GROWTH PERFORMANCE AND NUTRIENT UTILISATION OF *Clarias gariepinus*, Burchell, 1822 JUVENILES FED PROCESSED BAOBAB (*Adansonia digitata* L.) SEED MEAL BASED DIETS

BY

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ABSTRACT

Unconventional and underutilised protein-rich feedstuffs such as Baobab (*Adansonia digitata*) Seed Meal (BSM) could effectively replace the expensive soyabean meal, except for the presence of antinutritional factors. Processing has been reported to reduce BSM antinutrients, increase its nutritional composition and enhance its utilisation by animals. However, information on utilisation of processed BSM in the culture of *Clarias gariepinus* is limited. The growth performance and physiological response of *C. gariepinus* juvenilesfed differently processed BSM-based diets were therefore investigated.

Crude protein (CP; %), saponins (mg/100g) and oxalates (mg/100g) of Raw (RBSM), Autoclaved (ABSM), Toasted (TBSM), Soaked in Water (SWBSM), Soaked in Liquor (SLBSM), Soaked in Alkali (SABSM) and Soaked in Pulp (SPBSM) samples were determined using standard methods. Apparent Digestibility Coefficient for CP (ADC_{CP};%) of the meals by *C. gariepinus* (n=315, 18.54±0.04g, 15 fish $tank^{-1}$) were determined using standard procedures. Each of the two processed BSM with best ADC_{CP} were made into 12 isonitrogenous (36% CP) diets. These were used to replace soyabean meal at 0 (control), 20, 40, 60, 80 and 100% for growth trial. *Clarias gariepinus* (n=495, 24.04±0.25g, 15 fish $tank^{-1}$) were randomly allotted in triplicates to treatments in a 2×6 factorial arrangement in completely randomised design and fed twice daily at 5% body weight for 16 weeks. Mean Weight Gain (MWG; g) and Food Conversion Ratio (FCR) were calculated. Blood (5 mL) was sampled to determine Packed Cell Volume (PCV; %) and Alanine Aminotransferase (ALT; IU/L). Immunocompetence indices: Catalase (U/mg protein) and respiratory burst activity (H_2O_2;U/mg protein) were assessed using standard methods. Data were analysed using descriptive statistics, ANOVA and Polynomial regression at $\alpha_{0.05}$.

The CP ranged from 22.8±0.1 (TBSM) to 26.8±0.1 (SABSM). Saponins (61.7±2.9) and oxalates (171.7±10.4) were significantly least in TBSM, and highest in SABSM (213.3±5.8) and RBSM (276.7±10.4), respectively. Highest ADC_{CP} were recorded in SABSM (85.8±0.4) and SPSBM (81.32±0.10) while the least was obtained in SLBSM (59.4±1.9). The MWG were 102.1±22.3 and 97.3±32.6 while FCR were 1.9±0.2 and 1.9±0.3 in SPBSM and SABSM, respectively. Significantly highest MWG (152.9±12.2) and least FCR (1.6±0.1) were recorded in 20% SABSM while least MWG (59.1±9.3) and highest FCR (2.2±0.3) were obtained in 100% SABSM. Optimal dietary inclusion level of SPBSM and SABSM relative to MWG were 7.7% (R^2=0.5) and 13.4% (R^2=0.7), respectively. The PCV were 22.0±3.4; 21.7±4.2 and ALT 34.1±9.8; 31.3±7.0 in SPBSM and SABSM, respectively. The PCV ranged from 16.0±1.4 (60% SPBSM) to 25.5±6.4 (40% SPBSM) while ALT varied from 29.0±4.1 (0% BSM) to 38.7±13.9 (60% SPBSM). Least and highest H_2O_2 (48.1±9.1, 48.4±10.0) andcatalase(18.2±2.3, 18.9±2.0) were obtained in SPBSM and SABSM, respectively. Catalase varied from 17.5±1.5 (20% SABSM) to 20.5±2.0 (100% SABSM) while H_2O_2 ranged from 41.8±7.1 (20% SABSM) to 57.0±6.3 (100% SPBSM).

Baobab seed meal based diets soaked in alkali and pulp were best digested by *Clarias gariepinus.* Soyabean meal could be replaced optimally at 13.4% and 7.7% inclusion for soaked in alkali and pulp baobab seed meal, respectively for improved fish growth and health.

Key words: Baobab seed meal, *Clarias gariepinus*, Nutrient digestibility
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CERTIFICATION

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA: Analysis of Variance

AOAC: Association of Official Analytical Chemists

FAO: Food and Agricultural Organisation

pH: Hydrogen ion concentration

R^2: Co-efficient of Determination

%: Percentage

Chemical Symbols

°C: degree celcius

g: gramme

kg: kilogramme

mm: millimeter

±SD: Standard deviation

CHAPTER ONE

INTRODUCTION

1.0 Background to the study

Fisheries and aquaculture are vital sources of food, income and livelihoods for many people, and aquaculture remains the fastest growing industry in food sector in the world (FAO, 2016). In Nigeria, aquaculture is a good option to bridge the gap between fish supply and demand. Nigeria is blessed with about 1.75 million hectares of wetland that is suited for aquaculture and production capacity of over three million metric tonnes annually (Ajani, 2019).

Different factors like socio-economic, technical, political, physio-chemical and biological must be considered to obtain sustainable aquaculture development in Nigeria (Adikwu, 1999). The growth of aquaculture in Nigeria is faced with various limitations, including: high cost of feed, water quality and disease infestation (Dauda *et al*., 2015). Growth in aquaculture depends on cost-effective and sustainable feed resources (Moutino *et al*., 2017).

High cost of feed is commonly known as a key factor affecting the development of aquaculture in many developing countries (FAO, 2016). Feed is the most expensive item in semi-intensive and intensive fish culture (Aderolu *et al*., 2009). Fish growth is influenced by feed intake, genetics and environmental factors; and feed intake is considered the main factor that affects fish growth (Asuwaju *et al*., 2014).

The use of less expensive sources of protein that provide satisfactory growth is advantageous for diet manufacturers and aquaculture producers. Cost effective diets are essential for successful fish farming and it depends on the accessibility and digestibility of the feed ingredients (Abu *et al*., 2009). Ayinla (2003) reported that at least 60% of the

total cost of fish production in Africa is accountable to feed, which to a great extent determines profitability of fish farming enterprise.

Nutrient requirements of fish depend on age, species and environmental condition. The essential nutrient requirements of fish are proteins, lipids, carbohydrates, vitamins and minerals. In fish feed formulation, protein and energy requirements of the species under culture practices is predominantly considered above all other nutrients (Olufeagba *et al*., 2002 in Abu *et al*., 2009). Information on the protein requirement of fish is crucial for the formulation of a well-balanced artificial diet for economical fish feeding (Abu *et al*., 2009). Hence, protein component in aquaculture diets is the single most expensive portion and important dietary nutrient.

In Nigeria, like other developing countries of the world, protein of animal origin is expensive. Thus, efforts to alleviate the inadequacy in protein supply have been directed towards the utilisation of readily available and inexpensive feedstuffs, which are plant proteins (Yusuf *et al*., 2008). Soyabean meal protein is the most commonly used plant protein in feeds in African aquaculture for omnivorous fish species, such as tilapias and catfishes (Fagbenro *et al.,* 2013).

Soybean meal is a good alternative for animal products because of their good aminoacid profile (Mambrini *et al*., 1999); protein quality and moderately consistent nutritional composition (Ghadge *et al*., 2009). It is one of the best plant protein feedstuffs with good protein quality that can replace or substitute fish meal in diets of various fish species (Fagbenro *et al.,* 2013). High competition by man, fish and various domesticated animal feed producers for soybean meal has restricted its utilisation in fish feed industries (Fagbenro *et al*., 2013).

In order to expand and increase aquaculture productivity in many developing countries, cheaper unconventional protein sources that can replace soyabean meal need to be sought after for the production of inexpensive feed of good nutritional quality (Jimoh *et al*., 2014). One of such plants that have the potential of being inexpensive, locally available and nutritionally dense is Baobab (*Adansonia digitata*) (NRC, 2008).

Baobab is widespread in Africa and its seed is one of the lesser-known, ignored and underexploited vegetables that have high protein and caloric content (Yusuf *et al*., 2008). Baobab tree is widely found in Africa (FAO, 1988), and its seeds are neither usually consumed nor used in Nigeria. Antinutrients contained in the seed (Osman, 2004) are capable of being eliminated or reduced by a range of processing methods (Ezeagu *et al*., 2005a).

1.1 Justification for the study

Baobab seed has considerable quantity of protein and energy (Mwale *et al*., 2008) but contains antinutrients like oxalate, saponins, phytate and tannin (Osman, 2004; Belewu, 2008). The suitability and best utilisation of the seed as a feed ingredient are restricted by the occurrence of antinutrients (Yusuf *et al*., 2008), some of which can be reduced by processing (Ezeagu *et al*., 2005a). Prior to its utilisation, thorough evaluation is needed (Ezeagwu *et al*., 2005b) to ensure that the seed is not toxic to animals.

Various degrees of success have been reported on utilisation of baobab seed as feed ingredients for various kinds of animals. Yusuf *et al*. (2008) reported that baobab seed meal at 33.33% level could replace soybean meal with no deleterious effect on rats. Oladunjoye *et al.* (2014) reported that growing rabbits can utilise 15% baobab pulp and seed meal in their diets. Ezeagu *et al*. (2005a) reported that animals fed HCl-extracted baobab seed meal recorded lesser feed intake compared to those fed raw and cooked. Ezeagu *et al*. (2005b) also reported that rats fed HCl-extracted baobab meal had lower packed blood cell volume, haemoglobin and red blood cells.

Another avenue for exploiting the full potentials of non-conventional seeds as feed was mapped by exploring the impending ability of processing methods to decrease the antinutrients and increase the nutritional composition of the seed. Anene *et al*. (2012) who observed a decline in growth rate of *Clarias gariepinus* as the inclusion level of baobab increased processed the meal by soaking it in water for 24 hours, followed by boiling for an hour at 105°C and then oven drying at 60°C. Hassan *et al*. (2015) also reported a decline in growth of *Clarias gariepinus* at substitution of baobab seed meal level above 10.0 % in their diet.

The reduction in development rate of the fish as the substitution of baobab seeds amplified as reported by Anene *et. al*., (2012) and Hassan *et. al*. (2015) could be as a result of long period of cooking and subsequent loss of some of the nutrients in the seed due to the processing method adopted by the researchers. Processing methods improve the composition of baobab seed meal. However, there is also a gap in knowledge on the effect of ingestion of baobab seed meal on fish health.

In evaluating the effect of processed baobab seed meal to possibly replace soyabean meal in *Clarias gariepinus* feed, the effectiveness of processing methods, like soaking, autoclaving and toasting on baobab seed composition, the growth, nutrient utilisation and health of *Clarias gariepinus* fed differently processed baobab seed based diets were investigated.

1.2 Objectives of the study

The general objective of this study was to evaluate the growth and feed utilisation by *Clarias gariepinus* juveniles fed differently processed baobab seed meal based diets.

The specific objectives of this study were to:

i. determine the chemical composition of differently processed baobab seed meals,
ii. evaluate the digestibility of differently processed baobab seed meals by *Clarias gariepinus* juveniles,
iii. investigate the effects of different inclusion levels of baobab seed meal in *Clarias gariepinus* diets,
iv. determine the cost implication of replacing soyabean meal with baobab seed meal in *Clarias gariepinus* diets.

CHAPTERTWO

2.0 LITERATURE REVIEW

2.1 Utilisation of plant protein sources

2.1.1 Utilisation of plant protein sources in fish diets

The importance of the development of non-human-food grade feed resources whose growth can cope with the rapid growth of aquaculture sector has been stressed (Tacon and Forster, 2001). Efforts have been made on the use of many seeds (plant) as protein sources for different cultured fish species. Numerous studies have been conducted on seeds like soybean, sesame, rape seed, cotton seed, peanut, wheat and corn gluten meal (Francis *et al*., 2001a). All these materials are becoming prohibitively costly for their continued use for preparation of fish feeds (Muthukumar and Kandeepan, 2009).-

Researchers have reported the use of underutilised and unconventional plants that have useful nutrients as an alternative to commonly used feed ingredients. Fagbenro *et al*., (2005) reported that 60% of soya bean meal could be replaced by rosselle seed meal in *Clarias gariepinu* diets.Anti-nutrients in feed of plant origin have negative effects on its utilisation and intake; anti-nutritional factor limits the utilisation of plants and reduces nutrient utilisation and/or food intake in man and animals. (Shanthakumar *et al*., 2008). Cooked and mechanically defatted sesame seed meal can replace soybean meal at 25% in *Clarias gariepinus* diet (Jimoh *et al*., 2011).

Antinutrients

Anti-nutritional factors are chemical compounds produced in natural food and / or feedstuffs by the normal metabolism of species and by different mechanisms which exert effect contrary to optimum nutrition (Soetan and Oyewole, 2009). They are highly biologically active and commonly found in feeds obtained from plants. They are also known as ‘secondary metabolites’ in plants.

Antinutritional factors in plant

Antinutrients in plants are compounds produced by plants for their protection and other natural functions but reduce the utilisation of nutrients, especially proteins, vitamins, and minerals by animals. They prevent maximum use of the nutrients available in a feed and reduce its nutritive value. The quantity of the chemicals that are consumed determines if it will be harmful or beneficial to man and animal health (Ugwu and Oranye, 2006).

Tannins

Tannin binds to and precipitates proteins and various other organic compounds like starch, cellulose and minerals. It is commonly found in plants and act as defence mechanism against pathogens and herbivores. It belongs to the phenolic class of secondary metabolite in plants. Tannin is called 'tannins' because of its traditional use in preserving animal hides and transforming into leather by using plant extracts. It affects nutrients, palatability, utilisation and digestion due to its ability to form complexes with numerous types of molecules like protein, carbohydrates, polysaccharides and some digestive enzymes (Habtamu and Negussie, 2014).

Phytate

Phytate is the salt of phytic acid regarded as the primary storage compound for phosphorus, inositol and inorganic phosphate ions which are used in the energy metabolism of the plant (Enneking and Wink, 2000 as cited by Duodu *et al*., 2017). It is present in plants, animals and soil (Mueller, 2001). It is naturally occurring compounds that stores trace elements in plants and occurs as a mineral complex that is insoluble at physiological pH values.

It acts as an antinutrient in monogastric animals because of the absence of phytate degrading enzymes in their stomach. It affects the utilisation of minerals like calcium, copper, magnesium, manganese, iron and zinc in human and monogastric animals (Mueller, 2001). It also affects protein digestibility and digestive enzymes.

Oxalate

Oxalic acid forms water soluble salts with Na^{+}, K^{+}, and $NH4^{+}$ ions, binds with Ca^{2+}, Fe^{2+}, and Mg^{2+} and makes them unavailable to animals. Conversely, Zn^{2+} seems to be

comparatively impervious. Oxalates exist as potassium oxalate in plants with a cell sap of about pH 2; soluble sodium oxalate and insoluble calcium and magnesium oxalates in sap of around pH 6. Accumulation of the insoluble calcium oxalate results in kidney stones (Nachbar e*t al*., 2000).

Oxalates affect the bioavailability and utilisation of calcium in the bones and tissues of animals by forming insoluble calcium oxalate (Ojinnaka *et al*., 2013). Oxalates make nutrients unavailable to the body by binding with them in the gastrointestinal tract (Noonan and Savage, 1999) during digestion. Consumption of oxalic acid-rich food regularly can result to nutritional deficiencies and irritation to the lining of the gut. Adubiaro *et al*. (2011) stated that excess oxalate in feed could cause gastro-intestinal irritation, formation of kidney stones and muscular problem. However, oxalic acid is not a major problem to ruminant animals (Oladimeji *et al*., 2000).

Saponins

Saponins are secondary compounds that are generally present in plant kingdom. They are referred to as antinutrient due to their negative impact on animal growth (Liener, 2003). Saponins reduce bioavailability of nutrients and enzyme activity by hindering the activities of many digestive enzymes, such as trypsin and chymotrypsin (Liener, 2003). Biological effects of saponins on animals (Adubiaro *et al*., 2011) include erythrocyte haemolysis, reduced growth and low nutrient absorption and bile acid metabolism.

Lectins

Lectins are mostly found in legumes and some certain oil seeds. They are versatile protein that binds specifically to carbohydrate. They have the capability to directly bind to the intestinal muscosa, interacting with the enterocytes and interfering with the absorption and transportation of nutrients (particularly carbohydrates) during digestion and causing epithelial lesions within the intestine. (Boehm and Huck, 2009 as cited in Habtamu and Negussie, 2014). Their consumption can also result to reduced growth and increased incidence of bacterial infection.

2.1.2 Processing methods used in reducing or eliminating the effect of anti-nutritional factors

Soaking in various solutions followed by autoclaving or cooking reduces total phenolics of both white and black varieties of mucuna seeds (Siddhuraju *et al*., 2001). Cooking and autoclaving efficiently reduced phytate content, total phenolics content but increased the tannin content of the seed. Rise in tannin content might be as a result of condensation of free phenolics, while the reduction in the antinutrient may be due to leaching out of phenolic compounds. Dehulling of the seed coat was reported to reduce 80% of total phenolics in mucuna seeds (Siddhuraju *et al*., 1996) and soaking followed by irradiation (Adebowale, *et. al*., 2005).

High intake of phytic acid-rich diets by animals reduces the accessibility of essential minerals and growth of animals. However, inclusion of phytase in meals that have phytic acid enhanced the digestibility of phosphorous and growth rate (Siddhuraju *et al*., 2001). Dry heat treatment and autoclaving reduced the phytic acid content in *M. pruriens* seeds (Siddhuraju *et al*., 1996). Phytic acid content in *M. pruriens* seeds soaked in distilled water reduced more than those soaked in sodium bicarbonate solution (Vijayakumari *et al*., 1996). Decortication reduced the phenolics and tannins in mucuna because they were concentrated in the seed coat (Vijayakumari *et al*., 1996).

Nkafamiya *et al*. (2007) reported the presence of some anti-nutritional factors like phytate, oxalate, tannins and saponins in baobab seed cake. Igboel *et al*. (1997) reported that roasting, hot water and alkali treatment reduced the tannin and amylase inhibitor activity in baobab seed. Innocentia *et al*. (2014) reported that toasting at150˚C was more effective in reducing antinutrients in baobab than 120 and 100˚C.

2.2 Baobab distribution and utilisation

Baobab (*Adansonia digitata*) largely grows as an uncultivated tree in the Sahelian, Soudanian zones, Soudano –Sahelian, sub-Saharan Africa, Madagascar and Australia (Wickens and Lowe, 2008). It is mostly found in the northern part of Nigeria (Ezeagwu, 2005a). It belongs to the family Malvaceae and is found mostly in the savannah of sub-Saharan Africa.

Baobab tree produces an average of 200 kg of fruits per year. Baobab pod is 20-35 cm long and 10-13 cm wide when mature. It is oval and tapers a little towards the end. The pericarp of the fruit is covered with hair. The seeds are enclosed with whitish acidic pulp (De-Caluwe *et al*., 2010).

Baobab trees are indigenous to Nigeria, and the seeds are readily available, especially around the middle-belt and some parts of the far north (Nkafamiya *et al*., 2007). It is called "kukah" by many ethnic groups in Nigeria (Alhassan *et al*., 2016).The limited usage of baobab seed in Nigeria made it a non-conventional feedstuff of choice for farm animals.

Baobab Leaves

Baobab leaves contain 60-70% carbohydrate, 13-15% protein, 4-10% fat (De-Caluwe *et al*., 2010). It is abundant in vitamins A and C and can be rated 'good' in terms of protein content (WHO standards) because of its high values in five essential amino acids. Baobab leaf is a very good source of calcium, iron, potassium, magnesium, manganese, molybdenum, phosphorus and zinc (Yazzie *et al*., 1994).

Baobab Pulp and seeds

Studies have shown that baobab seed has a substantial amount of protein, energy and minerals (Osman, 2004), hence, it serves as a potential protein source in tropical and subtropical region. Baobab pulp that surrounds the seed is rich in calcium, hence, it serves as a calcium supplement. Nkafamiya *et al*., (2007) and Osman *et al*., (2004) stated that crude protein content of 20-36% and energy content of 3000 - 4500Kcal/kg is present in baobab seed meal. The seeds contain vitamins and minerals. Phosphorous, calcium and magnesium are the main mineral elements in baobab seeds (Nkafamiya *et al*., 2007)

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Fibre from the baobab bark is used in making rope, basket nets, snares and fishing lines. The roots and bark can be used to make dye (Dovie *et al*., 2003). Baobab bark and sliced lime fruit is boiled with maize liquor in Nigeria and drank to prevent post-coital seminal expulsion. Baobab flowers are used to fasten the expulsion of the foetus in Benin (Codjia *et al*., 2001 in Habtamu and Negussie, 2014). The fruit pulp is consumed in Mali and Benin to promote lactation (Gustad *et al*., 2004; Codjia *et al*., 2001, in Habtamu and

Negussie, 2014). The root-bark and leaves were active against the bacteria *B. subtilis*, *E. coli*, *Mycobacterium phlei*, *S. aureus* and *Streptococcus faecalis* but resistant to *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and the fungus *C. albicans* (Anani *et al*., 2000).

2.3. Baobab seed as a feed meal

2.3.1. Effects of different processing methods on nutritional composition of baobab seed

Crude protein and crude fibre reported by Sola-Ojo *et al*. (2011) was higher those reported by Ezeagu (2005a) and Anene *et.al*. (2012). Crude protein of 37.63% reported by Sola-Ojo *et al*. (2011) was higher than 16.6% and 18.4% reported by Ezeagu (2005a) and Anene *et al*., (2012), respectively. This could be as a result of difference in varieties of the baobab seed and processing methods that were used in the preparation of the meals.

Anene *et al*., (2012) prepared baobab seed meal by soaking (24 hours) in water, boiling (1 hour) and then oven drying at 60°C. Ezeagu *et al*. (2005) that obtained crude protein of 16.60% used cooking and acid extracted method to process the seeds. The period of cooking (1 hour) used by Anene *et al*. (2012) could have lowered the crude protein (18.40) of the seeds. The seeds might have lost some of its nutrients as a result of the period of cooking involved in the processing method that was adopted by Anene *et al*. (2012).

Soaked and cooked grains have reduced major and trace elements (El Maki *et al*., 2007). Soluble components could be leached into soaking and cooking water. (Yagoub and Abdalla, 2007). Cooking results in loss of trace elements compared to soaking due to transformation of insoluble constituents of the meal into soluble ones by heat. On the contrary, soaking and cooking have been stated to enhance the dietary composition of seeds like crude protein (Yagoub and Abdalla, 2007).

Innocentia *et al*. (2014) reported that roasting at 150^{o}C improved ash, crude protein, crude fiber, water and reduced the fat, moisture, anti-nutrient of baobab seed. He also reported that roasting at 150^{o}C for 30 minutes was the best compared to roasting at 100^{o}C and 120^{o}C for 30 minutes respectively. Processing methods may reduce nutrients and minerals

present in baobab seed meal and affect the growth of the animal fed the seeds. However, Parkouda *et al*., (2012) reported variability in physical characteristics and nutrient content of baobab (*Adansonia digitata* L.) fruits in the West African Sahel.

2.3.2. Effects of processing on antinutritional factors in baobab seed meal

The most excellent way to realize the complete potential of non-conventional seeds as feed is by researching into traditional uses of the seed and by exploring the possible advanced ways to categorise, quantify and minimise the toxic contents in it (Vijayakumari *et al*., 1996). Innocentia *et al*. (2014) reported a reduction in oxalate, phytate and tannin in baobab seed toasted at 100, 120 and 150˚C.

Some anti-nutrients in baobab seeds can be reduced by de-hulling, which involves boiling, rubbing by hand, sun drying, and then removal of the seed coat. The de-hulled seeds can then be fermented to enhance protein digestibility and lower the trypsin inhibition activity, although tannin content would rise (Addy *et al*., 1995).

2.3.3 Effects of differently processing baobab seed meal and cake on animals

Researches on use of baobab seed as animal feed have generated different reports. Reduction in growth of experimental animals was reported as the inclusion level of baobab seed in feed increased (Anjos, 2005; Mwale *et al*., 2008; Chimvuramahwe *et al*., 2011). Baobab seed meal can be used to substitute soybean meal at 33.33% level with no deleterious effect on the growth rate of rats (Yusuf, 2008).

Rat fed raw, cooked and acid-extracted baobab meals reduced in weight. The low weight gain was attributed to loss of nutrients during the processing or low amino acid composition of the seeds (Ezeagu *et al*., 2005a). Poor feed conversion ratio reported in chicken fed 100% inclusion level of baobab seed meal was related to antinutrients in the seed (Igboeli *et al*, 1997) which may not have been removed considerably by the processing method employed. Chimvuramahwe *et al*. (2011) and Anene *et al*. (2012) also observed poor performance of animals fed baobab seed meal and stated that the depression in growth could be attributed to anti-nutritional factors, such as oxalate, phytate, saponins and tannins reported to be present in baobab seed meal (Nkafamiya *et al*., 2007).

On the contrary, similar feed conversion and nutrient digestibility was reported by rabbits fed baobab seed meal and control diets. Also, 15% of the meal can be included in rabbits diets with no negative effect on growth and digestibility. This could be as a result of antioxidant action of baobab pulp in the meal. Inclusion of the meal reduced feed and production cost, hence, increased the profit. (Oladunjoye *et al.,* 2014).

According to Oladunjoye *et al*., (2014), feed intake was not influenced by inclusion of baobab seed meal in the diets of rabbits. They stated that feed conversion by the rabbits that received 5% and 10% baobab seed meal compared favourably with that of the control, but poor feed conversion was observed in those that were fed diets that contained 15% of the meal. Hassan *et al*. (2015) also indicated that baobab seed meal can substitute soyabean meal at 10% in diets of *Clarias gariepinus* juveniles without compromising their growth.

2.4 *Clarias gariepinus*

Clarias gariepinus (African mud catfish) is the most cultured fish in Nigeria (Sogbesan and Ugwumba, 2006). It is hardy, possesses good growth and high quality taste. (Sogbesan and Ugwumba, 2008). It can eat an array of food ranging from Zooplankton to fish (Olaosebikan and Raji, 1998). It is mainly found in tropical swamps, rivers and lakes. It is widely distributed throughout Africa.

C. gariepinus has a scaleless slimy skin, with dark pigments in the lateral and dorsal parts of the body. It turns lighter in colour when exposed to light. Its body is long with dorsally flattened head enclosed by bony plates. It has four pairs of simple barbells in its mouth. Its dorsal and anal fins are very long and spineless. Its dorsal fins have about eight rays and anal fin of about 50-56 rays reaching almost to the caudal fin. The caudal fin is a single rounded lobe. It is a popular culture specie in Nigeria and omnivorous. It can also be found in the wild (Ugwumba, *et al*., 1995).

Clarias species is a commercially important catfish because of its good quality as food, ability to grow fast especially in intensive culture system, prolific nature, hardiness (it can withstand handling and environmental stress), resistance to diseases and many other adverse conditions that can kill fish.

2.5 Evaluation of fish health

Blood is a good indicator in determining the health of an organism (Joshi *et al*., 2002). Red blood cell and white blood cells vary between species, individual, and in the same individual according to condition and health (Jean, 1993 in Sola-Ojo *et al*., 2011), and are also influenced by diets. Haematology of different species of fish has been studied, and varied normal value ranges have been established. Deviation from the normal haematological values may indicate a disturbance in the physiological processes (Raiza-paiza *et al*., 2000 in Akinrotimi *et al.*, 2011).

Sogbesan *et al*., (2007) reported that higher inclusion of harvested tadpoles' meal had a negative effect on haematological and blood chemistry of *Heterobranchus longifilis*. He reported a rise in clotting time, white blood cell andblood glucose which indicated deferred clotting ability of the fish blood and poor immune system of the fish. He also stated that the rise in white blood cells could be as a result of low immunity of the fish as a result of anti-nutritional factors in the feed or imbalance amino acid composition of the diets (Sogbesan *et al*., 2007).

There were no appreciable diagnostic clinical changes in the blood parameters as a result of inclusion of baobab seed meal in the experimental animals' diets (Sola-Ojo *et al.* (2011); Oladunjoye *et al*. (2014); no sign of abnormality during the experimental period Ezeagu *et al*. (2005b) and visceral organs were not altered (Chimvuramahwe *et al*., 2011).

Assessments of blood biochemical parameters are important in evaluating the health of many vertebrates including, fish. Several fish biologists used it to detect cellular damage (Tavares-Dias *et al*. (2008); Popoola *et al*. (2018). Chemical substances like protein, hormones, enzymes, lipids present in serum provide information on tissues and organs in fish body. The biochemical are used to assess the toxicity Singh *et al*. (2010); diseases in fishes and farm animals and also reveals physiological conditions of the organs and tissue of organisms (Obamanu *et al*., 2009).

Water quality is one of the main factors that causes variation in fish biochemical parammeters, because of their close association to the environment (Popoola *et al*., 2018).

