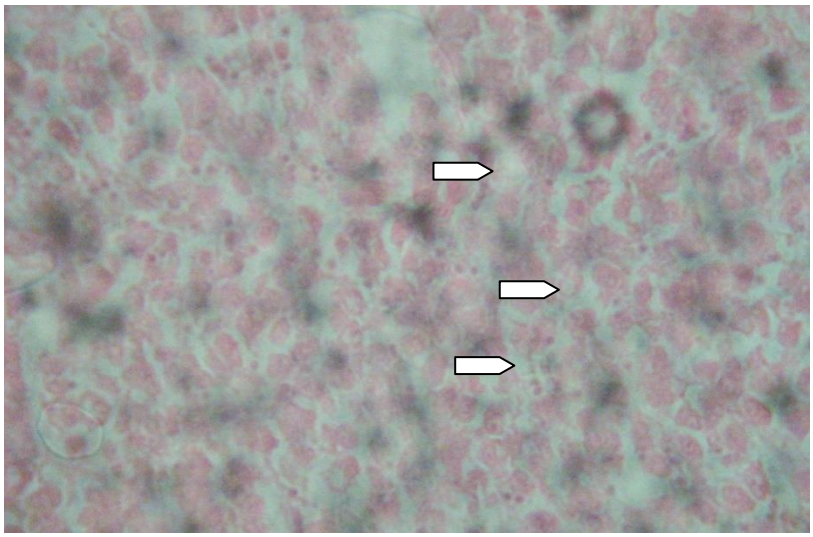

Plate 9: Photomicrograph of a section of liver of *C. gariepinus* fed 10% RMM showing vacuolation of hepatocytes

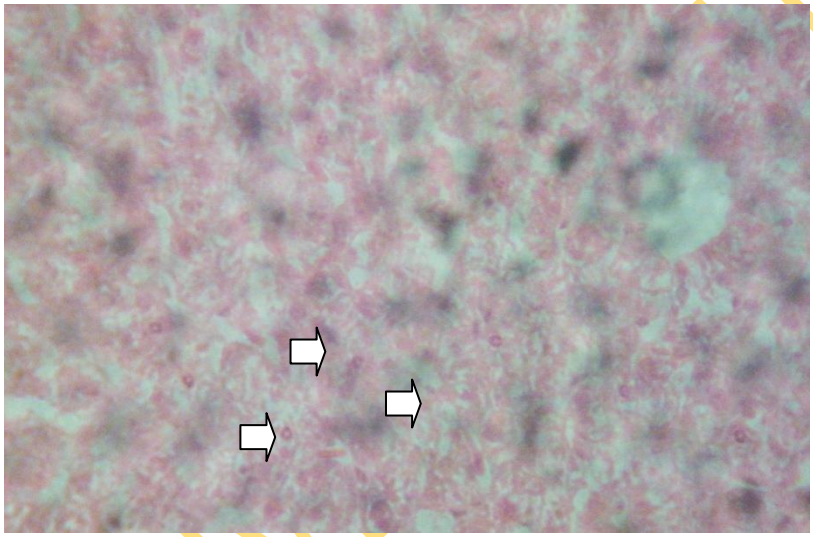

Plate 10: Photomicrograph of a section of liver of *C. gariepinus* fed 30% RMM showing moderate diffuse vacuolation of hepatocytes

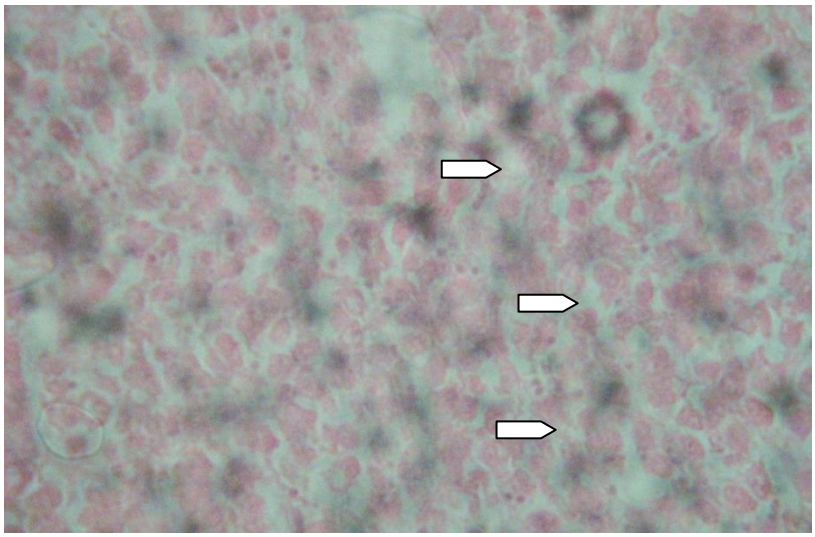

Plate 11: Photomicrograph of a section of liver of *C. gariepinus* fed 20% RMM showing marked diffuse vacuolation of hepatocytes

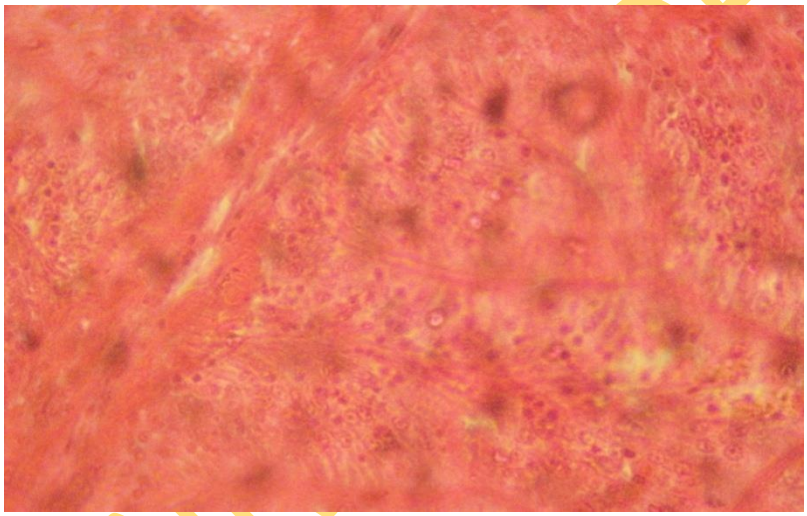

Plate 12: Photomicrograph of a section of kidney of *C. gariepinus* fed 0% RMM showing normal cells.

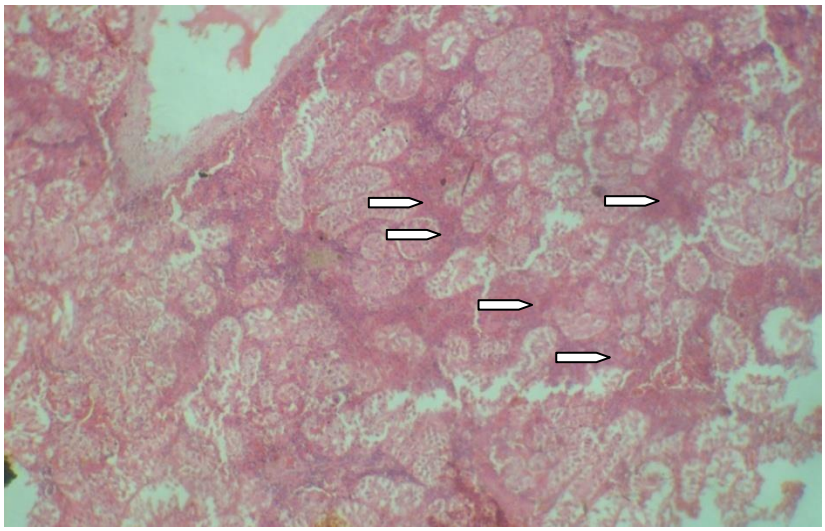

Plate 13: Photomicrograph of a section of kidney *C. gariepinus* fed 10 and 2 0% RMM showing marked congestion.

4.2.7 Water Quality Parameters of the Experimental Setup.

There was no significant difference in the values of temperature and ammonia in all the treatments. p^H values ranged from highest (7.00±0.00) in diet containing 30% RMM to the lowest (6.50±0.00) in control diet. Highest dissolved oxygen value (2.1±2.20mg/l) was recorded in control followed by 9.00±0.30mg/l in diet containing 10% RMM, 8.87±0.00mg/l in diet containing 20% RMM 6.10±0.92mg/l in diet containing 30% RMM (Table 15). Dissolved oxygen decreased as inclusion level increased.

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Table 15: Mean water Quality Parameters values of RMM Based Diets

Parameters	Diet 1 (0% CMM)	Diet 5 (10% CMM)	Diet 6 (20% CMM)	Diet7 (30% CMM)	SEM
Temperature (^{0}C)	26.5^a	27.0^a	26.3^a	27.1	0.31
pH	6.5^a	6.80^a	6.60^a	7.00^a	0.05
Dissolved oxygen (mg/l)	12.2^a	9.00^b	8.87^b	6.10 ^{c}	1.03
Ammonia (mg/l)	1.47 ^{a}	0.50^a	2.00^a	1.40^a	0.31

Means with the same superscripts along the same row are not significantly different ($p>0.05$).

EXPERIMENT 3: USE OF COOKED MUCUNA AS SUBSTITUTE FOR SOYBEAN

4.3.1: Proximate Composition (%DM) of Experimental feed

Crude protein value in all treatments is approximately 40% (Table 16). Highest ether extract value (10.3) was obtained in diet containing and the least (7.9) was diet containing 20% CMM. Crude fibre values were 2.6, 1.9, 3.9 and 6.5 in diets containing 0, 10, 20 and 30% CMM respectively. Ash content decreased from 11.9 to 11.8, 10.8 and 10.5 in diets containing 0, 30, 20 and 10%% CMM respectively. The moisture content was least (4.6) in diet containing 0% CMM followed by 5.5 in diet containing 30% CMM, 5.7 in diet containing 20% CMM and highest (10.5) in diet containing 10% CMM.

4.3.2 Growth Response and Nutrient Utilization of *C. gariepinus* fed CMM Based Diets.

The growth response and nutrient utilization of *C. gariepinus* fed CMM based diets is presented in Table 17. Mean weight gain (MWG) was significantly higher in control diet than the other treatments. MWG values decreased as inclusion level increased. The highest MWG (20.98g) was obtained in diet containing 0% CMM while the lowest (11.29g) was recorded in diet containing 30% CMM. However, percentage weight gain was highest (83.4%) in fish fed diet containing

Table 16: Proximate Composition (%DM) of CMM Experimental feed

Proximate Composition	Diet 1 (0% CMM)	Diet 5 (10% CMM)	Diet 6 (20% CMM)	Diet7 (30% CMM)
Crude Protein (%)	39.99	40.04	39.99	40.03
Ether Extract (%)	8.7	10.33	7.89	8.00
Crude Fibre (%)	2.6	1.86	3.93	6.52
Ash (%)	11.9	10.48	10.78	11.76
Moisture (%)	4.6	10.48	5.68	5.46

0% CMM followed by fish fed diet containing 10% CMM (74.8%), fish fed diet containing 0% CMM (72.81% and least (59.9) in fish feed diet containing 30% CMM (59.9%). SGR value was highest (11.91) in fish fed diet containing 0% CMM followed by those fed diet containing 10% CMM (10.99), 20% CMM (8.99) and least (9.19) in fish fed diet containing 30% CMM. Thus, the values decreased as the inclusion level increased. Survival rate (SR) was significantly higher ($p<0.05$) in diet containing 30% CMM than that of containing 0 and 10% which both recorded 86% survival rate, diet containing 20% CMM had the least value of 83%.

The best feed conversion ratio (FCR) value (2.81) was obtained in diet containing 0% CMM, followed by 3.04 in diet containing 10% CMM, 4.17 in diet containing 30% and worst (4.82) in diet containing 20%. Feed Conversion Efficiency (FCE) values ranged from 35.59 in diet containing 0% CMM to 23.97 in diet containing 20% CMM. FCE values in diets containing 10 and 20% CMM showed no significant difference from each other but their values were significantly different ($p<0.05$) from those of other treatments. PER value (0.50) for diet containing 0% CMM was not significantly different ($p>0.05$) from that of diet containing 10% CMM (0.45). However, PER values for diet containing 20% (0.29) and 30% (0.28) were significantly different ($p<0.05$) from that of the diets containing 0 and 10%. Highest PPV value (0.28) was recorded in fish fed diet containing 30% CMM followed by those containing 0% (0.16), 10% (0.11) and least in fish fed diet containing 20%.

Nitrogen metabolism values were 788.12, 721.95, 572.30 and 564.69 for the diets containing 0, 20 and 30% respectively while ADG values were 0.23 (0%), 0.21 (10%) and 0.18 (20% and 30%). The feed intake was highest (66.77) diet containing 0%, followed by 66.41 (10%), 59.19 in diet containing 20% and 54.14 in diet containing 30%. The growth pattern of

experimental fish fed CMM based diets is represented in Figure 2. Growth in both control and diet 5 were significantly better than what was obtained in diets containing 20 and 30%.

Table 17: Growth and Nutrient Utilization of *Clarias gariepinus* fed CMM Based Diets.

