GROWTH PERFORMANCE AND PROTEIN DIGESTIBILITY IN *Clarias gariepinus*, BURCHELL, 1822 FED SOYABEAN MEAL BASED DIETS SUPPLEMENTED WITH AMINO ACID AND PROTEASE

BY

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Abstract

Soyabean meal (SBM) could be a suitable replacement for the expensive fishmeal but for its low digestibility in fish. Appropriate dietary supplements of amino acid and protease can improve digestibility of SBM based diets. However, information on supplemental Dietary Amino Acid (DAA) and protease in such diets for optimum growth in cultured fish is limited. Therefore, growth performance and amino acid digestibility in *Clarias gariepinus* fed SBM based diets supplemented with DAA and protease were investigated.

Soyabean grains processed by roasting and solvent (n-hexane) extraction were assessed for Crude Protein (CP), lysine and methionine content using standard methods. Six diets containing varied combinations of lysine+methionine (g/kg) supplementation were formulated for each of Roasted SBM (RS) and Solvent extracted SBM (SS): RS_1, SS_1 (Control: without supplementation), RS_2, SS_2 (0+10), RS_3, SS_3 (2.5+7.5), RS_4, SS_4 (5.0+5.0), RS_5, SS_5 (7.5+2.5) and RS_6, SS_6 (10+0) respectively. Furthermore, protease (ppm/kg) was supplemented in each of RS and SS: RS_0, SS_0: (control: without supplementation), RS_{100}, SS_{100} (100), RS_{200}, SS_{200} (200), RS_{300}, SS_{300} (300), RS_{400}, SS_{400} (400) and RS_{500}, SS_{500} (500), respectively. *Clarias gariepinus* (n=1440; 19.70±0.20g) were fed to satiation with the diets for 12 weeks in triplicate in a completely randomised design. Final weight (FW, g) and Feed Conversion Ratio (FCR) were calculated. Blood (5mL) was sampled for Packed Cell Volume (PCV, %) and Alanine Amino Transaminase (ALT, IU/L). True Methionine (TM) and True Lysine (TL) digestibility were determined using standard procedures. Data were analysed using descriptive statistics, polynomial regression and ANOVA at $\alpha_{0.05}$.

The CP, lysine and methionine were 52.5±2.8%, 58.5±2.5%; 2.3±0.0%, 2.8±0.1% and 0.5±0.0%, 0.6±0.01% in RS and SS, respectively. Least FW 32.60±3.59 and 34.8±1.2 were recorded in RS_6 and SS_2, respectively while RS_5 (36.4±4.6) and SS_1 (43.4±4.0) had the highest. The FCR ranged from 3.0±0.7 (RS_5), 2.3±0.2 (SS_3) to 3.8±0.5 (RS_1) and 3.1±0.3 (SS_2). The PCV were 21.0±1.7, 27.0±2.7 and ALT 19.3±5.0, in RS_1 and RS_5, respectively. The PCV and ALT ranged from 22.7±3.8, 23.0±2.0 to 25.7±1.5, 31.0±3.6 in SS_1 and SS_5, respectively. Significantly least (90.9±0.3) and highest (92.9±0.4) TL were obtained in RS_2 and RS_4, respectively while it ranged from 86.2±1.0 (SS_4) to 95.9±0.2 (SS_5). Optimal FW were 35.7 and 35.0g at 0.6+0.4g/100g

supplementation in RS (R^2=0.8) and SS (R^2= 0.7). The FW were least in RS_{100} (31.2±3.4) and SS_0 (32.0±0.7), highest in RS_{400} (46.9±9.25) and SS_{400} (44.6±2.9), FCR least in RS_{400} (1.4±0.3) and SS_{400} (1.6±0.2), highest in RS_{100} (2.8±0.6) and SS_0 (2.8±0.0). The PCV were 23.5±5.0 (RS_{500}) and 27.0±1.0 (RS_0), ALT 20.3±0.6 (RS_{100}), 34.7±13.3 (RS_{400}) TM 77.8±0.2 (RS_{500}), 93.5±0.0 (RS_{200}) and TL 92.7±0.1, (RS_{300}), 96.2±0.0 (RS_{100}). In SS, PCV ranged from 22.7±4.6 (SS_{500}) to 31.3±4.0 (SS_0), ALT 20.0±1.0 (SS_0) to 26.3±3.1 (SS_{400}), TM 84.3±0.1 (SS_{100}) to 95.8±0.2 (SS_0) and TL 87.7±0.1 (SS_{100}) to 95.9±0.1 (SS_{200}). Optimal FW were 42.0 and 41.0g at 400 ppm/kg supplementation in RS (R^2=0.6) and SS (R^2=0.7), respectively.