Heavy metals intake by fish varies depending on ecology, metabolism, pollution level, food and sediments. Fish accumulates metals in tissues through absorption (Balogun and Ajani, 2018). Some of the effects of heavy metals contaminantion in fish are kidney damage and poor reproductive capacity (Shabanda and Itodo, 2012). Alkaline phosphate, aspartate aminotransferase, alkaline amino transferase are good physiological parameters and stress indicators (Popoola *et al*., 2018).

Histological study is a rapid method for the detection of pollutant and toxic effects on various tissues of fish (Simonato *et al*., 2008). Histological alteration can be used as biological marker in the assessement of the impact of pollutants in marine and fresh water organisms and ecological health (Zhou, 2006). Liver is a good indicator of nutritional pathology due to its function in metabolism of the food products from the digestive system. Histological alterations have been reported on animals fed herbal dietary supplements like ginger peel (Ashade *et al*., 2014) and *Garcinia kola*(Akinrinde *et al*., 2015)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 *Sources and processing of test ingredient*

Baobab fruits were collected from the vicinity of the University of Ibadan, Oyo State, Nigeria. The fruits were harvested at mature stage which was indicated by the hard brown colour of the ectoderm. The seeds were removed from the pods and processed by autoclaving, toasting and soaking in different solutions (water, wood ash, maize liquor and baobab pulp with water). The differently processed baobab seeds were hammer-milled, sieved using sieve of 2.5mm mesh size, ground and re-sieved (0.5mm) to remove the seed coat and obtain baobab seed meal. Baobab seeds were sorted, washed and sun dried to attain constant moisture content. The seeds were milled and sieved to obtain Raw Baobab Seed Meal (RBSM). The baobab seed meals were processed as follows:

Autoclaving: Some washed and dried baobab seeds were autoclaved (Steam–pressure disinfecting apparatus, Model YX-280A) at 121 ^{o}C, 15MPa for 30 minutes, and dried in an oven at a temperature of 70 ^{o}C for three hours as modified (Falaye *et. al*., 2014). The seeds were milled and sieved to produce autoclaved baobab seed meal, ABSM**.**

Toasting: Some portions of washed and sun dried baobab seeds wereoven dried(Laboratory oven, Model: NL-9023A) up till a temperature of 150^{0} C for 30 minutes (Innocentia *et. al*., 2014). The meal produced was designated as Toasted Baobab Seed Meal, TBSM.

Soaking in acidic medium: Part of the dried seeds were soaked in maize liquor for 72 hours, washed, sundried and roasted at 70^{0}C for 30 min as modified (Sola Ojo *et. al*., 2013). The meal produced was designated as Soaked in Liquor Baobab Seed Meal, SLBSM.

Soaking in water: Some of the dried seeds were soaked in water for 72 hours, sundried and roasted at 70 ^{0}C for 30 minutes (modified method of Sola-Ojo *et al*., 2013). The meal was designated as Soaked in Water Baobab Seed Meal, SWBSM.

Soaking in alkali: Part of the dried seeds were soaked in alkali medium (5% wood ash) for 72 hours, sundried and roasted at 70 ^{0}C for 30 minutes as modified (Sola-Ojo *et. al*., 2013). The baobab seed meal produced was designated as Soaked in Alkali Baobab Seed Meal (SABSM).

Soaking in pulp: Parts of the seeds were soaked in water with baobab pulp for 72 hours (Sola-Ojo *et al*. 2013), sundried and roasted at 70 ^{0}C for 30 minutes (72 hrs).The meal produced was designated as Soaked in Pulp Baobab Seed Meal, SPBSM.

3.2 *Determination of Chemical composition of experimental ingredients*

Differently processed baobab seed meals were analysed for proximate, amino acid, mineral and anti-nutrients composition.

3.2.1*Proximate analysis*

The percentage of crude protein, crude fibre, moisture content, dry matter, ash content, fat content and nitrogen free extract of raw and processed baobab seed meals were determined by using the method described by AOAC (2005).

Crude protein: This was done by Kjeldahl procedure described by AOAC, (2005). Selenium tablet was added to mixture of the sample (0.5g) and sulphuric acid (10mL) and the mixture was heated in a fume cupboard (digestion). Distilled water was added to the mixture in a volumetric flask and made up to 100mL. 5 millilitres of the diluted digest was mixed with 5 mL 40% NaOH solution in a Kjedahl distillation apparatus. The mixture was distilled into 5 mL boric acid (40% concentration) which contained an indicator (methyl red). 5 mL of the distillates was titrated against 0.05 N H_2SO_4. A reagent blank was also digested, distilled and titrated.

$$\% N = \frac{(\text{mL standard acid} - \text{mL blank}) \times N \text{ of acid} \times 1.4007}{\text{Weight of samples (g)}}$$

Crude Protein = % Nitrogen in the samplesx 6.25.

Crude fibre: Samples were defatted with petroleum ether using Soxhlet extractor. Two grams of defatted samples were weighed into 600 mL beaker and 100 mL trichloroacetic acid was used for digestion. The mixtures were boiled in 150 mL of 1.25 H_2SO_4 solution for 30 minutes underreflux. The boiled samples were washed in several portions of hot distilled water and once with methylated spirit. After washing in several portions of water, the samples were allowed to drain dry before being transferred quantitatively to weighed crucible where it was dried in the oven at 105 ^oC to a constant weight. It was then taken to a muffle furnace where it was burnt to ash at 600 ^oC (5 hours). The incinerate sample (ash) was put in a desiccator to cool and to be weighed. The weight of the fibre was determined by difference and calculated as a percentage of the samples analysed as follows:

Crude fiber (%) = $[(W_2-W_3) \div$Weight of sampe$] \times 100$

W_2 = Weight of the sample + crucible after washing, boiling and drying

W_2 = Weight of the sample ash+ crucible

Moisture content and dry matter were determined by gravimetric method described by AOAC, 2005. 2g of each of samples into a can of a known weight. The samples and crucible/can were transferred into the oven (100^oC) to dry for 24 hours. The samples and crucible were then removed from the oven and put in a desiccator to cool for 10 minutes. The weights of the following were recorded:

% Dry matter = $[(W_3 - W_0) / (W_1 - W_0)] \times 100/1$

% Moisture = $[(W_1 - W_3) / (W_1 - W_0)] \times 100/1$ Or 100 - % DM.

Where; Can = W_o,

Can and samples prior to drying = W_1

Can and oven dried samples = W_3

Ash: This was done by the furnaces incineration gravimetric method (AOAC, 2005). 2grams of the samples were weighed into a can and placed in the muffle furnace set at 550^oC for four hours. It was exposed to atmosphere to cool to about 100^oC, then, cooled in a dessicator. The weight obtained was used to calculate the ash content.

% Ash content = (Weight of Ash ÷ Weight of sample before incineration) x 100

Fat content: 5g of the sample was wrapped in whatman filter paper and put in thimble, then placed in a soxhlet flask and put into weighed extraction flask containing 200mL petroleum ether. The reflux flask was connected to a water condenser. The petroleum

ether was heated and vapourised into the reflux flask. This was repeated for four hours and the oil content was collected in the flask and dried at 60˚ C to remove the residual solvent. It was cooled in a dessicator and weighed (Soxhlet extraction method)

Fat content= $[(W_2-W_1) \div \text{Weight of sample}] \times 100$

W_1= Weight of flask

W_2= Weight of flask + oil extract

% Nitrogen free extract (NFE) = 100 - % (Ash content of sample + Crude fiber in the sample + Moisture + Fat content + Crude protein of sample).

3.2.2*Mineral analysis*

The compositions of minerals (Na, K, P, Fe, Zn, Co, Mg, Ca and Zn) in baobab seeds were determined using atomic absorption spectrophotometer (AAS model: Perkin Elmer 2380, USA 1976) according to AOAC (2005) method. The samples were digested into solutions by wet digestion, using a mixture of conc. nitric, perchloric and sulphuric acids in the ratio 9:2:1 respectively. Fe, Zn, Co, Mg, Ca and Mn concentration were determined by AAS, (Alpha 4 model, Buck Scientific Ltd USA).

Sodium and potassium were determined using atomic emission spectrometer (200-A model, Buck Scientific Ltd UK) while colorimetric method was used to determine phosphorus. Phosphorus was determined by the Vanado-molybdate colorimetric method. The ash of sample was digested using 2MHCl. 5mL of 2MHCl was used to digest two grams of ash of sample, and heat to dryness in a heating mantle. Five mL of 2MHCl was added, heat to boil and filtered. Ten millilitres of the filtrate was pipetted into 50mL standard flask, and 10mL of vandate-molybdate yellow solution was added, and the flask marked up with distilled water. The concentration of phosphorus was obtained by measuring the Optical Density (OD) or absorbance of the solution in a colorimeter at a wavelength of 40 nm.

3.2.3 *Amino acid analysis*

Preparation of the samples by Hydrolysis

A gram of well ground sample was weighed into a 50 mL laboratory bottle, 25 mL hydrochloric acid-phenol reagent and heated at 110^{o}C with a loosely applied screw top for

one hour. > Then quickly tighten the screw top and hydrolyse for a further 23 hours in the heating oven at 110^{o}C.

Determination

Two millilitres of the above hydrolysate was pippetted into 30 mL test tube. Ten millilites of buffered nihydrin reagent added, heat in a boiling bath for 15 minutes, cool to room temperature and three ml of 50% Ethanol added immediately. 0-5µg/mL working standard amino acids were prepared from each standard solution of amino acids to get the gradient factor from the calbration curve for each amino acid to get the gradient factor from the calibration curve for each amino acid. The working standards were heated with the buffered ninhydrin reagent as done with the sample hydrolysate above. The absorbance or transmittance of samples buffered heated hydrolysate and working standards were measured at the wavelengths of colour developed by each amino acid.

3.2.4 *Anti-nutrient content evaluation*

Standard methods were used for the quantification of antinutritional compounds. Phytate in the sample was determined using the bipyrimidine colorimetric method, and oxalate was also determined by method described by the Onwuka (2005). The tannin content of the sample was determined by Folin-Denis colorimetric method (Harborne, 1993). Saponin was evaluated by double solvent extraction gravimetric method (Harborne, 1993).

Determination of Tannins

The tannin content of the sample is determined by Folin-Denis Colorimetric method (Harborne, 1993). A weighed sample (5.0g) is mixed with distilled water in the ratio of 1:10 (w/v). The mixture is shaken for 30min at room temperature and filtered to obtain the extract. A standard tannin acid solution is prepared, 2ml of the standard solution and equal volume of distilled water were dispersed into a separate 50ml volumetric flask to serve as standard and reagent blank respectively. Then 2mls of each of the sample extracts is put in their respective flasks and labeled. The content of each flask is mixed with 35ml distilled water and 1ml of the Folin-Denis reagent is added to each. This is followed by 2.5mL of saturated Na_2CO_3 solution. Then each flask is diluted to the 50ml mark with distilled water and incubated for 90min at room temperature. Their absorbance is measured at 760nm in a colorimeter with the reagent blank at zero. The tannin content is calculated as shown below

% Tannin = 100/w x au/as x c/1000 x vt/va

w = weight of sample; au = absorbance of test sample; as = absorbance of standard tannin solution; c = concentration of standard tannin solution; vt = total volume of extract; va = volume of extract analyzed.

Determination of Saponin

This is done by the double solvent extraction gravimetric method (Harborne, 1993). 5.0g of the processed sample is mixed with 50mL of 20% aqueous ethanol solution and incubated for 12h at a temperature of 55^0C with constant agitation. After that, the mixture is filtered through what man No 42 grades of filter paper. The residue is re-extracted with 50ml of the ethanol solution for 30min and the extracts weighed together.

The combined extract is reduced to about 40mL by evaporation and then transferred to a separating funnel and equal volume (40mL) of diethyl ether is added to it. After mixing well, there is partition and the outer layer is discarded while the aqueous layer is reserved. This aqueous layer is re-extracted with the ether after which its pH is reduced to 4.5 with drop wise addition of dilute NaOH solution.

Saponin in the extract is taken up in successive extraction with 60ml and 30ml portion of named butanol. The combined extract (ppt) is washed with 5% NaCl solution and evaporated to dryness in a previously weighed evaporation dish. The saponin is then dried in the oven (at 60^0C removes any residual solvent); cooled in a desiccators and re-weighed. The saponin content is calculated as shown below;

$$\% \text{ saponin} = \frac{W2 - W1}{W}$$

W = Weight of sample used; W1 = Weight of empty evaporation dish; W2 = Weight of dish + Saponin extract.

Determination of Phytate

Phytate in the sample is determined using the bipyrimidine colorimeter method described by Onwuka (2005). A weighed sample (2g) is soaked in 50mL of 0.2N HCl solution and shaked for 30min in a machine shaker. It is filtered to obtain the extract. A portion of the extract (0.5mls) is dispensed into a test tube and 1ml of acidified ferrous ammonia

sulphite solution is added to it. The tube is stoppered and boiled in water bath for 30min. It is then cooled in ice water for 15minutes and allowed to reach room temperature. The mixture is centrifuged at 3000 rpm for 5min and the supernatant is collected for analysis. 1ml of the supernatant is mixed with 1.5mL of 2.2 Bipyridine solutions.

A standard solution of phytate is prepared and diluted to a chosen concentration. 1ml of the standard solution is treated the same way as the sample extract as described above. The absorbance of the standard and the sample were read in a spectrophotometer at a wavelength of 519nm. A reagent blank is used to set the instrument at zero. The formula below is used to calculate the phytate content.

% Phytate = 100/w x au/as x c/100 x Vt/Vs

Where au = Absorbance of sample; as = Absorbance of standard solution

c = Concentration of the standard; Vt = Total volume of extract

Va = Volume of extract analyzed.

Determination of Oxalate

5g of the sample is weighed into a 100ml beaker, 20ml of 0.30N HCl is added and warmed from 40–50^{0}C, using magnetic hot plate and stirred for one hour. It is extracted three times with 20ml of 0.30N HCl and filtered into a 100ml volumetric flask. The combined extract is diluted to 100ml mark of the volumetric flask. The oxalate is estimated by pipetting 5ml of the extract into a conical flask and made alkaline with 1.0mL of 5N ammonium hydroxide. A little indicator paper is placed in the conical flask to enable us know the alkaline regions. It is also made acid to Phenolphthalein (2 or 3 drops of this indicator added, excess acid decolourises solution) by dropwise addition of glacial acetic acid. 1.0ml of 5% $CaCI_2$ is then added and the mixture was allowed to stand for 3h after which it is then centrifuged at 3000rpm for 15min.

The supernatants were discarded and the precipitates washed 3 times with hot water with thorough mixing and centrifuging each time. Two milliliters of 3N H2SO4 is added to each tube and the precipitates dissolved by warming in a water bath (70 – 80^{0}C). The content of all the tubes is carefully poured into a clean conical flask and titrated with freshly prepared 0.05M KMnO4 at room temperature until the first pink colour appeared

till the solution became colourless. The solution is then warmed to 70 – 80^0C and titrated until a permanent pink colour that persisted for at least 30 seconds is attained.

Data were subjected to one-way analysis of variance (ANOVA) (Steel*et al*., 1997). Means were separated using Duncan multiple range test at 5% level of probability.

3.3 Digestibility of baobab seed meal by *Clarias gariepinus*

A preliminary study carried out to evaluate the biological value, digestibility and mean weight gained of weanling winstar strain male albino rats fed raw and differently processed baobab seed meal showed that raw and differently processed baobab seed meals had similar biological value. It also reflected significantly the same digestibility values in rats fed raw and processed baobab seed meal based diets except rats fed soaked in liquor baobab seed meal based diets which recorded lowest value (98.54%). Negative mean weight gain were recorded in rats fed raw and processed baobab seed meal based diets except those fed soaked in alkali baobab seed meal based diet which recorded a positive value (2.03±3.61).

Experimental unit and design

Raw, toasted, autoclaved, soaked in water, soaked in liquor, soaked in pulp, soaked in alkali baobab seed meal were tested for digestibility using *Clarias gariepinus*. The processed seeds were used to formulate fish diets (**Table** 3.1). Reference diet was also formulated using soybean meal. The treatments were replicated thrice. Experimental fish were randomly allotted into experimental tanks during the course of investigation.

Experimental fish for digestibility study

360 *Clarias gariepinus* juveniles of average weight 15.0±5.0g were procured from a reputable fish farm in Ibadan and acclimatised for three weeks in Genetics and Breeding Research Unit, Department of Aquaculture and Fisheries, University of Ibadan, Ibadan. The fish were fed with a sample diet, after which they were starved for 2-3 days prior to feeding with the experimental diets such that the fish would have been rid of undigested materials sample diet from their guts.

After acclimatisation, 15 juvenile fish were randomly selected and stocked in circular tank (55 litre volume) containing 35 litres of water each. The experimental fish were fed with the experimental diet at 5% of their body weight (morning and evening) for 56 days. Remnant feeds were cleaned 30 minutes after feeding to prevent feeds from contaminating the faeces.

Faeces of fish fed experimental diets were collected eight hours after feeding for digestibility test. The faeces were siphoned by using siphoning tube (2mm size) in plastic bottles, sun-dried and stored in air-tight container at 20 ^{o}C before analysis (Falaye *et al.,* 2014). Chromic oxide levels in diets and faeces were evaluated by digesting them with nitric acid, oxidising Cr_2O_3 to Cr_2O_7 with perchloric acid. They were digested at 120-150 ^{o}C until the solution turned yellow, and thick white fumes appear. The solution was cooled for five to ten minutes. Beaker was rinsed three to four times into 100mL volumetric flask and made up to mark with ddH_20 (distilled de-ionised water). Standard solution was prepared using the chromic oxide and calibrated using the volumes; 0 (blank), 2, 4, 6, 8 and 10mls of the chromium standard. Absorption of standard solutions was read at 440nm. The standard curve was plotted and used to estimate the concentration of chromium (Furukawa and Tsukahara, 1966).

The Apparent Digestibility Coefficient (ADC) of nutrients and energy of the reference diet (RD) and test ingredients were calculated according to the following equations (Fagbenro *et al*., 2017):

ADCnutrient= 100-100[(% Cr_2O_3diet/% Cr_2O_3faeces) x (% nutrient or energy in faeces/ % nutrient or energy in diet)]

ADC: apparent digestibility coefficient (%);

% Cr_2O_3 diet: % chromic oxide percentage in diet;

% Cr_2O_3 faeces: % chromic oxide in faeces.

Sampling of fish

The fish were observed daily. Dead fish were recorded for evaluation of survival rate. Subsequently, the fish in each experimental tank were weighed once in a week to access their growth and weight gain and adjust feed rations accordingly.

3.4 Fish culture condition and water quality analysis

Water samples were taken and evaluated for selected parameters (dissolved oxygen, pH, temperature, nitrate, nitrite, and ammonia) once in a week in line with standard methods (APHA, 2005). Water was changed using the static renewal method every two days.

Table 3.1 Gross composition of experimental diets fed to *Clarias gariepinus* for digestibility study

Ingredients (%)	Control	RBSM	TBSM	SPBSM	ABSM	SLBSM	SWBSM	SABSM
Fish meal	29.00	29.00	29.00	29.00	29.00	29.00	29.00	29.00
Soybean meal	30.00	13.50	13.70	12.50	11.10	10.90	12.40	10.90
Yellow maize	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Baobab Seed Meal	-	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Wheat offal	17.50	14.00	14.00	14.00	16.00	16.00	14.00	16.00
Vitamin premix	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Oyster shell	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Palm oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mollasses	13.60	3.60	3.40	4.60	4.00	4.20	4.70	4.20
Chromium oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

RD: Reference Diet RBSM: Raw Baobab Seed Meal TBSM: Toasted Baobab Seed Meal
ABSM: Autoclaved Baobab Seed Meal SPBSM: Soaked in Pulp Baobab Seed Meal SLBSM: Soaked in Luquor Baobab Seed Meal
SWBSM: Soaked in Water Baobab Seed Meal SABSM: Soaked in Alkali Baobab Seed Meal

3.5 Effects of different inclusion levels of baobab seed meal on growth of *Clarias gariepinus*

The best two processing methods from digestibility study were used to compound fish feed. The processed test ingredients were used to replace soybean meal at 0%, 20%, 40%, 60%, 80%, and 100%.

Experimental unit and design for growth study

Fish were randomly allotted into experimental tanks in triplicates to treatmentusing completely randomised design. The experiment comprised eleven treatments which were replicated thrice.

Analysis of experimental diets

Proximate composition of test ingredients, experimental diets and fish flesh were evaluated by method of AOAC (2000). Raw and processed seeds were analysed at the beginning of the feeding trial. Experimental fish were analysed before and at the end of the experimental trial.

Experimental fish for growth study

Clarias gariepinus juveniles (n=600) of average weight of 15.0±5.0g were procured from a reputable fish farm in Ibadan and acclimatised for three weeks at the Genetics and Breeding Research Unit, University of Ibadan, Nigeria. The fish were fed with a commercial feed after which they were starved for 2-3 days prior to feeding with experimental diet so that the fish were rid of undigested commercial feed from the guts.

After acclimatisation, 15 juvenile fish were randomly selected and stocked in circular tank of 55cm diameter and 36cm depth(55 litre volume). The water was maintained at 27cm depthcontaining 35 litres of water each. The test ingredients were ground, sieved with sieve of 0.5mm mesh size and mixed with other ingredients to formulate the respective test diets (**Table** 3 and **Table** 4). Isonitrogenous diets (36 % CP) were formulated to meet the crude protein requirement of *Clarias gariepinus* (NRC, 1993). The fish were fed 5% of their body weight daily (8:00-9:00am and 5:00-6:00pm), and the feeding ration was adjusted as the fish increased in weight. The experiment lasted for 16 weeks.

Sampling of fish

The fish were monitored daily for mortality. Dead fish were removed and recorded for determination of survival rate. Subsequently, the fish in each experimental tank were weighed once in a week to access their growth and weight gain and adjust feed rations accordingly.

3.6 Fish growth analysis

Fish growth parameters were determined according to the procedure of Ridha and Cruz (2001):

Weight gain (g) = Final weight of fish (W_2) – Initial weight (W_1).

Average daily gain (g) = Weight gain (g) ÷ Days of feeding.

Specific growth rate (SGR % day) = 100 x (In W_2 – In W_1/ T_2-T_1),

where: T_2-T_1 represents the experimental periods in days.

Nutrients' utilisations were also determined:

Protein efficiency ratio (PER) = Weight gain (g) ÷ Protein intake (g).

Protein productive value (PPV) = 100 (Protein gain / Protein fed), where Protein fed (PF) = Total feed consumed X % crude protein in feed.

Feed efficiency ratio (FCR) = Fish weight gain (g) ÷ Feed intake (g)

Feed conversion ratio = Weight of feed fed (g) ÷ Fish weight gain (g)

Survival rate = (Number of fish remaining at the end of the experiment ÷ Number of fish at the beginning of the experiment) x 100

3.7 Fish culture condition and water quality analysis

Water samples were taken and evaluated for selected parameters (dissolved oxygen, pH, temperature, nitrate, nitrite and ammonia) once in a week in line with standard methods (APHA, 2005). Water was changed using the static renewal method every two days.

3.8 Carcass analysis

At the beginning of the feeding trial, six fish were randomly selected, dried and homogenised for proximate analysis. Two fish from each replicate, resulting to six fish per treatment were pooled together at the end of the feeding trial, then homogenised and their proximate compositions determined (AOAC, 2000).

Table 3.2 Gross composition of soaked in alkali baobab seed meal based experimental diets fed to *Clarias gariepinus*

Ingredients (%)	Inclusion levels of soaked in alkali baobab seed meal					
	0%	20%	40%	60%	80%	100%
Fish meal	32.00	32.00	32.00	32.00	32.00	32.00
Soya bean	19.00	15.20	11.40	7.60	3.80	-
Baobab seed meal	-	6.00	11.90	17.80	23.80	29.50
Maize	6.00	6.00	6.00	6.00	3.80	6.00
Wheat offal	23.00	21.00	20.00	20.00	22.00	22.00
Palm oil	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Oyster shell	0.70	0.70	0.70	0.70	0.70	0.70
Molasses	13.80	13.60	12.50	10.40	6.20	4.30
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	100
Calculated crude protein (%)	36.10	35.79	35.58	35.50	35.67	35.59

Table 3.3. Gross composition of soaked in pulp baobab seed meal based experimental diets fed to *Clarias gariepinus*

Ingredients (%)	Inclusion levels of soaked in pulp baobab seed meal					
	0%	20%	40%	60%	80%	100%
Fish meal	32.00	32.00	32.00	32.00	32.00	32.00
Soyabean	19.00	15.20	11.40	7.60	3.80	-
Baobab seed meal	-	6.50	13.10	19.60	26.10	32.60
Maize	6.00	6.00	6.00	6.00	6.00	6.00
Wheat offal	23.00	21.00	20.00	22.00	22.00	22.00
Palm oil	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Oyster shell	0.70	0.70	0.70	0.70	0.70	0.70
Molasses	13.80	13.10	11.30	6.60	3.90	1.20
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	100
Calculated crude protein (%)	36.10	35.77	35.53	35.68	35.55	35.47

3.9 Plasma and serum biochemistry of *Clarias gariepinus* fed baobab seed meal based diets

Three fish per tank were randomly selected (nine fish per treatment) from each dietary treatment for tissue haematological, serum biochemical analysis and histopathological studies.

3.9.1 *Haematology*

Haematological analysis of the experimental fish was done before the commencement of the experiment and at the end of the experiment. At the end of the experiment, three fish per tank were randomly selected (nine fish per treatment) from each dietary treatment for haematological analysis. The blood samples were collected as described by Stoskopf (1993) and Joshi *et al*. (2002) from the caudal peduncle of the fish. The blood samples were dispensed into tubes containing lithium hepanin anticoagulant and then taken to the laboratory.

Haematology procedure

Packed cell volume (PCV) determination

3/4 of microhaematocrit (heparinised) capillary tube was filled with blood from appropriately labeled whole blood in a heparine tube by capillary action one end of the tube was sealed with plastercine. The tube was spinned at 1,200 rpm in microhaematocrit centrifigue for five minutes (Jain, 1986). The PCV was read using microhaematocrit reader and expressed as a percentage of the total blood volume.

Haemoglobin concentration determination

The concentration of haemoglobin (Hb) was determined by cyanohaemoglobin method (Jain, 1986). Briefly 20µL blood was mixed with four millilitres of modified drabkin's solution, volume made up to one litre with distilled water and pH adjusted to 7.0. The haemoglobin concentration was read with autospectrophotometer (spectrum lab 23A) at a wavelength of 540 nm. The actual value of Hb concentration was extrapolated from a standard haemogblobin curve. For the solution to work perfectly, the drabkin's solution was kept in a dark cupboard after preparation before usage.

Determination of total red blood

Differential WBC counts were evaluated manually (Zinkl, 1986), by the use of Neubauer haemocytometer according to Jain (1986) and the number of cells was expressed as 10^{12}Rbc per litre of blood and 10^{9} white blood cells per litre of blood respectively. 1mL of RBC diluting fluid (Daciers fluid) was taken into test tube. 0.05 µL of whole blood was added into the Daciers fluid to keep and preserve the shape of the cells. This was gently mixed and allowed to stand, and it was counted at X40 magnification under the microscope.

Differential white blood cell (WBC) Counts

Differential WBC counts were evaluated manually as described by Zinkl, (1986). Freshly prepared Giemsa stain solution was used. The stain was used at 10% with distilled water. Thin blood smear was done using clean glass slide. Capillary tube was filled with whole blood. This was done by capillary action. A drop of blood was poured on the clean glass slide, and then a spreader was used to evenly spread the blood at angle 45^{0} to make a very good thin blood smear. This was allowed to dry under room temperature, then fixed with methanol (Analar grade) and allowed to dry; it was then immersed in giemsa stain solution for 45 minutes.

After staining, it was allowed to dry, then viewed under the microscope at x100 magnification using Leica Galen III Microscope to identify and count blood cells present. Thin blood smear stained with Giemsa as described by Jain (1986) and Kelly (1984) was used; 200 white blood cells were enumerated and differentiated.

3.9.2 *Blood biochemistry*

The blood collected from the sampled fish was also used for plasma biochemical analysis. The blood samples were dispensed into tubes containing lithium hepanin anticoagulant and then taken to the laboratory. The following parameters were determined by using commercial kits (AMES Blood Analyser and Randox Laboratory Limited, UK), following the procedure described by the manufacturer: total protein, albumin, globulin, creatine, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase.

3.9.3 *Oxidative stress*

The blood and liver collected from the sampled fish were used to determine the following pro-oxidant and anti-oxidant analysis:

Total protein concentration: Serum of the fish samples was used to evaluate the protein concentration by employing Biuret method as described by Gornall *et al.* (1949) with some modifications. Distilled water and eight grams of sodium hydroxide were mixed and made up to 1 litre (with distilled water). Distilled water (2mL) was used to dissolve 20 mg of the serum bovine albumin to produce a solution of 10mg protein/ml (Standard). 500 mL of 0.2 M NaOH was used to dissolve three grams of $CuSO_4$ and $5H_2O$. The mixture was added to nine grams of Sodium tartarate and five grams of potassium iodide, KI (BDH Chemicals, England) and made up to a litre.