Parameters	Diet 1 (0% CMM)	Diet 5 (10% CMM)	Diet 6 (20% CMM)	Diet7 (30% CMM)	SEM
Initial Mean weight (MW)(g)	6.60 [a]	6.60 [a]	6.60 [a]	6.60 [a]	-
Final Mean weight gain (MW) (g)	27.58 [a]	24.71 [b]	18.22 [c]	17.89 [c]	1.21
Mean weight gain (MWG) (g)	20.98[a]	18.11[a]	11.62[b]	11.29[b]	1.21
% Weight gain	317.87[a]	274.39[a]	176.06[b]	171.06[b]	18.39
Specific weight gain (SGR)	11.91[a]	10.99[a]	8.44[b]	8.19[b]	0.02
Feed conversion Ratio (FCR)	2.81[a]	3.04[a]	4.82[b]	4.17[b]	0.02
Feed conversion efficiency (FCE)	35.59[a]	30.89[a]	29.97[b]	23.97[b]	-
Percentage survival (% SR)	86 [a]	86[a]	83 [a]	90[a]	-
Protein efficiency ratio (PER)	0.50 [a]	0.45 [a]	0.29[b]	0.28[b]	0.03
Apparent net protein utilization (AP.NPU)	15.95	9.15	10.63	17.70	-
Protein productive value (PPV)	0.16 [b]	0.09	0.11	0.28	-
Nitrogen metabolism (Nm) x (10)	788.12	721.95	572.30	564.69	-
Average daily growth (ADG)	0.23	0.22	0.18	0.18	-
Feed consumed/ fish (g)	58.95[a]	58.63[a]	50.49 [b]	47.10[c]	0.26

Means with the same superscripts along the same row are not significantly different (p>0.05).

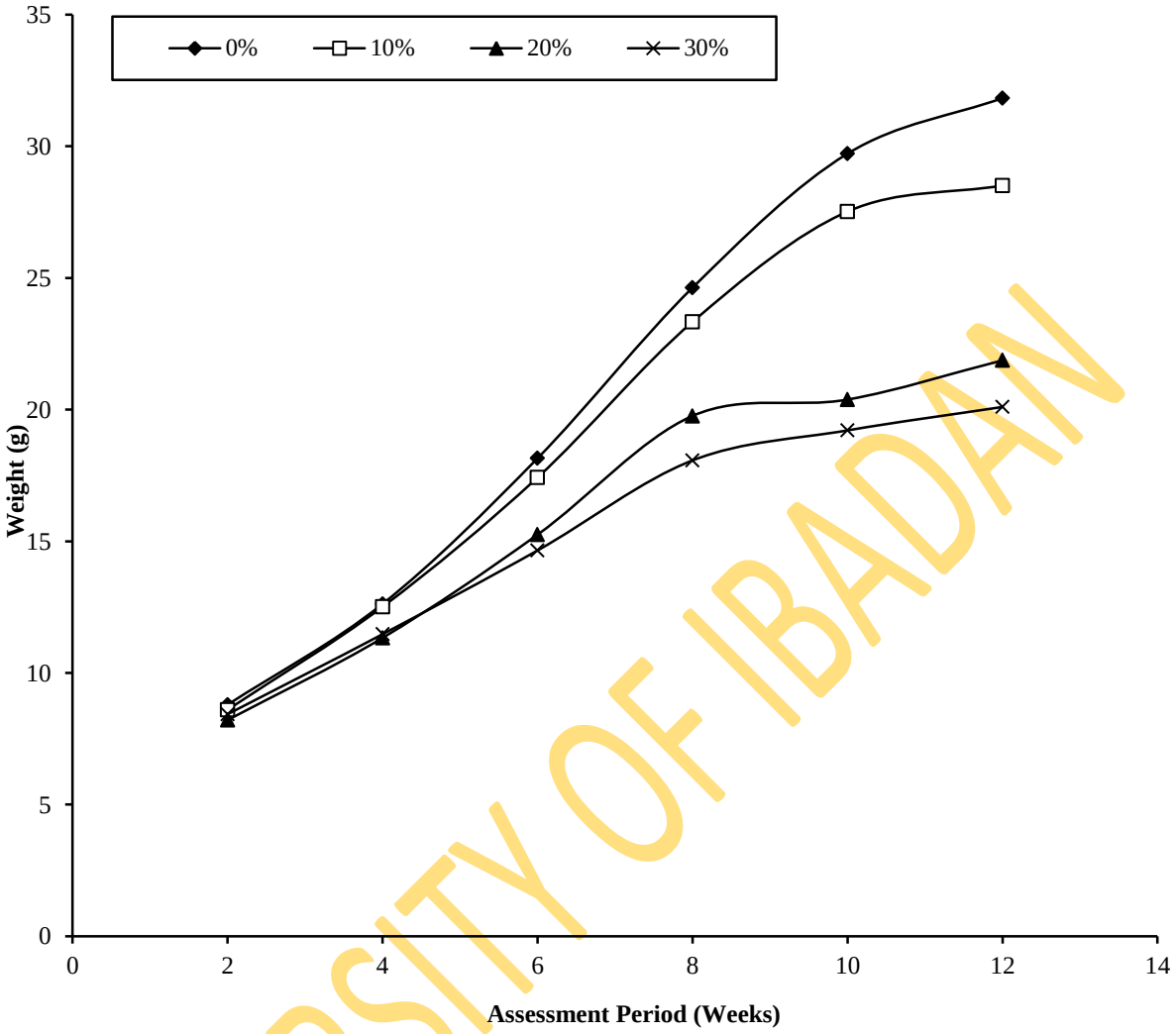

Figure 2 : Weight gain of *Clarias gariepinus* fed varying levels of cooked mucuna seed meal.

4.3.2 Carcass Composition of the *C. gariepinus* fed CMM Based Diets

Table 18 shows the carcass composition of *C. gariepinus* fed CMM based diets. As in RMM based diets, the highest carcass CP (59.7) was recorded in diet containing 30% CMM followed by diet containing 0% CMM (59.00), 20% (56.87) and lowest (56.28) in diet containing 10%. All these were significantly higher than the initial value. Ether extract also decreased from initial 22.05 to 5.73 in diet containing 0%, 8.67 in diet containing 10%, 10.20 in diet containing 20% and 7.75 in diet containing 30%. Carcass fibre increased from nil at the beginning of the study to 10.04, 12.70, 11.40 and 20.18 in diets containing 0, 10, 20 and 30% CMM respectively. Ash content increased from initial 10.86 to 21.20 in diet containing 0% CMM, 15.26 in 10% CMM, 24.36 in 20% CMM and 16.16% in 30% CMM. Moisture content decreased from initial value of 19.96 to 10.84 in diet containing 0% CMM, 7.85 in diet containing 10% CMM, 7.55 in diet containing 20% CMM and 8.33 in diet containing 30% CMM.

4.3.3 Haematological Assessment of fish fed CMM Based diets

PCV values in control, diets containing 20 and 30% CMM (27.00±0.58, 28±2.00 and 37.66±3.51 respectively) rose above the initial value (23) at the end of the trial (Table 19). However, there was a slight decrease in PCV value of the diet containing 0% CMM (22.33. There was no significant difference in initial WBC value and that obtained in diet containing 20% CMM at the end of the study but significant difference existed between initial WBC value and those of diet containing 10% (1.46±4.54) and 30% CMM (1.44 ±4.76). RBC values ranged from 3.10±0.15 in diet containing 20% CMM to 3.47±0.50 in diet containing 30% CMM

Haemoglobin (Hb) values in all treatments increased significantly above the initial 3.8g/L. The highest Hb value, 12.47±1.36g/L was recorded in diet containing 30% CMM followed 9.2±1.38g/L in diet containing 20% CMM, 9.03±0.15g/L in diet containing 0% CMM and

7.4±3.12g/L in diet containing 10% CMM. MCHC values decreased relative to the initial value of 37g/L. There was no significant difference in the MCHC values in all treatments. The highest (33.46±3.45) and lowest (32.56±2.13) values were obtained in diet containing 0 and 20% CMM respectively. Highest MCH value (36.86±8.60) was recorded in diet containing 0% CMM followed by 32.40±9.42 in 10% inclusion, 28.13±1.74 in 20% and 25.60±1.39pg in diet containing 0% CMM.

Table 18: Carcass Composition of *Clarias gariepinus* fed CMM Based Diets

Parameters	Initial	Control (0% CMM)	Diet 5 (10% CMM)	Diet 6 (20% CMM)	Diet 7 (30% CMM)
Crude Protein (%)	52.62	59.00	56.28	56.87	59.7
Ether Extract (%)	22.05	5.73	8.67	10.20	7.75
Crude Fibre (%)	-	10.04	12.70	11.40	20.18
Ash (%)	10.86	21.20	15.26	24.36	16.16
Moisture (%)	19.96	10.84	7.85	7.55	8.33

Table 19: Haematological and Serum Assessment of fish fed CMM Based Diets

Parameter	Initial	Diet 1 (0% CMM	Diet 5(10% CMM)	Diet 6 (20% CMM)	Diet 7 (30% CMM)	SEM
PCV (%)	23^{c}	27.00^{b}	22.33^{c}	28.00^{b}	37.66^{a}	2.22
WBC($x10^{3}$mm)	2.9^{a}	1.83^{b}	1.46^{b}	3.77^{a}	1.44^{b}	0.19
RBC($x10^{12}$/L)	1.7^{a}	3.53^{a}	3.10^{a}	3.30^{a}	3.47^{a}	0.18
Haemoglobin (g/L)	3.8^{c}	9.03^{b}	7.4^{b}	9.2^{b}	12.47^{a}	0.76
MCHC (g/L)	37^{a}	33.46^{b}	33.26^{b}	32.56^{b}	33.06^{b}	0.53
MCH (pg)	23^{c}	25.60^{c}	32.40^{b}	28.13^{b}	36.86^{a}	3.05
MCV (fl)	70^{c}	76.47^{c}	74.3313^{c}	85.30^{b}	111.20^{a}	2.73
Lymphocytes (%)	93^{a}	75.00^{b}	100.00^{a}	100.00^{a}	76.66^{b}	2.40
Neutrophil (%)	7^{b}	18.33^{a}	0.66^{c}	0.00^{c}	0.00^{c}	1.24

Means with the same superscripts along the same row are not significantly different ($p>0.05$)

All these values were higher than the initial value (23). Similarly, MCV value (111.20±24.25) in diet containing 30% CMM was significantly higher than 85.30±3.98 in diet containing 20% CMM, 76.47±3.55 in diet containing 0% CMM, 74.33±28.04 in diet containing 10% CMM and 70.00±0.00 recorded initially. Conversely, diet containing 0% CMM recorded the lowest lymphocyte value 75.00±15.52) followed by 76.66±0.00 in diet containing 30% CMM and 100 in both diets containing 10 and 20% CMM. However, the initial value was 93.00.

The initial value of neutrophil was 7.00. The diet containing 0% CMM recorded highest value of 18.33±8.62, while diet containing 10% CMM recorded 0.66, 20 and diet containing 30% CMM recorded zero.

4.4.4 Plasma Biochemistry of *C. gariepinus* fed CMM based Diets

The highest blood glucose (44.00±16.09g/l) was obtained in diet containing 30% CMM followed by 39.00±10.00) in diet containing 20% CMM and least (31.00±4.00g/l) in diet containing 0% CMM. The initial value was 38g/l. Cholesterol values ranged from 9.66±0.35 in diet containing 0% CMM to 1.40±0.76 in diet containing 30% CMM. The value of cholesterol obtained in diet containing 10% CMM was significantly higher than other treatments. The highest total protein value (129.00±48.19g/l) was obtained in diet containing 0% CMM followed by 83. 67±4.53g/l in diet containing 30% CMM, 62.33±12.58g/l in diet containing 20% CMM and 44.67±4.24g/l in diet containing 10% CMM. Globulin value in diet containing 0% CMM (110.00±4.56) was significantly higher than 68.33±4.47 in diet containing 30% CMM, 45.33±13.87 in diet containing 20% CMM and35.00±0.71 in diet containing 10% CMM. These values were significantly higher than 12.00 obtained before the study commenced. Initial albumin value was 26.00. This was significantly higher than 18.67 obtained in diet containing 0% CMM, 9.33±4.24 in diet containing 10% CMM, 16.67±1.53 in diet containing 20% CMM

and 15.00±1.00 in diet containing 30% CMM. Globulin-albumin ratio value was highest (5.77) in diet containing 0% CMM followed by 4.46 in diet containing 30% CMM, 2.76 in diet containing 20% CMM and 2.63 in diet containing 10% CMM. Globulin-albumin values were significantly higher than the initial value, 0.46, obtained at the commencement of the study (Table 19).

Table 20: Plasma Biochemistry of *C. gariepinus* fed CMM based Diets

Parameter	Initial	Diet 1 (0% CMM)	Diet 2 (10% CMM)	Diet 3 (20% CMM)	Diet 4 (30% CMM)	SEM
Glucose	38 [b]	39.3	31.00 [c]	39.00 [b]	44.00[a]	5.67
Total Protein	38[e]	129[a]	44.67[d]	62.33[c]	83.67[b]	2.60
Cholesterol	ND	2.2[a]	9.66[b]	1.83[a]	1.40[a]	3.40
Globulin	12[e]	110 [a]	35.0[d]	45.33[c]	68.33[b]	2.89
Albumin	26[a]	18.67[b]	9.33 [c]	16.67[b]	15.00[b]	0.20
Glo-Alb. Ratio	0.46[c]	5.77[a]	2.63[b]	2.76[b]	4.46[a]	-

Means with the same superscripts along the same row are not significantly different ($p>0.05$).

4.3.5 Histological Changes in Organs of *C. gariepinus* fed CMM Based Diets

Histological examinations of organs of fish fed CMM based diets were also carried out. The results of the study are presented in Table 21. The brain in diet containing 10% CMM showed moderate spongiosis of the white matter (Plates 14 and 15) while diets containing 20 and containing 30% CMM showed marked spongiosis (Plate 16). There was no histological sign in the gill. The liver in diet containing 10% CMM showed moderate diffuse vacuolation of hepatocytes (Plates 17 and 18) while diet containing 20% CMM revealed marked diffuse vacuolation of hepatocytes (Plate 19). Fish fed diet containing 30% CMM on the other hand, showed mild diffuse vacuolation of hepatocytes (Plate 20). As in RMM based diets, the kidney also showed congestion of kidney cells (Plate 21).