Lysine+methionine and protease supplementation at 0.6+0.4g/100g and 400ppm/kg could optimally improve growth and digestibility in *Clarias gariepinus* fed soyabean meal based diets without negative impact on fish health.

Keywords: Supplemental amino acids, Soyabean meal, *Clarias gariepinus*, Protein digestibility

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CERTIFICATION

We certify that this work was carried out by OYEDOKUN, Oyeleye Jacob under our supervision in the Department of Aquaculture and Fisheries Management, Faculty of Renewable Natural Resources, University of Ibadan, Ibadan, Nigeria.

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DEDICATION

This work is dedicated to the glory of God Almighty, the entire family of Deacon (Engr) and Mrs. Samuel Oyedokun, my dearest wife and daughters (Oluwademiladeayo and Oluwagbemisoke).

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Introduction

1.1 Background of study

Fish production universally has being developing gradually in the last fifty years with fish supply at 3.2 percent annual average rate increase, overtaking world population growth at 1.6 percent while the world per capital fish consumption also increased from 9.9-19.2 kg between 1960 and 2014 (FAO, 2016). Aquaculture production globally developed at an average annual rate of 6.2 percent in the period between 2000 to 2012 (9.5 percent in 1990 to 2000), from 32.4 to 73.8 million tonnes (FAO, 2016). This notable improvement has been propelled as a result of increased population, incomes and urbanization which had been facilitated through fish production expansion and further resourceful channels of distribution. A similar geometric growth as stated above was also experienced in Africa albeit at a slow rate (Adedeji and Okocha, 2011).

FAO (2016) reported Nigeria as the second largest aquaculture producer in Africa and twenty first aquaculture producer in the world, with production output of over 313.2 thousand tonnes in 2014. Ozigbo *et al.* (2013) also acknowledged that Nigeria is one of the largest fish consumers with a total consumption of more than 2 million metric tonnes of fish annually. Reports from FDF (2014) estimated that, Nigeria fish production from aquaculture was expected to reach about 671,492 metric tonnes by the end of 2015. Yet, Nigeria is still left with the deficit of about 1.4 million tonnes. (FDF, 2014; Ozigbo *et al*., 2013). The deficit is being augmented with importation of more than 910,000 metric tonnes of fish and local catch was assessed to be 450,000 metric tonnes yearly (Ozigbo *et al*, 2013).

The aquaculture farms in Nigeria are still growing compared with large world market opportunity for its products and marketing. It is therefore imperative that aquaculture needs to be promoted to provide a viable socio-economic alternative to importation and capture fisheries. It also, reduces efforts as well as contributing to the gross domestic production (GDP), provide employment, which could be a principal source of livelihood

for Nigerians (Adedeji and Okocha, 2011). However, there are numerous constraints hindering aquaculture production and development in Nigeria.

The constraints faced by aquaculture in Nigeria include among others; inadequate quality fish seed for stocking ponds, dearth of information on modern technologies in aquaculture due to poor extension services, lack of viable cooperative societies, poor infrastructural facilities, poor funding by government and high cost of fish feed (Ugwumba and Nnabuife, 2008). Fish feed production has posed lot of threat to aquaculture development in Nigeria due to the ever increasing cost of fish feed ingredients. Fish production depends on the augmentation of adequate feeding practices to meet up with nutritional requirements of the desired species. Also, fish production economically dependent on adequate supply of lower feeds cost with adequate nutritional quality. Feed is the major cost in fish industry; it represents about 40-70% of the variable cost of commercial aquaculture operation for many fish species such as *Clarias gariepinus* (Jamu and Ayinla, 2003). Likewise, Udo *et al*. (2011) noted that cost of fish feed contributed about 40-60% of recurrent cost in major aquaculture farms.