Appropriate dilutions of experimental samples (livers) were prepared with distilled water (diluted10 times to reduce sensitivity range) and used to estimate the protein in the samples following the procedure described for the standard. Mixtures of the standard and test samples were incubated at room temperature for 30 minutes, and their absorbance were read at 540 nm. Distilled water was used as blank. The actual amount of protein in the fraction of the samples was then extrapolated from the standard curve and multiplied by 10.

Estimation of nitric oxide (NO) in serum sample: Tissue supernatants (20 µL of) and Griess reagent (200 µL) were mixed and incubated at room temperature for 20 minutes. AAS was determined at 490 nm by micropipette plate reader. NO in the sample was evaluated from a sodium nitrite ($NaNO_2$) standard curve (Olaleye *et al.,* 2007).

Estimation of serum myeloperoxidase (MPO): Serum sample (70 µL) was mixed with mixture of O-dianisidine and H_2O_2 (2,000 µL). Absorbance was recorded at 0, 30 and 60 seconds at 460 nm wavelengths.

MPO = [(Reading at 60 seconds- Readings at 0 seconds) nm ÷ mg protein] x 10

Estimation of GSH level: Tissue sample (0.5 ml) and precipitating solution (0.5 ml) were mixed in the tubes and centrifuge for five minutes at 4,000 rpm. Absorbance of mixture of Ellman's reagent (4.5 mL) and the centrifuged mixture (0.5 mL) was determined against blank as distill water at 412 nm. The GSH was evaluated by using the standard curve.

Malondialdehyde (MDA): Tris-KCl (1.6 mL), 0.5 mL of 30% TCA. and then 0.4 ml of test samples were put into test tubes. Also, 0.5 mL of 0.75% TBA was added to the mixture immediately. These were incubated in water bath at 80°C for 45mins, cooled in ice and centrifuged at 3,000 rpm for 15 minutes. Its absorbance and the blank (distilled water) were obtained at 532 nm.

Molar extinction coefficient of 1.56×10^5 $M^{-1}Cm^{-1}$ was used in calculating MDA.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance X volume of mixture}}{E_{532nm} \text{ X volume of sample X mg protein}}$$

Superoxide dismutase (SOD) activity: 800 µL of Tris-KCl buffer, 250 µL of tri chloroacetic acid (TCA), 250 µL of thiobarituric acid (TBA), and 200 µL of sample were added into the test tube subsequently. The mixture was incubated for 45 minutes (80˚C) in water bathe, cooled and centrifuged for five minutes at 4, 000 rpm. Absorbance was measured against a reference blank of distilled water at wavelength 490 nm.

Determination of gluthathione-s-transferase activity: 10 µl of sample and 140 µL of phosphate buffer saline, then, 10 µL of GSH, and then, 50 µL of CDNB (1- chloro, 2,4-dinitrobenzol) were added as stated. Half plate was read first; then the other half at wavelength of 405nm. Setting blanks for comparison (Habig *et al.,* 1974).

Catalase activity: 2.95 mL of phosphate buffer containing 19 mM of hydrogen peroxide was pipetted into a one cm quartz cuvette and 50 µL of sample was added. The mixture was speedily upturned to mix. Absorbance (240 nm) was obtained every 15 seconds for three minutes in a spectrophotometer (Claiborne, 1985).

$$\text{Catalase activity} = \frac{\Delta A_{240}/\text{min} \times \text{rxn vol. dilution faction}}{0.0436 \times \text{sample vol} \times \text{mg protein}}$$

$$= \mu\text{mole } H_2O_2/\text{min/mg protein}$$

3.10 *Histopathological indices*

Three fish per tank were randomly selected from each treatment and dissected after the experiment. Samples of the gill, liver and kidney were removed from the fish, fixed in 10% neutral buffered formalin, and labelled. The appropriately labelled samples were processed in automatic tissue processor, embedded in paraffin wax and sectioned at five microns on a rotary microtome mounted on glass slides. The stepwise protocol for the automatic tissue processor for histological examination slide was described (Akpokodje *et al*., 2005).

The procedure is presented as follows:

Fixation: The general principle in handling and fixation of specimen was that the fixative should be 10 to 20 times the volume of the specimen. The specimens were fixed for at least 24 hours before the commencement of dehydration.

Dehydration: It is the process of removing the inherent water content of a given specimen of tissue in a gradual way considering osmotic dynamics. Dehydration was done by automated means, using the automated tissue processor (Shandon-Elliot^{R}).

Clearing: It involves the removal of the alcohol (Ethanol) that the tissues had bathed in and to initiate and complete a process that will make cells transparent at microscopic level.

Infiltration: Molten paraffin wax was used for infiltration to support the tissues for subsequent stage of sectioning. Paraffin wax permeates into the tissues to fill up vacuoles that have been left by dehydration.

Embedding: The tissues were carefully and consciously positioned in the orientation they were sectioned. The embedded tissues were left until the wax solidified.

Sectioning: This was done on a quint-essential piece of histological equipment called a microtone, which cuts only a very thin slice of the original tissue at a preset thickness, for instance, 4µ.

The produced sections were floated out in a floating water bath. Satisfactory section(s) were picked up with frosted edge microscope glass slides. The slides carrying the sections were labeled with a pencil, arranged in a slide carrier for staining in oven 40 ^{0}C for 30 minutes.

Staining: The slides were stained in Haematoxyline and Eosin.

Examination and description of microscopic specimens:Slides (sections) were examined by the naked eye, then thorough examination of whole section using low power (*4) objective lens (of Olympus microscope camera) so as to appreciate the main structural patterns, and identification of normal tissue was done. Abnormal areas were subjected to further examination, still under low power and medium power (*10) objective, and doubtful cells or structures were further examined under higher (*40) objective.

Description: The pathological changes observed in the microscopic examination were described accordingly based on histological structure of the tissues. Photomicrographs were taken with the aid of computerised digital camera (Amscope MU900).

3.11 Cost implication of incorporating baobab seed meals in the diets of *Clarias gariepinus*

The price and quantity of raw materials required to formulate the diets were used to calculate the cost/kg of each diet. The economic conversion ratio was determined according to Piedecausa *et al*. (2007) as follows:

Gross revenue=Final weight gain (Kg) × Feed cost per Kg

Economic conversion ratio (₦/kg) = Cost of diet (₦/kg) x Feed conversion ratio

Feed conversion ratio = Feed consumed (g) / Fish weight gain (g)

3.12 Statistical analyses

Data were subjected to descriptive statistics, regression and analysis of variance (ANOVA) (Steel*et al*., 1997). Means were separated using Duncan multiple range test (p=0.05).

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical composition of differently processed baobab seed meal

4.1.1 *Proximate composition of processed baobab seed meals*

Proximate composition of differently processed baobab seed (**Table** 4.1) indicated that soaked in water baobab seed meal had the highest moisture content (MC) of 8.22±0.01% while the least (6.84±0.05%) was obtained in toasted baobab seed meal. The MC in all the processed meals were different ($p<0.05$). Crude Protein (CP) of processed baobab seed meal was significantly different ($p<0.05$).

The crude proteins recorded in soaked in alkali baobab meal (SABSM), 26.78±0.13% and soaked in liquor baobab seed meal (SLBSM), 26.78±0.13% were similar ($p<0.05$). The highest CP of 26.78±0.13% was recorded in SLBSM and SABSM, respectively, while toasted baobab seed meal (TBSM) had the least (22.84±0.13%). However, CP of 22.84±0.13% obtained from TBSM was lower compared to raw baobab seed meal (23.13±0.12%).

Crude fat ranged from 19.00±0.15% in TBSM to 21.23±0.06% in ABSM. Also, SPBSM recorded the highest crude fibre of 17.60±0.03%, while SWBSM had the lowest, 11.80±0.03%. Highest crude ash of 8.01±0.025% was recorded in SLBSM, while the least of 6.49±0.01% was in SPBSM. Nitrogen free extract ranged from 23.01±0.19% was recorded in SLBSM to 29.98±0.11% in TBSM.

Table 4.1. Proximate composition of differently processed baobab seeds

Parameters %	RBSM	TBSM	ABSM	SPBSM	SLBSM	SWBSM	SABSM
Moisture content	7.14 ± 0.01^{b}	6.84 ± 0.05^{a}	7.41 ± 0.02^{c}	7.56 ± 0.02^{d}	7.77 ± 0.02^{e}	7.93 ± 0.01^{f}	8.22 ± 0.02^{g}
Crude protein	23.13 ± 0.12^{b}	22.84 ± 0.13^{a}	26.42 ± 0.13^{d}	24.45 ± 0.13^{c}	26.78 ± 0.13^{e}	24.59 ± 0.1^{c}	26.78 ± 0.13^{e}
Crude fat	19.22 ± 0.03^{b}	19.00 ± 0.15^{a}	21.23 ± 0.06^{f}	20.31 ± 0.01^{d}	20.82 ± 0.02^{e}	19.89 ± 0.0^{c}	20.79 ± 0.01^{e}
Crude fibre	14.79 ± 0.02^{d}	14.79 ± 0.02^{d}	12.00 ± 0.02^{b}	17.60 ± 0.03^{e}	13.60 ± 0.04^{c}	11.80 ± 0.0^{a}	12.00 ± 0.02^{b}
Ash	6.90 ± 0.01^{c}	6.55 ± 0.12^{b}	6.88 ± 0.01^{c}	6.49 ± 0.01^{a}	8.01 ± 0.02^{f}	7.27 ± 0.02^{e}	7.22 ± 0.02^{d}
NFE	28.82 ± 0.07^{f}	29.98 ± 0.11^{g}	26.05 ± 0.03^{d}	23.60 ± 0.16^{b}	23.01 ± 0.19^{a}	28.52 ± 0.12^{e}	24.98 ± 0.17^{c}

Values with different superscripts on the same row are significantly different ($p < 0.05$) NFE: Nitrogen Free Extract
RBSM: Raw Baobab Seed Meal TBSM: Toasted Baobab Seed Meal ABSM: Autoclaved Baobab Seed Meal
SPBSM: Soaked in Pulp Baobab Seed Meal SLBSM: Soaked in Luquor Baobab Seed Meal SWBSM: Soaked in Water Baobab Seed Meal
SABSM: Soaked in Alkali Baobab Seed Meal

4.1.2 Mineral composition of differently processed baobab seed meal

Macro minerals contents of differently processed BSM are shown in **Table** 4.2. The highest total phosphorus (0.74±0.00%) was obtained in SABSM, while the least was in SPBSM (0.48±0.001%). Calcium values ranged from 4.36±0.00% recorded in RBSM to 7.61±0.06% in SWBSM. The highest magnesium of 2.01±0.00% was obtained in ABSM; and SABSM had the highest potassium of 3.79±0.00%. Sodium values ranged from 0.03±0.00% in TBSM to 0.44±0.00% in SWBSM.

Micro minerals composition of differently processed baobab seed meal (**Table** 4.3) showed that the highest manganese of 283.25±0.35 mg/kg was obtained from SPBSM while RBSM had the least value of 20.48±0.04 mg/kg. The highest iron and copper of 3403±0.00 mg/kg and 24.10±0.00 mg/kg, respectively were recorded in SLBSM, while the lowest values were recorded in TBSM (1205±0.00 and 17.93±0.00 mg/kg). Zinc values ranged from 18.50±0.00 mg/kg recorded in SWBSM to 24.10±0.00 mg/kg in SLBSM.

Table 4.2.Macro mineral composition of differently processed baobab seed meal

Parameters %	RBSM	TBSM	ABSM	SPBSM	SLBSM	SWBSM	SABSM
Total phosphorus	0.530 ± 0.00^{b}	0.72 ± 0.00^{f}	0.69 ± 0.00^{e}	0.48 ± 0.00^{a}	0.53 ± 0.00^{c}	0.58 ± 0.00^{d}	0.74 ± 0.00^{g}
Calcium	4.36 ± 0.00^{a}	6.21 ± 0.00^{c}	6.24 ± 0.00^{c}	5.71 ± 0.00^{b}	7.19 ± 0.00^{e}	7.61 ± 0.06^{f}	6.42 ± 0.03^{d}
Magnesium	1.82 ± 0.00^{d}	2.39 ± 0.003^{f}	2.67 ± 0.00^{g}	2.01 ± 0.00^{e}	0.86 ± 0.01^{b}	0.76 ± 0.00^{a}	1.33 ± 0.00^{c}
Potassium	3.06 ± 0.00^{a}	3.36 ± 0.01^{bc}	3.38 ± 0.00^{c}	3.65 ± 0.00^{d}	3.65 ± 0.07^{d}	3.29 ± 0.01^{b}	3.79 ± 0.00^{e}
Sodium	0.11 ± 0.01^{a}	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.44 ± 0.00^{b}	0.21 ± 0.243^{a}

Values with different superscripts on the same row are significantly different ($p < 0.05$) RBSM: Raw Baobab Seed Meal
TBSM: Toasted Baobab Seed Meal ABSM: Autoclaved Baobab Seed Meal SPBSM: Soaked in Pulp Baobab Seed Meal
SLBSM: Soaked in Lquor Baobab Seed Meal SWBSM: Soaked in Water Baobab Seed Meal SABSM: Soaked in Alkali Baobab Seed Meal

Table 4.3.Micro mineral composition of differently processed baobab seed meal

Parameters mg/kg	RBSM	TBSM	ABSM	SPBSM	SLBSM	SWBSM	SABSM
Manganese	20.48±0.04^{a}	29.50±0.0^{d}	45.05±0.07^{f}	283.25±0.35^{g}	38.75±0.36^{e}	21.50±0.00^{b}	22.25±0.35^{c}
Iron	1262.50± 3.54^{b}	205.00±0.00^{a}	1485.00±0.00^{d}	2292.50±3.54^{e}	3402.50±3.54^{f}	1292.50±3.54^{b}	1372.50±3.54^{c}
Copper	19.35±0.01^{b}	17.93±0.04^{a}	20.33±0.04^{c}	21.28±0.04^{e}	24.08 ±0.04^{g}	21.03±0.04^{d}	21.88±0.04^{f}
Zinc	19.95±0.00^{c}	20.12±0.04^{d}	22.43±0.04^{f}	21.88±0.04^{e}	24.08±0.04^{g}	18.53±0.04^{a}	19.48±0.04^{b}

Values with different superscripts on the same row are significantly different ($p < 0.05$)
RBSM: Raw Baobab Seed Meal
TBSM: Toasted Baobab Seed Meal
ABSM: Autoclaved Baobab Seed Meal
SPBSM: Soaked in Pulp Baobab Seed Meal
SLBSM: Soaked in Lquor Baobab Seed Meal
SWBSM: Soaked in Water Baobab Seed Meal
SABSM: Soaked in Alkali Baobab Seed Meal

4.1.3 Amino acids profile of differently processed baobab seed meal

The amino acids profile of differently processed baobab seed meal are represented in **Table** 4.4. Methionine, cystine, methionine+cystine, lysine, histidine, threonine, arginine, isoleucine, leucine, valine, phenylalanine, glycine, serine, proline, alanine, aspartic acid, and glutamic acid values recorded close.

Table 4.4. Amino acids profile of differently processed baobab seed

Indices	RBSM	TBSM	ATSM	SPBSM	SLBSM	SWBSM	SABSM
Methionine	0.31	0.31	0.35	0.33	0.37	0.32	0.34
Cystine	0.39	0.36	0.42	0.41	0.46	0.41	0.42
Methionine + cystine	0.70	0.67	0.77	0.74	0.83	0.73	0.76
Lysine	1.06	0.93	1.15	1.10	1.24	1.10	1.16
Threonine	0.64	0.62	0.70	0.65	0.74	0.66	0.69
Arginine	2.31	2.22	2.56	2.37	2.72	2.38	2.55
Isoleucine	0.76	0.74	0.84	0.78	0.88	0.78	0.82
Leucine	1.40	1.35	1.53	1.43	1.62	1.44	1.51
Valine	1.04	1.00	1.14	1.05	1.20	1.06	1.12
Histidine	0.50	0.48	0.55	0.51	0.58	0.51	0.64
Phenylalanine	1.00	0.96	1.09	1.02	1.16	1.02	1.07
Glycine	1.00	0.97	1.09	1.01	1.16	1.03	1.09
Serine	1.07	1.03	1.18	1.11	1.26	1.11	1.17
Proline	0.76	0.75	0.84	0.76	0.88	0.77	0.80
Alanine	0.93	0.90	1.01	0.94	1.08	0.96	1.00
Aspartic acid	1.90	1.84	2.09	1.94	2.23	1.96	2.07
Glutamic acid	4.80	4.65	5.30	4.97	5.63	4.97	5.25

RBSM: Raw Baobab Seed Meal
TBSM: Toasted Baobab Seed Meal
SPBSM: Soaked in Pulp Baobab Seed Meal
SWBSM: Soaked in Water Baobab Seed Meal
ABSM: Autoclaved Baobab Seed Meal
SLBSM: Soaked in Liquor Baobab Seed Meal
SABSM: Soaked in Alkali Baobab Seed Meal

4.1.4 Antinutritional factors in differently processed baobab seed meal

Oxalates, phytate and saponins level recorded for the raw and processed baobab seed meals were significantly different ($p<0.05$) as presented in **Table** 4.5. Highest saponin of 4.21±0.20 % was recorded in SPBSM while the lowest (0.53±0.13) was recorded in RBSM. Oxalates ranged from 1.81±0.07 in SABSM to 3.60±0.10 in TBSM. Oxalate level in SABSM was lower ($P<0.05$) than RBSM. The SLBSM had lowest phytates of 8.62±0.04% while TBSM recorded the highest value of 10.21±0.06 %.

Table 4.5. Antinutrients in processed baobab seed meals

Indices %	RBSM	SABSM	SLBSM	SPBSM	SWBSM	TBSM	ABSM
Oxalate	2.42± 0.08^{b}	1.81± 0.07^{a}	3.19± 0.05^{d}	3.20± 0.02^{d}	2.87± 0.06^{c}	3.60± 0.10^{e}	2.42± 0.05^{b}
Phytate	9.28± 0.13^{d}	9.51± 0.14^{e}	8.62± 0.04^{a}	9.05± 0.05^{c}	9.75± 0.08^{f}	10.21± 0.06^{g}	8.81± 0.12^{b}
Saponin	0.53± 0.13^{a}	3.50± 0.11^{e}	3.90± 0.02^{f}	4.21± 0.20^{g}	2.31± 0.02^{c}	2.00± 0.05^{b}	3.20± 0.05^{d}

Means with different superscripts on the same row are significantly different ($p < 0.05$) RBSM: Raw Baobab Seed Meal TBSM: Toasted Baobab Seed Meal ABSM: Autoclaved Baobab Seed Meal SPBSM: Soaked in Pulp Baobab Seed Meal SLBSM: Soaked in Liquor Baobab Seed Meal SWBSM: Soaked in Water Baobab Seed Meal SABSM: Soaked in Alkali Baobab Seed Meal

4.2 Growth, nutrients utilisation and digestibility of differently processed baobab seed meal based dietsfed*Clarias gariepinus* juveniles

Growth, nutrients utilisation and digestibility of differently processed BSM based diets by the experimental fish are presented in **Table** 4.6. Mean Weight Gain (MWG) and Specific Growth Rate (SGR) recorded in control diets and processed baobab seed meals were not significantly different (P>0.05). The highest MWG and SGR of 43.83±2.29g and 2.17±0.07%, respectively in SABSM were similar (p>0.05) to 41.56±1.50 and 2.10±0.05 in fish fed with soyabean meal diets. The least MWG and SGR of 33.51±7.39 and 1.83±0.26 respectively were recorded in fish fed RBSM.

Feed intake ranged from 818.46±54.76 in fish fed RBSM to 1020.33±68.82 in those fed SPBSM. Related (P>0.05) Feed Conversion Ratio (FCR) was recorded in fish fed soyabean meal and processed baobab seed meal diets. The least feed conversion ratio of 1.65±0.01 was obtained in SABSM, while TBSM (1.96±0.33) had the highest. Protein Efficiency Ratio (PER) and Feed Efficiency Ratio (FER) recorded were similar (p>0.05). Also, PER and FER varied from 1.27±0.01 and 0.47±0.11, respectively in TBSM to 1.64±0.01 (PER) and 0.61±0.00 (FER) in those fed SABSM. The RBSM had the least SR (66.67±18.86 %), while the highest (96.67±4.71%) was obtained in those fed SLBSM.

The highest apparent protein digestibility ($ADC_{Protein}$) value of 85.79±0.41% was recorded in fish fed SABSM, while the least of 59.44±1.90% was in those fed SLBSM. The SPBSM and TBSM recorded similar (p>0.05) $ADC_{Protein}$ values of 81.32±0.00 % and 81.40±0.11%, respectively. Apparent energy digestibility values ranged from 51.60±0.11% recorded in fish fed SPBSM to 72.36±0.17% in those fed soyabean meal. Apparent dry matter digestibility values ranged from 21.45±0.01% recorded in fish fed SLBSM to 53.20±.25% in those fed soyabean meal. Fish fed soyabean meals recorded the highest apparent crude fat digestibility value of 75.26±1.62% while those fed SPBSM had lowest value (56.76±2.77%).

Table 4.6: Growth and digestibility of *Clarias gariepinus* juveniles fed differently processed baobab seed based diets

Indices	RD	SLBSM	RBSM	SPBSM	ABSM	TBSM	SWBSM	SABSM
IMW(g)	18.57±0.00	18.46±0.00	18.55±0.02	18.51±0.08	18.57±0.05	18.58±0.02	18.53±0.00	18.53±0.00
FMW(g)	60.13±1.50	53.61±2.76	52.06±7.37	58.43±0.06	57.40±0.11	53.94±12.74	57.72±6.86	62.39±2.29
MWG(g)	41.56±1.50	35.15±2.76	33.51±7.39	39.92±0.14	38.84±0.07	35.35±12.76	39.18±6.86	43.85±2.29
SGR(g)	2.10±0.05	1.90±0.10	1.83±0.26	2.05±0.01	2.02±0.00	1.88±0.43	2.02±0.21	2.17±0.07
FI(g)	$795.46±128.85^{a}$	$860.52±49.10^{abc}$	$818.46±54.76^{ab}$	$1020.33±68.8^{c}$	$968.73±11.25^{bc}$	$914.31±29.53^{abc}$	$886.08±63.96^{abc}$	$938.30±57.88^{abc}$
PI(g/day)	$5.26±0.85^{a}$	$5.69±0.32^{abc}$	$5..41±0.36^{ab}$	$6..74±0.45^{c}$	$6.40±0.07^{bc}$	$6.04±0.19^{abc}$	$5.85±0.42^{abc}$	$6.20±0.38^{abc}$
FCR	1.95±0.17	1.70±0.12	1.73±0.41	1.83±0.07	1.92±0.02	2.18±0.50	1.92±0.02	1.65±0.01
PER	1.40±0.12	1.60±0.11	1.61±0.38	1.48±0.06	1.41±0.01	1.27±0.29	1.41±0.02	1.64±0.01
FER	0.52±0.05	0.59±0.04	0.60±0.14	0.55±0.02	0.52±0.01	0.47±0.11	0.52±0.01	0.61±0.00
SR (%)	66.67±18.86	96.67±4.71	96.67±4.71	93.33±9.43	86.67±0.00	83.33±14.14	80.00±18.86	86.67±9.43
$ADC_{Crude\ protein}$ (%)	$77.43±0.18^{c}$	$59.44±1.90^{a}$	$64.57±1.00^{b}$	$81.32±0.10^{e}$	$79.86±0.20^{de}$	$81.40±0.11^{e}$	$79.10±0.60^{cd}$	$85.79±0.41^{f}$
ADC_{Energy} (%)	$72.36±0.17^{h}$	$53.86±0.27^{b}$	$62.09±0.36^{e}$	$51.60±0.11^{a}$	$60.95±0.01^{d}$	$69.13±0.20^{g}$	$58.72±1.01^{c}$	$67.80±0.03^{f}$
$ADC_{Crude\ fat}$ (%)	$75.26±1.62^{d}$	$57.54±3.60^{a}$	$66.75±2.68^{bc}$	$56.76±2.77^{a}$	$64.45±0.49^{b}$	$71.16±1.27^{cd}$	$66.40±1.85^{bc}$	$72.71±2.93^{d}$
$ADC_{Dry\ matter}$ (%)	$53.20±0.25^{g}$	$21.45±0.01^{a}$	$39.16±0.26^{d}$	$21.47±0.04^{a}$	$35.89±0.17^{c}$	$50.24±0.07^{f}$	$30.70±0.06^{b}$	$44.31±0.04^{e}$

Values with different superscripts on the same row are significantly different ($p<0.05$)
IMW: Initial Mean Weight Gain
FMW: Final Mean Weight Gain
MWG: Mean Weight Gain
SGR: Specific Growth Rate
FI: Feed Intake
PI: Protein Intake
FCR: Feed Conversion Ratio
PER: Protein Efficiency ratio
FER: Feed Efficiency Ratio
%SR: Survival Rate
$ADC_{PROTEIN}$: Apparent Protein digestibility
ADC_{ENERGY}: Apparent digestibility of energy
ADC_{FAT}: Apparent fat digestibility
$ADC_{DRY\ MATTER}$: Apparent dry matter digestibility
RD: Reference Diet
SPBSM: Soaked In Pulp Baobab Seed Meal
SWBSM: Soaked In Water Baobab Seed Meal
SLBSM: Soaked In Luquor Baobab Seed Meal
RBSM: Raw Baobab Seed Meal
ABSM: Autoclaved Baobab Seed Meal
TBSM: Toasted Baobab Seed Meal
SABSM: Soaked in Alkali Baobab Meal

4.3: Rank of mean weight gain and apparent digestibility coefficient of crude protein of *Clarias gariepinus* fed differently processed baobab seed meal based diets

The highest digestibility for crude protein (85.79+0.41) was recorded in fish fed SABSM, followed by 81.40+0.11 in those fed TBSM (**Table** 4.7). The third best digestible processed baobab seed meal in terms of crude protein was obtained in fish fed SPBSM (81.32+0.10). Fish fed SABSM had the highest mean weight gained of 43.85±2.29followed by soyabean meal (41.56±1.50), then 39.92±0.14 in those fed SPBSM. However, fish fed TBSM was ranked 6thin terms of mean weight gain among the fish that ate soyabean meal, raw and the six differently processed baobab seed meal.

Table 4.7: Rank of mean weight gain and apparent digestibility coefficient of crude protein of *Clarias gariepinus* fed differently processed baobab seed meal based diets

Ranks	Indices	MWG(g)	Ranks	Indices	$ADC_{Crude\ protein\ (\%)}$
1st	SABSM	43.85±2.29	1st	SABSM	85.79+0.41
2nd	RD	41.56±1.50	2nd	TBSM	81.40+0.11
3rd	SPBSM	39.92±0.14	3rd	SPBSM	81.32+0.10
4th	SWBSM	39.18±6.86	4th	ABSM	79.86+0.20
5th	ABSM	38.84±0.07	5th	SWBSM	79.10+0.60
6th	TBSM	35.35±12.76	6th	RD	77.43+0.18
7th	SLBSM	35.15±2.76	7th	RBSM	64.57+1.00
8th	RBSM	33.51±7.39	8th	SLBSM	59.44+1.90

$ADC_{CRUDEPROTEIN}$: Apparent digestibility of Protein
MWG: Mean Weight Gain
RD: Reference Diet
SPBSM: Soaked In Pulp Baobab Seed Meal
SWBSM: Soaked In Water Baobab Seed Meal
SLBSM: Soaked In Luquor Baobab Seed Meal
RSBM: Raw Baobab Seed Meal
ABSM: Autoclaved Baobab Seed Meal
TBSM: Toasted Baobab Seed Meal
SABM: Soaked in Alkali Baobab Meal

4.4 Fish culture condition and water quality of the water used during the experiment

Water quality parameters of the water used in the course of the experiment is illustrated in **Table** 4.10. pH, temperature, dissolved oxygen (DO), ammonia, nitrite and nitrate were within the range of 7.00±0.10-7.23±0.10, 26.20±0.00-28.00±0.20 ^{o}C, 3.90±0.10-4.87±0.12 mg/L, 4.00±0.00 -8.00±0.00 mg/L, 0.00±0.00 -0.17±0.29 mg/L and 0.00±0.00 -4.17±1.44 mg/L, respectively.

Table 4.8. Water quality parameters of the water for experimental trial

Indices	Range
pH	7.00±0.10-7.23±0.15
Dissolved oxygen	3.90±0.15-4.87±0.12ppm
Temperature	26.20±0.10-28.00±0.20˚C
Ammonia	4.00±0.31-8.00±0.00 PPM
Nitrite	0.00±0.00-0.17±0.29 ppm
Nitrate	0.00±0.00-4.17±1.44 ppm

4.5 Growth and nutrient utilisation of *Clarias gariepinus* fed soaked in alkali baobab seed meal

Growth and nutrient utilisation of *Clarias gariepinus* juveniles fed SABM are presented in **Table**4.8. The FMW (177.03±11.89g),MWG (152.93±12.24g) and SGR (1.66±0.07) of fish fed 20% SABSM recorded significantly higher ($p<0.05$) values compared to those fed 0% BSM, 40% SABSM, 60 % SABSM, 80 % SABSM and 100% SABSM. Fish fed 100% SABSM recorded significantly lowest ($p<0.05$)FMW, MWG and SGR values of 83.3±9.08 g, 59.12±9.29 g and 1.03±0.10, respectively. SR recorded in experimental fish fed 0% BSM, 61.54±20.35% was similar ($p>0.05$) to those fed 20% SABSM (61.54±7.69 %). The lowest SR, 20.51±11.75% was recorded in fish fed 100% SABSM.