4.3.6 Water Quality Parameters of the Experimental Setup.

There was no significant difference in the values of temperature and ammonia in all the treatments. Dissolved oxygen was lowest (7.79±0.60mg/l) in diet containing 20% CMM and highest in the diet containing 0% CMM while highest p^H (7.00±0.0) and lowest values were obtained in diet containing 30% CMM and diet containing 0% CMM respectively (Table 22).

Table 21: Histological Changes in Organs of Fish fed CMM Based Diets

Organs/Tissues	Histological signs	Diet 1 (0%CMM)	Diet 5 (10% CMM)	Diet 6 (20%CMM)	Diet 7 (30% CMM)
Brain	Moderate spongiosis of the white matter	-	+	-	-
	Marked spongiosis of the white matter	-	-	+	+
Gill	Mild clumping of the secondary lamella	-	-	-	-
	Marked congestion and oedema	-	-	-	-
	Severe oedema	-	-	-	--
Liver	Mild diffuse vacuolation of hepatocytes	-	-	-	+
	Moderate diffuse vacuolation of Hepatocytes	-	+	-	-
	Marked diffuse vacuolation of hepatocytes	-	-	+	-
	Marked widespread vacuolation hepatocytes	-	-	-	-
	Focal marked centrilobular vacuolation of hepatocytes	-	-	-	-
Kidney	Marked congestion	-	+	+	-

Legends: - No visible lesions + Observed lesion

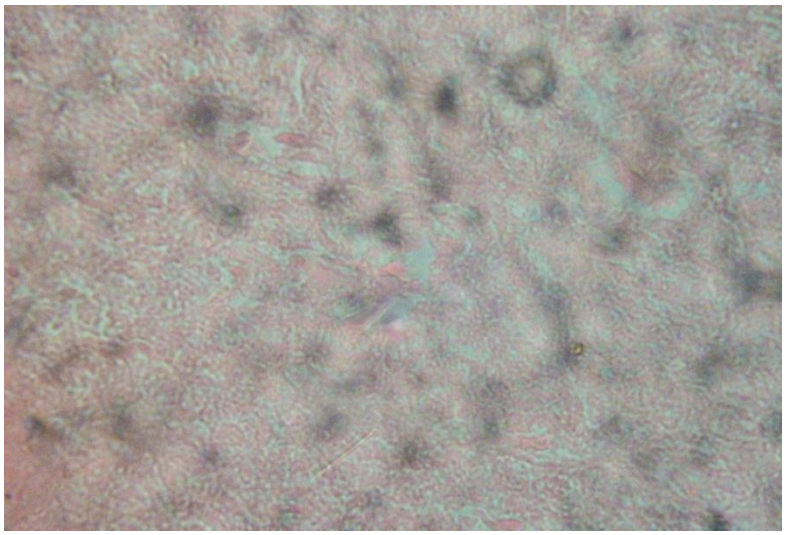

Plate 14: Photomicrograph of a section of brain of *C. gariepinus* fed 0% CMM showing normal cells

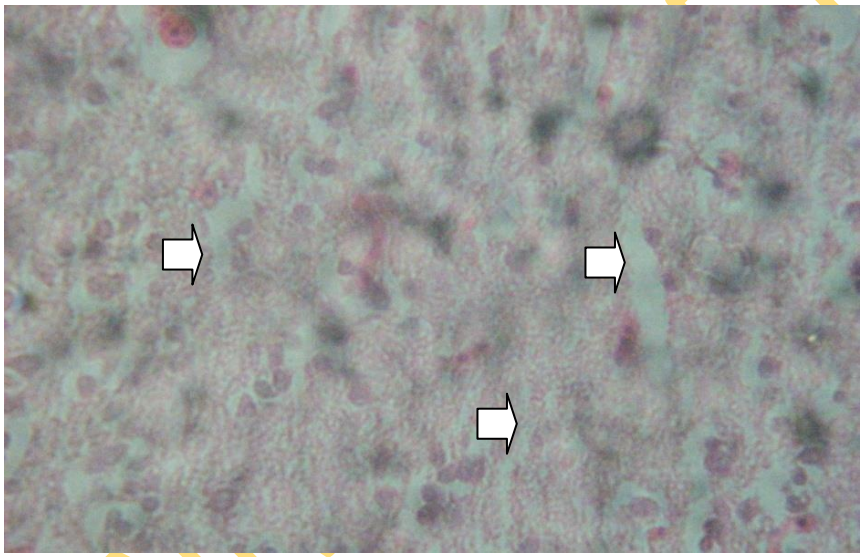

Plate 15: Photomicrograph of a section of brain of *C. gariepinus* fed 10% CMM showing moderate spongiosis of the brain cells

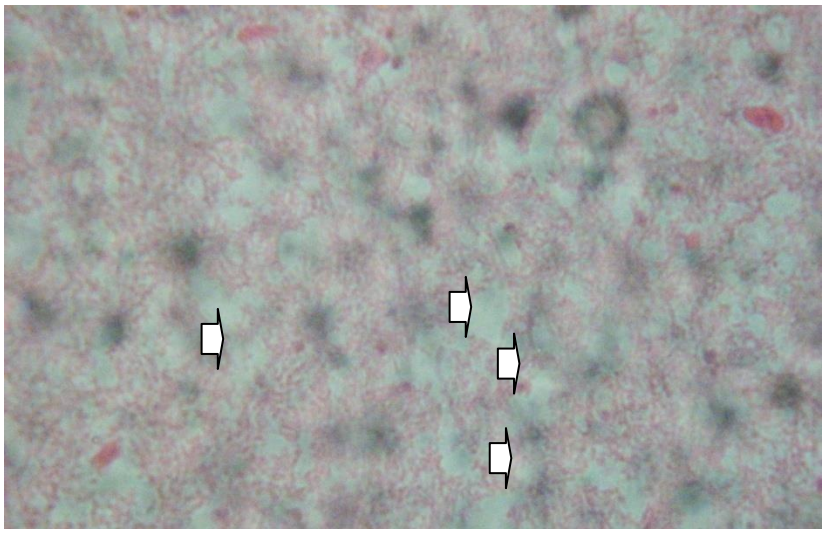

Plate 16: Photomicrograph of a section of brain of *C. gariepinus* fed 10, 20 and 30% CMM showing marked spongiosis of the white matter

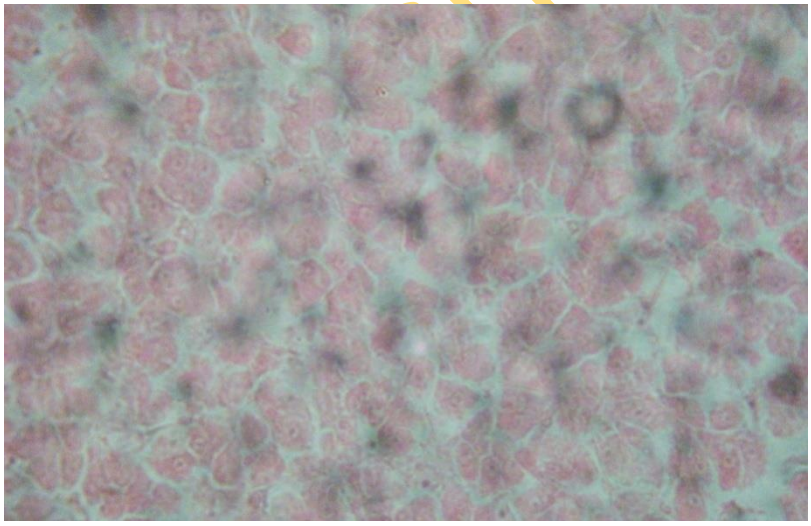

Plate 17: Photomicrograph of a section of liver of *C. gariepinus* fed 0% CMM showing normal cells.

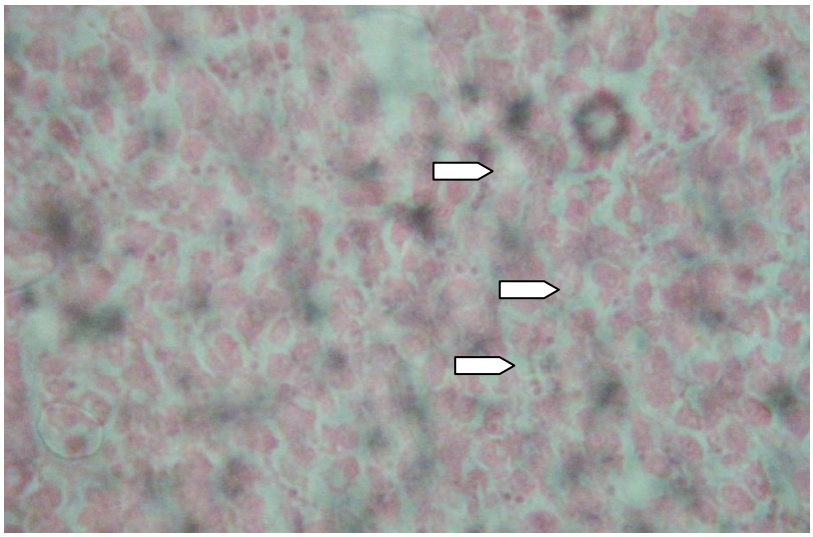

Plate 18: Photomicrograph of a section of liver of *C. gariepinus* fed 30% CMM showing mild diffuse vacuolation of hepatocytes

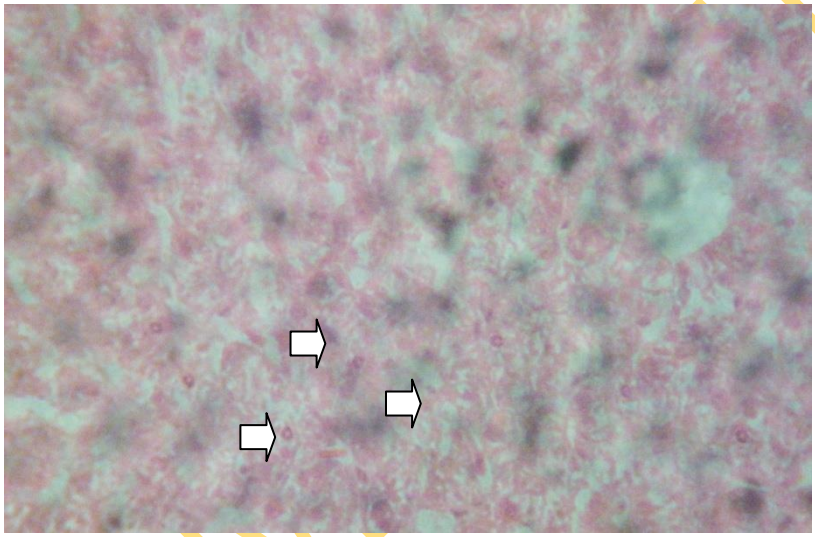

Plate 19: Photomicrograph of a section of liver of *C. gariepinus* fed 10% CMM showing moderate diffuse vacuolation of hepatocytes

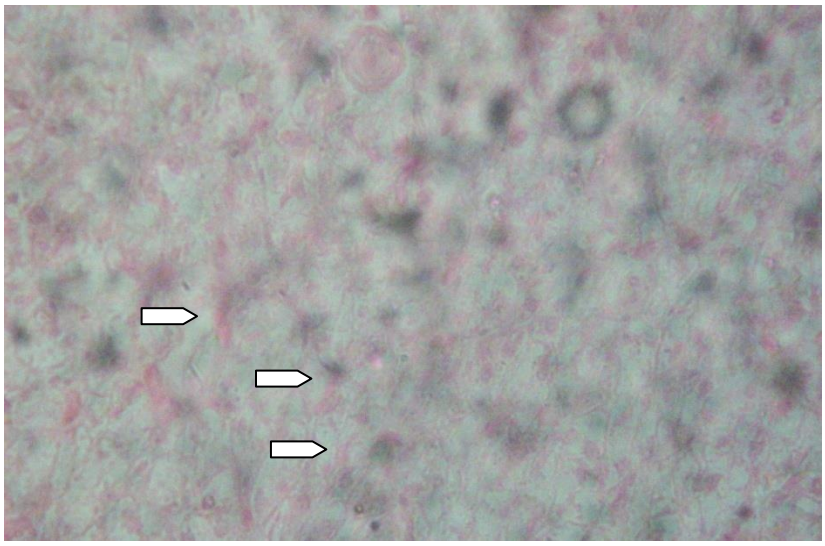

Plate 20: Photomicrograph of a section of liver of *C. gariepinus* fed 20% CMM showing marked diffuse vacuolation of hepatocytes

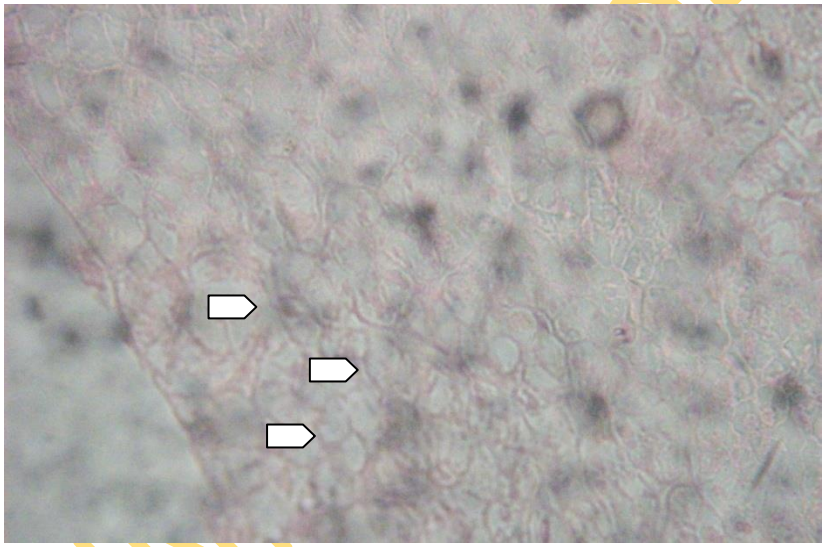

Plate 21: Photomicrograph of a section of liver of *C. gariepinus* fed 20% CMM showing marked widespread vacuolation of hepatocytes