Feed production costs are driven by the price of fishmeal, a significant protein source in fish feed. Also, it competition in the livestock industry for micro nutrient, macro nutrient and essential amino acids used in feed production has contributed to it scarcity and high cost. Therefore, non-conversional feeds ingredients (such as soyabean meal, groundnut meal, palm kernel meal, cotton seed meal etc.) has been investigated by scholars (Adeniji, 2008; Ajani *et al*., 2016) and fish farmers in order to reduce cost of production by substituting the expensive fish meal with plant proteins. The non-conversional ingredients are cheaper than fish meal and they are available and accessible in Nigeria.

Soyabean cake is commonly used plant protein ingredient in livestock feeds globally especially in Nigeria. Their demands are high due to their expansive usage in industries (Carrillo *et al*., 2012). Soyabean cake have been extensively used to replace fish meal diet due to their agreeable flavour which makes it highly palatable (Adeniji, 2008). Though fish meal is highly digestible than soyabean meal, aquaculture nutrition research is trending toward decrease and total removal of fish meal (Craig, 2004). The substitution

of fish meal with plant protein or grain by-products are significant for the development of cheaper fish feeds and improving the nutritional quality (Ajani *et al*., 2016).

The presence of trypsin inhibitor (anti- nutritional factors) make soyabean not easily digested and used up by fish when equated with animal protein sources (Siddiqui *et al.*, 2013). Researches have been focused on digestion improvement, nutritional quality and enhancement of optimum performance of fish species using appropriate processing methods. These include solvent extraction (Subuh *et al.,* 2002), roasting (Marty *et al.,* 1994), and micronisation (Woodworth *et al.,* 2001). Each of the processing methods has been shown to inactivate antinutritional factors and improve digestibility and utilization of plant protein and energy sources, but it remains low compare to fish meal (Drew *et al*., 2005). Although, success have been recorded in the complete substitution of fish meal with differently processed soyabean meal in the diet of fish (Wu *et al*., 2004; Ajani *et al*., 2016). Reports on the effect of complete substitution of fish meal with soyabean meal in *Clarias gariepinus* diet still remained scanty.

Soyabean cake is the most acceptable plant protein source due to better amino acid profile (Ogbonna *et al*, 2014) but it is deficient in methionine and have low cystein (Davies and Ezenwa, 2010). The limiting amino acids in soyabean cake limit their inclusion in fish diet to 45% (Eyo, 2003; Siddiqui *et al*., 2013), despite that, it is ready availabile. Dietary supplementation of amino acids provides new strategies to the development of balanced amino acid fish feeds that can balance ecological effects on cultured fish species, increase performance, and profitability of the aquaculture industry (Li *et al*., 2009). Methionine and Lysine are the main limiting and essential amino acids in soyabean based diets for fish.

These two amino acids are required for effective feed consumption, growth rate and carcass composition. Lysine is the most essential amino acids when replacing fish meal with plant protein sources during commercial fish feeds production (Mai *et al*., 2006). Also, its limitation in fish feed affects performance and carcass quality *Clarias gariepinus* (Rezae *et al*., 2004). In many fish diets, the first limiting amino acid is usually methionine especially those having higher inclusion of plant protein sources, such as groundnut cake, soyabean meal and corpa meal (Mai *et al.,* 2006). Methionine also, acts

as lipotropic agents and its role as an amino acid in balancing crude protein (Hesabi *et al*., 2006).

Findings have revealed that lysine and methionine supplementation in *Clarias gariepinus* diet improved feed quality, performance, digestibility and profitability of the aquaculture investors. Osti and Pandey (2004) found that supplemental amino acid (lysine and methionine) in low protein and high protein diet (without lysine and methionine supplementation) on broiler chicken performance indicated that they improved feed consumption, increased body weight of broiler and reduces cost of broiler production. Nwanna *et al.* (2012) posited that supplementation of DL- methionine in *Cyprinus carpio* diets significantly improved protein digestibility, Final weight, feed conversion efficiency and carcass quality. Thu *et al*. (2009) earlier reported similar observations when lysine utilisation efficiency at marginal lysine intake in *Oncorhynchus mykiss* fry was assessed. Fagbenro *et al*. (1998; 1999) also observed that inclusion of methionine and lysine could enhance Final weight of *Clarias gariepinus*. Ajani *et al*. (2016) found that complete substitution of fish meal with soyabean meal is achievable when methionine is supplemented in *Oreochromis niloticus* diet. Ochang *et al*. (2007) reported that amino acid requirement of fish diet could be improved in the plant protein based diet when it is supplemented with dietary lysine and methionine. Therefore, there is need to improve the utilisation of dietary lysine and methionine in plant protein based diet for *Clarias gariepinus.*