Table 4.9: Growth and nutrients utilisation of soaked in alkali baobab seed mealby *Clariasgariepius*juveniles

Indices	Control	SABSM 20	SABSM 40	SABSM 60	SABSM 80	SABSM 100
IW(g)	360.61±9.64	361.40±10.43	358.07±5.83	364.30±11.99	367.53±10.53	362.75±8.90
FW(g)	1098.30±320.82^{bc}	1408.97±98.40^{c}	755.83±135.68^{abc}	657.40±527.22^{a}	621.27±314.47^{a}	226.87±145.90^{a}
IMW(g)	24.04±0.64	24.09±0.70	23.87±0.39	24.29±0.80	24.50±0.70	24.18±0.59
FMW(g)	138.83±6.99^{ab}	177.03±11.89^{b}	114.33±6.70^{ab}	113.16±21.62^{ab}	102.32±12.97^{ab}	83.31±9.08 ^{a}
MWG(g)	114.79±6.36^{ab}	152.93±12.24^{b}	90.46±6.64^{ab}	88.87±22.40^{ab}	77.82±13.53^{ab}	59.12±9.29^{a}
FCR	2.06±0.13	1.57+0.05	1.88+0.08	1.94+0.44	1.94+0.22	2.16+0+.28
SGR	1.46±0.02^{ab}	1.66±0.07^{b}	1.30±0.05^{ab}	1.27±0.20^{ab}	1.19±0.12^{ab}	1.03±0.10^{a}
PI (g/day)	7.36±2.12^{b}	6.11±0.51^{ab}	5.25±1.09^{ab}	3.96±2.82^{ab}	4.62±2.16^{ab}	2.13±1.35^{a}
PER	1.35±0.09	1.77±0.06	1.48±0.06	1.48±0.30	1.44±0.15	1.30±0.17
SR(%)	61.54±20.3^{b}	61.54±7.69^{b}	51.28±11.75^{ab}	41.03 ±31.09^{ab}	46.15±20.35^{ab}	20.51±11.75^{a}

Values with different superscripts on the same row are significantly different ($p < 0.05$)
IW: Initial Weight Gain
FW: Final Weight Gain
IMW: Initial Mean Weight Gain
FMW: Final Mean Weight Gain
MWG: Mean Weight Gain
SGR: Specific Growth Rate
FI: Feed Intake
PI: Protein Intake
FCR: Feed Conversion Ratio
PER: Protein Efficiency ratio
%SR: Survival Rate
SABSM: Soaked in alkali baobab seed based diets
0, 20, 40, 60, 80, 100: Replacement levels of soyabean meal with baobab seed meal at 0, 20, 40, 60, 80, and 100% replacement level respectively

4.6 Growth and nutrients' utilisation of *Clarias gariepinus* fed with different replacement levels of soaked in pulp baobab seed meal based diets

The growth and nutrients utilisation by *Clarias gariepinus* juveniles fed SPBSM are represented in **Table** 4.9. The FW, FMW, MWG, and SGR recorded in fish fed 20%SPBSM were higher than those fed control diets (0% BSM). The FMW, MWGand SGR of fish fed 40% SPBSM,60% SPBSM,80% SPBSM and 100% SPBSM were not significantly different ($p>0.05$). The FCR of fish fed diets 20% SPBSM was significantly ($P<0.05$) lower than those fed control diets (soyabean meal), 40, 60, 80 and 100 % replacement levels of SPBSM. Highest FER ($p<0.05$) was obtained in fish fed 20 % SPBSM. The SR of fish fed 0% BSM, 20% and 40% SPBSM were not significantly different ($P>0.05$). Lowest SR was obtained in fish fed 100%SPBSM.

Table 4.10: Growth and nutrients utilisation by *Clariasgariepius*juveniles fed soaked in pulp baobab seed based diets

Indices	Control	SPBSM 20	SPBSM 40	SPBSM 60	SPBSM 80	SPBSM 100
IW (g)	360.61±9.64	362.12±11.90	357.73±5.20	355.40±0.96	357.32±3.30	361.54±11.59
FW (g)	1098.30±320.82^{bc}	1331.17±355.07^{c}	772.50±205.61^{ab}	545.10±330.44^{a}	504.50±166.70^{a}	342.60±127.36^{a}
IMW (g)	24.04±0.64	24.14±0.79	23.85±0 .35	23.60±0.06	23.82±6 .22	24.10±0.77
FMW (g)	138.83±6.99^{b}	165.88±3.38^{c}	106.41±13.8^{a}	117.39±6.6^{a}	114.78±9.39^{a}	112.91±10.93^{a}
MWG (g)	114.79±6.36^{b}	141.74±2.99^{c}	82.57±14.16^{a}	93.70±6.63^{a}	90.95±9.30^{a}	88.81±10.95^{a}
FCR	2.06±0.13^{b}	1.58±1.00^{a}	1.94+0.20^{b}	1.92+0.14^{b}	1.94+0.04^{b}	1.96+0.05^{b}
SGR	1.46±0.02^{b}	1.61±0.02^{c}	1.24±0.12^{a}	1.33±0.05^{a}	1.31±0.07^{a}	1.29±0.08^{a}
PI (g/day)	7.36±2.12^{b}	6.01±1.20^{ab}	5.68±1.33^{ab}	3.77±2.17^{ab}	3.54±0.10^{ab}	2.46±0.89^{a}
PER	1.35±0.09^{a}	1.76±0.11^{b}	1.44±0.16^{a}	1.45±0.10^{a}	1.44±0.03^{a}	1.42±0.04^{a}
SR (%)	61.54±20.35^{b}	61.54±15.39^{b}	56.41±16.01^{b}	35.90±22.21^{ab}	33.33±8.88^{ab}	23.08±7.69^{a}

Values with different superscripts on the same row are significantly different ($p < 0.05$) IW: Initial Weight Gain FW: Final Weight Gain
IMW: Initial Mean Weight Gain FMW: Final Mean Weight Gain MWG: Mean Weight Gain
SGR: Specific Growth Rate FI: Feed Intake PI: Protein Intake
FCR: Feed Conversion Ratio PER: Protein Efficiency ratio %SR: Survival Rate SPBSM 20: 20% Replacement Level of Soyabean Meal with Soaked in Pulp Baobab Seed Meal
SPBSM 40: 20% Replacement Level of Soyabean Meal with Soaked in Pulp Baobab Seed Meal SPBSM 60: 20% Replacement Level of Soyabean Meal with Soaked in Pulp Baobab Seed Meal
SPBSM 80: 20% Replacement Level of Soyabean Meal with Soaked in Pulp Baobab Seed Meal SPBSM 100: 20% Replacement Level of Soyabean Meal with Soaked in Pulp baobab Seed Meal

4.7.1 Regression of weight gain and dietary inclusion of soaked in pulp baobab seed meal based diets

Relationship between weight gained by *Clarias gariepinus* fed varying replacement levels of soaked in pulp baobab seed meal and the varied replacement levels is presented in **Figure** 4.1. The relationship was highly significant ($p<0.01$) and represented by the order 5 polynomial regression line. The equation of the graph is: $f(x)= 0+25.613x^4-1.470x^3+0.033x^2-0.0003291x+0.000001182$ and the optimum replacement level of soyabean meal with soaked in pulp baobab seed meal is 14 % (Figure 1). Regression coefficient, R^2 is 0.72.

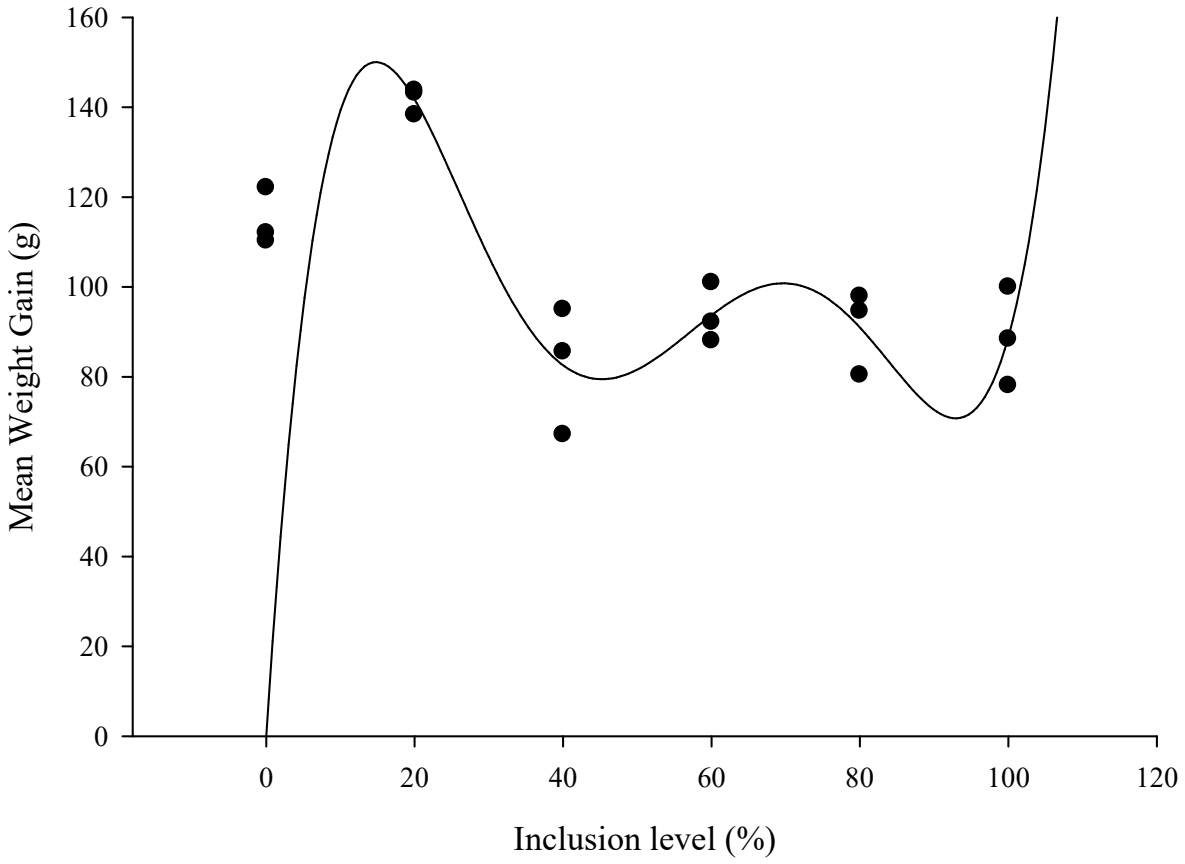


Figure 4.1: Relationship between dietary inclusions of soaked in pulp baobab seed meal based diets (%) and mean weight gain by *Clarias gariepinius* fed soaked in pulp baobab seed based diets

4.7.2 Regression of weight gain and dietary inclusion of soaked in alkali baobab seed meal based diets

Relationship between weight gained by *Clarias gariepinus* fed dietary inclusion of soaked in alkali BSM based diets and different replacement levels of soaked in alkali baobab seed meal is represented in **Figure** 4.2. The relationship was highly significant ($p<0.01$), weight gained had a polynomial relationship with varied replacement levels of soaked in alkali baobab seed meal based diets.Polynomial relationship of degree 5 between dietary inclusions of soaked in alkali baobab seed based diets (%) and mean weight gain (g) by *Clarias gariepinius* fed SABSM show that optimum inclusion level of SABSM is 16 %. The polynomial regression line equation of weight gain (y) on dietary inclusion of soaked in alkali baobab seed meal based diets (x) is: $f(x)$ $=0+26.155x^4-1.448x^3+0.032x^2-0.000308x+0.00000109$. R^2 value is 0.83

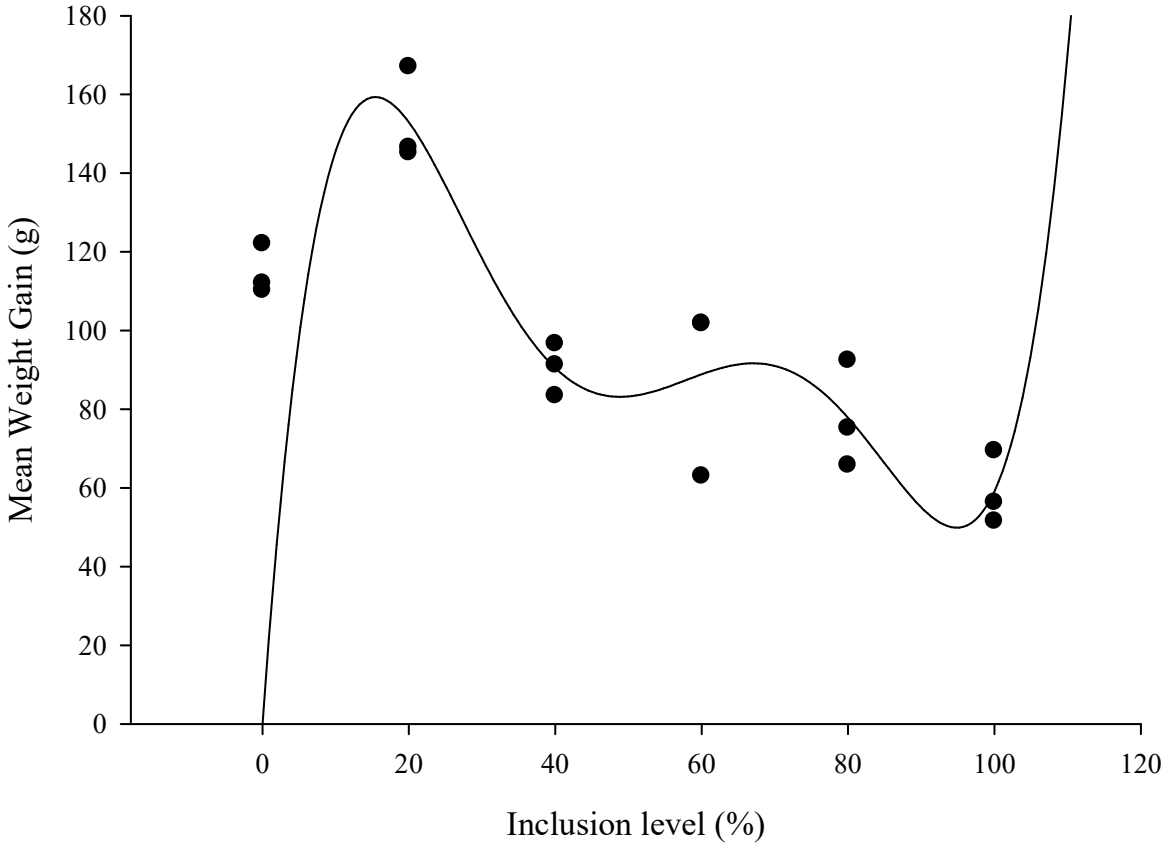


Figure 4.2: Relationship between dietary inclusions of soaked in alkali baobab seed meal based diets (%) and mean weight gain by *Clarias gariepinius* fed soaked in alkali baobab seed based diets

4.8. Haematology of *Clarias gariepinus* fed soaked in alkali baobab seed meal based diets

Haematological indices of fish fed SABSM are presented in **Table** 4.11. The PCV,HB,RBC, lymphocytes, heterocytes, monocytes, oesinophils, and basophils were not significantly different (P>0.05).The WBC count of fish fed control diets recorded the highest value of 16375.00±247.49x10^3µL while those fed 80%SABSM recorded least value of 11500.00±707.11x10^3µL. Highest plateletsvalue of 340,000.00±113137.09µL was recorded in fish fed control diet while the least value of 133333.33±11547.01µLwas obtained in those fed 100% SABSM.

Table 4.11: Haematological indices of *Clarias gariepinus* juvenilesfed soaked in alkali baobab seed meal based diets

Indices	Control	SABSM 20	SABSM 40	SABSM 60	SABSM 80	SABSM 100
PCV (%)	23.50±3.53	24.33±5.77	20.50±3.54	21.00±1.41	22.00±2.83	19.00±6.08
HBg/dl	7.85±1.20^{ab}	7.93±1.96^{ab}	6.50±1.13^{ab}	6.80±0.28^{ab}	7.20±0.7^{ab}	5.90±2.17^{ab}
RBC(x10^{6}µL)	1.50±0.38	1.93±1.15	1.36±0.22	1.26±0.03	1.40±0.12	1.63±0.80
WBC(x10^{3}µL$^{)}$)	16375.00±247.49^{b}	14683.33±1353.08^{ab}	14525.00±459.62^{ab}	14875.00±601.04^{ab}	11500.00±707.11^{a}	14093.33±2505.72^{ab}
Platelets(µL)	340000.00± 113137.09^{c}	202666.67± 43924.18^{ab}	272000.00± 33941.13^{bc}	171000.00± 69296.47^{ab}	195333.33± 21501.94^{ab}	133333.33± 11547.01^{a}
Lymphocytes %	65.50±0.71	64.00±4.36	71.00±0.00	66.50±4.95	69.00±3.46	67.33±6.03
Heterocytes%	24.00±2.83	28.67±4.93	21.00±1.41	25.00±4.24	24.00±1.73	25.67±6.81
Monocytes%	3.50±0.71	2.33±1.16	3.00±1.00	3.67±0.58	3.33±0.58	3.33±0.58
Oesinophils%	5.50±2.12	4.67±0.58	5.33±0.58	3.33±2.08	3.33±2.08	3.67±3.22
Basophills %	0.50±0.71	0.33±0.58	0.33±0.58	0.33±0.58	0.33±0.58	0.00±0.00

Values with different superscripts on the same row are significantly different ($p < 0.05$) PCV: Packed Blood Cell Volume
RBC: Red Blood Cell Volume Hb: Haemoglobin level WBC: White Blood Cell Volume
0, 20, 40, 60, 80, 100: Replacement levels of baobab seed meal with soyabean meal at 0, 20, 40, 60, 80, and 100% replacement level respectively
SABSM: Soaked in alkali baobab seed based diets

4.9. Haematology of *Clarias gariepinus* fed soaked in pulp baobab seed meal based diets

Haematological indices of fish fed SPBSM are presented in **Table** 4.12. The least PCV of 16.00±1.41 % was recorded in fish fed 60% SPBSM while the highest of 25.150±6.36 %was obtained in those fed 40% SPBSM. Hb ranged from 4.70±0.42 g/dL in *Clarias gariepinus* fed 60% SPBSM to 8.35±1.91 g/dL in those fed 40% SPBSM. Highest WBC value of 16375.00±247.49x10^3µL was recorded in fish fed control diets while the least was obtained in 80% SPBSM.

Least lymphocytes value of 65.50±0.71% was recorded in fish fed control diets while those fed 80% SPBSMrecorded the highest value of 73.00±0.00 %. Heterocytes ranged from 21.33±0.58 % and 21.33±2.31 % obtained in 80 and 100% SPBSM, respectively to 31.00±0.00 % in *Clarias gariepinus* fed 20 % SPBSM.

Table 4.12. Haematology of *Clarias gariepinus* fed different replacement levels of soaked in pulp baobab seed meal based diets

Indices	Control	SPBSM 20	SPBSM 40	SPBSM 60	SPBSM 80	SPBSM 100
PCV (%)	23.50 ± 3.53^{ab}	23.33 ± 1.53^{ab}	25.50 ± 6.36^{b}	16.00 ± 1.41^{a}	22.50 ± 0.71^{ab}	21.00 ± 4.24^{ab}
Hb g/dl	7.85 ± 1.20^{b}	7.60 ± 0.87^{b}	8.35 ± 1.91^{b}	4.70 ± 0.42^{a}	7.10 ± 0.14^{ab}	6.75 ± 1.49^{ab}
RBC($x10^{6}$)	1.50 ± 0.38	1.61 ± 0.15	1.25 ± 0.17	1.31 ± 0.28	1.27 ± 0.06	1.35 ± 0.32
WBC($x10^{3}$ul)	16375.00 ± 247.49^{c}	15066.67 ± 503.32^{bc}	12433.33 ± 1686.22^{ab}	15850.00 ± 636.40^{c}	10566.67 ± 665.83^{a}	14250.00 ± 2650.94^{bc}
PlateletsµL	$340000.00\pm 113137.09^{c}$	133000.00 ± 6082.76^{a}	$195666.67\pm 36909.80^{ab}$	$238000.00\pm 25455.84^{abc}$	$228666.67\pm 12423.10^{ab}$	$251333.33\pm 67892.07^{bc}$
Lymphocytes%	65.50 ± 0.71^{a}	68.00 ± 6.00^{ab}	72.00 ± 5.66^{ab}	73.00 ± 0.00^{b}	72.67 ± 1.53^{b}	70.67 ± 1.16^{ab}
Heterophils%	24.00 ± 2.83^{a}	31.00 ± 0.00^{b}	30.00 ± 2.83^{b}	21.67 ± 0.58^{a}	21.33 ± 0.58^{a}	21.33 ± 2.31^{a}
Monocytes%	3.50 ± 0.71	3.33 ± 1.16	4.33 ± 1.53	4.00 ± 1.00	3.33 ± 0.58	3.33 ± 1.53
Oesinophils%	5.50 ± 2.12^{b}	4.67 ± 2.52^{ab}	3.33 ± 3.05^{ab}	1.00 ± 0.00^{a}	2.33 ± 0.58^{ab}	3.67 ± 2.89^{ab}
Basophils %	0.50 ± 0.71^{ab}	0.33 ± 0.58^{ab}	0.00 ± 0.00^{a}	0.33 ± 0.58^{ab}	0.00 ± 0.00^{a}	1.00 ± 0.00^{b}

Values with different superscripts on the same row are significantly the different ($p<0.05$) PCV: Packed Blood Cell Volume
RBC: Red Blood Cell Volume Hb: Haemoglobin level WBC: White Blood Cell Volume
0, 20, 40, 60, 80, 100: Replacement levels of soyabean meal with soaked in pulp baobab seed meal at 0, 20, 40, 60, 80, and 100% replacement level respectively
SPBSM: Soaked in pulp baobab seed based diets

4.10. Plasma biochemical indices of *Clarias gariepinus* fed soaked in alkali baobab seed meal based diets

Plasma biochemical indices in*Clarias gariepinus*fed Soaked in Alkali Baobab Seed Meal(SABSM)based diets represented in **Table** 4.13 showed that total protein, albumin, globulin, A:G ratio, aspartate aminotransferase (AST), alkaline aminotransferase (ALT), alkaline phosphate (ALP), bilirubin, creatinine were not significantly different (P>0.05).

Table 4.13: Plasma biochemical indices of *Clarias gariepinus* fed soaked in alkali baobab seed meal based diets

Indices	Control	SABSM 20	SABSM 40	SABSM 60	SABSM 80	SABSM 100
Total protein(µmol/L)	5.57±0.40	5.83±0.58	5.83±0.57	5.93±0.64	6.33±0.83	6.37±0.64
Albumin(g/dL)	1.43±0.31	1.47±0.31	1.43±0.12	1.70±0.10	1.93±0.42	1.97±0.29
Globulin(g/dL)	4.13±0.67	4.37±0.31	4.40±0.66	4.23±0.74	4.40±0.53	4.40±0.36
A:G	0.33±0.12	0.27±0.06	0.33±0.58	0.40±0.10	0.43±0.06	0.30±0.17
AST(U/L)	210.00±26.00	210.33±32.71	186.00±9.54	108.67±11.85	227.33±42.19	221.33±25.70
ALT(U/L)	29.00±4.58	29.67±2.89	32.00±8.72	38.00±12.12	25.00±8.66	34.00±8.19
ALP(U/L)	359.33±147.41	411.67±95.04	422.00±21.63	446.67±43.02	298.67±80.05	323.67±99.40
BUN(mmol/L)	10.80±0.66	11.47±0.04	11.50±1.41	11.43±1.15	12.17±1.37	11.43±0.75
Creatinine(µmol/L)	0.60±0.10	0.60±0.10	0.53±0.06	0.67±0.06	0.60±0.10	0.67±0.06

Values with different superscripts on the same roware significantly different ($p < 0.05$) AST: Aspertate aminotransferase ALT: Alkaline aminotransferase AG: Albulin Globulin ratio ALP: Alkaline phosphate BUN: Blood urea nitrogen

Control, SABSM 20, SABSM 40, SABSM 60, SABSM 80, SABSM 100 = Replacement levels of soyabean meal with soaked In alkali baobab seed meal at 0, 20, 40, 60, 80 and 100%, respectively.

4.11. Plasma biochemical indices of *Clarias gariepinus* fed soaked in pulp baobab seed meal based diets

Plasma indices of *Clarias gariepinus*fed soaked in pulp baobab seed mealbased diets are represented in **Table** 4.14. The total protein, globulin, A:G ratio, aspartate aminotransferase (AST), alkaline aminotransferase (ALT), alkaline phosphate (ALP), bilirubin, creatinine of fish fed SPBSM were not significantly different (P>0.05).The albumin was significantly different at (p<0.05) and ranged from 1.27±0.42 g/dL in 60% SPBSMto 1.87±0.64 g/dLobtained in fish fed 20 % SPBSM.

Table 4.14: Plasma biochemical indices of *Clarias gariepinus* fed soaked in pulp baobab seed meal based diets

Indices	Control	SPBSM 20	SPBSM 40	SPBSM 60	SPBSM 80	SPBSM 100
Total protein(µmol/L)	5.57±0.40	6.23±0.78	5.33±0.29	5.20±1.15	6.00±0.40	5.67±0.11
Albumin(g/dL)	1.43±0.31^{ab}	1.87±0.64^{ab}	1.33±0.25^{ab}	1.27±0.42^{a}	1.57±0.06^{ab}	1.43±0.32^{ab}
Globulin(g/dL)	4.13±0.67	4.37±0.15	4.00±0.17	4.00±0.85	4.47±0.35	4.13±0.37
A:G	0.33±0.12	0.40±0.17	0.30±0.10	0.23±0.12	0.30±0.00	0.30±0.10
AST(U/L)	210.00±26.00	207.00±25.87	195.33±5.03	195.00±18.19	205.33±0.58	192.00 ±9.54
ALT(U/L)	29.00±4.58	30.33±11.93	32.00±7.00	38.67±13.87	38.33±5.86	36.00±1.00
ALP(U/L)	359.33±147.41	385.00±106.02	425.67±113.36	336.67±113.18	347.67±120.50	384.00±32.14
BUN(mmol/L)	10.80±0.66	12.47±1.85	10.47±0.23	10.97±1.65	11.17±0.32	11.40±1.48
Creatinine(mmol/L)	0.60±0.10	0.60±0.17	0.60±0.00	0.70±0.10	0.67±0.56	0.63±0.15

Values without superscripts on the same row are similar ($p > 0.05$)
T. PROTEIN: Total Protein
AST: Aspertate aminotransferase
ALT: Alkaline aminotransferase
AG: Albulim Globulin ratio
ALP: Alkaline phosphate
BUN: Blood urea nitrogen
0, 20, 40, 60, 80, 100: Replacement levels of soyabean meal with baobab seed meal at 0, 20, 40, 60, 80, and 100% replacement level respectively
SPBSM: Soaked in pulp baobab seed based diets

4.12Oxidative stress indices of *Clarias gariepinus* fed soaked in alkali baobab seed meal based diets

Oxidative stress indices of fish fed soaked in alkali baobab seed meal based diets shown in **Table** 4.15 were significantly different ($p<0.05$). Least total protein of 0.39±0.05 was recorded in fish fed 100% SABSMwhile the highest(0.45±0.05) was obtained in 20% SABSM. Hydrogen peroxide ranged from 41.81±7.10 in fish fed 20% SABSM to 55.07±10.84 in 100% SABSM. Least MDA of 1.60±0.21was recorded in fish fed 40% SABSM while those fed 80% SABSM (2.80±1.23) recorded the highest value. Least catalase and superoxide dismutase(17.45±1.52 and 15.94±1.44)were obtained in fish fed 20% SABSMwhile the highest (20.53±1.97 and 18.92±2.80, respectively)were recorded in those fed 100% SABSM.

Highest GSH of 121.31±5.94 was recorded in fish fed 20% SABSM while 100% SABSM had theleast (99.32±8.21). GST ranged from 0.09±0.10 recorded in fish fed 20% SABSM to 0.26±0.20 in those fed 100% SABSM. Least MPO and NO(21.11±1.60 and 0.39±0.04, respectively)were obtained in fish fed 40% SABSM.