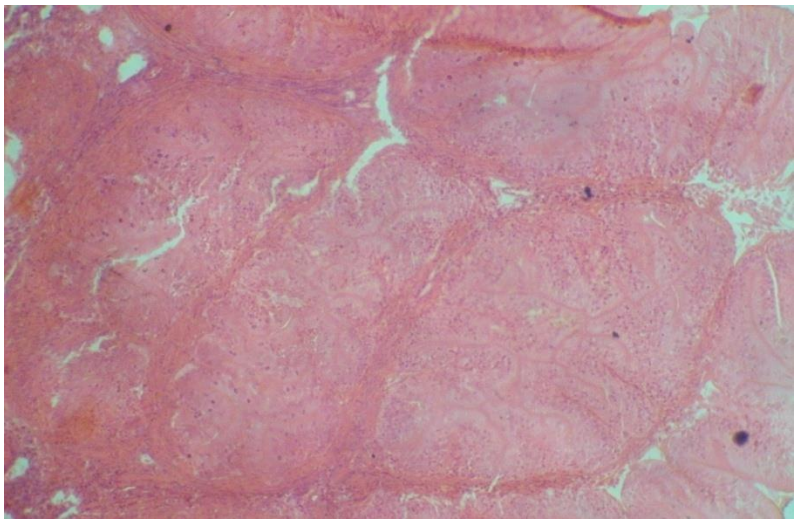

Plate 22: Photomicrograph of a section of kidney of *C. gariepinus* fed 0% CMM showing normal cells

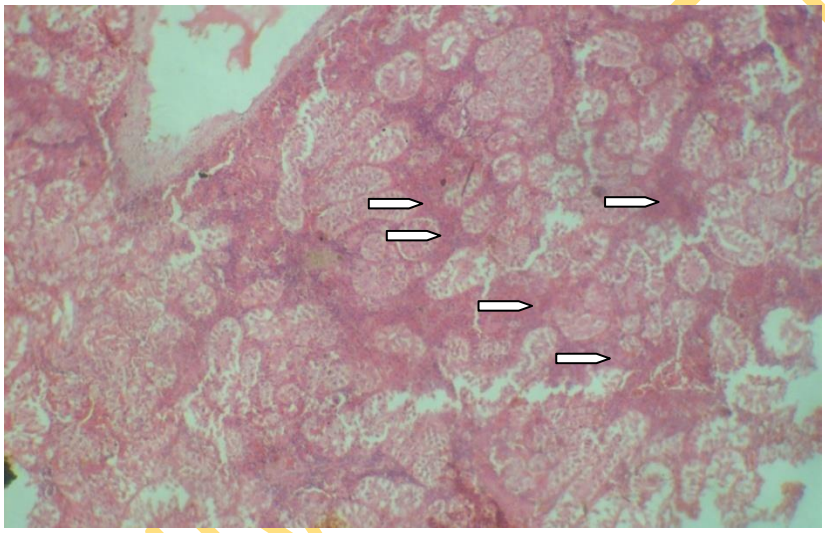

Plate 22: Photomicrograph of a section of kidney of *C. gariepinus* fed 10 and 20% CMM showing marked congestion.

Table 22: Mean Water Quality Parameters values of CMM Based Diets

Parameters	Diet 1 (0% CMM)	Diet 5 (10% CMM)	Diet 6 (20% CMM)	Diet 7 (30% CMM)	SEM
Temperature (^{O}C)	26.5^{a}	26.7^{a}	26.5^{a}	26.5±0.58^{a}	0.40
p^{H}	6.50^{a}	6.60^{a}	6.96^{b}	7.00±0.00^{b}	0.06
Dissolved oxygen (mg/l)	12.1^{a}	11.9^{a}	7.70^{b}	8.40±0.60^{b}	1.39
Ammonia (mg/l)	1.47^{a}	0.40^{a}	0.40^{a}	0.40±0.41^{a}	0.40

Means with the same superscripts along the same row are not significantly different (p>0.05).

EXPERIMENT 4: USE TOASTED MUCUNA MEAL AS A SUBSTITUTE FOR SOY BEAN

4.4.1 Proximate Composition (%DM) of Experimental feed

There was only a slight variation in the values crude protein in all treatments (Table 12). Ether extract recorded the highest value (9.6) in diet containing 30% TMM, followed by 8.7 in diet containing 30% TMM, 8.0 in diet containing 20% TMM and least (7.6) in diet containing 10% TMM. Crude fibre values ranged between 2.56 in diet containing 30% TMM to 1.8 in diet containing 20% TMM.

4.4.2 Growth Response and Nutrient Utilization of *C. gariepinus* fed TMM Based Diet

The result of growth response and nutrient utilization parameters of *C. gariepinus* fed TMM based diets is presented in Table 23. The highest mean weight gain (MWG), 20.98g was obtained in diet containing 0% TMM followed by 16.00g in diet containing 20% TMM, 15.47g in diet containing 10% TMM and least (14.03g) in diet containing 30% TMM. Percentage WG in diet containing 30% TMM (82.72) was higher than that of any other treatments which were 74.45, 73.70 and 72.81 for diets containing 10, 20 and 0% TMM respectively. Highest percentage survival rate (93%) was obtained in diet containing 20%. The diet containing 0% recorded 86% survival while diets containing 10 and containing 30% TMM recorded 83% and 76% survival respectively. SGR values were 11.91, 9.53, 9.79 and 7.67 for diet containing 0, 10, 20 and 30% TMM respectively. Best FCR value (2.81) was obtained in diet containing 0% followed by 3.34 in diet containing 20% TMM, 3.74 in diet containing 10% and 4.54 in diet containing 30%. PER values were 0.50, 0.35, 0.38 and 0.26 for diets containing 0, 10 20 and 30% TMM respectively. PPV value (0.20) in diet containing 10% TMM was highest and 0.06 in

diet containing 20% TMM was lowest. Other values, 0.16 in diet containing 0% and 0.12 in diet containing 30% TMM fell within these extremes.

Nitrogen metabolism (Nm) value, 543.25 in 30% TMM was significantly lower than 652.77 in diet containing 20% TMM, 631.33 in diet containing 10% TMM and 788.12 in diet containing 0% TMM. Generally, Nm values decrease as inclusion level increase.

Table 23: Proximate Composition (%DM) of TMM Experimental feed

Proximate Composition	Diet 1 (0% TMM)	Diet 8 (10% TMM)	Diet 9 (20% TMM)	Diet 10 (30% TMM)
Crude Protein (%)	39.99	40.04	39.99	40.03
Ether extract (%)	8.67	7.62	8.00	9.63
Crude Fibre (%)	2.55	2.15	1.80	2.56
Ash (%)	11.88	12.00	12.0	9.00
Moisture (%)	4.36	4.74	5.00	5.99

Table 24: Growth and Nutrient Utilization of *Clarias gariepinus* fed TMM Based Diets.

Parameters	Diet 1 (0% TMM)	Diet 8 (10% TMM)	Diet 9 (20% TMM)	Diet 10 (30% TMM)	SEM
Initial MW (g)	6.6 [a]	6.6 [a]	6.6 [a]	6.6 [a]	
Final MW (g)	27.58 [a]	20.78[b]	21.71[b]	16.96 [c]	1.21
MWG (g)	20.98 [a]	14.18 [b]	15.11[b]	10.36[c]	1.21
% WG	317.87[a]	214.85 [b]	228.94[b]	156.97[c]	18..39
SGR	11.91[c]	9.53[b]	9.79[b]	7.67[a]	0.02
FCR	2.81[d]	3.74 [b]	3.34 [c]	4.54[a]	0.02
FCE	35.59	26.75	29.97	21.90	-
%SR	86 [b]	83 [b]	93 [a]	76 [c]	-
PER	0.50 [a]	0.35 [b]	0.38 [b]	0.26[b]	0.31
APP.NPU	15.95	17.70	17.00	33.38	-
PPV	0.16 [a]	0.20 [a]	0.06[b]	0.12 [b]	-
Nm (x10)	788.12 [a]	631.33 [b]	652.77 [b]	543.25 [c]	-
ADG	0.23 [a]	0.18 [a]	0.19 [a]	0.17 [a]	-
Feed Consumed (g/fish)	58.95 [a]	53.01 [b]	50.41[b]	47.03 [c]	0.26

Means with the same superscripts along the same row are not significantly different (p>0.05).

Best Average daily growth (ADG) value (0.23) was obtained in diet containing 0%, followed by 0.19 in diet containing 20%, 0.18 in diet containing 10% and worst (0.17) in diet containing

30%. Feed consumed per fish were 66.77, 60.14, 57.10 and 53.27 for diets containing 0, 10, 20and 30% respectively.

The growth pattern of the experimental fish fed TMM based diets is presented in Figure 3.

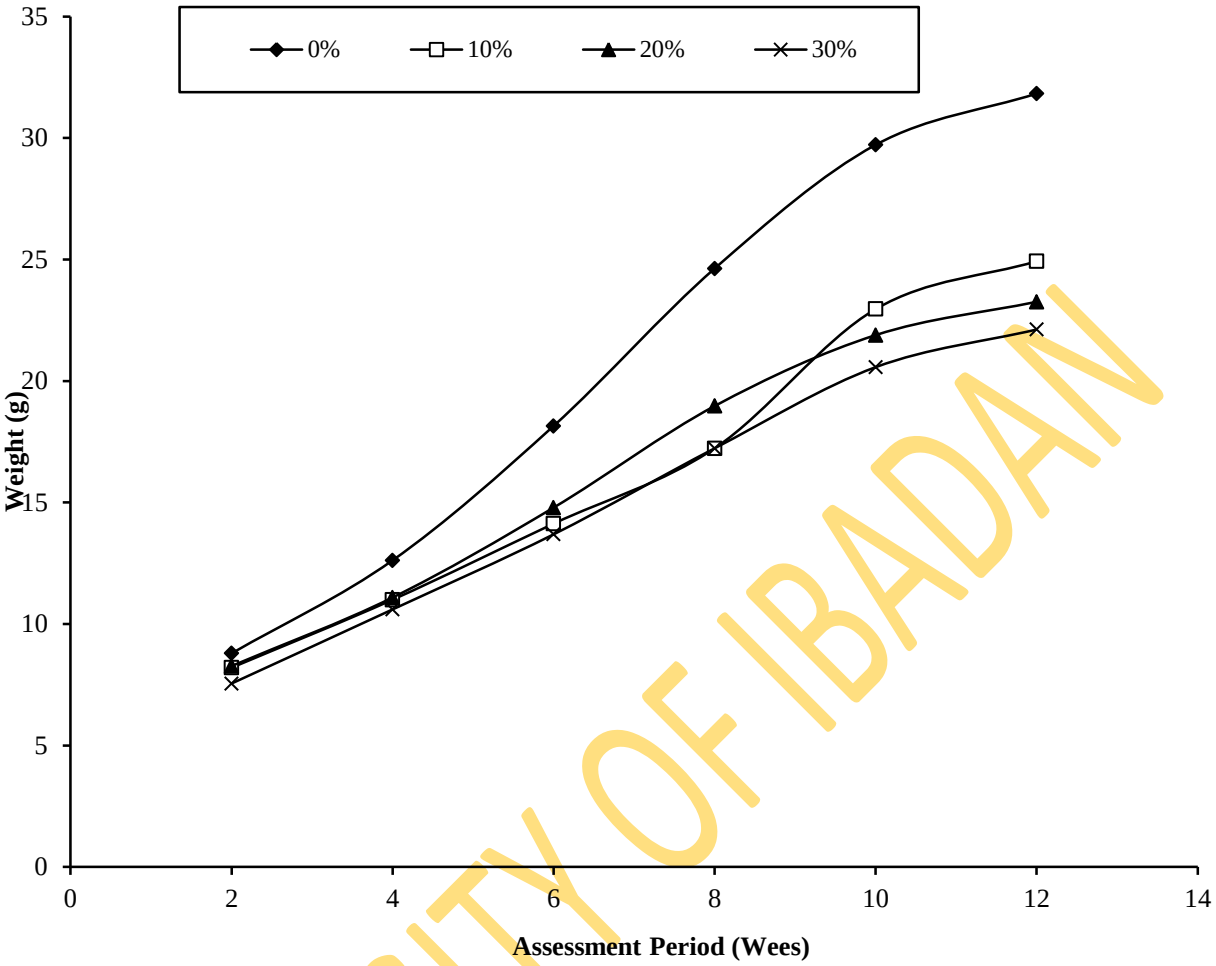

Figure 3 : Weight gain of *Clarias gariepinus* fed varying levels of toasted mucuna seed meal.

4.4.2 Carcass Composition of the *C. gariepinus* fed TMM Based Diets

The carcass composition of fish fed TMM based diets is presented in Table 25. Highest CP value (63.7) was recorded in diet containing 10% followed by 60.81 in diet containing 20%, 59.00 in diet containing 0% and lowest (57.40) in diet containing 20%. Ether extract values were lower from the initial 22.05 except in diet containing 20% whose value was slightly higher (22.43). The lowest value recorded was 5.73 in diet containing 0% followed by 14.33 in diet containing 30%, 16.98 in diet containing 10% and 22.43 obtained in diet containing 20%. Crude fibre in containing 0% was 10.04 as against 13.92 in containing 10%, 15.24 in diet containing 20% and 9.10 in diet containing 30%. Ash content was least (14.35) in diet containing 20% and highest (21.69) in diet containing 30%. Moisture content in all treatments was lower at the end of the study than at the beginning. However, the lowest moisture content (7.54) was obtained in diet containing 10% followed by 9.57 in diet containing 30%, 9.7 in diet containing 20% and 10.84 in diet containing 0%.