The digestibility efficiency and feed utilization in the body of animals depends on feed quality and growth of fish which depends on the presence of either endogenous or exogenous enzymes (Thu *et al*., 2009). Introduction of exogenous enzymes in the livestock industry has helped to improve the nutritive value of animal feed by reducing manure's nutrient, which have high environmental beneficts in areas with concentrated production. Several reseachers have documented the environmental advantages of using exogenous enzymes such as xylanase, phytase and protease in animal diets (Nagaraju and Nielsen, 2011; Oxenböll *et al*., 2011) in either pig or poultry diets. These enzymes have been established to increase the digestibility of poorly digested intakes than those properly digested diets (Scott *et al*., 2001). Whereas exogenous protease supplementation

in livestock diet has been shown as a beneficial approach to enhanced nutritional value of soyabean meal (Oxenböll *et al*., 2011). There is therefore the need for aquaculturist to explore the possibility of augmenting the maximum use of the nutrients contained in plant protein supplemented with dietary protease.

Dietary exogenous protease is still at infant stage in aquaculture sector but several works have been carried out on protease and other enzymes (phytase, carbohydrase, amylase) in livestock sectors with the objective of assessing the action of an enzyme or combinations of enzymes on broilers performance (Cowieson *et al*., 2003; Cowieson and Adeola, 2005; Iwaniuk *et al*., 2008). Results from the studies showed improvement in digestion and utilization of nutrients in animal production apart from making diet formulation more flexible and cost effective. Romero *et al*. (2013) reported that dietary proteases inclusion in maize-soya-based diets increased the digestibility of protein by improving protein hydrolysis and inactivating anti-nutrients such as trypsin inhibitors (Ghazi *et al*., 2002). Similar studies on cultured fish could probably enhance it feed efficiency.

Therefore this project was aimed at assessing the performance and digestibility of *Clarias gariepinus* fed processed soyabean based diets supplemented with dietary amino acid and protease.

1.2 Problem Statement

In Nigeria, *Clarias gariepinus* have been the most widely cultured fish species. They reach table size in relatively short time, hardy in nature, easy to breed in captivity and acceptable (Fagbenro *et al.,* 2012). The need to increase production of this fish species is imperative but this desire has a drawback resulting from high cost of commercial feeds and environmental issue resulting from undigested nutrients discharge from fish farms into the environment (Adeniji, 2008). To achieve rapid progress and achievement in fish culture therefore depends on obtainability of good quality and relatively cheap feed (Eyo, 2003; Udo *et al*., 2011)

The rapid growth in fish culture and poultry production globally indicated that crisis could precipitate in the aquaculture and livestock feed industries in the near future (Udo *et al*., 2011). Human's food is not included in this consideration because livestock, fish

and humans can consume the basic food commodities during emergencies or time of scarcity. Feedstuffs are eaten first by humans thus, alternative sources of fish feed through non-conventional means is imperative. The most critical feedstuffs are those that would supply protein and essential amino acid. Fish meal comprise of complete EAAs profile required to meet the protein requirements of fish species. There are reports of plant protein sources usage as alternative to fish meal in the growth of *Clarias gariepinus (*Adeniji, 2008).

The sources of plant protein are the most frequently used legumes in fish feed formulation such as soyabean. The main limitation in the use of soyabean meal is to ascertain the best processing methods suitable for the optimum performance of fish species. These include solvent extraction (Subuh *et al.,* 2002), roasting (Marty *et al.,* 1994), and micronisation (Woodworth *et al.,* 2001).