Table 4.15: Oxidative stress indices of *Clarias gariepinus* fed soaked in alkali baobab seed meal based diets

Indices	Control	SABSM 20	SABSM 40	SABSM 60	SABSM 80	SABSM 100
TP(µ/mol)	0.44 ± 0.06^{ab}	0.45 ± 0.05^{b}	0.42 ± 0.02^{ab}	0.44 ± 0.03^{ab}	0.43 ± 0.07^{ab}	0.39 ± 0.05^{a}
H_2O_2(g/mol)	42.12 ± 5.90^{a}	41.81 ± 7.10^{a}	44.64 ± 7.05^{ab}	52.69 ± 7.44^{bc}	54.79 ± 12.11^{c}	55.07 ± 10.84^{c}
MDA (U/mgprotein	1.92 ± 0.47^{ab}	1.78 ± 0.24^{ab}	1.60 ± 0.21^{a}	2.77 ± 1.67^{b}	2.80 ± 1.23^{b}	2.70 ± 1.42^{b}
Catalase (U/mg protein)	18.23 ± 1.48	17.45 ± 1.52	18.51 ± 1.15	18.34 ± 1.20	20.36 ± 3.14	20.53 ± 1.97
GSH (U/mg protein)	105.89 ± 12.20^{a}	121.31 ± 5.94^{b}	105.73 ± 9.29^{a}	104.64 ± 12.84^{a}	102.33 ± 9.36^{a}	99.32 ± 8.21^{a}
SOD (U/mg protein)	16.74 ± 1.81^{a}	15.94 ± 1.44^{a}	16.76 ± 0.85^{a}	16.51 ± 1.10^{a}	17.18 ± 2.86^{ab}	18.92 ± 2.80^{b}
GST (U/mg protein)	0.11 ± 0.06^{ab}	0.09 ± 0.10^{a}	0.18 ± 0.10^{ab}	0.11 ± 0.10^{ab}	0.17 ± 0.14^{ab}	0.26 ± 0.20^{b}
MPO (U/mg protein)	64.38 ± 16.58^{b}	31.07 ± 12.73^{ab}	21.11 ± 1.60^{a}	27.18 ± 4.31^{a}	30.28 ± 15.78^{a}	54.68 ± 15.03^{b}
NO	0.58 ± 0.14^{d}	0.56 ± 0.12^{cd}	0.39 ± 0.04^{a}	0.52 ± 0.12^{bcd}	0.44 ± 0.08^{ab}	$0.47\pm0.05s^{abc}$

Values with different superscripts on the same row are significantly different ($p < 0.05$) TP: Total protein H_2O_2: Hydrogen peroxide MDA: Malanodehydehide GSH: Gluthathione SOD: Superoxide dismutase GST: Gluthathione-s-transferaseMPO: Myeloperoxidase NO: Nitrogen oxide
Control, SABSM 20, SABSM 40, SABSM 60, SABSM 80, SABSM 100 = Replacement level of soyabean meal with soaked in alkali baobab seed meal at 0, 20, 40, 60, 80 and 100%, respectively.

4.13Oxidative stress indices of *Clarias gariepinus* fed soaked in pulp baobab seed meal

Total protein, catalase, superoxide dismutase, glutathione-s-transferase, melanoperoxidase and nitrogen oxide analysed (**Table** 4.16) in fish fed soaked in pulp baobab seed meal were not significantly different ($p>0.05$). Total protein ranged from 0.44±0.06 µ/mol recorded in fish fed control diets to 0.47±0.08 µmol in those fed 20% SPBSM. Least H_2O_2(41.06±5.53 g/molwas recorded in fish fed 60 % SPBSMwhile100 % SPBSM had the highest(57.01±6.34 µ/mol). MDA range from 1.15±0.08 U/mg protein in fish fed 80 % SPBSM to 3.03±0.82 U/mg protein in those fed 100 % SPBSM. Catalase ranged from 17.75±2.78 U/mg protein in fish fed 20 % SPBSM to 18.93±4.26 U/mg protein in those fed 40 % SPBSM.

There was significant difference ($p<0.05$) in GSH recorded in fish fed control diet and different replacement levels of baobab seed meal. The GSH ranged from 100.33±7.05 U/mg protein in 40 % SPBSM to 111.62±6.64 U/mg protein in fish fed 100 % SPBSM. GST recorded ranged from 0.09±0.12 U/mg protein in fish fed 20 % SPBSM to 0.19±0.15 U/mg protein in 40 % SPBSM. Least MPO(38.38±14.51 U/mg protein) was obtained in fish fed 60 % SPBSM while those fed control diets had the highest(64.38±16.58 U/mg protein). The NO ranged from0.42±0.03in fish fed 40 % SPBSMto 0.62±0.18 recorded in 20 % SPBSM.

Table 4.16: Oxidative stress indices of *Clarias gariepinus* fed soaked in pulp baobab seed meal based diets

Indices	Control	SPBSM 20	SPBSM 40	SPBSM 60	SPBSM 80	SPBSM 100
TP(μ/mol)	0.44±0.06	0.47±0.08	0.44±0.06	0.45±0.04	0.45±0.09	0.46±0.05
H_2O_2(g/mol)	42.12±5.90^{a}	46.13±4.48^{a}	55.04±10.12^{b}	41.06±5.53^{a}	47.89±8.51^{a}	57.01±6.34^{b}
MDA(U/mg protein)	1.92±0.47^{c}	1.35±0.25^{ab}	2.06±0.46^{c}	1.78±0.28^{bc}	1.15±0.08^{a}	3.03±0.82^{d}
Catalase(U/mg protein)	18.23±1.48	17.75±2.78	18.93±4.26	18.08±2.31	17.87±4.40	18.51±1.20
GSH(U/mg protein)	105.89±12.20^{ab}	110.92±6.52^{b}	100.33±7.05^{a}	108.42±7.29^{ab}	111.23±4.95^{b}	111.62±6.64^{b}
SOD(U/mg protein)	16.74±1.81	15.56±2.71	16.60±2.42	15.91±1.63	16.42±3.19	15.54±1.82
GST(U/mg protein)	0.11±0.06	0.09±0.12	0.19±0.15	0.15±0.15	0.18±0.10	0.17±0.21
MPO(U/mg protein)	64.38±16.58	62.80±27.80	61.52±38.24	38.38±14.51	50.56±16.61	51.39±17.42
NO	0.58±0.14^{bc}	0.62±0.18^{c}	0.42±0.03^{a}	0.47±0.09^{ab}	0.48±0.07^{abc}	0.49±0.10^{abc}

Values with different superscripts on the same row are significantly different ($p < 0.05$) TP: Total protein H_2O_2: Hydrogen peroxide MDA: Melanohydehyde GSH: Gluthathione SOD: Superoxide dismutase GST: Gluthathinone-s-transferaseMPO: Melanoperoxidase NO: Nitrogen monooxide

Control, SPBSM 20, SPBSM 40, SPBSM 60, SPBSM 80, SPBSM 100 = Replacement level of soyabean meal with soaked In pulp baobab seed meal at 0, 20, 40, 60, 80 and 100%, respectively.

4.14 Proximate composition of fish fed soaked in pulp baobab seed meal based diets Fish fed varied replacement levels of soaked in pulp baobab seed meal had different proximate compositions. Crude protein ranged from 60.45±0.05 % in fish fed 60 % SPBSM to 65.30±0.13 in control (**Table** 4.17). The highest moisture content (7.81±0.01%)was obtained in fish fed soyabean meal, while 100 % SPBSM (6.47±0.03 %) had the least. The least crude fat of 16.31±0.07 % was observed in control diets, while those fed 60 % SPBSM had the highest (23.00±0.04 %).

Table 4.17: Proximate composition of *Clarias gariepinus* fed soaked in pulp baobab seed based diets

Indices	Control	SPBSM 20	SPBSM 40	SPBSM 60	SPBSM 80	SPBSM 100
% M. content	7.81±0.01^{f}	7.01±0.02^{e}	6.60±0.03^{b}	6.92±0.04^{d}	6.68±0.02^{c}	6.47±0.03^{a}
% Crude protein	65.30±0.13^{f}	64.39±0.00^{d}	64.96±0.05^{e}	60.45±0.05^{a}	60.86±0.00^{c}	60.67±0.06^{b}
% Crude fat	16.31±0.07^{a}	17.62±0.02^{b}	20.05±0.01^{c}	23.00±0.04^{f}	22.04±0.01^{e}	21.74±0.07^{d}
Ash	10.57±0.07^{d}	10.98±0.04^{e}	8.36±0.02^{a}	9.63±0.03^{b}	10.42±0.02^{c}	11.11±0.02^{f}

Means±SD on the same row with different superscript are significantly different ($p<0.05$).
Control, SPBSM 20, SPBSM 40, SPBSM 60, SPBSM 80 and SPBSM 100 = Replacement levelof soyabean meal with soaked in pulp baobab seed meal at 0, 20, 40, 60, 80 and 100%, respectively.

4.15 Proximate composition of fish fed soaked in alkali baobab seed meal based diets

Proximate compositions of fish fed varied replacement levels of soaked in alkali baobab seed meal were significantly different ($p<0.05$). Crude protein ranged from 55.66±0.05 % recorded in fish fed 80 % SABSM to 65.30±0.13 in control (**Table** 4.18). The highest moisture content (8.32±0.01 %)was obtained in 40 SABSM, while 100 % SPBSM (6.47±0.03 %) had the least. The least crude fat of 16.31±0.07 % was observed in control diets, while those fed 100 % SABSM had the highest (25.96±0.01 %).

Table 4.18: Proximate composition of *Clarias gariepinus* fed soaked in alkali baobab seed meal based diets

Indices	Control	SABSM 20	SABSM 40	SABSM 60	SABSM 80	SABSM 100
% M. content	7.81±0.01^{e}	7.19±0.02^{c}	8.32±0.01^{f}	7.52±0.01^{d}	6.57±0.01^{a}	6.92±0.02^{b}
% Crude protein	65.30±0.13^{e}	61.79±0.05^{d}	56.14±0.02^{b}	60.86±0.00^{c}	55.66±0.05^{a}	56.14±0.02^{b}
% Crude fat	16.31±0.07^{a}	20.09±0.03^{c}	23.72±0.02^{e}	18.40±0.01^{b}	23.10±0.02^{d}	25.96±0.01^{f}
Ash	10.57±0.07^{a}	10.93±0.04^{b}	11.81±0.01^{c}	13.22±0.02^{d}	14.67±0.03^{e}	10.91±0.12^{b}

Means±SD on the same row with different superscript are significantly different ($p<0.05$).
Control, SABSM 20, SABSM 40, SABSM 60, SABSM 80 and SABSM 100 = Replacement level of soyabean meal with soaked in alkali baobab seed meal at 0, 20, 40, 60, 80 and 100%, respectively.

4.16 Histo-pathology of some organs of the *Clarias gariepinus* fed soyabean meal and varied inclusion levels of baobab seed meal (Plates 4.1 – 4.18)

The details of changes in each organ are as follows:

The histo-pathology in gills of Clarias gariepinus gills fed soyabean meal and varied inclusion levels of baobab seed meal based diets

Histology of gills of *Clarias gariepinus* fed diets soyabean meal, 0% level of soaked in pulp baobab seed meal and soaked in alkali baobab seed meal (**Plate**4.1.); 20 % SPBSM and 20 % SABSM (**Plate** 4.2.); 40 % SPBSM and 40 % SABSM (**Plate**4.3.); 60 % SABSM (**Plate** 4.4.), 80 % SABSM (**Plate** 4.5.) and 100 % SABSM (**Plate** 4.6.) showed no observable lesion. Mild lamellae hyperplasia was observed on gills of *Clarias gariepinus* fed 60% SPBSM (**Plate** 4.4), 80% SPBSM (**Plate** 4.5.) showed lamellae necrosis, while those fed 100% (**Plate** 4.6.) SPBSM showed moderate lamellae hyperplasia.

Histo-pathology of *Clarias gariepinus* fed baobab seed meal based diets

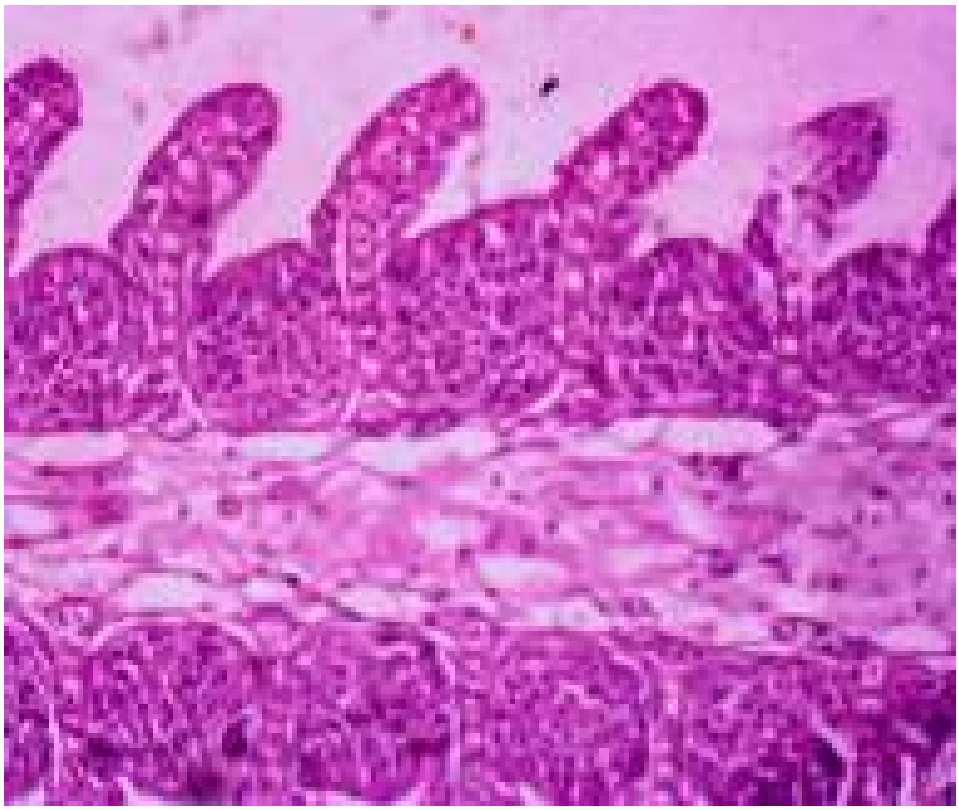
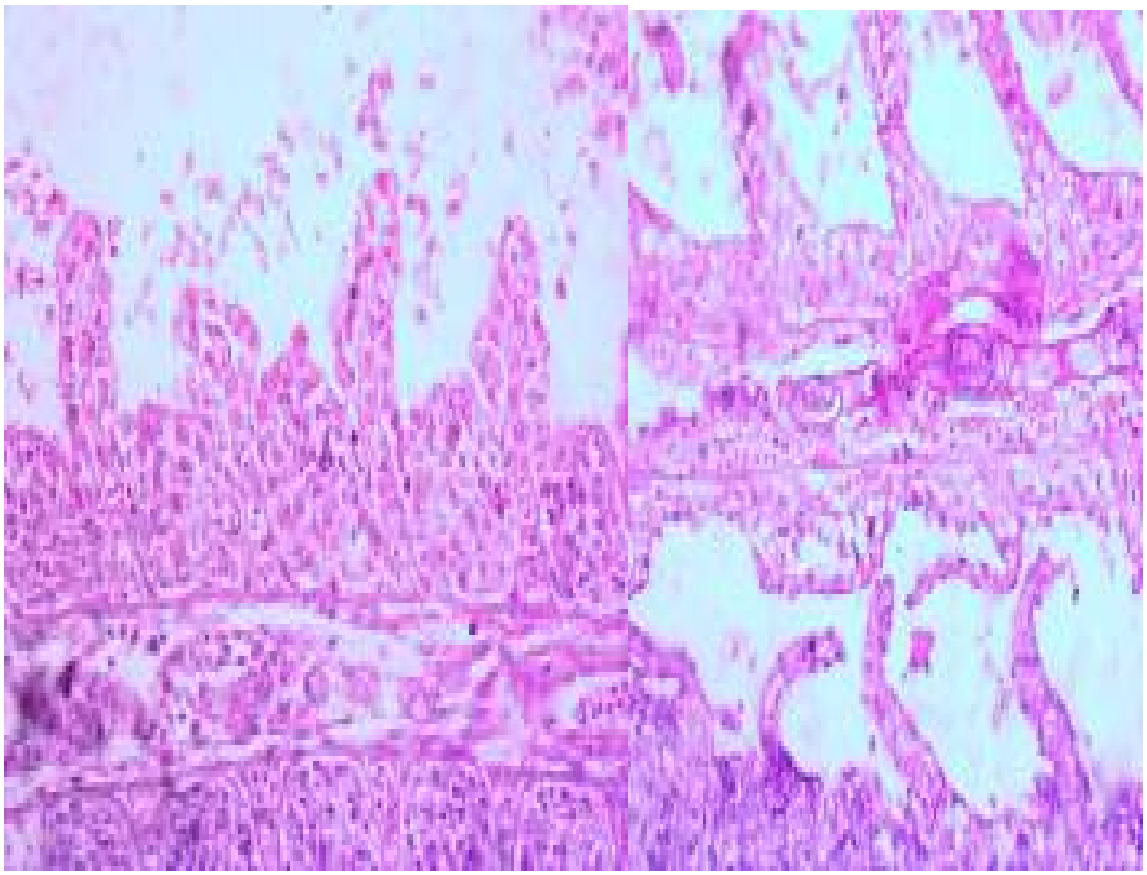
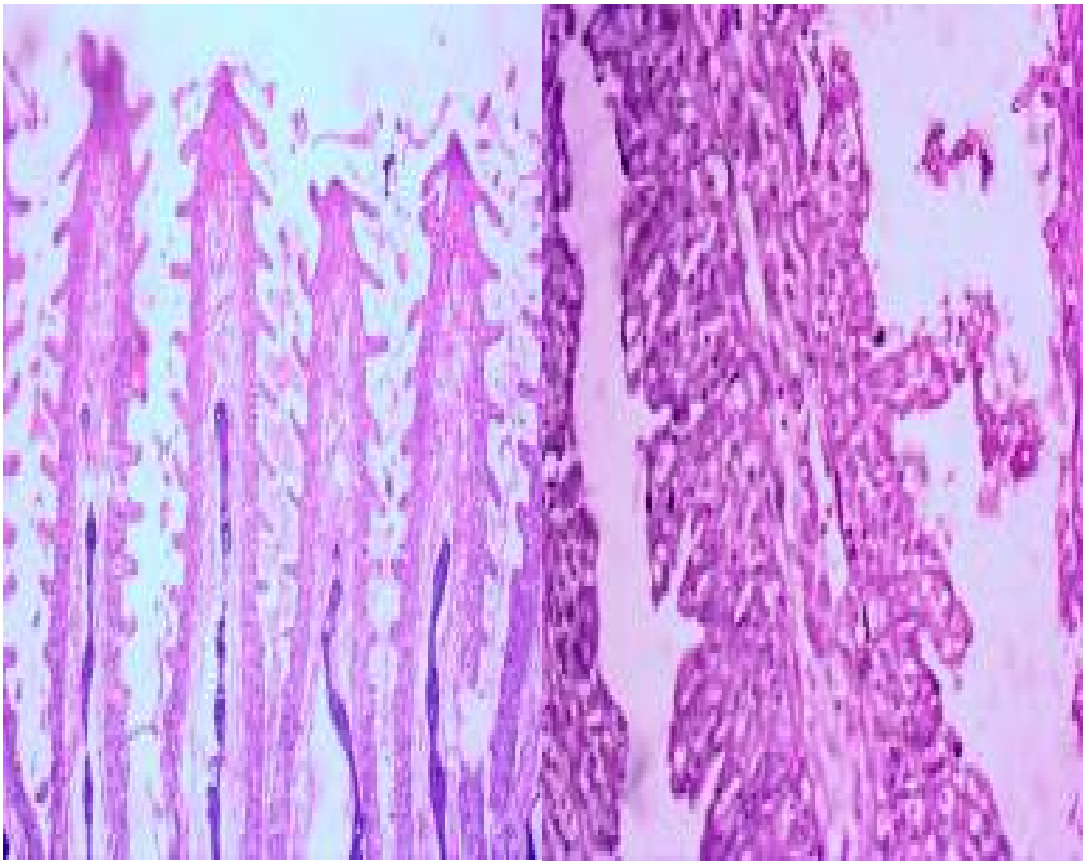


Plate4.1. Histological section of gills of *Clarias gariepinus* fed soyabean meal (0% soaked in pulp baobab seed meal and soaked in alkali baobab seed meal) showed no observable lesion. (X 400)



(a) (b)

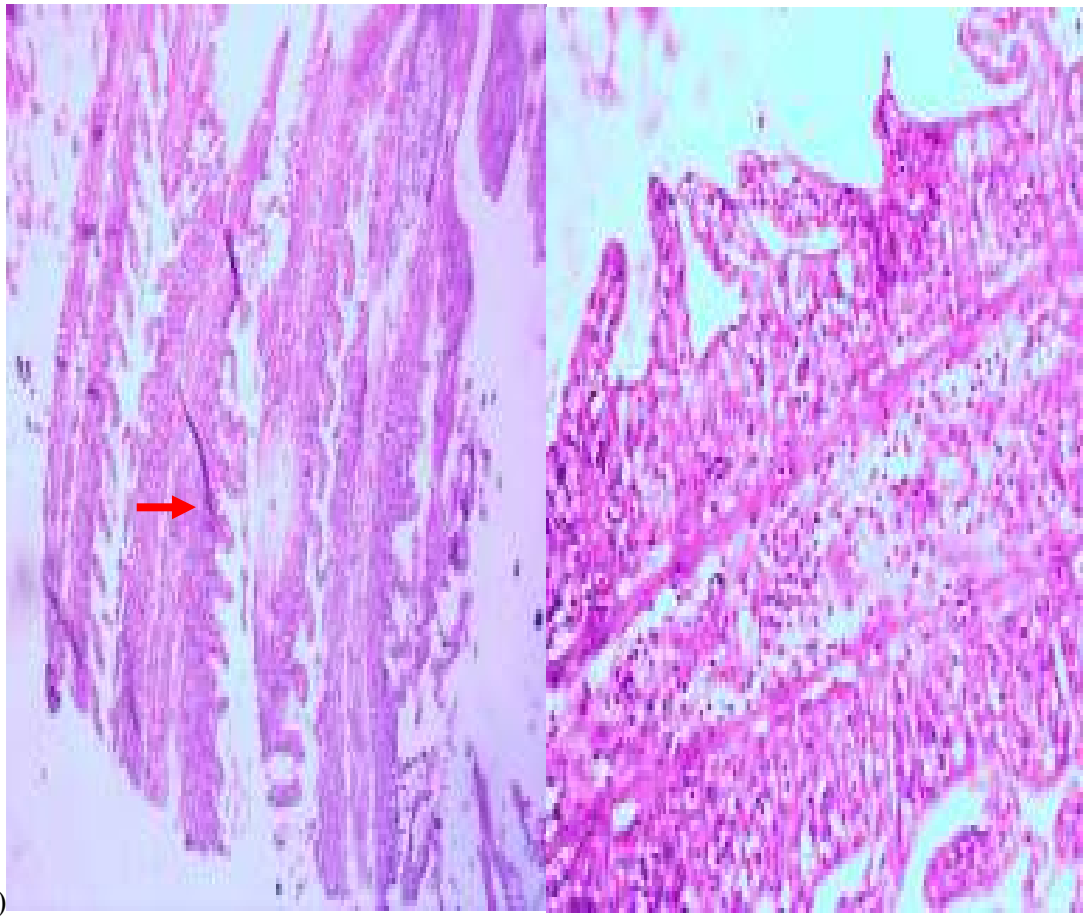
Plate4.2. Histological section of gills of *Clarias gariepinus* fed (a) 20% soaked in pulp baobab seed meal (showing no observable lesion) (b) 20% soaked in alkali baobab seed meal (showed no observable lesion). (X400)



(a) (b)

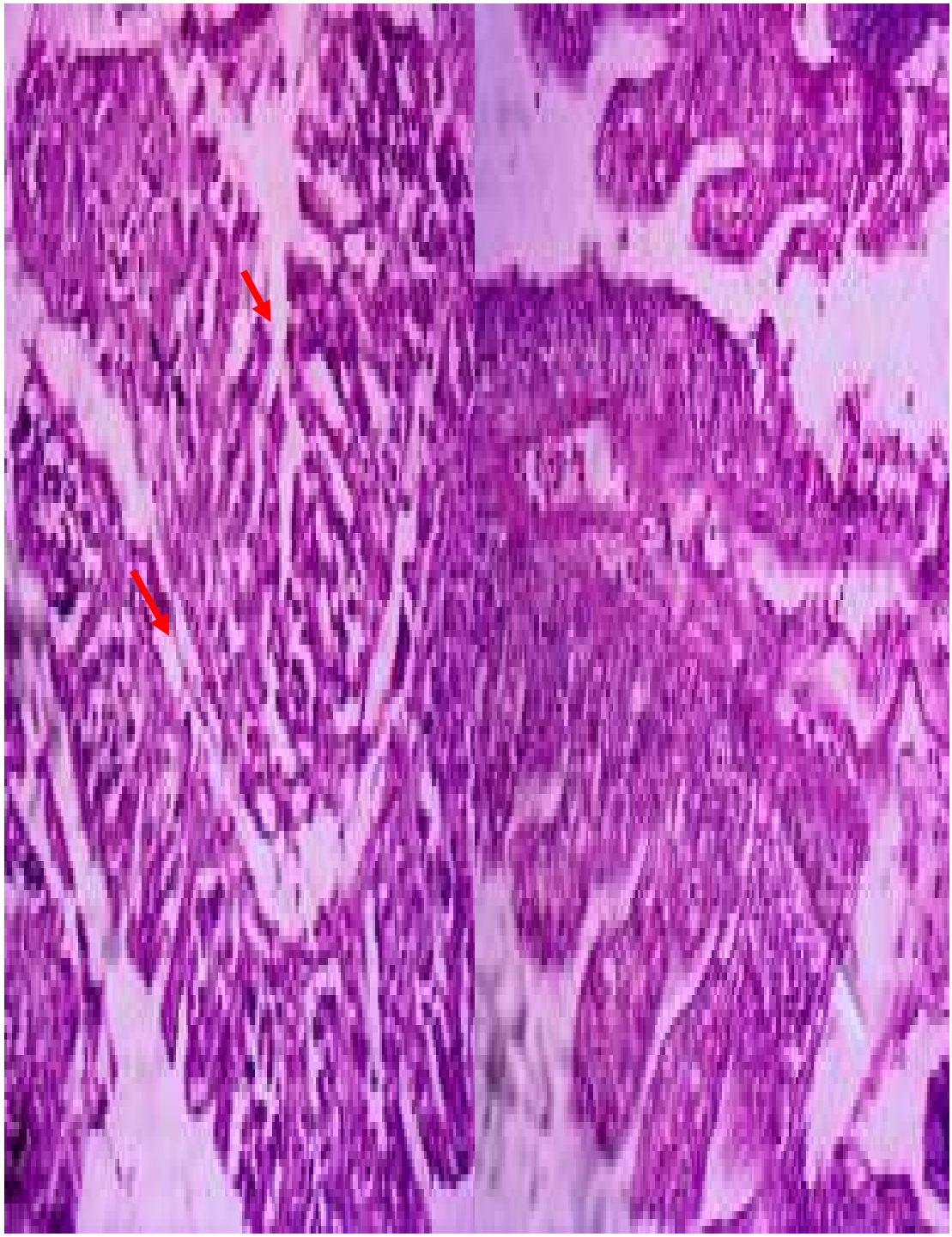
Plate4.3. Histological section of gills of *Clarias gariepinus* fed (a) 40% inclusion of soaked in pulp baobab seed meal (showing no observable lesion) (b) 40% inclusion of soaked in alkali baobab seed meal showing no observable lesion. (X 400)

(a)



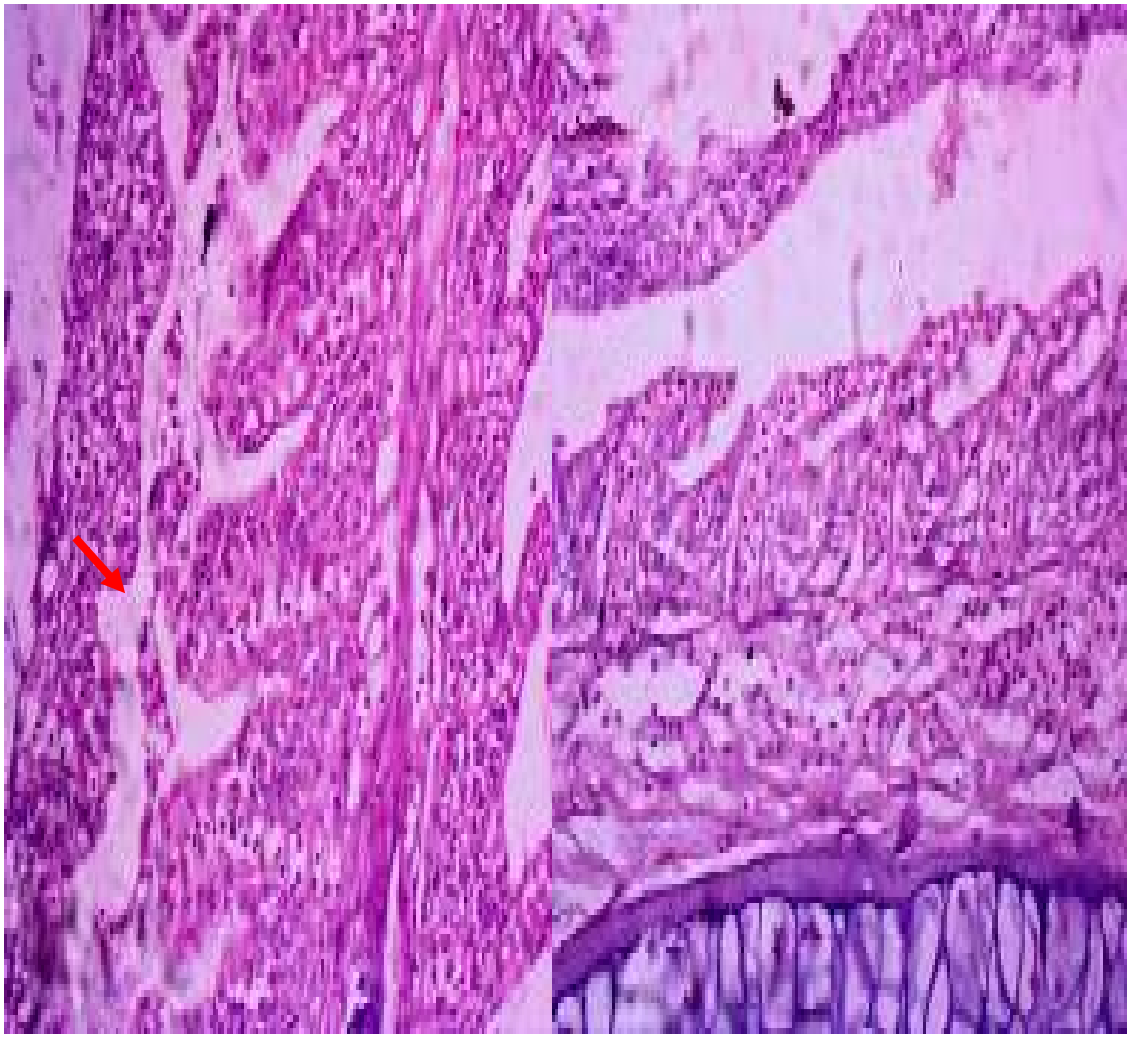
(a) (b)

Plate 4.4. Histological section of gills of *Clarias gariepinus* fed (a) 60% inclusion of soaked in pulp baobab seed meal (showing mild lamellae hyperplasia) (b) 60% inclusion of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



(a) (b)

Plate4.5. Histological section of gills of *Clarias gariepinus* fed (a) 80% inclusion of soaked in pulp baobab seed meal (showing lamellae necrosis) (b) 80% inclusion of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



(a) (b)

Plate4.6. Histological section of gills of *Clarias gariepinus* fed (a) 100% inclusion of soaked in pulp baobab seed meal (showed moderate lamellae hyperplasia) (b) 100% inclusion of soaked in alkali baobab seed meal showing no observable lesion. (X 400)

The histo-pathology of livers in Clarias gariepinus fed soyabean meal and varied inclusion levels of baobab seed meal based diets

Histology of liver of *Clarias gariepinus* fed soyabean meal (**Plate** 4.7); 20 % SPBSM and 20 % SABSM (**Plate** 4.8); 40 % SPBSM and 40 % SABSM (**Plate** 4.9); 60 % SPBSM and 60 % SABSM (**Plate** 4.10), 80 % SABSM (**Plate** 4.11), 100 % SABSM (**Plate** 4.12) showed no observable lesion. Swelling and vacuolation of hepatocyteswere observed in the liver of fish fed 80% SPBSM (**Plate** 4.11), and those fed 100% SPBSM (**Plate** 4.12) showed patchy hepatocellular necrosis.