Haematological Assessment of fish fed TMM Based diets

PCV values in all treatments were significantly higher than the initial value (23.00). There was however no significant difference in the values among the treatments (Table 27). The highest value (33.66±1.53) was obtained in 30% inclusion while the lowest value (27.00±0.58) was obtained in diet containing 0%. Highest WBC value (6.67±0.23x10^{3}mm) was obtained in 30%, followed by 5.20±2.97 in 10% inclusion, 1.83±2.32 in diet containing 0% and 1.01±1.29 in 20% inclusion. The lowest RBC followed value 2.76±0.35x10^{12}/L was obtained in 20% inclusion followed by 3.53±0.15 and 3.53±0.38x10^{12}/L in containing 0% and diet containing 20% respectively, the highest value3.56±0.50x10^{12}/L was obtained in diet containing 10%. All these values were higher than initial value (1.7 x10^{12}/L). Hb values ranged from 1.20±0.50g/L in

diet 10 to 9.80±0.89g/L in diet containing 10% TMM. Hb values in all treatments were significantly different from the initial value of 2.8g/L. MCHC values 33.46±3.45, 33.20±1.78, 32.66±2.00, and 38.223±3.30g/L were recorded in diets containing 0, 10, 20 and 30% respectively. MCHC values in control, diet containing 10 and 20% were significantly lower than the initial value of 37.00g/L. The highest MCH value 36.20±2.10pg was recorded in diet containing 20% followed by 31.86±2.5pg in diet containing 30%, 29.73±3.26pg in diet containing 10% and 25.60±1.39pg in the diet containing 0% TMM. MCV values ranged from 76.47±3.55fl in control diet to 108.93±6.88fl in diet containing 20% TMM. Lowest lymphocyte value was obtained 75.00±5.52 in diet containing 0% and all other treatments recorded 100.00±0.00fl. Neutrophil value, 18.33±8.62 recorded in diet containing 0% TMM was significantly higher than 7.0 (initial) and 0.00 (for both diets containing 10 and 30).

Table 25: Carcass Composition of *Clarias gariepinus* fed TMM Based Diets

Parameters	Initial	Diet 1 (0% TMM)	Diet 8 (10% TMM)	Diet 9 (20% TMM)	Diet 10 (30% TMM)
Crude Protein (%)	52.62	59.00	63.7	60.81	57.4
Ether extract (%)	22.05	5.73	16.98	22.43	14.33
Crude Fibre (%)	-	10.04	13.92	15.24	9.10
Ash (%)	10.86	21.20	18.14	14.35	21.69
Moisture (%)	19.96	10.84	7.54	9.77	9.57

Table 26: Haematological and Serum Assessment of fish fed TMM Based Diets

Parameters	Initial	Diet 1 (0% TMM)	Diet 8 (10% TMM)	Diet 9 (20% TMM)	Diet 10 (30% TMM)	SEM
PCV (%)	23^{b}	27.00^{a}	32.00^{a}	30.00^{a}	33.66^{a}	2.04
WBC ($x10^{3}$mm)	2.9^{b}	1.83^{b}	5.20^{a}	1.01^{b}	6.67^{a}	2.95
RBC ($x10^{12}$/L)	1.7^{b}	3.53^{a}	3.56^{a}	2.76^{a}	3.53^{a}	0.15
Haemoglobin (g/L)	3.8^{b}	9.03^{a}	7.63^{a}	9.80^{a}	1.20^{c}	0.45
MCHC (g/L)	37^{a}	33.46^{b}	33.20^{b}	32.66^{b}	38.23^{a}	0.48
MCH (pg)	23^{c}	25.60^{b}	29.73^{b}	36.20^{a}	31.86^{b}	1.34
MCV (fl)	70^{c}	76.47^{c}	89.53^{b}	108.9^{a}	95.77^{b}	3.32
Lymphocytes (%)	93^{a}	75^{b}	100.00^{a}	100^{a}	100^{a}	2,54
Neutrophil (%)	7^{b}	18.33^{a}	0.00^{c}	23.33^{a}	0.00^{c}	1.24

Means with the same superscripts along the same row are not significantly different ($p>0.05$).

4.6.1 Plasma Biochemistry of *C. gariepinus* fed TMM based Diets

Blood glucose value was highest (44.00±16.09) was recorded in diet containing 30% followed by 39.33±9.24, 39.00±10.00 and 31.00 in diets containing 10, 20 and 30% TMM respectively. The initial value was 38.00. Total protein values in all treatments were significantly different from each other. The highest value (129.00±48.19) being in diet containing 0% followed by 83.67±4.53 in diet containing 30%, 62.33±12.58 in diet containing 20% and 44.67±4.24 in diet containing 10%. There was no significant difference in cholesterol values in all treatments. The highest value was 2.2±0.78 in containing 0% while the lowest value was 1.43±0.55 in diet containing 10% TMM (Table 25).

Significant difference existed between globulin values in all treatments. However, there was no significant difference between values obtained in diets containing 20 and 30% TMM. The lowest globulin value (35.00±7.37 was recorded in diet containing 10% TMM while the highest value (110.00±45.56) was obtained in the diet containing 0% TMM. Initial albumin value (26) was significantly higher than what was obtained in all treatments at the end of the study. The highest value (18.67±2.52) was recorded in diet containing 0% followed by 16.67±0.58 in diet containing 30%, 13.33±3.51 in diet containing 10% and 13.33±1.53 in diet containing 0%. Globulin-albumin ratio values in all treatments were significantly higher than the initial value of 0.46. 5.77 was recorded in control diet, 3.00 in diet containing 10% TMM, 4.12 in diet containing 20% TMM and 3.37 in diet containing 30% TMM.

4.7.3 Histological Changes in Organs of *C. gariepinus* fed TMM Based Diets

The brain in all treatments except control showed marked spongiosis of the white matter. No other histological sign was observed in the cells. Feeding CMM based diets did not have any negative effect in the gill and kidney. However, the liver in diet containing 10% TMM showed

marked diffuse and focal marked centrilobular vacuolation of hepatocytes while those fed diet containing 20% TMM recorded marked diffuse vacuolation of hepatocytes (Table 28).

Table 27: Plasma Biochemistry of *C. gariepinus* fed TMM based Diets

Parameter	Initial	Diet 1 (0% TMM)	Diet 8 (10% TMM)	Diet 9 (20% TMM)	Diet 10 (30% TMM)
Glucose	38 [b]	39.33±9.24 [b]	31.00±4.00 [c]	39.00±10.00 [b]	44.00±16.0[a]
Total Protein	38 [e]	129±48.19[a]	44.67±4.24[d]	62.33±12.58[c]	83.67±4.53[b]
Cholesterol	ND	2.2±0.78[a]	1.43±0.55[a]	2.17±0.50[a]	1.57±0.49[a]
Globulin (Glo)	12[d]	110.00±45.56[a]	35.00±7.37[c]	54.33±20.55[b]	56.33±[b]
Albumin (Alb)	26 [a]	18.67±2.52[b]	13.33±3.51[c]	13.33±1.53[c]	16.67±0.58[b]
Glo-Alb. Ratio	0.46[c]	5.77[a]	3.00[b]	4.12[a]	3.37[b]

Table 28: Histological Changes in Organs of Fish fed TMM Based Diets

Organs/Tissues	Histological signs	Diet 1 (0% TMM)	Diet 8 (10% TMM)	Diet 9 (20% TMM)	Diet 10 (30% TMM)
Brain	Moderate spongiosis of the white matter	-	-	-	-
	Marked spongiosis of the white matter	-	+	+	+
Gill	Mild clumping of the secondary lamella	-	-	-	-
	Marked congestion and oedema	-	-	-	-
	Severe oedema	-	-	-	-
Liver	Mild diffuse vacuolation of hepatocytes	-	-	-	+
	Moderate diffuse vacuolation of Hepatocytes	-	-	-	-
	Marked diffuse vacuolation of hepatocytes	-	+	+	-
	Marked widespread vacuolation hepatocytes	-	-		-
	Focal marked centrilobular vacuolation of hepatocytes	-	+	-	-
Kidney	Marked congestion	-	-	-	-

Legends: - No visible lesions + Observed lesion

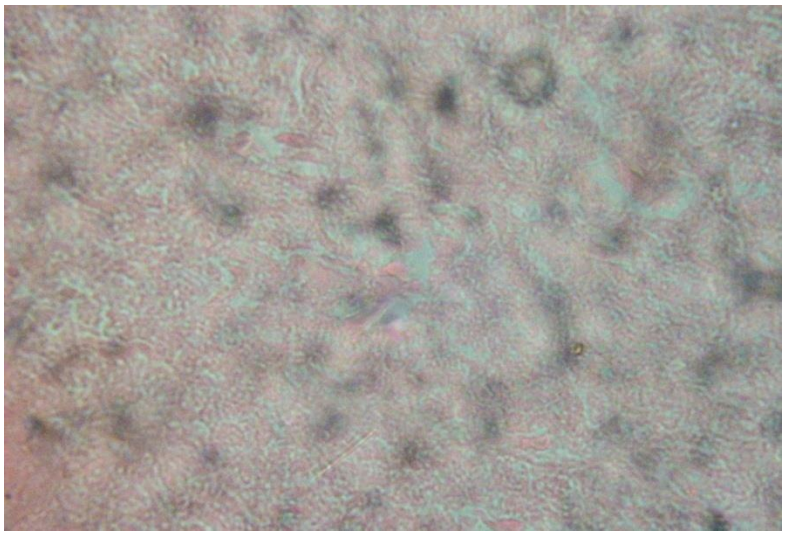

Plate 22: Photomicrograph of a section of brain of *C. gariepinus* fed TMM showing normal cells

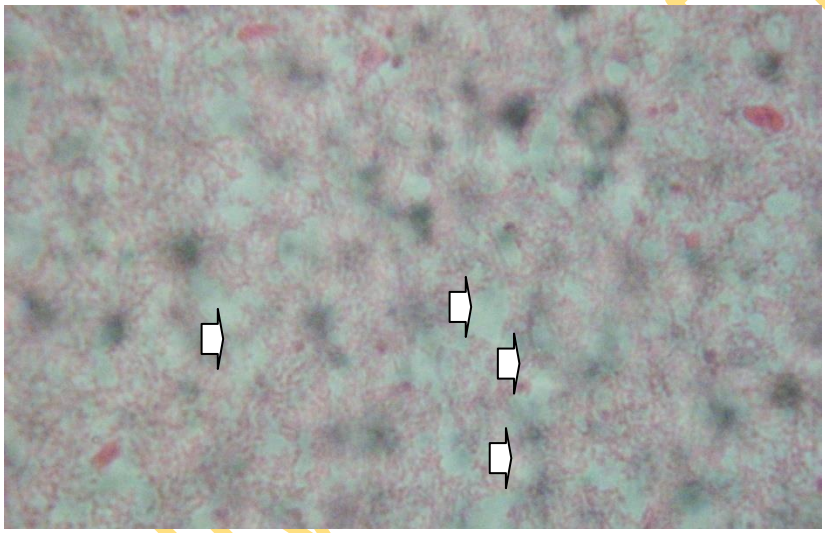

Plate 23: Photomicrograph of a section of brain of *C. gariepinus* fed 10, 20 and 30% TMM showing marked spongiosis of white matter

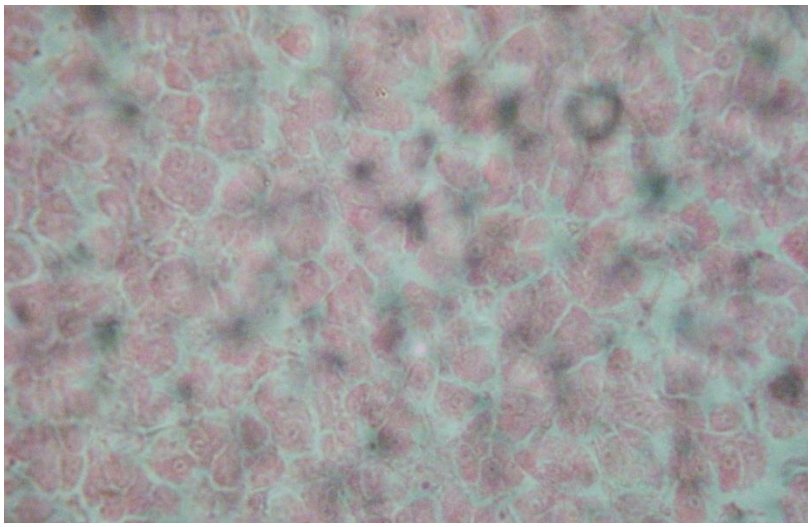

Plate 24: Photomicrograph of a section of liver of *C. gariepinus* fed 0% TMM showing normal cells

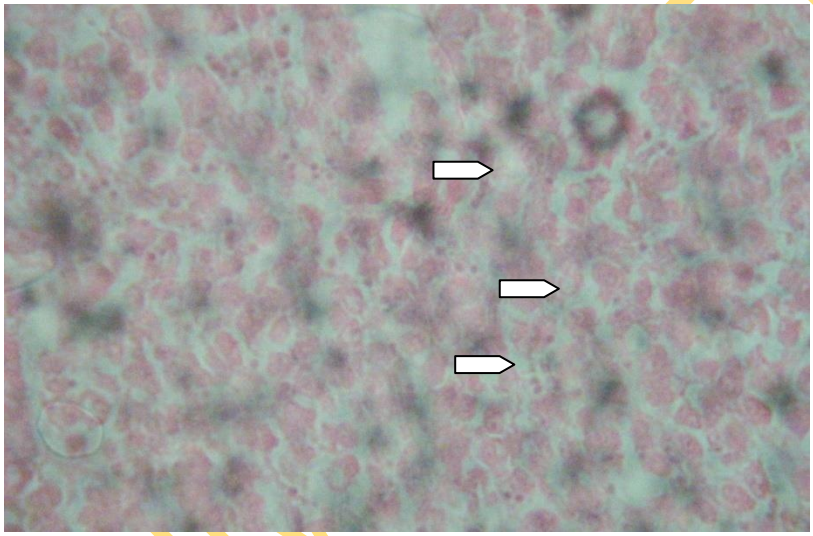

Plate 25: Photomicrograph of a section of liver of *C. gariepinus* fed 30% TMM showing mild diffuse vacuolation of hepatocytes