Rackis *et al.* (1986) and NRC (1994) reported that heat treatment of soyabean without conceding the nutritional value, inactivates anti-nutritional factors, improves nutritional factors, improves amino acid balance, reduce fibre content and increase energy when equated with other oilseed. Therefore, the use of soyabean cake in aquaculture diet is important as a result of its nutritional value and cost friendly compared to other plant sources (Tidwell and Allan, 2002). Also, there have been reports on improvement in the nutrient digestibility of soyabean, but limited reports have been documented on their amino acid digestibility in *Clarias gariepinus* (Gatlin and Hardy, 2002; Zhou *et al.,* 2006). Therefore, there is the need to ascertain the availability of limiting amino acid and optimum digestibility of legume oilseeds, particularly, soyabean when fed to *Clarias gariepinus*.

The potential negative effects of nitrogen and phosphorus dischargred from aquaculture systems to the environment have been reported and needed considerable attention (Gatlin and Hardy, 2002). This negative effect is as a result of undigested nutrients in the excretion. Gatlin and Hardy (2002) also ascertained that more efficient utilisation of dietary phosphorus and nitrogen from feed nutrients would contribute to decreasing the environmental impact in aquaculture. Exogenous phytase have been extensively studied for its implication in the diets of livestock and aquaculture (Akpoilih *et al*., 2016).

Though, it has been stated to have significantly improved dietary phosphorus and calcium utilisation (Nwanna and Schwarz, 2007; Akpoilih *et al.,* 2016). Augspurger and Baker (2004) noted that phytase supplementation could not improve nitrogen utilisation.

Similar reports have shown that dietary protease could improve the utilisation of nitrogen rich feed ingredient, make diet formulation more flexible and cost effective in livestock production (Lemme *et al.,* 2004; Freitas *et al.,* 2011). Supplementing dietary exogenous protease in poultry feed has been proven to be a useful approach to increase nutritional potential of soyabean meal (Cowieson and Adeola, 2005). Pettersson and Pontoppidan, (2013) also concluded that dietary exogenous protease improves amino acid digestibility, has positive impact on environment by enhancing protein digestibility and helps reducing nitrogen excretion in fish farms. Reports on protease in aquaculture especially with reference to *Clarias gariepinus* have been very scanty. Also, there is dearth of information on the environmental impact in reducing nitrogen excretion in ponds and cost effectiveness in *Clarias gariepinus* production (Guggenbuhl *et al*., 2012). Therefore, there is need for more study to provide baseline information on effect of protease requirement of *Clarias gariepinus*. This study was intended to contribute to providing information on protease activity level needed for *Clarias gariepinus* growth and amino acid digestibility when fed soyabean based diet.

1.3 Justification of the study

The advent of dietary exogenous enzymes has encouraged lot of consideration by farmers and researchers in the field of animal production over the years. Researches are ongoing to ascertain its efficacy in animal production and aquaculture sector. Thus far, results from its application in poultry production have shown tremendous improvement in nutrient utilisation and digestion of feeds (Cowieson and Adeola, 2005). The common dietary enzymes already investigated include phytase, protease and carbohydrase (Cowieson and Adeola, 2005).

Studies have shown that carbohydrase supplementation improved energy and dry matter digestibility in monogastric animal nutrition (Nortey *et al.*, 2007; Li *et al*., 2009). In poultry, supplemental carbohydrase improved energy utilisation in corn-soyabean meal

diets (Yang *et al*., 2010). Also, protease have been supplemented in swine and poultry diets regularly for decades as an enzyme admixtures comprising of glucanases, pectinases, amylases, xylanases and other activities (Cowieson and Adeola, 2005). Protease has been used alone in soyabean meal treated with *Bacillus subtillis* with the aim of reducing the harmful effect of proteinaceous antinutrient in weaner piglets fed soyabean (Caine *et al*., 1997). Rooke *et al*. (1998) noted body Final weight and feed conversion ratio improvement when acid fungal (Aspergillus) and alkaline bacterial (Bacillus) protease were supplemented in broiler and piglet diets.

Phytase has been a staple compliment of livestock feeds to improve phosphorus availability in oilseeds and grains by dephosphorylation of myo-inositol hexakisphosphate (phytate) (Cromwell *et al*., 1993). Research findings with Rainbow trout (Vielma *et al*. 2000) and *Clarias gariepinus* (Li and Robinson, 1997) demonstrated phytase effectiveness in increasing the availability of phosphorus in fish. Though, these studies established the effect of rearing water temperature on efficacy and optimal dietary phytase level. Li and Robinson (1997) discovered that cost of supplementing phytase to *Clarias gariepinus* diets was almost equivalent to the savings related with removal of dietary inorganic phosphorus.