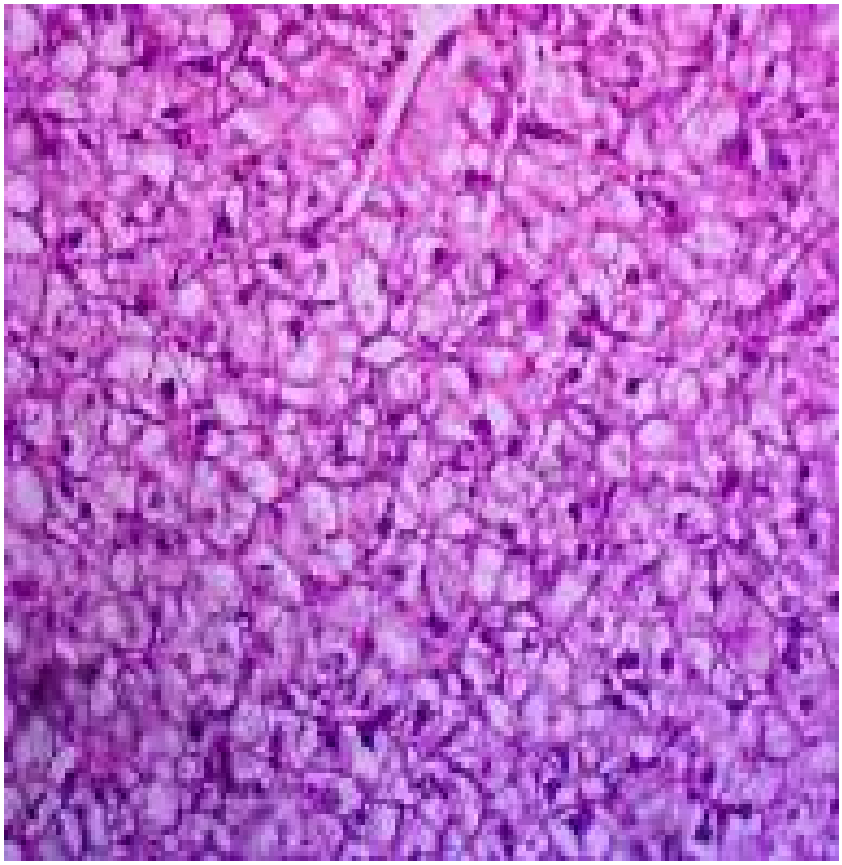


Plate4.7. Histological section of liver of *Clarias gariepinus* fed soyabean (0% baobab seed meal) meal showing no observable lesion. (X 400)

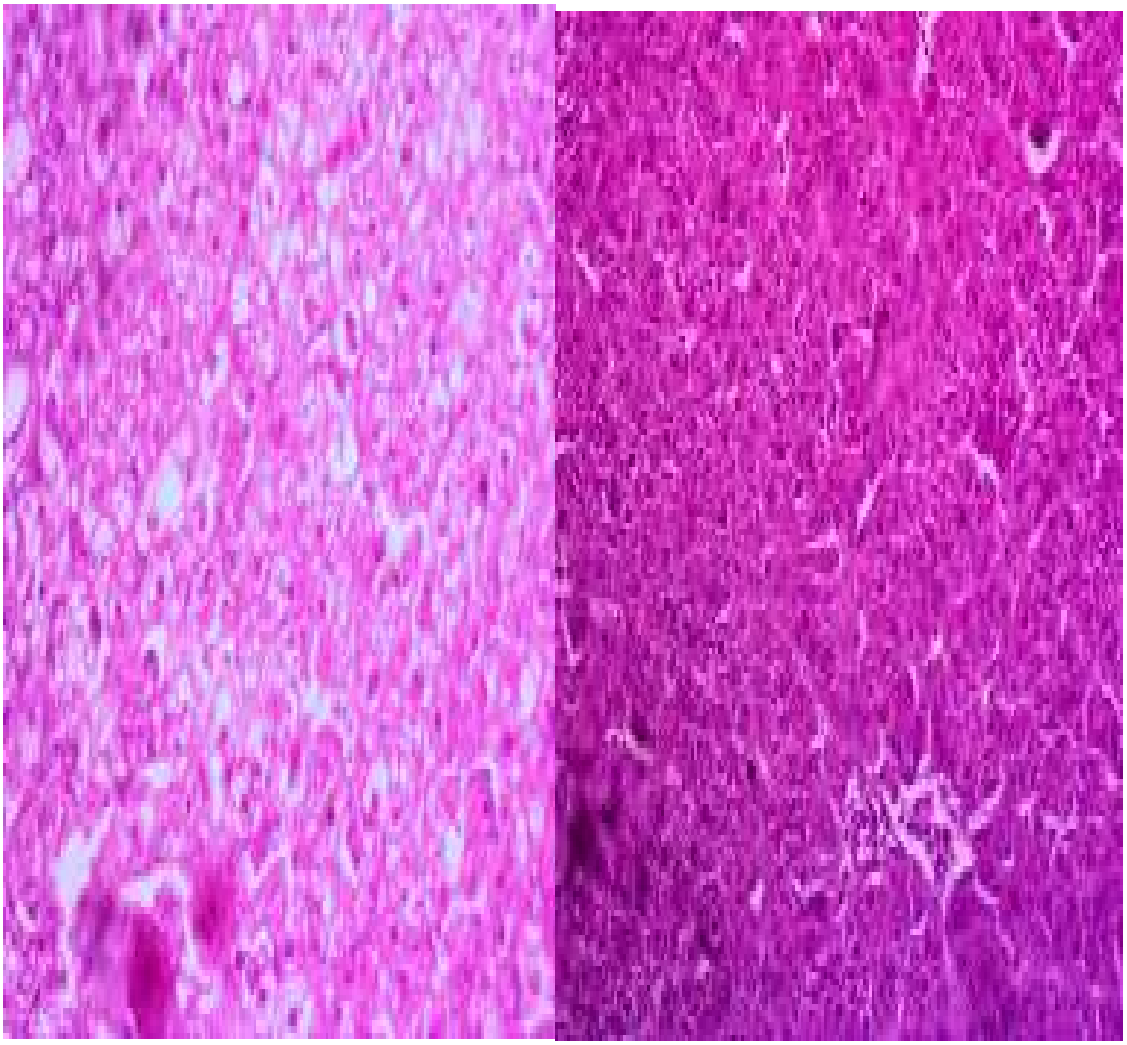
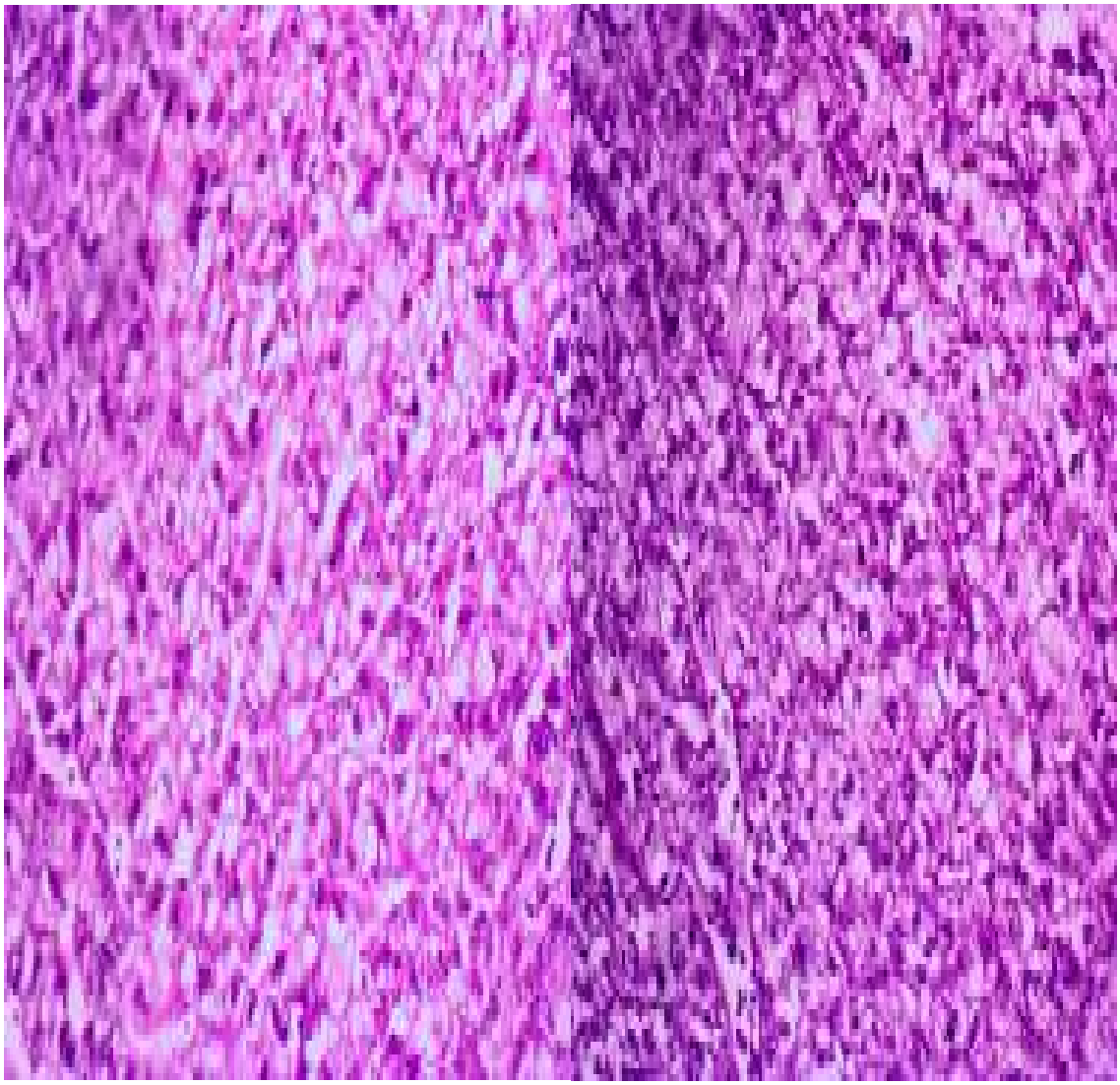
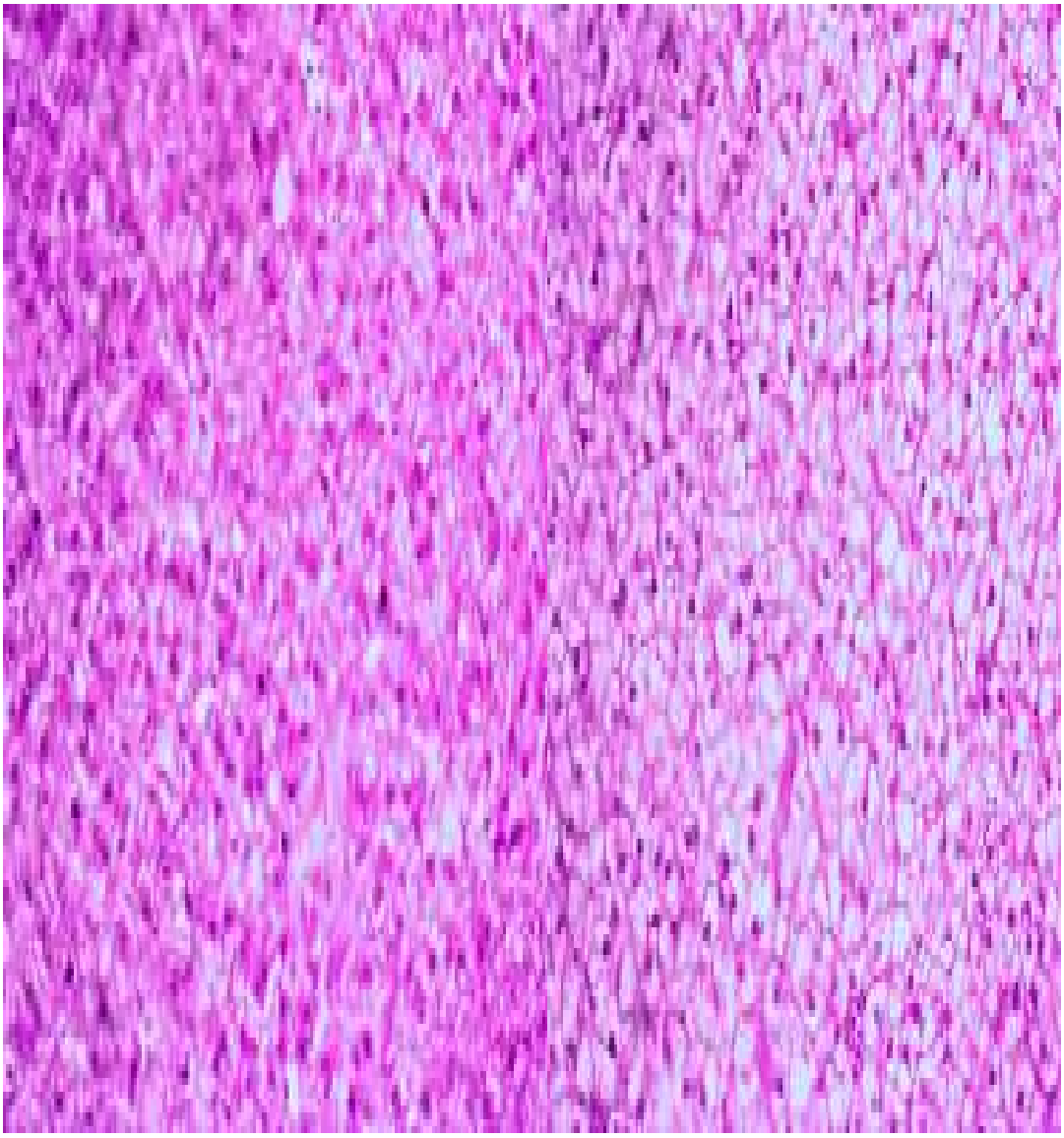


Plate4.8. Histological section of liver of *Clarias gariepinus* fed (a) 20% inclusion of soaked in pulp baobab seed meal (showing no observable lesion) (b) 20% inclusion of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



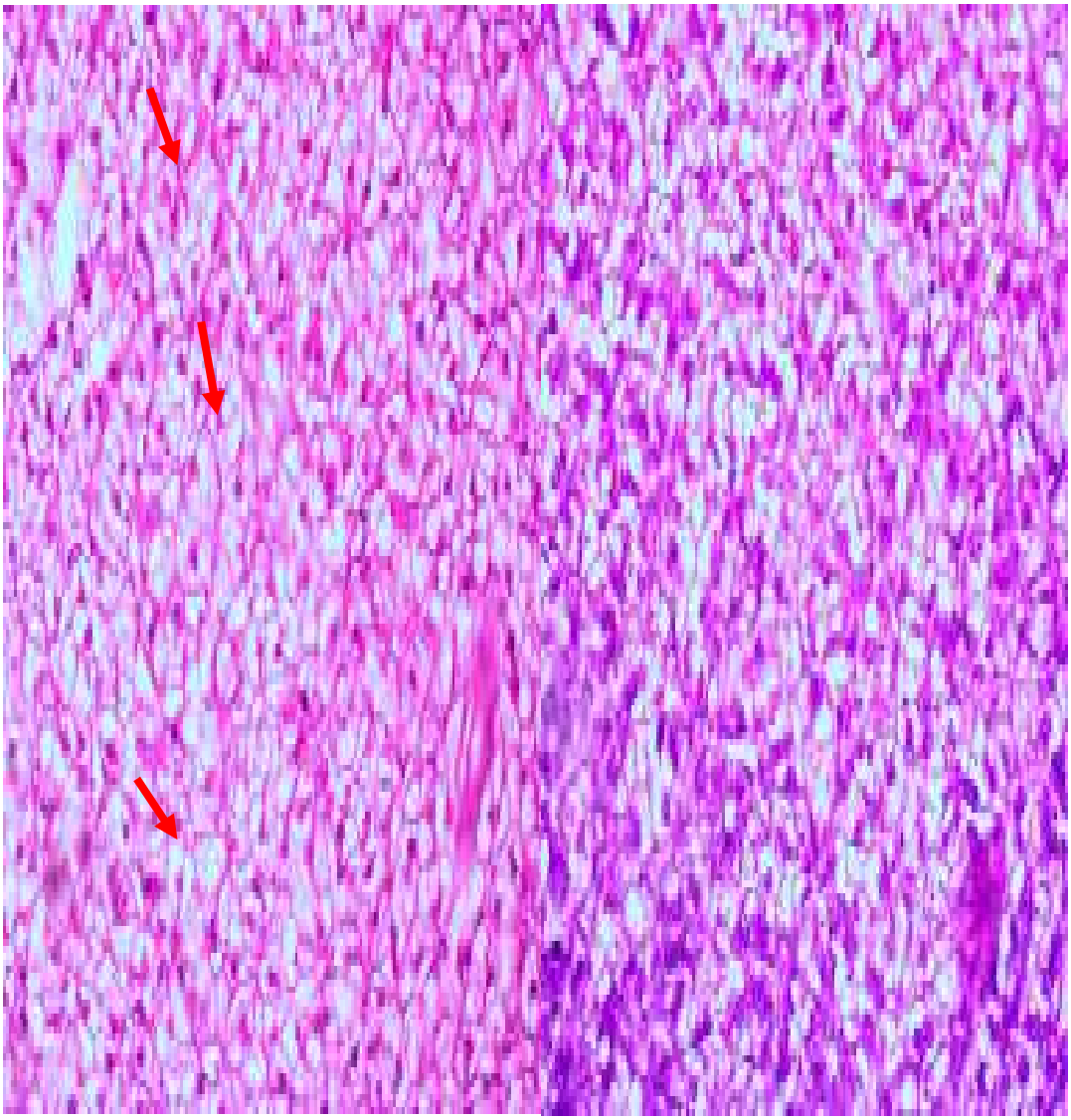
(a) (b)

56Plate4.9. Histological section of liver of *Clarias gariepinus* fed (a) 40% inclusion of soaked in pulp baobab seed meal (showing no observable lesion) (b) 40% inclusion of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



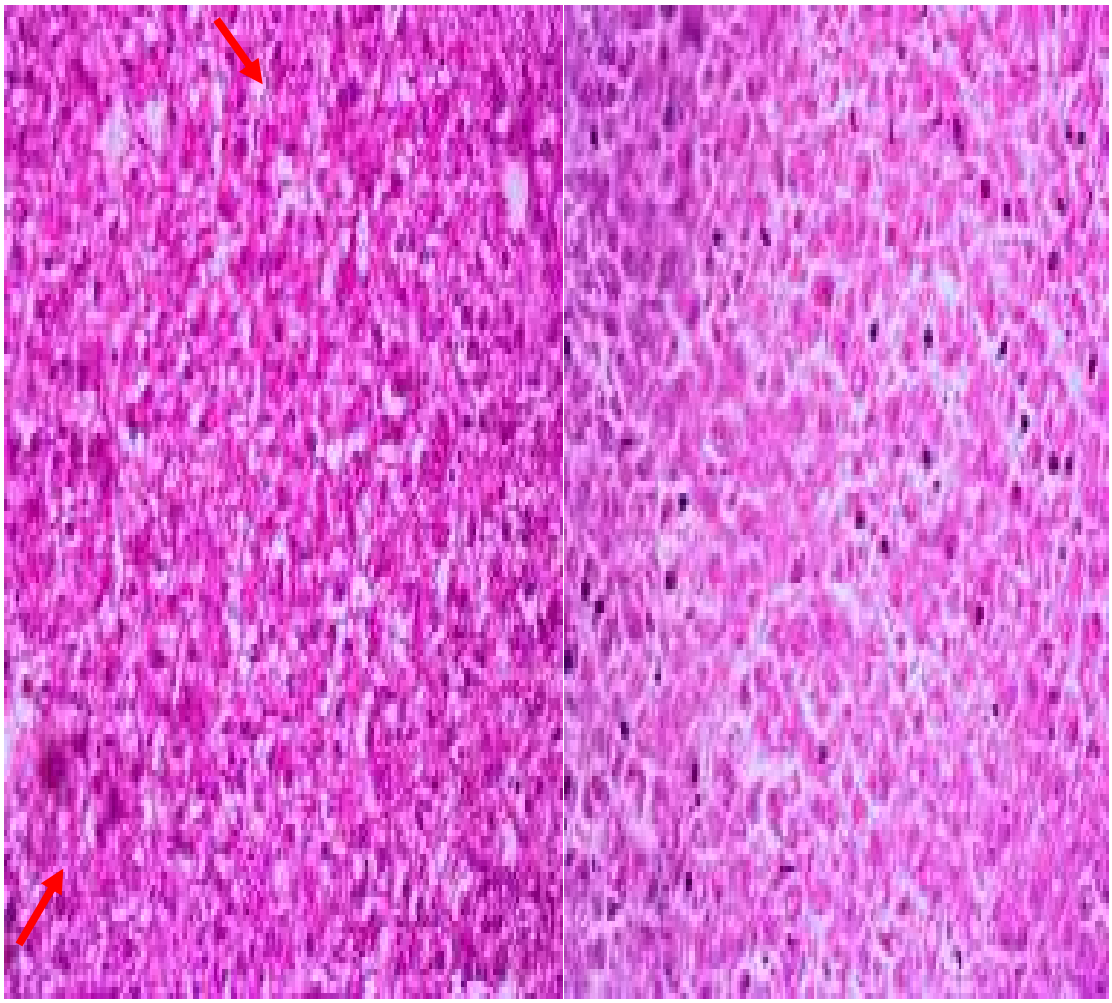
(a) (b)

Plate4.10. Histological section of liver of *Clarias gariepinus* fed (a) 60% inclusion of soaked in pulp baobab seed meal (showing no observable lesion). (b): 60% inclusion of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



(a) (b)

Plate4.11. Histological section of liver of *Clarias gariepinus* fed (a) 80% inclusion of soaked in pulp baobab seed meal (showing swelling and vacuolation of hepatocytes) (b): 80% inclusion of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



(a) (b)

Plate4.12. Histological section of liver of *Clarias gariepinus* fed diets that contained (a) 100% substitution level of soyabean meal with soaked in pulp baobab seed meal (showing patchy hepatocellular necrosis) (b): 100% substitution level of soyabean meal with soaked in alkali baobab seed meal showing no observable lesion. (X 400)

The histo-pathology of kidneys of Clarias gariepinus fed soyabean meal and varied inclusion levels of baobab seed meal based diets

Histology of kidney of *Clarias gariepinus* fed soyabean meal, 0% baobab seed meal (**Plate** 4.13); 20 % SPBSM and 20 % SABSM (**Plate** 4.14); 40 % SPBSM and 40 % SABSM (**Plate** 4.15); 60 % SPBSM and 60 % SABSM (**Plate** 4.16); 80 % SPBSM and 80 % SABSM (**Plate** 4.17) and 100 % SABSM (**Plate** 4.18) showed no observable lesion. Tubular epithelial necrosis was observed in fish fed diets 100% SPBSM (**Plate** 4.18).