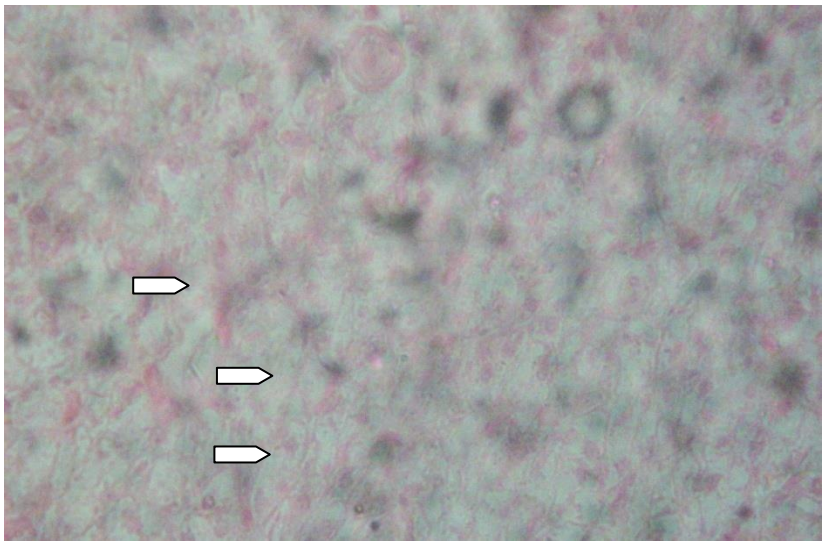

Plate 26: Photomicrograph of a section of liver of *C. gariepinus* fed 10 and 20% TMM showing marked diffuse vacuolation of hepatocytes

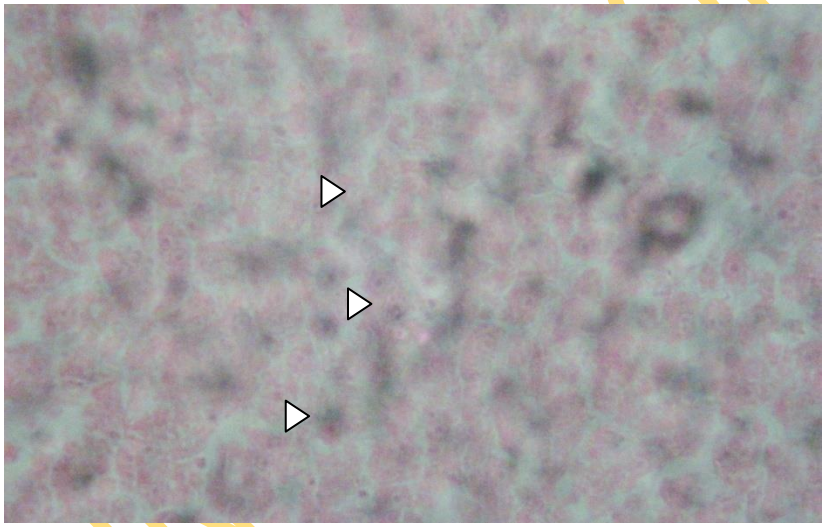

Plate 26: Photomicrograph of a section of liver of *C. gariepinus* fed 10% TMM showing marked centriolbular vacuolation of hepatocytesl

Water Quality Parameters of the Experimental Setup

Dissolved oxygen values decreased as inclusion level increased. The highest value was 12.10±2.20mg/l in control while the lowest was 8.47±2.5mg/l in diet containing 30% TMM. Ammonia level in diet containing 20% TMM was significantly higher than in all other treatments.

Table 29: Mean Water Quality Parameters values of TMM Based Diet

Parameters	Diet 1 (0% TMM)	Diet 8 (10% TMM)	Diet 9 (20% TMM)	Diet 10 (30% TMM)	SEM
Temperature (^{O}C)	26.5^{a}	26.7^{a}	27.0^{a}	27.2^{a}	0.40
P^{H}	6.50 ^{a}	7.00^{a}	7.00^{a}	7.00^{a}	0.06
Dissolved oxygen (mg/l)	12.1^{a}	10.4^{b}	9.33^{bc}	8.47^{c}	1.33
Ammonia (mg/l)	1.47^{b}	0.47^{b}	0.33^{b}	2.67^{a}	0.40

CHAPTER FIVE

5.0 DISCUSSION

EXPERIMENT 1

5.1 Effects of Processing on the Proximate Composition and L-DOPA Content of Mucuna

5.1.1 Effects of Processing on the Proximate Composition *of* Mucuna Seed Meal

The results of the proximate composition of Mucuna obtained in this study showed that its nutrient composition compares favourably with other legumes and are within the range reported by Oyenuga(1968), Hasim and Idris (1977), Kay (1979), Ukachukwu and Obioha (1997a), Emenalon and Udedibie (1998), Camara *et al.* (2003) and Ogunji *et al* (2003). The results are consistent with the submission of Sridhar and Bhat (2007) who reported that Mucuna seeds consisted high protein, high, carbohydrates, high fibre, low lipids and minerals. It met the requirement of essential amino acids.

Processing Mucuna into meals using different methods produced slight reductions in the crude protein, fat and fibre in the current study, this according to Emenalom and Udedibie (1998) is due to solubilization of some nitrogenous compounds during cooking. Ogunji *et al (2003)* reported that processing led to the deactivation of some of the nutrients and anti-nutrients in legumes. The results obtained in this study differs slightly from that of Osuigwe (2007) who reported that cooking Mucuna for sixty minutes improved the protein content by 6.6% and that of Emenalom and Udedibie (1998) who reported that toasting Mucuna seeds led to an increase in its protein content. The differences observed in this study may be due to differences in period of cooking and methods of processing employed. However, Ani (2008) reported a decrease of 1.7% in protein of cooked *Mucuna pruriens* as compared to raw and concluded that the reduction was

due to leaching of soluble protein into the cooking water. Also, Ani (2002) showed that cooking of castor oil bean led to the solubilisation of and removal of some nitrogenous substances in the castor oil bean. The result obtained in the current study agrees with the results of these workers. The mineral components of the seed in the current work are similar to that reported by Iyayi and Egharevba (1998) and Ukachukwu *et al* (2002) and Ukachukwu and Obioha (1997). Dahouda *et al (2009*) reported that Mucuna seed processing reveals changes in ash, NFE, CP, Crude fibre and EE contents which is similar to the observation in this study. The relatively high content of Nitrogen Free Extract seems to suggest that the seeds can be used in a semi-intensive setting to supply nutrients to fish. *Mucuna utilis* in this study contains sufficient nutrients and can be used as feed ingredient in the diet of *Clarias gariepinus.*

5.1.2 L-DOPA content of *Mucuna* seed meal.

The L-DOPA content of raw *Mucuna* seed meal is consistent with the reports by Bell and Janzen (1971), Vadivel and Janardhanan (2000) and Ezeagu *et al., (*2003). L-DOPA, a non-protein amino acid is potentially toxic on consumption and not easily de-toxified. This is a major constraint to its use in fish feed even though MSM contains a rich source of protein and carbohydrate along with an adequate concentration of minerals. Afolabi *et al.* (1985), Ravindran and Ravindran (1988) reported that L-DOPA is the biggest obstacle confronted in the utilization of Mucuna beans and Sridhar and Bhat (2007) reported that the presence of this compound is a major impediment to consider it as food and feed. Intoxication on consumption of Mucuna is related to the quantity of L-DOPA (Janardhanan and Lakshmanan (1985).

In the current study, processing reduced the quantity of L-DOPA as reported by Carsky *et al.* 1998) and Egounlety (2003). Janardhanan *et al* (2003) reported a reduction in L-DOPA content of 18%- 20% of five accessions of *M. utilis* subjected to various processing methods and

concluded that repeated boiling in water and decanting is more effective than any other method of processing. However, toasting at 130^0C in the present study seemed to be more effective than boiling and decanting reported by these authors. Result of the present work agrees with the submission of other workers (Siddhuraju *et al.*1996, Dossa *et al.*1998, Bressani 2002, Gilbert 2002, Diallo and Berhe 2003),that no one single method can be conclusively considered best but detoxification could employ a combination of methods. According to Tacon and Jackson (1985), Pfeffer et al. (1995) and Fagbenro (1999) the anti-nutritional factors in legume seeds require inactivation by heat processing, roasting or autoclaving, before inclusion in fish feeds. Siddhuraju and Becker (2001) reported that a single processing technique was ineffective on the reduction of anti-nutritional factors and the enhancement of nutrient assimilation in feeds containing legume seeds. The development of new combined processing technologies of legume seeds permits higher use in fish feeds (Gouvia *et al.* (1993). Future trials with Mucuna should therefore consider as many methods as possible so that the problem posed by this anti-nutrient can be surmounted so that Mucuna seeds can be listed among the long array of fish feed ingredients.

EXPERIMENT 2

5.2 Use of RMM as substitute for soybean meal

Mean weight gain in the control diet was significantly better than other treatments. Fish grew best on control diet. Growth generally decreased as inclusion level increased in all treatments. Osuigwe (2003) also reported similar result when he fed *M. cochinchinensis* to *Heterobranchus longifilis* and related his findings to the presence of an anti-nutritional factor detected in Mucuna. Similarly, the replacement of 20-40% of the total dietary protein in the diets by raw Mucuna seeds also produced low growth performance in carp (Siddhuraju and Becker

2001). This reduction in growth performance was also attributed to the presence of various anti-nutrients present in Mucuna seed meal. A report by Dahouda *et al (2009*) indicated that raw seed significantly impaired feed intake and growth in guinea fowl but processing improved the condition. These authors therefore discouraged the use of raw MSM but recommended an inclusion of up to 20% processed MSM to replace soybeans in adult guinea fowl. The results obtained in this study however showed that C. *gariepinus* can tolerate up to 20% inclusion of RMM. Growth within the first four weeks was similar in all treatments (Figure 1). This is likely due, at least in part, to the fact that fish were just acclimatizing to the diets and the change in diet probably affected the palatability and therefore the consumption rate by the fish at this period. It could also be due to reduced flavour. Fagbenro *et al.* (2010) reported depressed growth in clariid fish fed unprocessed sunflower and sesame seed meals at inclusions beyond 30% and related the cause to the presence of anti-nutritional factors in these seeds.

The FCR value in diet containing 30% RRM was significantly higher than the values in the other treatments indicating a poorer conversion of nutrient into tissues. The non significant difference between FCR in treatments 0, 10 and 20% RMM showed that fish were able to digest and convert the diets into tissues with the same degree of efficiency. Thus, fish can tolerate up to 20% RMM based diets.

SGR and Nm values in diets containing 10 and 20% RMM were not significantly different from each other but were significantly different from that of 0 and 30%. PER in diets containing 0, 10 and 20% RMM were not significantly different from each other. PER is known to be regulated by the non protein energy input of the diet and it is a good measure of the protein sparing effect of lipid and/or carbohydrate (Lie *et al.*1989; Tibbets *et al.*, 2005). Fagbenro *et al.* (2010) reported depressed growth in clariid fish fed sunflower and sesame seed meals at inclusions beyond 30%

and related the cause to the presence of anti-nutritional factors in these seeds. In the current study, the significantly poor nutrient utilization reported in fish fed 30% RMM could also be blamed on the presence of a wide range of anti-nutritional factors that has been reported to be present in the seed (Ghosal *et al*., 1971, Duke 1981, Vadivel and Janadharnan 2000, Francis *et al.* 2001, Carmen 2002).

Carcass protein and crude fibre increased in all treatments but the body fat dropped significantly. Sotolu (2010) reported higher values of fish carcass protein and lipid than initial values in fish fed *Leucaena leucocephala* seed meal and attribute his observation to different utilization levels of the diets and Fafioye *et al.* (2005) further observed that fish carcass composition recorded higher protein content in *C. gariepinus* fed processed soybean meals than the traditionally raw and toasted soybean. This indicates that there was protein synthesis and increased tissue production in the fish. This agrees with Cho *et al.* (1985), Richter *et al.* (2003) and Adesina (2008), who observed that when proper feeding commence in fish growth relatively increased.

PCV, RBC, Hb and MCV at the end of the trial rose significantly above the initial value. Sotolu (2008) made similar observation in *C. gariepinus* fed *Leuceana leucocephala.* Svobodova *et al.* (1994) also reported that significant increases in erythrocyte count was found in carp after toxic exposure to organophosphorus pesticides and concluded that toxic substances can significantly damage the heamopoetic system of fish. According to Ajani (2005) and Kori-Siakpere and Ubogu (2008), high WBC count means release of more cells to maintain homeostatis while low RBC is a common stress response. Thus increasing or decreasing numbers of white blood cells are a normal reaction to a toxicant demonstrating the effect of the immune system under toxic conditions. Effects of feeding-MSM based diets on haematological

parameters in *C. gariepinus* in the present study included increased level of erythrocyte number, haemoglobin concentration and haematocrit values. These observations might indicate a compensatory erythropoiesis, which resulted in production of RBC to recompense the older ones that are rapidly destroyed due to decrease in blood's carrying capacity. This observation is in agreement with reports by Mazon *et al.* (2002) and Oshode *et al.* (2008). Increase in haematocrit is an indication of a stress response causing RBC swelling or haemo-concentration due to plasmatic volume reduction (Wilson and Taylor, 1993). WBC plays an important role in the immune system of living organisms. An unusually high WBC count might indicate hypersplenism, inflammation, trauma and stress (Nordenson, 2004). Ayoola (2011) observed that there was an increase in the WBC of *C. gariepinus* fed poultry waste and attribute this to increase in the production of leucocytes in the haematopoietic tissue of the kidney and the spleen. The consequence of this is the suppression of the immune system and increased susceptibility to disease. MCV and MCH increased significantly above the initial values. The MCV gives an indication of the status or size of the red blood cells and reflects an abnormal or normal cell division during erythropoiesis. The decrease in MCV indicates that the RBC has shrunk. This agrees with the findings of Alwan *et al*. (2009).