Despite the successes recorded from the use of dietary exogenous protease in livestock, its exploration and usage in aquaculture sector is still at infant stage. Presently, scanty report is available on the supplementation of protease in aquaculture sector (Cowieson and Adeola, 2005) compared with phytase. Therefore, study could be conducted to provide information on protease supplementation in aquaculture on *Clarias gariepinus*. This study was therefore targeted among others at assessing the effect of dietary protease supplementation on protein utilisation and amino acid digestibility of *Clarias gariepinus* fed soyabean based diet.

1.4 Main Objective of Study

To assess the effect of dietary supplement of protease and amino acid on growth performance and essential amino acid digestibility in *Clarias gariepinus* fed plant protein based diets.

1.4.1 Specific Objectives

- To determine the effect of processing on chemical composition of soyabean meal.
- To determine the effect of feeding processed soyabean meal based-diets supplemented with lysine and methionine on the performance of *Clarias gariepinus*.
- To assess the growth performance and amino acid digestibility by *Clarias gariepinus* fed soyabean based-diet supplemented with exogenous protease.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutrient requirement of *Clarias gariepinus*

The dietary protein of *Clarias gariepinus* (*C. gariepinus*) has been extensively studied. The optimal protein levels in *C. gariepinus* diets are affected by some factors, including fish size and age, non-protein energy in the feed, dietary protein quality and source, feeding levels, natural food availability and culture conditions (Kaushik, 2000; Wu *et al.,* 2004). Proteins, carbohydrates, lipids and vitamins are the main constituents in the diet of fish which are imperative for growth and source of energy (Wu *et al.,* 2004). In recent years, fish nutrition has advanced with the development of balanced commercial diets that enhance optimum fish growth and health (Kaushik, 2000).

Helfrich and Craig (2002) indicated that nutrition is the main factors among others prompting the capacity of fish to reach genetic potential for reproduction, growth and longevity. Efficient production and growth of fish in the culture systems depends entirely on feeding complete feed at appropriate rate with due considerations to the dietary requirements of fish which ought not to be exceeded (Wu *et al.,* 2004). The nutrient requirements of *Clarias gariepinus* are based on informations available on various least-cost feeds of adaptable quality that are available in the market throughout the region (Wu *et al.,* 2004).

Protein is an important nutrient for fish growth and maintenance, it also serve as the basic component of animal tissues. During maintenances, the fish needs to replace worn-out tissues with proteinous products such as internal epithelial cells; hormones and enzymes, which are essential for the appropriate functioning of the body, and are rapidly recycled. Requirement of protein is apparent as crude protein constitute 45-47% (tissues dry matter) (Kaushik, 2000). According to Jobling (1994), proteins are comprised of nitrogen (15-18%), carbon (50-55%), oxygen (21-23%), sulphur (0-4%) and hydrogen (6-8%).

Several reports have been documented on the basic requirement of nutrient need by *Clarias gariepinus* (Faturoti *et al.,* 1986; Kaushik, 2000; Wu *et al.,* 2004). Machiels and Henken (1985) reported that *Clarias gariepinus* fed varying crude protein purified feed ingredientst needs dietary protein content that is more than 40% for maximum growth regardless of dietary energy level. Ufodike and Ekokotu (1986) reported that *Clarias gariepinus* diet formulated to 50.2% protein level performed better in term of growth than diets formulated to 38.0% or 62.9% protein. Ayinla (1988) reported protein requirements of *Clarias gariepinus* at the different growth phases as follows: fingerling to juvenile (37.5%), juvenile to adult (32.5%) and fingerling to adult (35.5%).

However, several reports (Akinwole and Faturoti 2007; Adewolu and Adamson 2011; Hecht 2013) vary with the report of Faturoti *et al*. (1986) and Ayinla (1988) which indicated that fingerling stages of *Clarias gariepinus* required 31 – 40% Crude Protein; juveniles 31 – 40% crude protein; adults, 40% crude protein and broodstock, 40% crude protein. Furthermore, Keremah and Beregha (2014) noted that 35% crude protein resulted in best growth performance for *Clarias gariepinus* fingerlings in the study when compared with other varying levels of crude protein in the study.