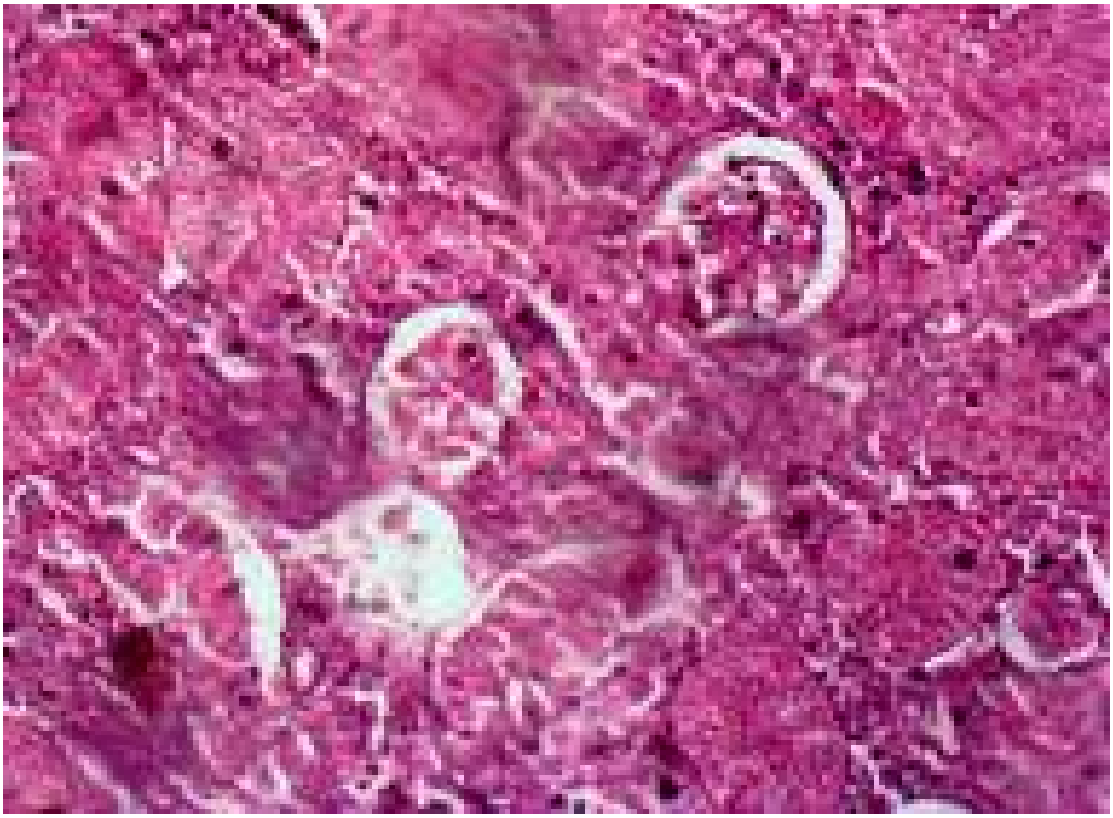
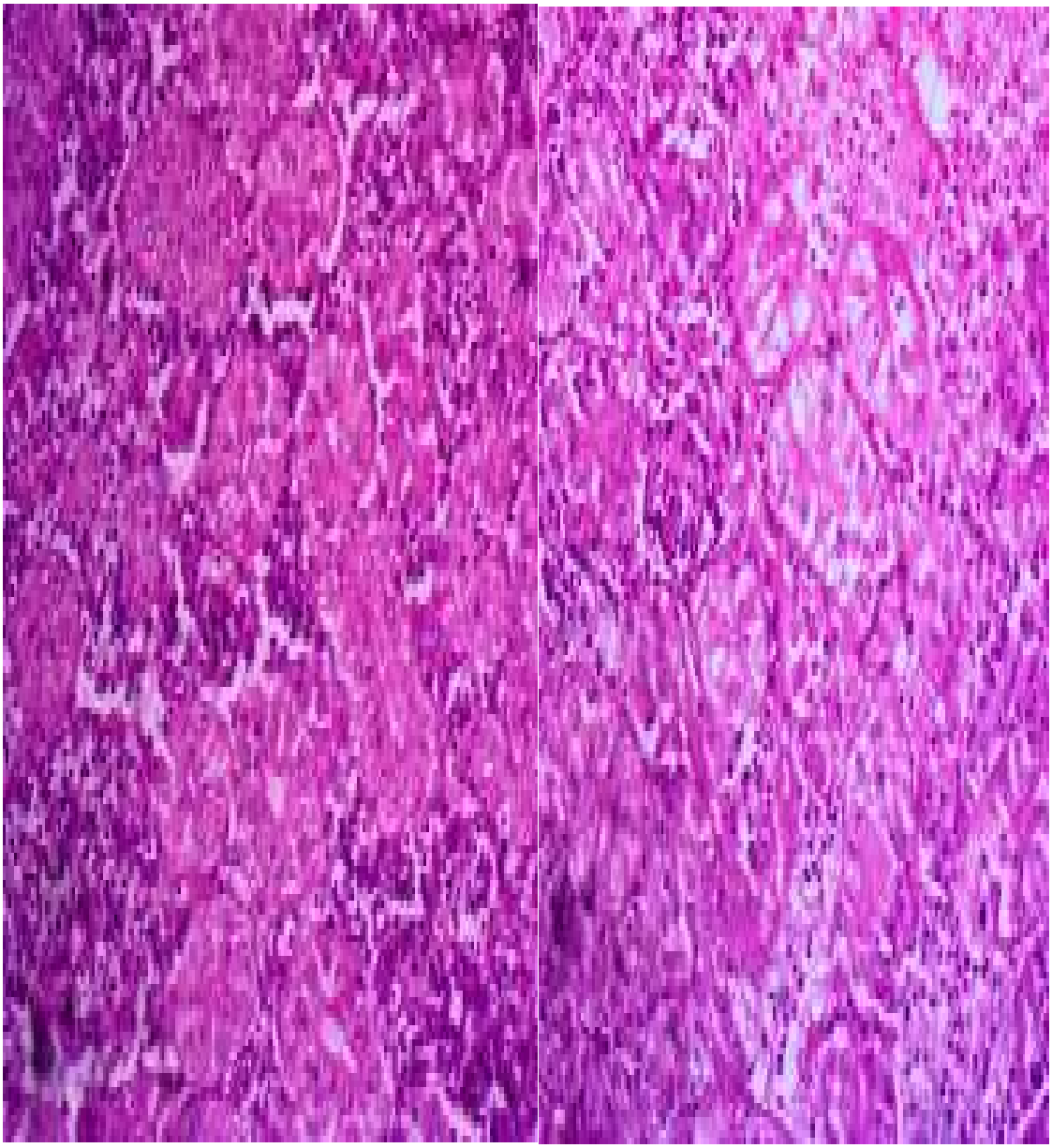
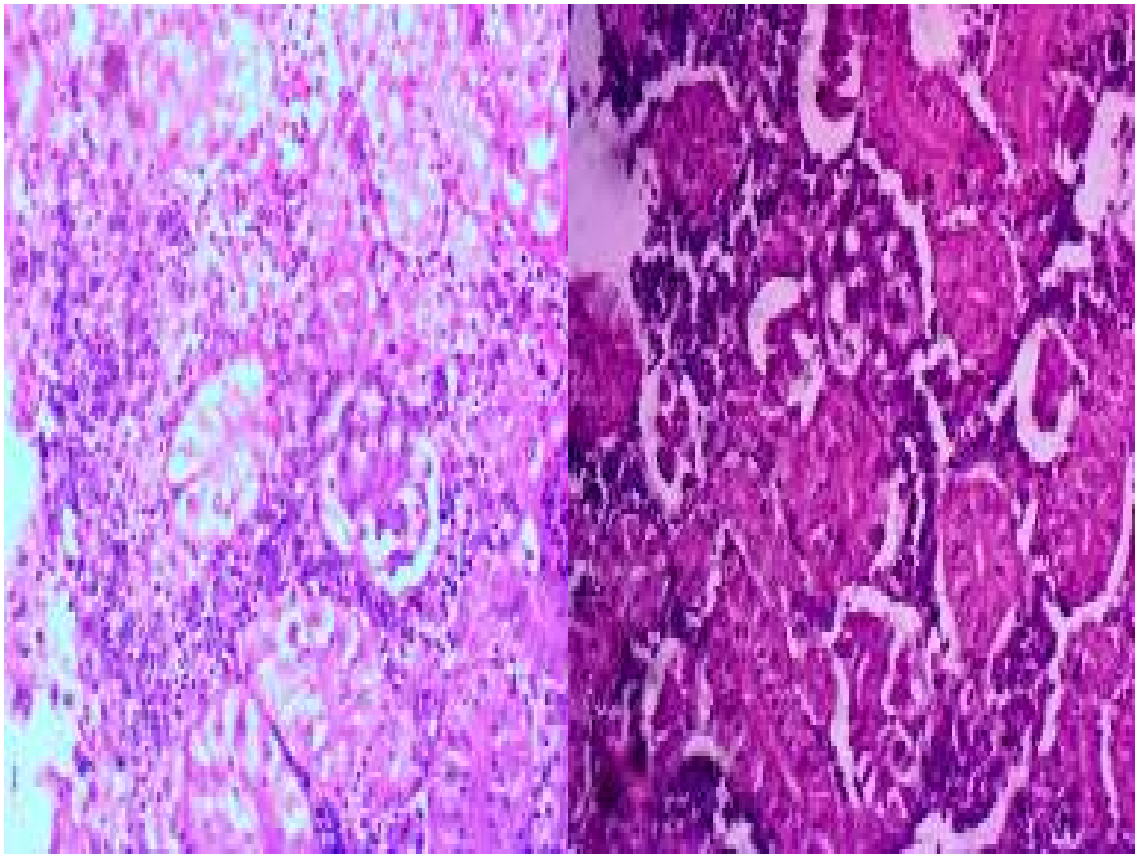


Plate4.13. Histological section of kidney of *Clarias gariepinus* fed soyabean meal (0% baobab seed meal) showing no observable lesion. (X 400)



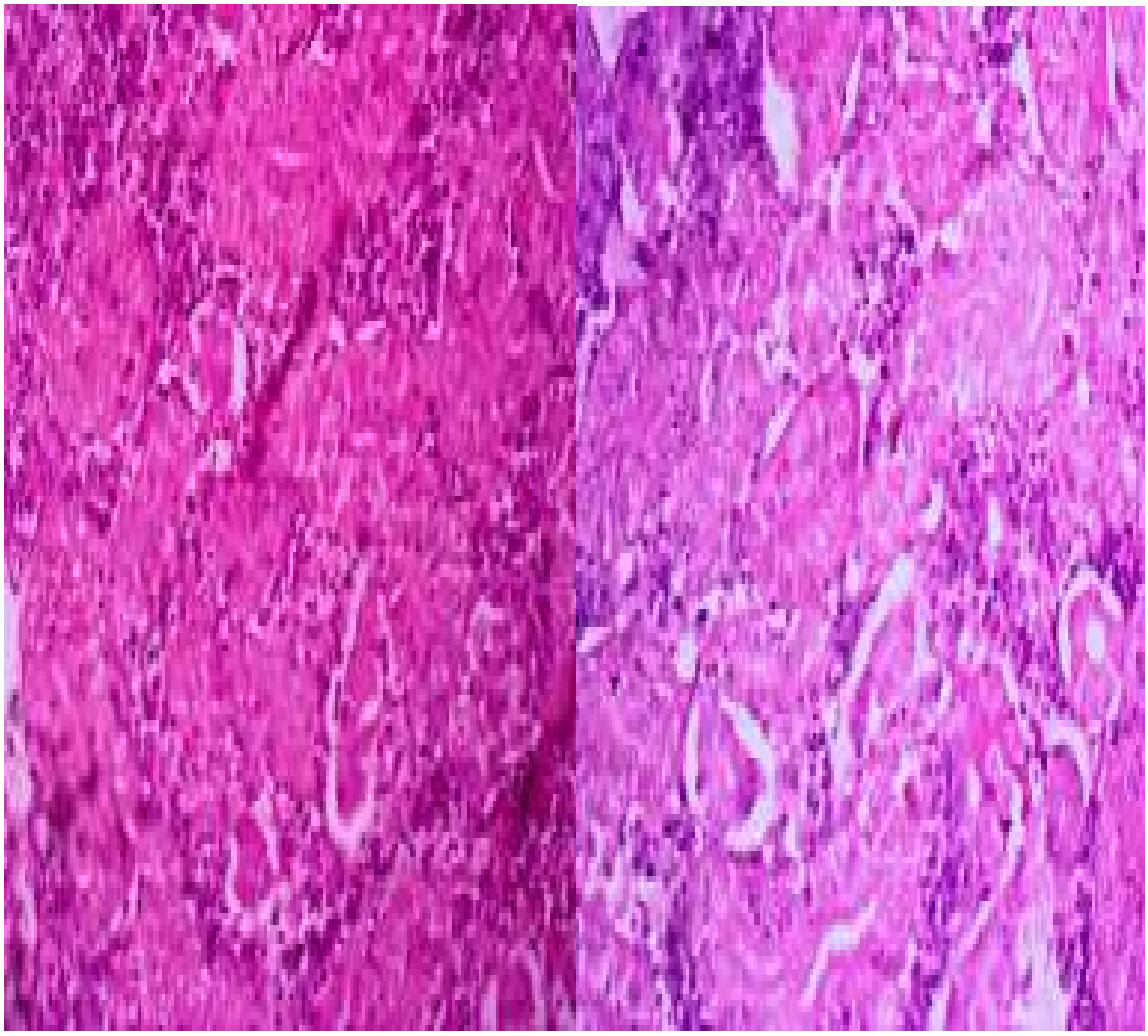
(a) (b)

Plate4.14. Histological section of kidney of *Clarias gariepinus* fed (a) 20% inclusion level of soaked in pulp baobab seed meal (showing no observable lesion) (b): 20% inclusion level of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



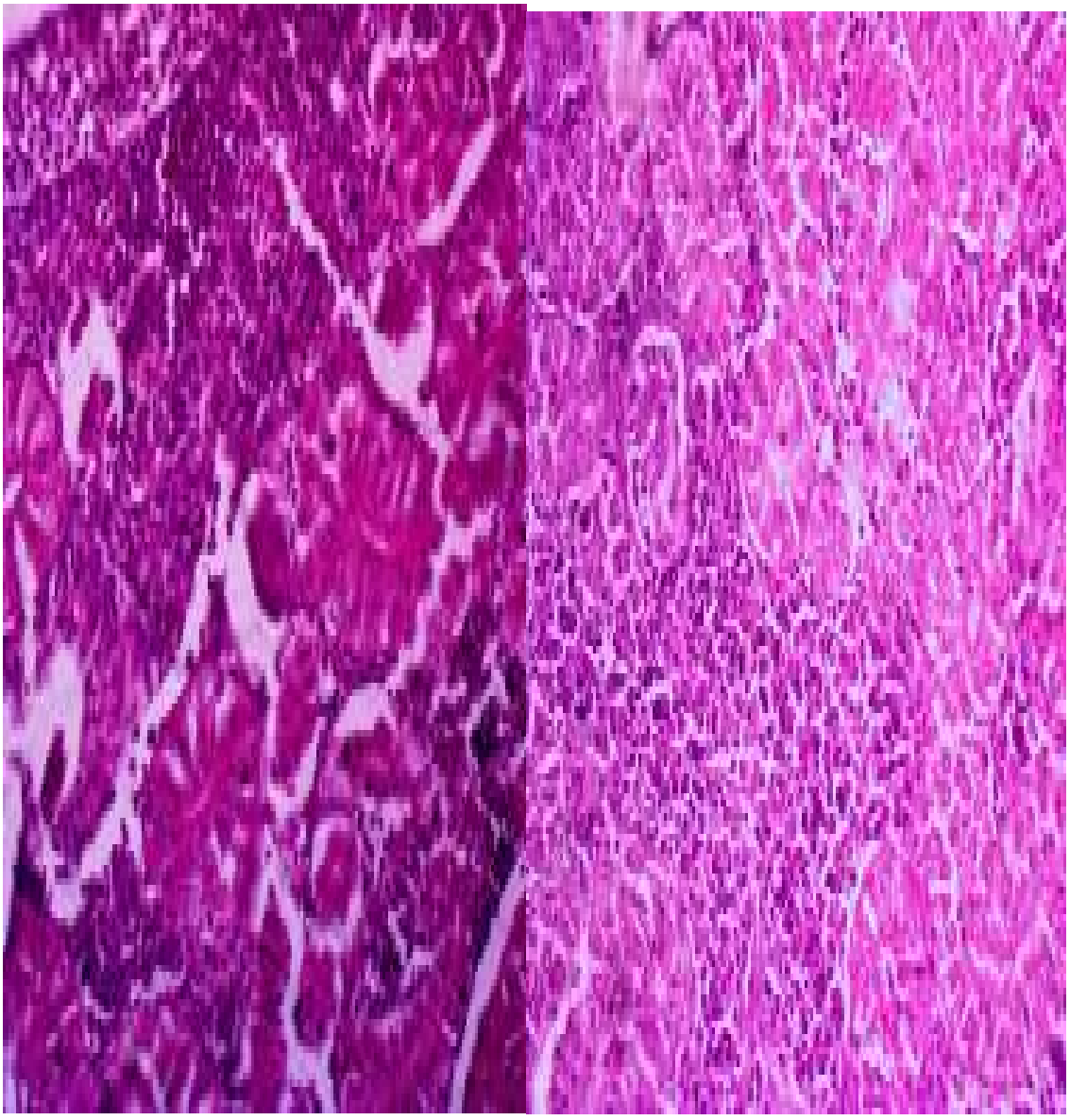
(a) (b)

Plate4.15. Histological section of kidney of *Clarias gariepinus* fed (a) 40% inclusion level of soaked in pulp baobab seed meal (showing no observable lesion) (b): 40% inclusion level of soaked in alkali baobab seed meal showed no observable lesion. (X 400)



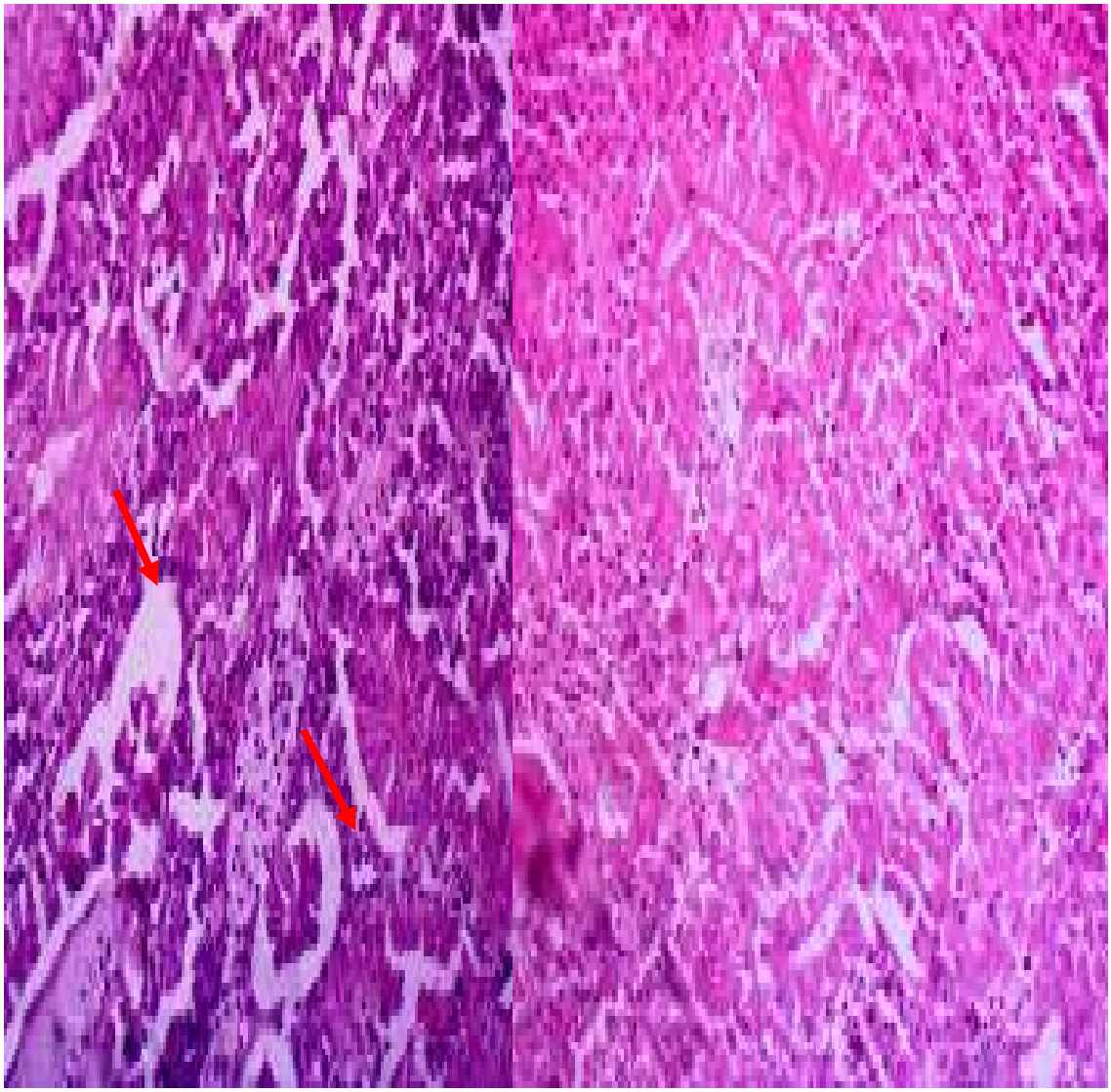
(a) (b)

Plate4.16. Histological section of kidney of *Clarias gariepinus* fed (a) 60% inclusion level of soaked in pulp baobab seed meal (showing no observable lesion) (b): 60% inclusion level of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



(a) (b)

Plate4.17. Histological section of kidney of *Clarias gariepinus* fed (a) 80% inclusion level of soaked in pulp baobab seed meal (showing no observable lesion) (b): 80% inclusion level of soaked in alkali baobab seed meal showing no observable lesion. (X 400)



(a) (b)

Plate4.18. Histological section of kidney of *Clarias gariepinus* fed (a) 100% inclusion level of soaked in pulp baobab seed meal (showing tubular epithelial necrosis) (b): 100% inclusion level of soaked in alkali baobab seed meal showing no observable lesion. (X 400)

4.17. Summary of histopathological changes observed in the gill, liver and of *Clarias gariepinus* fed soaked in pulp baobab seed based diets

Histopathology of gill, kidney and liver of *Clarias gariepinus* fed soaked in pulp baobab seed meal at 0, 20 and 40 % did not show any lesion. Mild lamellae hyperplasia was noticed in fish fed 60 % SPBSM. At 80 % inclusion of SPBSM, hyperplasia was observed on the gill; swelling and vacuolation of hepatocytes were also noticed. Mild lamella hyperplasia, necrosis of liver cells and mild tubular necrosis were observed at 100 % inclusion of SPBSM (**Table** 4.19).

Table 4.19. Summary of Histopathological changes observed in the gill, kidney and liver of *Clarias gariepinus* fed soaked in pulp baobab seed based diets

Dietary Treatments (%)	Organs	Necrosis	Hyperplasia	Swelling	Vacuolation
SPBSM 20	Gill	-	-	-	-
	Liver	-	-	-	-
	Kidney	-	-	-	-
SPBSM 40	Gill	-	-	-	-
	Liver	-	-	-	-
	Kidney	-	-	-	-
SPBSM 60	Gill	-	½	-	-
	Liver	-	-	-	-
	Kidney	-	-	-	-
SPBSM 80	Gill	-	+	-	-
	Liver	-	-	-	-
	Kidney	-	-	+	+
SPBSM 100	Gill	-	½	-	-
	Liver	+	-	-	-
	Kidney	½	-	-	-

Note: - = Completely absent, ½=Mild, +=Present, ++=Severe

4.18 Summary of histopathological changes observed in the gill, kidney and liver of *Clarias gariepinus* fed soaked in alkali baobab seed based diets

Histopathology of gill, kidney and liver of *Clarias gariepinus* fed soaked in pulp baobab seed meal at 0, 20, 40, 60, 80 and 100 % did not show any lesion. (**Table 4.20**). There were no vacuolation, necrosis, hyperplasia and swelling on the organs of fish observed.

Table 4.20. Summary of histopathological changes observed in the gill, kidney and liver of *Clarias gariepinus* fed alkali baobab seed based diets

Dietary Treatments (%)	Organs	Necrosis	Hyperplasia	Swelling	Vacuolation
SABSM 20	Gill	-	-	-	-
	Liver	-	-	-	-
	Kidney	-	-	-	-
SABSM 40	Gill	-	-	-	-
	Liver	-	-	-	-
	Kidney	-	-	-	-
SABSM 60	Gill	-	-	-	-
	Liver	-	-	-	-
	Kidney	-	-	-	-
SABSM 80	Gill	-	-	-	-
	Liver	-	-	-	-
	Kidney	-	-	-	-
SABSM 100	Gill	-	-	-	-
	Liver	-	-	-	-
	Kidney	-	-	-	-

Note: - = Completely absent, ½ = Mild, + = Present, ++ = Severe

4.19 Cost implication of replacing soyabean meal with varied replacement levels of soaked in alkali baobab seed meal based diets

Lowest economic conversion ratio (ECR) was recorded in fish fed 20 % soaked in alkali baobab seed meal based diets (**Table** 4.21). Gross revenue obtained in fish fed 20% soaked in alkali baobab seed meal based diets was higher ($p<0.05$) those fed 40 %, 60 % and 80% SABSM. The lowest gross revenue was recorded in fish fed 100 % SABSM. The ECR obtained in fish fed 20 % SABSM (586.10±19.13) is lower than 773.34±47.43 in those fed soyabean meal.

Table 4.21Cost implication of replacing soyabean meal with varied replacement levels of soaked in alkali baobab seed meal based diets

Indices	Control	SABSM 20	SABSM 40	SABSM 60	SABSM 80	SABSM 100
Feed cost (₦/Kg)	376.43	373.57	370.35	366.16	362.64	362.64
GR	658.98± 192.49^{b}	845.38± 59.04^{b}	453.50± 81.41^{ab}	394.44± 316.33^{ab}	372.76± 188.69^{ab}	136.12± 87.54^{a}
FCR	2.06±0.13^{b}	1.58±1.00^{a}	1.94+0.20 ^{b}	1.92+0.14 ^{b}	1.94+0.04 ^{b}	1.96+0.05^{b}
ECR	773.34± 47.43	586.10± 19.13	696.47± 28.84	711.98± 160.98	705.03± 79.88	778.44± 103.09

Values with different superscripts on the same row are significantly different ($p<0.05$)
GR: Gross Revenue ECR: Economic Conversion Ratio
Control, SABSM 20, SABSM 40, SABSM 60, SABSM 80, SABSM 100 = Replacement level of soyabean meal with soaked in alkali baobab seed meal at 0, 20, 40, 60, 80 and 100 % replacement levels respectively

4.20 Cost implication of replacing soyabean meal with varied replacement levels of soaked in pulp baobab seed meal based diets

Gross revenue and economic conversion ratio recorded in fish fed varied replacement levels of soaked in pulp baobab seed meal based diets significantly different ($p<0.05$). Fish fed 20 % SPBSM had the highest gross revenue (₦798.70±213.04) while those fed 100 % SABSM (₦205.56±76.42) recorded the least. Lowest economic conversion ratio (ECR) was recorded in fish fed 20 % soaked in pulp baobab seed meal based diets (**Table** 4.22). The ECR obtained in fish fed 20 % SPBSM (589.96±35.76) was lower ($p<0.05$) than those fed soyabean meal.

Table 4.22Cost implication of replacing soyabean meal with varied replacement levels of soaked in pulp baobab seed meal based diets

Indices	Control	SPBSM20	SPBSM 40	SPBSM 60	SPBSM 80	SPBSM 100
Fish cost (₦)	376.43	373.57	370.35	366.16	362.64	362.64
GR (₦)	658.98±192.49^{bc}	798.70±213.04^{c}	463.50±123.37^{abc}	327.06±198.26^{ab}	302.70±100.02^{ab}	205.56±76.42^{a}
FCR	2.06±0.13^{b}	1.58±1.00^{a}	1.94+0.20^{b}	1.92+0.14^{b}	1.94+0.04^{b}	1.96+0.05^{b}
ECR	773.34± 47.43^{b}	589.96± 35.76^{a}	719.96± 73.41^{b}	703.88± 51.79^{ab}	702.05± 14.95^{ab}	703.60± 19.53^{ab}

Values with different superscripts on the same row are significantly different ($p<0.05$)
GR: Gross Revenue ECR: Economic Conversion Ratio
Control, SPBSM 20, SPBSM 40, SPBSM 60, SPBSM 80, SPBSM 100 = Replacement level of soyabean meal with soaked in pulp baobab seed meal at 0, 20, 40, 60, 80 and 100%, respectively.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Chemical composition of raw and processed baobab seed meals

5.1.1 Proximate composition of differently processed baobab seed meals

Crude protein of baobab seed meals improved as a result of processing, except toasted baobab seed meal (TBSM). Reduction in moisture content, crude protein, crude fat, and ash of TBSM might be due to the temperature (150^{o}C) used in toasting the seeds. The temperature might have denatured the protein. Salazar-Villanea *et al.* (2018) reported a change in the secondary structural conformation of protein in kidney bean, lentils, chickpea and soyabean as a result of thermal processing. Saulawa *et al.* (2014) also observed a similar reduction in crude protein of toasted baobab seed meal while Innocentia *et al*. (2014) reported a rise in crude protein of baobab seeds roasted at 150^{o}C.

Crude protein of the processed Baobab Seed Meals (BSM) obtained in this research was lesser than the value recorded by Sola-Ojo (2011); Yusuf *et al*., (2008); higher than 16.6% reported by Ezeagu (2005), 18.4% by Anene *et al*. (2012) but related to 28.85% obtained by Saulawa *et al*., (2014) and 20.13% reported by Danbature (2014). Discrepancy in crude protein might be because of the difference in the fibre level in the meal, baobab seed meals with reduced fibre level have high crude protein content.

Processing increased the crude fat of BSM except the toasted meals (TBSM). Increased in crude fat of autoclaved, soaked in pulp, soaked in alkali, soaked in liquor and soaked in water BSM compared to raw BSM is similar to findings reported by Duodu *et al*. (2018) who obtained a rise in crude fat of autoclaved, soaked and fermented cotton and groundnut seed meals. Processed baobab seeds had reduced crude fibre, except SPBSM. Toasting BSM at 150^{0}C lowered crude protein and ash but increased the nitrogen free extract of the

seed. Best crude protein was reported in seed soaked in alkali and those soaked in maize liquor.

5.1.2 Mineral composition of differently processed baobab seed meals

Processing enhances calcium and potassium levels in baobab seed. This is similar to findings observed by Innocentia *et al*. (2014) in roasted baobab seed meal but in discrepancy with that of Saulawa *et al*. (2014) who reported a reduction in mineral composition of processed baobab seed meal. The TBSM, ABSM and SPBSM had higher magnesium compared to RBSM. High value of iron and copper recorded in SLBSM could be as a result of the medium in which the seeds were soaked.