Neutrophil in dietscontaining 0 and 10% RMM were significantly higher than the initial value. However, neutrophil in diets containing 30% RMM and 10% CMM were zero. Foster (2011) reported that when total nuetrophil increases it is a sign of bacterial infection or a form of stress. The cause of the increase in this study could not be ascertained but it is unlikely to be associated with bacterial infection as there was no sign of such infection in the fish used in the study. There was no significant difference between initial lymphocytes value and diets

containing 10, 20 and 30% RMM. This further confirms the fact that feeding RMM based diets did not likely lead to secondary infection in the fish

Fish fed processed MSM-based diets showed various histo-pathological changes in the brain, gill, and liver. These changes are indications of toxic effects of feeding MSM-based diets to *C. gariepinus* since no histological change was observed in the diet containing 0% MSM. This observation is in agreement with the report of Iyayi *et al.* (2006) who reported that broiler birds fed heated and raw Mucuna bean meals produced varied histological signs in their organs. The brain of the fish fed Mucuna-based diets showed moderate to marked spongiosis of the white matter in the cerebellum. This is similar to the observation of Adesina (2008) who reported mild congestion of the cerebellum in *Oreochromis niloticus* juveniles exposed to fresh root-bark extract of *Moringa oleifera* and attributed this observation to toxic effect of the substance on the organ and tissues. Some authors have attributed the toxicity of Mucuna to the presence of L-DOPA (Afolabi *et al*. 1985, Josephine and Janardhanan, 1992). Carew *et al.* (2002) and Iyayi *et al.* (2006) identified L-DOPA as the neurotoxic agent in Mucuna and that unprocessed raw velvet beans (Mucuna) caused neurotoxicity and behavioural changes in humans. The results obtained in the present study tend to support these findings.

Feeding MSM-based diets to *C. gariepinus* resulted to various lesions in the liver cells of the fish. The lesions ranged from mild diffuse vacuolation of the hepatocytes to focal marked centrilobular vacuolation of hepatocytes while the liver of the diet containing 0% MSM showed normal morphology. This observation agrees with the work of Jha (2004) who reported remarkable lesions in the liver of *C. batrachus* exposed to surf and that of Ayoola (2008) on the effects of glyphosate in *C. gariepinus.* Omitoyin *et al.* (2006) also reported similar observation in fish exposed to Lindane. Ukachukwu *et al.* (2003) reported that diets containing raw beans

caused wide area of periportal necrosis with some mononuclear cell infiltration in the liver of broilers, while the centrilobular areas showed vacuolation and degeneration of hepatocytes. Ayoola (2008) opined that vacuolation of liver cells is an evidence of fatty degeneration. In the present study, lesions observed in the liver may probably have resulted from the excessive work done by the fish to get rid of toxicants from its body during the process of detoxification by the liver. Hepatocytes in the periportal areas suffer most from toxic insults. In this situation, toxic substances present in *Mucuna* beans must have been responsible for the histopathological changes found in the liver sections.

The gill in the diets containing 0% CMM and TMM diets had normal morphology of the hayaline cartilaginous rods in each filament, while the gill of fish fed RMM diets showed histological changes which included mild clumping of the secondary lamella, marked congestion and oedema and severe oedema. According to Fafioye *et al.* (2004), these histopathological changes in the gills are similar to epithelial damages caused by cadmium. The alterations produced may probably lead to several physiological stresses in the fish. Histological changes were observed only in the kidney of fish fed RMM but not in any other.

The water quality parameters of the experimental set up was maintained within the range recommended by Boyd (1979), Viveen *et al.* (1985), Omitoyin (1995) and Omitoyin (2006). Olapade (2009) reported a temperature range of 26.4 - 27.7 for *C. gariepinus* cultured in earthen ponds. Boyd and Litchkoppler (1979) noted that warm water fish grow best at temperature between 25^0C and 30^0C. This is similar to a range of 26.3 - 27.2 recorded in this study. It can then be concluded that whatever negative changes or abnormality observed in this study may not be linked to water quality parameters of the experimental set up as adequate care was taken to maintain ideal ranges.

EXPERIMENT 3

5.3 Use of CMM as substitute for soybean

Fish growth was generally poor among fish fed diets containing 20 and 30% CMM despite their comparatively high apparent digestibility values. Nwanna (2003) also recorded progressively low weight gain in experimental fish as the inclusion of fermented shrimp head waste increased in the diet of *C. gariepinus* increased. Fagbenro (1996) however opined that other than looking at growth responses, the digestibility of nutrients in feedstuffs could be used to assess the suitability and nutritive value of feedstuffs or diets in fishes. This is because the digestibility of individual ingredient is the diet is considered as one of the important factors affecting the growth of fish (Cho *et al.* 1985, De Silva *et al.* 1996).

FCR value in diet containing 10% CMM was better but not significantly different ($p>0.05$) from that containing 0%. SGR and PER decreased as inclusion level decreased following the pattern recorded in experiment 1. This means that fish were able to utilize the nutrients furnished by the diets even better than the control diet. Faturoti and Lawal (1986) recorded similar results in *Oreochromis niloticus* fed a combination of supplementary feed and organic manure. Cooking improved the performance of fish fed at 10% inclusion. Inclusion of 10% CMM in the diet of *C. gariepinus* in this study resulted to similar results as that of 0%. It can be concluded that cooking improved the nutrient utilization by the fish. The improvement in the body weight gain and feed efficiency could be explained by the fact that feed intake by fish, in relation to its weight gain, tends to increase as it grows rapidly with improved feed efficiency. Belewu and Olajide (2010) also made similar observation in sheep fed graded levels of *Mucuna* meals. Also, Siddhuraju and Becker (2003) reported that use of processed *Mucuna* seeds improved the growth performance and nutrient utilization of tilapia compared with that of raw

seeds and that values obtained were found to be similar to the results obtained with a fishmeal-based control diet. Siddhuraju and Becker (2002) reported that partial replacement of fish meal with *Mucuna* beans after prolong soaking of cracked seeds in various solutions followed by autoclaving for short time improved the growth performance and feed utilization. They then attributed their result to the improvement of the structural changes, palatability and digestibility of available major nutrients such as proteins and carbohydrates in addition to the higher reduction/inactivation of anti-nutrients during the hydrothermal process. *Mucuna* seed meal therefore tends to hold great potential as feed ingredient for fish and other livestock however the seeds require some form of processing in order to maximize its feed potentials in fish and livestock nutrition.

Carcass protein, crude fibre and ash in experimental fish were significantly higher at the end of the feeding trial than the initial in all treatments. Similar observation was made by Fafioye *et al* (2005). However, the carcass lipid, and moisture dropped significantly. Adebayo (2010) reported a decrease in carcass fat in both *Heterobranchus bidorsals, C. gariepinus* and Hetero-clarias fed dietary phosphorus supplement and Robinson *et al.* (2001) reported that fish fed to satiation contain more fat than fish fed at restricted levels.

PCV value in diet containing 10% CMM fell below the initial but not significantly different from it. However, PCV values in other treatments rose significantly above the initial value of 23. A decrease in PCV may show extent of the shrinking cell size due to treatment effect (Ahmad *et* al 1995; Atamanalp and Yanik, 2003). MCHC values in all the treatments dropped significantly below the initial value however the values did not differ significantly among treatments. Hb, RBC, MCH and MCV values recorded a significant increase above the

initial value in all treatments. Atamanalp *et al* (2002) also observed a significant increase in RBC count in *O. mykiss* exposed to cypermethrin.

Total protein levels increased and differed significantly among treatments. Dietary raw velvet bean caused several changes (including lower blood cholesterol) in blood chemistry of chickens (Carew *et al.* (2002) and rats (Iauk, *et al.* 1989*).*

Fish fed graded levels of CMM diets showed various histo-pathological changes in the brain, liver and kidney. Fish fed diet 5 showed moderate spongiosis of the white matter in the cerebellum of the brain while those fed diets 6 and 7 showed marked spongiosis. Iyayi *et al* (2006) also reported varied histological changes in broiler birds fed heated MSM diets and Adesina (2008) reported congestion in the brain (cerebellum) of *Oreochromis niloticus* exposed to fresh root-bark of *M. oelifera*. The histological changes observed in liver and kidney must have been caused by the ingestion of high percentage of CMM based diets which imposed stress on the organs above their capacity to cope. The liver and kidney have been identified as the sites that are mostly affected by 'toxic' substances in man and various clinical signs have been linked to liver and kidney detoxifying these toxic substances in man (Benjamin, 2009). This author reported that congestion of the kidney is the first stage in the development of kidney disease. Feeding of CMM based diets for twelve weeks in this study thus have negative influences on the liver and the kidney. This is a sign of high toxicity of the diets to the fish; further research is therefore needed to explore better way of processing that will reduce the toxic components in *Mucuna* seeds.

As discussed under RMM, adequate care was taken to maintain the water quality parameters within tolerable ranges for *C. gariepinus* and there was little variability among the values among treatments. The lowest mean value of dissolved oxygen (7.70 ± 0.60) recorded in

fish fed diet 20% CMM is higher than the optimum range recommended by Boyd (1979). Temperature values in all treatments were not significantly different from each other. Ajani (2007) reported that a temperature range of 27-31^{0}C as ideal for warm water fish. The minimum temperature of 26.5^{0}C and maximum of 27.1^{0}C recorded in this study fell within the acceptable recommended range.

EXPERIMENT 4

5.4 Use of TMM as substitute for soybean

Inclusion of 10-30% TMM in fish rations caused progressive reductions in growth with an extreme growth depression produced by feeding 30%. This is similar to what Flores *et al.* (2002) observed in pigs fed MSM based diets in replacement of soybean meal. They reported higher weight gain with toasted *Mucuna* than with cooked *Mucuna*. In the current study however, best growth was obtained only in 20 % toasted *Mucuna*. Growth at 30% inclusion was greatly reduced. This might be due to the fact that as inclusion level of MSM in the diet increased, the concentration of growth depressing factors also increased leading to a more pronounced effect on nutrient utilization by the fish. Ukachukwu *et al.* (2002) investigated the effect of different locally adaptable and cost effective methods of processing *Mucuna* bean for inclusion in broiler diets and reported that cooking the beans for 90 minutes was more effective in reducing toxic components than toasting or soaking them in order to reduce the constituent toxicant load and to produce meals that supported higher performance in broilers. Results obtained in both experiments 3 and 4 in this study tend to support their findings.

Nutrients were poorly utilized by fish fed 20 and 30% TMM. This was evidenced by comparatively low value for nutrient utilization parameters when compared to what was obtained in experiments 2 and 4. Ukachukwu (2002) reported that experimental trials on pigs in southern

Benin showed that supplementing pig diet with 100g day^{-1} of roasted or cracked and soaked (overnight) *Mucuna* seeds for 12 months produced weight gains 30-50% better than the control. However, fish fed. Omitoyin (195) observed that even though feather meal contained high crude protein content (85%), it did not have commensurately high digestibility and nutrient utilization in fish indicating its nutritional poor status. The observation in the present study agrees with this observation.

Carcass protein improved in all treatments above the initial value. However the improvement recorded in 30% inclusion was the least. Fish were therefore able to utilize feeds fed at varying degrees. This is in line with report of Alegbeleye (2005) who reported an increase in protein level of *O. niloticus* fed autoclaved cottonseed meal and attributed it to loss of soluble solids like gossypol, carbohydrates and lipids to leaching during autoclaving. Anti-nutritional factors in *Mucuna* in this study must have been reduced through toasting of the seeds.

PCV, RBC and Hb values of fish in all tested diets were not significantly different from each other, these values however differed significantly from the initial fish. Church and Pond (1974) observed that haematological traits especially PCV and Hb values correlated with the nutritional status of the animal and the influence of diet on haematological trait is very strong. Ajani (2005) reported that low PCV and RBC values in extensive system were associated to stress due to high stocking and poor nutrient quality of feed. These parameters in the present study were much higher than the initial, thus confirming the ability of the quality of the experimental diets to support normal physiological functions in the tested fish. The initial MCHC values for all the diets were of the same average and within the same range (33-38%). This indicates adequate dietary energy for all the fish. Similar observation were made by Iyayi *et al.* (2006) when they fed raw and processed velvet bean-based diets to broilers but contrary to their

report, fish in the current study did not show haematological features that indicated macrocytosis or regenerative anaemia indicated by low RBC value. MCV values Initial lymphocyte value differed significantly from the treatments but there was no significant difference among the treatments.

Glucose value in diet containing 30% TMM was significantly higher than both initial and other treatments while that of the diet containing 10% TMM was significantly lower. Total protein in fish fed tested diets differed significantly from each other and from the initial fish. Ajani (2007) on the other hand observed a gradual decrease in plasma protein with increased stocking density and attributed this to the severity of stress leading to osmotic imbalance. There was no significant difference in cholesterol values in the treatments. Globulin values in diets containing 20 and 30% TMM recorded no significant difference but were significantly different from other treatments and the initial fish. The initial albumin value was significantly higher than what was obtained in the treatments and values also varied significantly among treatments.