2.2 Plant protein sources: Alternative to fishmeal in *Clarias gariepinus* diet

Fishmeal substitution with plant proteins has shown to decrease growth performance in omnivorous and carnivorous species (Hecht 2013). Introduction of plant protein ingredients such as cotton (Gossypium sp.) seed meal, sunflower (*Helianthus annuus*) cake, wheat (*Triticum aestivum*) middling's, soyabean, corn, and cotton seed meal in fish diet have shown to reduce fish growth rate when compared with fishmeal (Nyina-Wamwiza *et al*., 2007; Li *et al*., 2009). Likewise, leaf meal of *Amaranthus spinosus* (Adewolu and Adamson 2011), inclusion in fish diet also had an adverse effect on growth rate compared to fish meal diets. The reduction in growth rate of fish fed plant proteins could be as a result of the following reasons: anti-nutritional factors (ANFs), imbalanced amino acid composition or poor digestibility (Tiril *et al*., 2008). Substitution of plant proteins in fish meal based diet could also have adverse influence on feed palatability and consequently decrease feed intake (Nyina-Wamwiza *et al.,* 2007, Tiril *et al.,* 2008).

Research findings had been reported to have tackled the earlier challenges as growth rate of *C. gariepinus* were improved (Akinwole and Faturoti 2007; Ogbonna *et al*., 2014). Supplementation of processed plant protein ingredients such as soyabean meal (Ogbonna *et al*., 2014), *Leucaena leucocephala* seed meal (Sotolu and Faturoti, 2009), sesame seed meal (Fagbenro *et al*., 2010), groundnut cake (Davies and Ezenwa, 2011), fermented sickle pod seed meal, moringa meal (Dienye and Olumuji, 2014), water hyacinth meal (Sotolu, 2010*), Moringa oleifera* leaf meal (Dienye and Olumuji, 2014) and cooked *Jatropha curcas* seed meal (Jimoh *et al*., 2016) improved growth performances in *C. gariepinus*.

Different inclusion rates of the plant protein have been suggested by researcher to improve growth rate, improved digestibility, increase palatability of feed and inactivates anti nutrient in the feed. Dienye and Olumuji (2014) informed that 10% inclusion of *Moringa oleifera* leaf meal to be suitable to substitute fishmeal in *Clarias gariepinus* diet with adverse toxicological effect. Also, Enyidi *et al*. (2017) reported that the combination of soyabean meal and bambara nut meal could favourably substitute more than 60% of fish meal in feed for *Clarias gariepinus* with better feed conversion ratio. Other plant protein sources have been used to substitute soyabean meal in the presence of fishmeal in the diet. Findings revealed that boiled and cooked *Jatropha curcas* seed meal could substitute soyabean meal up to 30% and 25%, respectively (Jimoh *et al*., 2016). Also, substitution of sunflower and sesame seed meal with soyabean meal up to 45% improved growth, nutrient utilisation, carcass quality (crude protein) in *Clarias gariepinus* and reduced feeding cost (Fagbenro *et al.,* 2010). Soyabean meal being a good substitute to fish meal and have been assessed in quite a large number of nutritional studies with a better results of about 90% maximum level of inclusion with fish meal, based on fish species. According to, Gatlin and Hardy (2002), diets with 90% substitution of soyabean meal for fish meal in red drum resulted in lower performance and decreased feed efficiency. Ajani *et al*. (2016) report that soyabean meal could partly substitute fishmeal up to 46.7% and could substitute it completely when supplemented with methionine in *Oreochromis niloticus* diet without any negative effect on feed utilization, growth and health status of the fish.

2.3 Soyabean meal

Soyabean meal is obtained from soyabean seeds that are exposed to various treatment or processing methods, (such as cakes, expellers) which are the by-product from the oil industry are used in animal nutrition. Processing methods which does not involves extraction of oils are boiling, flaking, roasting of soyabean seeds and the processing that involves extraction of oil from the seed are mechanical method and by solvent extraction (Van Eys *et al*., 2004). Soyabean meal characteristically contains higher level of protein and amino acids and has consistent nutritional value.