Increased manganese level was observed in processed baobab seed meal. High manganese observed in SPBSM could be as a result of the pulp in which the baobab seeds were soaked. The values of mineral composition observed in this experiment are higher than the values recorded by Saulawa *et al*. (2014) and Adedayo and Sani (2015).

5.1.3 Amino acids profile of differently processed baobab seed meals

Toasting, autoclaving and soaking used in this experiment did not affect the amino acid composition of baobab seed meal. Methionine, cystine, methionine+cystine, lysine, histidine, threonine, arginine, isoleucine, leucine, valine, phenylalanine, glycine, serine, proline, alanine, aspartic acid and glutamic acid recorded in the raw baobab seed meal were similar to those in processed meal. Methionine, cystine and lysine content in the baobab seed meal reported in this study were lower than the requirement for African catfish (NRC, 1993).

5.1.4 Antinutritional factors in differently processed baobab seed meals

Processing reduced the oxalates levels in SABSM. SABSM had the lowest oxalates. Soaking in maize liquor reduced the phytate level in baobab seed meal. Balogun, 2013 also reported poor reduction of phytic acid for all the processing methods he used for Bauhinia seed with the exception of the soaked meal.

Phytate content in raw and processed BSM reported in this study are higher than the values reported by Olaitan *et al.* (2014) who reported 2.27±0.31% phytate level. This may

be due to difference in the processing techniques that were used in the studies or seed coats that were removed by sieving in this study.Phytates level reported for raw and processed baobab seed meal were below 5 g kg-1 tolerant levels of phytic acid for tilapia reported by Francis *et al*., 2001. The saponins levels obtained in raw and processed baobab meals were lower than those reported for soyabean meal 600mg/100g respectively (El-Shemy *et al*., 2010 and Janjua, 2016).

Oxalate values in SABSM, RBSM and ABSM reported in this study are below 2.5% maximum oxalate content in a fish diet reported by Ulfert, 2015. The saponin levels in this study are also within the established tolerance limits of below 1g/kg of diets in commonly cultured fish (Francis *et al*., 2001a)

5.2 Growth, nutrients utilisation and digestibility of *Clarias gariepinus* juveniles fed with differently processed baobab seed meal based diets

Similar (P>0.05) mean weight gain (MWG) and specific growth rate (SGR) recorded in fish fed soyabean and differently processed baobab seed meals indicate that baobab seed meals do not have a depressing effect on *Clarias gariepinus's* growth. Highest MWG and SGR recorded in fish fed SABSM indicates that soaking in alkali is the best out of the processing methods that were used in this study. The least MWG and SGR recorded in fish fed with RBSM showed that processing increased the nutritional value of baobab seed.

The least FI observed in fish fed RBSM compared to those fed with processed baobab seed meals based diets shows that processing improved the palatability, acceptability and consumption of the seed by fish. Related FCR of fish fed with soyabean meal and processed baobab seed meals diets indicate that the inclusion of the experimental diets on the meal did not affect utilisation of the meals. The findings are not in disparity to that of Oladunjoye *et al.* (2014) who reported a similar feed conversion of baobab seed meal and the control diets by the rabbits. However, low FCR obtained in fish fed with SABSM pointed out that it is the best utilised meal. Similar (p>0.05) PER recorded in the experiment showed that raw and processed baobab seed meals can be well utilised by *Clarias gariepinus*.

High apparent protein digestibility values are an indicative of the ability of fish to digest protein (Fagbenro *et al*., 2017). High apparent digestibility coefficient ($ADC_{Protein}$) values recorded in SPBSM, TSBSM, ABSM and SWBSM and the highest in SABSM (**Table** 4.10) is an indication that fish utilised the protein in the meals better than SLBSM and RBSM which had the lowest values. The low $ADC_{Protein}$ in RBSM can be attributed to the positive effect of processing on BSM utilisation. The ($ADC_{Protein}$) values in SPBSM, TSBSM, ABSM, SWBSM and SABSM were close to 88.01% and 79.83% obtained by Falaye *et al*. (2014) for boiled and toasted lima beans meals.

The least apparent digestibility for protein, dry matter, energy and crude fat recorded in SLBSM is an indication that fish cannot utilise SLBSM. Highest apparent digestibility for energy similar to (72.5%) reported by Fagbenro *et al*. (2017) was recorded in fish fed soyabean meal. This is an implication that fish can utilise the energy in soyabean meal than BSM.

Highest apparent digestibility for dry matter, energy and fat obtained in fish fed soyabean meal indicates that fish utilises soyabean meal better than the experimental meals. This may be owed to the excessive fibre content of BSM compared to soyabean meal. This finding is contrast to that of Oladunjoye *et al.* (2014) who reported similar feed conversion and digestibility by the rabbits fed baobab seed meal and the control diets.
The fish fed TBSM had higher $ADC_{Protein}$ compared to those fed SPBSM but its MWG did not correspond with its $ADC_{Protein,}$ hence SPBSM was considered a better BSM than TBSM in diet of *Clarias gariepinus*.

5.3Rank of mean weight gain and apparent digestibility coefficient of crude protein of *Clarias gariepinus* fed differently processed baobab seed meal based diets

Fish fed SABSM had the highest apparent digestibility for crude protein followed by those fed TBSM, then those fed SPBSM. However, SABSM and SPBSM were used ranked as the best two processed baobab seed meal based diets because of reduced mean weight gain in fish fed TBSM compared to SPBSM. According to Omitoyin and Faturoti (2000), mean weight gain is a good indices in qualifying the quality of a feed.

5.4 Fish culture condition and water quality of the water used during the experiment

The pH, temperature, ammonia, nitrite and nitrate of 7.00±0.10-7.23±0.10, 26.20±0.00°C-28.00±0.20°C, 4.00±0.00 mg/L-8.00±0.00 mg/L, 0.00±0.00 mg/L-0.17±0.29 mg/L and 0.00±0.00 mg/L-4.17±1.44 mg/L, respectively fall within the normal range of water quality parameters in fish aquaculture described by Omitoyin, (2007) throughout the experiment.

Dissolve oxygen of 3.90±0.10 mg/L-4.87±0.12 mg/L recorded during the experiment was less than the optimum range of five mg/l recommended by Akinwole *et al*., (2017). He stated that low DO in culture water may also possibly be due to metabolic activities of the fish and use of oxygen in the decomposition of organic wastes from uneaten feeds and faecal droppings. African catfish can survive in culture water with DO level below 5mg/l due to the presence of air-breathing organ which enables them to utilise atmospheric oxygen when the dissolved oxygen is below the optimum level. This supports Oyewole *et. al.* (2008) report, which stated that African catfish can grow very well in culture water where DO goes down frequently below the optimum level.

5.5 Growth and nutrients' utilisation of *Clarias gariepinus* fed different replacement levels of soaked in alkali baobab seed meal based diets

Replacement levels of the soaked in alkali baobab seed meal in *Clarias gariepinus* diets affect their development and nutrients utilisation ($p<0.05$). Maximum mean weight gain was recorded in fish fed 20% SABSM while the least was in those fed 100% SABSM.

Mean weight gained of fish fed with 20% baobab seed meal was better than those fed with control diets.Reduction in MWG of fish fed with 40% BSM and above indicates that replication level of soyabean meal with baobab seed meal at 40% and above resulted in declined fish growth.

The decreased mean weight gain in fish fed control diets in relation to 20% SABSM is in accordance the with findings of Hassan *et al*. (2015) who reported that a rise in weight gained of fish fed 10% BSM compared to control could be related to the beneficial effect of BSM. This can be attributed to advantageous property of some anti-nutrients like saponins in little quantity in fish diets (Francis *et al*., 2001b). Hassan *et al*., (2015)

reported that the negative effect of antinutrients in BSM may not be felt at lower inclusion level, because the antinutients might still be within the range of their acceptable limit by fish. Lowest MWG and SGR recorded in fish fed with 100 SABSM indicates that high inclusion of soaked in alkali baobab seed have adverse effects on fish growth.

Highest ($P<0.05$) specific growth rate recorded in 20% SABSM compared to fish fed soybean meal, 40, 60, 80 and 100 % replacement levels of soyabean meal with baobab seed meal based diets indicated that the best replication level of soyabean meal with baobab seed meal is 20%. This is not accordance with the result of Anene *et al*. (2012) and Hassan *et al*. (2015) that observed similar specific growth of fish fed with 5- 25% and 10-30% inclusion levels of baobab seed meal, respectively in *Clarias gariepinus* diets.

Similar FCR in fish fed soyabean meal and different replacement levels of SABSM indicated that fish utilised SABSM comparably with soyabean meal. This report is in agreement with that of Oladunjoye *et al*. (2014) who stated that feed conversion by the rabbits that received five and 10% baobab seed meal compared favourably with those of the control.Low FCR indicates a good feed utilisation by the fish (Hassan *et al*., 2015).

Survival rate (SR) of fish fed control diets and 20% baobab seed meal were similar ($p>0.05$). Inadequate nutrients in 40, 60, 80 and 100% SABSM could have resulted in their lower SR. It could also be as result of decreased amino acid compositions of SABSM compared to soyabean meal or presence of high Mn and Fe in baobab seeds.

5.6 Growth and nutrients' utilisation of *Clarias gariepinus* fed with different replacement levels of soaked in pulp baobab seed meal based diets

Growth and nutrients' utilisation indices analysed were significantly different ($P<0.05$) in fish fed with control diets and varied replacements levels of SPBSM. Highest ($p<0.05$) MWG and SGR recorded in *Clarias gariepinus* fed with 20 % SPBSM compared to those fed soyabean meal based diets indicates that 20 % inclusion of SPBSM in *Clarias gariepinus* diet is the best. This is not in accordance with the report of Anene, (2012) who observed decrease in MWG at 25% inclusion of baobab seed meal. The disagreement might be the effect of the difference in processing means that were used in this study and that of Anene *et al*. (2012).

The least feed conversion ratio (FCR) value ($p<0.05$) in fish that received 20% SPBSM in comparison to those fed with control, 40%, 60%, 80%, 100% BSM, indicated that fish utilised SPBSM better at 20% inclusion level. Similar findings were obtained by Hassan *et al*., 2015 who reported a better level of utilisation of BSM diet at 10% inclusion as indicated by an excellent feed conversion ratio. High FCR in fish fed with 40, 60, 80 and 100 % SPBSM indicates that fish were unable to utilise the nutrients in the diets efficiently. This may be related to the antinutrients in the seed (Igboeli *et al*, 1997) which may not have been removed considerably by the processing method employed.

Low FCR in fish fed with 20% SPBSM compared to those fed soyabean meal is similar to the findings of Mwale *et al*. (2008), who also reported a lower FCR in fish fed five and 10% BSM compared to soyabean meal. High ($p<0.05$) FER in fish fed with 20 % SPBSM also showed that it was well utilised compared to control diets, 40, 60, 80 and 100 % SPBSM.

Highest survival rate ($p<0.05$) in fish fed with control diets, 20 % SPBSM and 40 % SPBSM compared to those fed with 60 % SPBSM, 80 % SPBSM, 100 % SPBSM could be an effect of high fibre, Fe, Mn and Cu level of soaked in pulp baobab seed meals.

5.7.1 Regression of weight gain and dietary inclusions of soaked in pulp baobab seed meal based diets

Polynomial regression of order 5 showed that optimum replacement level of soyabean meal with soaked in pulp baobab seed meal is 14%. This indicates that the high growth performance in fish fed soaked in pulp BSM can be obtained at 14% inclusion level. This is close to the findings of Hassan *et al.* (2015) who stated that inclusion of baobab seed meal at a level greater than 10% is deleterious to fish growth. However, it is not in accordance with the result of Yusuff *et al*. (2008) who recommended that baobab seed meal at 33.33% level can substitute soybean meal with no any decline on rat growth.

5.7.2 Regression of weight gain and dietary inclusion of soaked in alkali baobab seed meal based diets

Polynomial relationship of degree 5 between replacement levels of soaked in alkali baobab seed based diets (%) and mean weight gain by *Clarias gariepinius* fed SABSM showed that optimum inclusion level of SABSM is 16 % at R^2 of 0.83. This indicates that SABSM should be included at 16% in fish diet to obtain a maximum growth performance. This is not in line with 33.33% optimium inclusion level in rats' diets reported by Yusuf *et al.* (2008); variation in methods adopted in preparation of the meals and experimental animal used could be the cause of discrepancy.

5.8. Haematology of *Clarias gariepinus* fed soaked in alkali baobab seed meal based diets

Haematocrit is used as a universal indicator of fish health (NRC, 1993). Similar ($p<0.05$) PCV, haemoglobin, RBC and lymphocytes of fish fed control diets and various inclusion levels of SABSM is an indication that replacement of soyabean meal with soaked in alkali baobab seed meal does not have harmful effect on fish health. This is related to the report of Sola-Ojo *et al*., 2011 who stated that there were no clinical changes in the blood parameters of the layers fed baobab seed meal and the work of Ezeagwu, (2005) who reported that BSM did not affect fish blood. The PCV values recorded in this study fall within the normal range of 20-38% for fish reported by Erondu *et al*. (1993).

5.9. Haematology of *Clarias gariepinus* fed soaked in pulp baobab seed meal based diets

Lymphocytes comprise the leucocytes which help in producing antibodies that assist in protecting the body against infection. Changes in leucocyte could be as a result of stress (Gill and Pant, 1981). Least lymphocytes ($p<0.05$) obtained in fish fed with soyabean meal based diets compared to SPBSM is an indication that high replacement levels of SPBSM resulted in fish.

5.10. Plasma biochemical indices of *Clarias gariepinus* fed soaked in alkali baobab seed meal based diets

Similar ($p<0.05$) plasma indices of fish fed soaked alkali baobab seed meal are indications that effect of varied replacement levels of SABSM and soyabean meal on fish health is the same. Increased replacement level of soaked in alkali baobab seed did not affect ($p>0.05$) the plasma biochemical indices analysed in this experiment. This indicates that inclusion level of SABSM did not affect the fish health.

Similar values of blood urea nitrogen and creatinine reported in *Clarias gariepinus* fed soyabean and the experimental seeds indicate that SABSM did not affect the health of the fish. Urea and creatinine levels present an exact assessment of how healthy the kidneys are functioning (Ajeniyi and Solomon, 2014).

5.11 Plasma biochemical indices of *Clarias gariepinus* fed soaked in pulp baobab seed meal based diets

Total protein, globulin, A:G ratio, aspartate aminotransferase (AST), alkaline aminotransferase (ALT), alkaline phosphate (ALP), bilirubin, creatinine values of experimental fish fed control diets, 20, 40, 60, 80, and 100 % SPBSM were similar ($P>0.05$). Total blood protein content is a good indicator of dietary state of animals (Gad, 2007). Albumin and globulin are the main proteins which perform a key role in the immune response (Kaneko *et al*. 1997). Similar ($p>0.05$) total protein observed is an indication that baobab seed meals have no negative effect on the nutritional status of fish. Abdulazeez *et al*. (2016) also indicated that replacement of soyabean meal with baobab seed showed no detrimental consequence on haematological and serum biochemical parameters of broiler chicken.

The ALP, ALT and AST increase in blood when an organ is damaged (Tietz, 1986). Thus, high levels of ALP, ALT and AST in blood provide information on the damage of organs, especially, liver cells. Similar ($p>0.05$) levels of ALT and AST in the fish fed with the experimental diets and soyabean meal in this study indicates normal functioning of the fish organs.

Blood urea nitrogen reported in this study falls within the normal range of 7 to 20 mg/dL reported by Ajeniyi and Solomon, (2014). Creatinine indicates kidney damage or malfunction (Kumar, 2011). The creatinine falls within the normal range of 0.2-1.5mg/dl reported by Kumar, 2011. Blood urea nitrogen and creatinine were similar ($p<0.05$) in control diets and SPBSM. Abdulazeez *et al*. (2016) also obtained a corresponding result on feeding BSM to broiler chickens.

5.12 Oxidative stress indices of *Clarias gariepinus* fed varied replacement levels of soaked in alkali baobab seed meal based diets

Processing affects total protein, superoxide dismutase and myelenoperoxidase of fish fed soaked in alkali baobab seed meal based diets. Hydrogen peroxide, melanohydehyde, myelenoperoxidase and nitrogen oxide of fish fed with varied replacement levels of soaked in pulp baobab seed based diets are different ($p<0.05$). Fish fed with high inclusion level of baobab seed based diets recorded high H_2O_2 andMDA. It could be related to the existence of higher heavy metals like manganese, iron and copper in SPBSM. Olaifa *et al*. (2004) reported that fish could accumulate heavy metals from contaminated water, food or sediments. Manganese is an important micronutrient that plays a vital function as a component and co-activator of several enzymes in fish (Maage *et al*., 2000), but its excessive levels can trigger autoimmune responses (Gunter *et al*., 2006).

High catalase, superoxide dismutase and glutathione-s-transferase observed in fish fed 100 % soaked in alkali baobab seed meal based diets could be as a result of stress while the least values observed in 20 % SABSM indicates that low replacement level of SABSM in fish diet did not affect fish health.

5.13 Oxidative stress indices of *Clarias gariepinus* fed soaked in pulp baobab seed meal

Hydrogen peroxide (H_2O_2), malondiadehyde (MDA), mylenoperoxidase (MPO) and nitrogen monoxide (NO) of fish fed control diets, 20, 40, 60, 80 and 100 % SPBSM were not similar ($p<0.05$).High manganese in SPBSM could have instigated a rise in H_2O_2 and MDA recorded in fish fed 100 % SPBSM, thus, indicating that soaked in pulp at high inclusion level have adverse effects on fish health.

Extreme level of manganese in the fish can result to neurogenetic disorders by production of free radicals that can stimulate oxidative stress by disturbing the antioxidant defense system of the fish (Aschner *et al*., 2007). Cu, Mn and Fe in the meals exceeded the permissible limit of 10mg/kg, 6.61mg/kg and 150 mg/kg, respectively (WHO, 1989) for all food.

5.14 Proximate compositions of fish fed soaked in alkali baobab seed meal based diets

Fish fed with varied inclusion levels of soaked in alkali baobab seed meal based diets had different proximate compositions. Highest crude protein recorded in fish fed with control diets could be as a result of the better amino acids in soyabean meal compared to SABSM. The least crude fat in fish fed control diets and the highest crude fat obtained in fish fed 100% SABSM could be as a result of oil presence in SPBSM. Paul *et al.* (2018) also observed similar trend in *Oreochromis niloticus* different commercial feeds.

5.15 Proximate composition of *Clarias gariepinus* fed with soaked in pulp baobab seed based diets

High crude protein in fish fed with control diets could be as a result of increase amino acid composition of soyabean meal compared to soaked in pulp baobab seed meal based diets. The least crude fat observed in control might be due to its low crude fat level compared to SPBSM. Opiyo *et al.* (2014)also observed decrease in carcass lipid levels and high protein level of *Oreochromis niliticus* fed commercial feed with low crude fat.

5.16 Histo-pathological of some organs of the *Clarias gariepinus* fed soyabean meal and varied inclusion levels of baobab seed meals

Inclusion of SPBSM at higher levels could have consequently raised manganese levels beyond permissible limit. Hedayati *et al*., 2014 also reported epithelial lifting, hyperplasia, lamellar disfusion in fish (*Rutilus capsicus*) exposed to 60-300 mg/L of Mn. The severity of the damage to gill as the inclusion of SPBSM increased is similar to the result of Bose *et al*., (2013) who also reported that severity of damage to the gills depends on the concentration of the toxicant and the period of exposure. He observed a varied morphological changes and marked alterations in the epithelia in the gill tissue of

fingerlings of *Catla catla* treated with copper and ferrous sulphate. Iron is an essential element in many physiological processes (Valko *et al*. 2005) but the excessive uptake, or disturbances in its regulation, causes cellular injury (Orino *et al*. 2001).

Histopathological alterations observed in adult freshwater tilapia (*Oreochromis niloticus*) exposed to heavy metals were severe edema, hyperplasia, fusion and focal desquamation of the epithelial lining; and epithelial vacuolation of the secondary lamellae. Lifting, swelling, and hyperplasia of the gill epithelium are defence functions of the organ against heavy metals, as these alterations increase the distance across which waterborne irritants must diffuse to reach the bloodstream (Kaoud and El-Dahshan, 2010)

Histology of liver of *Clarias gariepinus* fed with soyabean meal and baobab seed meal showed no observable lesion, except those fed 80 % SPBSM which showed swelling and vacuolation of hepatocytes and those fed 100 % SPBSM that showed patchy hepatocellular necrosis. This is an indication that inclusion of soaked in pulp baobab seed meal at 80% level and above is toxic to fish.

Zinc are required in small quantities for the normal development and metabolism (Srivastava and Kaushik, 2001; Shukla et al., 2002), but if its level exceeds the physiological requirements, it can act as a toxicant. Excess zinc resulted in retardation of growth; and metabolic and pathological changes in various organs in fishes (Sharma and Sharma, 1994; Singh and Gaur, 1997).

Fish contaminated by heavy metals suffers pathological alterations, with consequent inhibition of metabolic processes, hematological changes, and decline in fertility and survival. Maharaja *et al*. (2016) reported that liver of *Lates calcarifer* exposed to lethal and sublethal concentration of copper exhibited swelling, hyperplasia, degeneration of blood vessels, vacuolisation, hypertrophy, necrosis and accumulation of blood vessels.

The absence of lesion in fish fed diets that contained 0 to 100% SABSM and 0 to 60 % SPBSM indicate that the levels of Fe, Mn, Cu and Zn in the diets are still within the acceptable limit. It could also be as a result of increased amount of soyabean meal or adequate aminoacid composition of the diet with consequent increase in feed intake. This

is in accordance with observation of Jacob *et al*., (2013) who reported that higher feed rations reduced the tissue burdens of some metals and ameliorate their toxicity on fish.

Liver showed more lesion than gill and kidney in this study. The concentration of cadmium, lead and copper was higher in liver compared to kidney tissue (Kaoud and El-Dahshan, 2010) when fish was exposed to heavy metals. Kamunde (2003) also observed more accumulation of copper in liver than gill. He reported that no Cu was accumulated in the plasma, kidney and gut. Liver has the ability to degrade toxic compounds but its regulating mechanism can be overwhelmed by increased concentration of the toxic compounds, and could consequently cause structural damage (Brusle and Anadom, 1996).

Tubular epithelial necrosis observed in fish fed 100 % SPBSM could be attributed to Mn, Fe and Cu toxicity of the soaked in pulp baobab seed at higher inclusion level.It could also be attributed to permissible limit (WHO, 1989) for Cu, Mn and Fe for all food (10mg/kg, 6.61mg/kg and 150 mg/kg, respectively) which were exceeded in SPBSM.

Kidney is involved with detoxification and excretion of pollutants but high accumulation of pollutants like zinc affects the detoxification mechanism of kidney and cause histological changes. Histopathological changes in the kidney observed at the exposure of fish (*Channa punctatus*) to zinc are accompanied by high zinc accumulation. Some of the changes include expansion, loss of cellular integrity, dilation, oedema and hypertrophied nuclei of renal tubules, vacuolization, disorganized blood capillaries and necrosis (Gupta and Srivastava, 2006). It has also been observed that if the concentration of heavy metals is very high in the tissue, it may cause severe structural damage (Kumari and Kumar, 1997).

5.17 Cost implications of feeding *Clarias gariepinus* juveniles with soaked in alkali baobab seed meal based diets

The reduction ($P>0.05$) in gross revenuein fish fed 20 % SABSM compared to soyabean meal might be as a result of variance in feed cost of soyabean meal and soaked in alkali BSM; and the production rate of the experimental fish given the diets. Similar result was obtained by Aderolu and Akpabio, (2009) who reported that a reduced price of mucuna bean meal compared to soyabean meal resulted in improved benefit cost ratio. Variance in

economic conversion ratio in fish that received different replication levels of soaked in alkali baobab seed meal obtained in the experiment could be attributed to their varied production rates and cost.

Optimium gross revenue obtained in 20 % SABSM and the least recorded in 100 % SABSM could be a consequence of reduced growth rate of fish that received higher inclusion level of the meals. This is in accordance with the findings of Mwale *et al*. (2008), Chimvuramahwe*et al*. (2011) and Chisoro *et al*. (2017) who reported reduction in profit as the inclusion level of baobab seed in feed increased.

5.18Cost implications of feeding *Clarias gariepius* juveniles with soaked in pulp baobab seed meal based diets

The highest gross revenue recorded in fish fed with 20% SPBSM indicated that replacement of soyabean meal withSPBSM at 20% was more profitable than those fed at 0, 40, 60, 80 and 100 % replacement levels. Toyosi *et al*. (2018) also reported that inclusion of fishmeal with 20% cassava leaf meal in the diets of *Clarias gariepinus* resulted in a better benefit cost ratio than when the fish were fed with either fishmeal or cassava diet alone. The result of this experiment is close to the findings of Oladunjoye *et al*. (2008) who reported lowest cost of production of grower rabbit at 10% inclusion of baobab seed meal. Chisoro *et al.* (2017) also reported that a partial replacement of soyabean meal with BSM was cost effective because it reduced production cost in poultry feed.

Lesser ECR in 20 % SPBSM compared to fish fed soyabean meal showed that fish fed with 20% SPBSM are better than those fed with control diets. Moutinho *et al*. (2017) also reported improved economic parameters at lower economic conversion ratio (ECR) when he included 50% non-ruminant meat and bone meal (MBM) in gilthead seabream diets. However, a rise in ECR at higher inclusion level of SPBSM showed a decline in profitability. Piedecausa *et al*. (2007) stated that least ECR value obtained when soyabean oil was included in sharpsnout seabream diet indicated the least expensive, best economic conversion ratio (ECR) and profitability.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Processing enhanced the nutritional composition of baobab seed by increasing their crude protein, mineral composition and reducing some of its antinutritional factors. Only toasted baobab seed meal showed decline in crude protein because of high temperature that was used in toasting the seed.

16% inclusion of soaked in alkali baobab seed meal showed an optimum increase in growth rate of fish. Soaked in pulp baobab seed meal at 14% had the best positive effect on growth and nutrient utilisation rate of experimental fish, while those fed with 100% had the least.

Soaked in alkali and pulp had similar effects on all the haematology indices observed in this study, except lymphocytes of the fish. Fish fed with soaked in pulp had higher lymphocytes value compared to those fed with soaked in alkali. The highest white blood cell count and platelets were reported in fish fed with soyabean meal. White blood cell counts and platelets of fish fed soyabean meal were high compared to those fed with varied inclusion levels of baobab seed meal.

Aspartate aminotranferase, alanine aminotransferase and alkaline phosphate of fish fed with soyabean meal and baobab seed meal were similar. Histopathology of fish fed with soaked in alkali and pulp indicates that fish fed with soaked in pulp at 60% and above level have adverse effects on fish health.

Inclusion of baobab seed meal at 20% has positive effect on growth performance, nutrient utilisation and health of fish. Soaked in alkali and pulp had similar effect on fish. Soaked in alkali at 20% had the best growth, nutrients' utilisation and economic impact on fish.

6.2 RECOMMENDATIONS

Based on the observations on the use of baobab seed meal as a fish feed ingredient in this study, the following are recommended:

1. Baobab seeds should be processed to enhance its nutritional composition;
2. Toasting, soaking in pulp and alkali treatments can be given to plant based aquafeed without compromising its digestibility by *Clarias gariepinus*;
3. Soaked in pulp and alkali baobab seed meal can be included at 14 % and 16 % respectively in *Clarias gariepinus's* diet without compromising its growth performance and nutrients utilisation;
4. 40% soaked in pulp baobab seed meal in *Clarias gariepinus's* diet is recommended for better health performance;
5. Soaked in alkali baobab seed meal can be used to replace soyabean meal in *Clarias gariepinus's* diet up to 80% inclusion level without any adverse effect on its health
6. For better economic efficiency, inclusion of 20% soaked in pulp and alkali baobab seed meal in *Clarias gariepinus*'s diet is recommended;
7. Further research on of reduction of fibre and improvement of aminoacid composition of baobab seed meal is recommended.

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CONTRIBUTIONS TO KNOWLEDGE

1. Autoclaving, soaking in alkali, pulp, water and liquor enhance nutritional composition of baobab seed meals by increasing its crude protein and reducing its antinutritional factors.
2 Soaked in pulp and alkali baobab seed meals are well digestible by *Clarias gariepinus* and also have the best growth performance compared to toasted, autoclaved, soaked in water and liquor baobab seed meals.
3 Optimum inclusion of soaked in pulp and alkali baobab seed meal were at 14 % and 16% respectively, in *Clarias gariepinus's* diet for improved growth and health.
4 Substitution of soyabean meal with soaked in pulp and alkali baobab seed meals in *Clarias gariepinus's* diet at 40% replacement level and above reduced its growth performance and nutrients utilisation.
5 Soaked in pulp and alkali baobab seed meal did not have any negative impact on *Clarias gariepinus's* health performance.
6 For better economic efficiency, inclusion of 20% soaked in pulp and alkali baobab seed meal in *Clarias gariepinus*'s diet is recommended.