Fish fed MSM-based diets showed various histological changes in the brain ranging from moderate spongiosis of the white matter in diet containing 10% RMM to marked spongiosis of the white matter in diets containing 20 and 30% CMM and10, 20 and 30% of both RMM and TMM. The gill showed mild clumping of the secondary lamella in diet containing 20% RMM, marked congestion, oedema and severe oedema in diet containing 10% RMM. The hepatocytes of the liver of fish fed the tested diets suffered mild diffuse vacuolation (diet containing 10% TMM.), moderate diffuse vacuolation (diet containing 30% TMM), marked diffuse vacuolation (diet containing 20% TMM.) and marked centrilobular vacuolation (diet containing 30% TMM.) while the kidney of fish fed diets containing 10% and 20% TMM suffered marked congestion. Wade *et al*. (2002) reported that following 96hr- toxicity assay of cassava (*Manihot esculenta*

Crantz) effluent on the Nile tilapia, histo-pathological examination of the kidney, gill and liver of the treated fish indicated damages, ranging from oedema and telangiectasis of the gill lamella and gill hyperplasia to vacuolation of the liver cells and necrosis. Adeyemo (2005) also made similar observations in *C. gariepinus* fed cassava mill effluent. Feeding graded levels of MSM-based diets in this study therefore resulted to histological lesions that may impair proper physiological functions the tested fish.

Generally, all values in water quality parameters fell within the recommended ranges by Viveen *et al.* (1985) and Omitoyin (2007).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

The results obtained from this study have shown that *Mucuna* possesses a number of attributes that qualifies it for consideration as feed ingredient and that it has a great potential as a feed ingredient for *C. gariepinus*. The proximate analysis of the seed ranks with other legumes in terms of nutrient composition especially with respect to its protein and mineral contents. Processing affected the nutrient composition as well as the quantity of L-DOPA.

Fish fed with up to 20% *Mucuna* seed meals (MSM) grew fairly well compared to that of SBM. Inclusion of MSM-based beyond this level led to comparatively low weight gain. It is therefore uneconomical to substitute SBM beyond this level.

Haematological studies of fish fed MSM-based diets also indicated that fish tolerated the diets to a good extent. However, histological examinations of fish organs like liver, brain, gill and kidney showed some toxic effects. These toxic effects were varied in different organs ranging from mild lesions to more marked ones. Organs of fish fed RMM diets were mostly affected. Further studies are required to ascertain which anti-nutritional factor(s) is/are responsible for these toxic effects. L-DOPA, the only anti-nutritional factor investigated in this study, has been previously identified as constraints to the use of Mucuna in the feed industry. This anti-nutrient is also suspected for the histological changes observed in the fish organs in the study. Further studies are also needed to identify better treatments hat will effect better utilization.

It could be concluded that *Mucuna* seeds has a good potential to be listed as a feed resource for *C. gariepinus,* however, processing methods used in this research did not affect its

level of acceptance to *C. gariepinus*. Further research is therefore required on how the quality of its nutrients could be improved and on how better detoxification that will remove (or at least reduce further) its anti-nutrients for better performance could be achieved.

6.2 RECOMMENDATIONS

M. utilis in this study has a good potential for being listed as a feed ingredient in the nutrition of *C.* gariepinus. However, it cannot at this time be competitive with soybeans rather it should be seen as a compliment not as a substitute. The problem of acceptability and safety should be solved and this takes long time. Information in this study should therefore be treated as preliminary. However, they provide a basis for further investigation and are intended as a stimulus for more consideration.

Research into methods of processing that will further eliminate L-DOPA and other toxic components of *M. utilis* should be intensified. Efforts should be concentrated on how it can be developed into new food and feed since it can adapt to a wide range of environmental conditions. This calls for multi-disciplinary research collaboration between nutritionists (fish, poultry and others in the field) and other scientists especially those in food technology.

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APPENDIX I

Result of ANOVA for Growth Parameters

Parameters	Df	MS	F	p-level
FCR				
Inclusion level	4	16.679	10.682	0.00001*
Processing Method	2	0.860	0.551	0.5837ns
Inclusion level x Processing Method	6	0.860	0.551	0.5833ns
Error	24	1,561		
SGR				
Inclusion level	3	29.438	18.860	0.000002*
Processing Method	2	0.431	0.276	0.761ns
Inclusion level x Processing Method	6	2.241	1.436	0.242ns
Error	24	1.561		
SR				
Inclusion level	3	76.852	0.503	0.684ns
Processing Method	2	52.778	0.345	0.711ns
Inclusion level x Processing Method	6	82.407	0.539	0.773ns
Error	24	152.778		
MWG				
Inclusion level	3	357.082	100.467	0.000000*
Processing Method	2	10.755	3.026	0.051603ns
Period	5	1237.118	348.069	0.000000*
Inclusion level x Processing Method	6	27.115	7.629	0.000000*

Inclusion level x Period	151	33.60133 .601	9.454	0.00000*
Processing Method x Period	10	1.7321.7 32	0.487	0.896168ns
Inclusion level x Processing method x Period	30	2.644	0.744	0.827164ns
Error	144	3.554		

APPENDIX II

Result of ANOVA for Haematology and Serum Parameters

Parameters	df	MS	F	p-level
PCV				
Inclusion level	3	115.732	8.316	0.000575*
Processing Method	2	61.194	4.397	0.024*
Inclusion level x Processing method	6	32.898	2.364	0.062
Error	24	13.917		
RBC				
Inclusion level	3	0.894	6.664	0.001972*
Processing Method	2	0.588	4.381	0.023884*
Inclusion level x Processing method	6	0.211	1.571	0.198814ns
Error	24	0.134		
WBC				
Inclusion level	3	119196	4.037	0.018577*
Processing Method	2	483719	1.638	0.215314ns
Inclusion level x Processing method	6	438245	1.484	0.225744ns
Error	24	295266		
MCHC				
Inclusion level	3	1.016667	1.126	0.358236ns
Processing Method	2	0.291944	0.323	0.726800ns
Inclusion level x Processing method	6	0.395278	0.438	0.846201
Error	24	0.902778		

MCV				
Inclusion level	3	1187.135	6.174	0.002911*
Processing Method	2	102.400	0.533	0.593857ns
Inclusion level x Processing method	6	249.535	1.298	0.295841ns
Error	24	192.266		
Albumin				
Inclusion level	3	73.435	4.421	0.13057*
Processing Method	2	3.028	0.182	0.834516
Inclusion level x Processing method	6	31.657	1.906	0.121102
Error	24			
Globulin				
Inclusion level	3	8153.296	7.716	0.000888*
Processing Method	2	64.333	0.061	0.941079ns
Inclusion level x Processing method	6	740.296	0.701	0.651855ns
Error	24	1056.694		
Neutrophil				
Inclusion level	3	486.926	2.772	0.063419*
Processing Method	2	97.694	0.556	0.580635ns
Inclusion level x Processing Method	6	173.732	0.989	0.454701ns
Error	24			
MCH				
Inclusion level	3	130.621	5.693	0.004323*
Processing Method	2	12.581	0.548	0.585009ns

Inclusion level x Processing Method	6	26.7242	1.165	0.357404ns
Error	24			
Glucose				
Inclusion level	3	19.037	0.165	0.916662ns
Processing Method	2	45.778	0.397	0.676785ns
Inclusion level x Processing Method	6	75.704	0.656	0.685057ns
Error	24			
Total protein				
Inclusion level	3	9741.064	8.130	0.000657*
Processing Method	2	36.694		0.969878ns
Inclusion level x Processing Method	6	951.065		0.583929ns
Error	24			
Haemmoglobin				
Inclusion level	3	19.777		0.010826*
Processing Method	2	2.709		0.539137ns
Inclusion level x Processing Method	6	1.843	0.431	0.850580ns
Error	24			
Lymphocytes				
Inclusion level	3	575.6296	3.509	0.030633*
Processing Method	2	103.083	0.628	0.542023ns
Inclusion level x Processing Method	6	178.269	1.087	0.39831ns
Error	24			

APPENDIX III

Result of ANOVA for Water Quality Parameters

Parameters	Df	MS	F	p-level
Dissolved Oxygen				
Inclusion level	3	34.622	7.886	0.000784*
Processing Method	2	4.377	0.997	0.383794ns
Inclusion level x Processing method	6	3.268	0.744	0.619506ns
Error	24			
pH				
Inclusion level	3	0.339	27.104	.000000*
Processing Method	2	0.0269	2.156	0.137737
Inclusion level x Processing method	6	0.910	7.281	0.000163
Error	24			
NH_3				
Inclusion level	3	0.642	0.435	0.729693
Processing Method	2	0.350	0.238	0.790343
Inclusion level x Processing method	6	2.636	1.788	0.144144
Error	24			

APPENDIX IV

Crude Protein Determination

Crude protein was determined by Kjeldalh method. Kjeldalh method involves three steps namely;

1. The digestion of sample with conc. H_2SO in the presence of a catalyst (Hg or Se) to convert all the nitrogen present to $NH_4)_2SO_4$
2. The titration of NH_3 from the digest by the addition of excess NaOH. The steam distillation of this NH_3 into boric acid.
3. Determination of theNH_3 liberated by back titration to the end point with standard HCl.

Procedure

A homogenous sample of the material was prepared. A small piece of smooth aluminum foil (approximately 2cm x 1cm) was weighed on a sensitive scale. 300mg of sample was added to the foil and reweighed. The sample was transferred to a labeled digestion tube and the foil was reweighed. A Kjeldahl tablet (Selenium) and 5mlof conc. H_2SO_4 were added and the tubes transferred to the digestion block and was switched to 420^{0C}. The sample was digested for 1 hour during which the tubes were rotated occasionally. The tubes were allowed to cool. About 20ml of deionised water was added to the tubes followed by 5ml of sodium thiosulphate (330mg/l $Na_2S_2O_3.H_2O$).

The mixture was distilled in a kjeldahl distillation unit. It was titrated in 30ml boric acid [plus 5 drops indicator (Boric acid is a very weak acid and does not in any way affect the indicator)] and was distilled against 0.2mHCl. The end point of titration was when the colour changed from blue to grey then for first hint to orange. This was compared to the obtained blank values (mean of 3 values). The results were tabulated as follows:

Sample name	Weight of foil	Weight of foil + sample (A)	Weight of foil after transfer (B)	Weight of sample (A-B = Titration value	Protein

% protein = $\frac{(\text{Titration} - \text{Blank}) \times 0.2 \times 14.007 \times 6.25 \times 100}{\text{Sample weight (mg) B}}$

APPENDIX V

Determination of lipid

Lipid was determined by using soxhlet extraction method.

Procedure: A soxhlet extraction system was assembled complete with reflux condenser and a small flask which has recently been previously dried in the oven and weighed on an analytical balance. 2g of the sample was transferred to a fat-free extraction thimble which was plugged lightly with cotton wool. The thimble was placed in the extractor and N-hexane 60 at 8^0Cwas added until it siphoned over once. More solvent was added until the barrel of the extractor (in this case 300ml) was half full, the extractor was transferred to the water bath. This was adjusted such that the solvent boiled gently, this was continued until the solvent siphoned over twice. The flask was detached when the solvent was just short of siphoning over. The barrel of the extractor was allowed to drain completely after which the content of the thimble was removed and dried on a clock glass (away from the source of flame). The entire solvent in the extractor was transferred into another unit was attached to the condenser and was distilled until the flask was virtually dry. The flask was detached and transferred to an oven where it was dried to constant weight. Oil content was calculated as follows:

Weight of clean dry flask = X^0

Weight of flask + dried ether extract = X^t

Weight of paper thimble = Y^0

Weight of paper thimble + samples = Y^t

$$\text{Crude lipid content (\%)} = \frac{(X^t - X^0)}{(Y^t - Y^0)} \times 100$$

APPENDIX VI

Crude Fibre Determination

Trichloroacetic acid method for crude fibre determination was used in this study.

Reagent: (Trichloroacetic acid digestion reagent)

A mixture of Glacial acid (500ml), 450ml water and concentrated nitric acid (50ml). Into this mixture was dissolved 20g trichloroacetic acid, industrial spirit.

Equipment: Air condenser, water, 500ml conical flask, number 15 whatman filter paper, refluxing apparatus, Desiccator, Silica dish.

Method

1g of the sample was weighed with a chemical balance into a 500ml conical flask. 1000ml of the trichloroacetic acid digestion reagent was washed and put inside the flask. The mixture was boiled and refluxed for exactly 40 minutes (i.e. from the time heating started). The flask was disconnected from the heater and cooled under a tap. The sample was pipetted and filtered through a whatman filter paper (150mm), it was then washed six times with hot water and later with industrial spirit. The filter paper was opened and the residue left was removed with a spatula and transferred to a weighed silica dish. The residue was dried overnight at 105^0C in an oven. It was allowed to cool in a desicator after which it was reweighed. The residue was ashed at 600^0C in a niffle furnace. It was allowed to cool and reweighed.

% fibre = difference in weight x100.

APPENDIX VII

Moisture Content Determination

Equipment: Silica dish, analytical balance, oven.

Method: Three Petri dishes were dried to constant weight in an oven, were cooled and weighed. Two grammes of the sample was weighed into each Petri and transferred into a drying oven set at 105^0C and dried to constant weight overnight. The samples were removed from the the oven and cooled in a desiccator for 20 minutes and weighed. The weight difference represented the moisture content of the samples.

$$\% \text{ moisture} = \frac{\text{weight of sample} - \text{weight of sample after drying}}{\text{Weight of sample}} \times 100$$

APPENDIX VIII

Ash Content Determination

Equipment: Porcelain crucibles, analytical balance, muffle furnace.

Procedure

From the residue from moisture content, 1g was weighed into a weighed and numbered porcelain crucible, the crucible was weighed with the residue and ashed in a muffle furnace at 450^0C for 12 hours. The crucibles were then taken out and allowed to cool in a desiccator and reweighed. The ash content was calculated with the following relationship:

Weight of clean, dry crucible = X(g)

Weight of clean, dry crucible plus sample = Y^0 (g)

Weight of clean, dry crucible plus ash = Y^1

Ash content of sample (%) = $\frac{Y^1 - X}{Y^0 - X} \times 100$

APPENDIX IX

Nitrogen Free Extract Determination

This was determined by summing up the % of moisture, ash, lipid extract, crude protein and crude fibre and subtracting this from the total (100).