Soyabean meal has been the commonly consumed protein source in livestock feed production globally most especially in fish diet (Shipton and Hecht 2005). It have been estimated that about 63% of all protein sources consumed by animals are from soyabean meal as estimated and up to 98 % of the plant protein utilised in poultry and aquaculture feeds emanate from soyabean meal (Soyabean meal INFO center, 2010). Soyabean products have been integrated into fish diet to cut down the quantity of fish meal used, which is costly and accounts for a bulk in the cost of production in aquaculture. Studies revealed that soyabean have been incorporated in aquaculture and other animal feeds (Fapohunda, 2012). The primary plant protein source in *C. gariepinus* diets is soyabean meal (Shipton and Hecht, 2005). Also, soyabean meal has achieved considerable success in replacing fish meal up to 40 %, particularly in feeds for omnivorous fish. Drew *et al*. (2005) reported that 50% of the diet containing heat-treated full-fat soyabean meal with half of the fish meal in the control diet improved the growth of carp at about 60 to 65% in the control diet. Soyabean meal is the most favorable feed stuff for complete or partly replacing fish meal in fish diet (Fapohunda, 2012). Commercially produced aquaculture feeds contain 25 % to 45 % crude protein (Eyo, 2001), while soyabean possesses not less than 45 % crude protein. Also, due to its excellent biological values in oil, high quality in calcium and phosphorus has made it the most preferred plant protein for fish feed (Fagbenro *et al*., 2003).

Nutritive value of soyabean meal is constrained by sulphur amino acids and tryptophan. Soyabean meal contained highly digestible protein and amino acids. FAO (2012) reported that utilisation and digestibility of protein fraction increases in a well processed soyabean meal when fed to fish species. FAO (2012) reported apparent protein digestibility of

soyabean meal by carp, trout and red sea bream have 90-93%. Soyabean meal possesses better amino acid profile when equated with cereal grain and even other legume seeds. The protein content in cereal grains and plant concentrates generally do not contain complete amino acid profile and contain incomplete proteins. They are normally lacking in most of the essential amino acids mainly methionine and lysine. Hence using them in fish diets may need supplementation from these essential amino acids (McDonald *et al*., 2001).

The use of soyabean products for aquatic animals has it own limitations which are as a result of varied crude protein, sulphur amino-acids, higher carbohydrate and low crude fat compared to fish meal (Ogbonna *et al*., 2014). The anti-nutritional factors (ANF) present in the seeds need to be addressed, though they are destroyed during processing of soyabean meal (Fapohunda, 2012).

2.3.1 Anti-nutrients in soyabean meal

Makkar *et al.* (1993) discribed anti-nutrients as "materials which by themselves or through their metabolic products arising in living systems interfere with feed utilisation and affect the health and production of animals". These substances are classified based on some factors such as protein utilisation in digestion (protease inhibitors, tannin and lectins), mineral utilisation (phytates, gossypol pigments, oxalates and glucosinates), anti-vitamins and other substances such as mimosine, mycotoxins, nitrate, cyanogens, phytoestrogens, saponin, alkaloids and photosensitising agents.

Soyabeans possesses various types of ANFs which could negatively disturb its nutritional value or be harmful to the fish health except they are well processed (Yasothai, 2016). Trypsin inhibitors, phytic acid, antigenic factors, oligosaccharides and lectins are the major ANFs in soyabeans, but lectins and trypsin inhibitors are heat labile which could be destroyed with heat (Li *et al.,* 2009; Song *et al.,* 2010). Liener (2000) further ascertained that adequate thermal treatment (Trypsin inhibitor) and enzyme suplemetation (Non-Starch Polysaccharide (NSP) enzymes, phytase) can inactivate or destroyed some ANFs that are of nutritional significance and some ANFs are not affected by any of these processing methods. Attaining a high quality soyabean meal suitable for

feeding animals and with quality nutritional value, suitable roasting condition including temperature, moisture content, duration of heating and shear force is needed. Under-processing could allow considerable amount of ANFs in soyabean meal remain active resulting in low quality meal. Over-processing of soyabean meal could reduce the nutritive value and rendered several essential amino acids (lysine and arginine) to be unavailable (Renner *et al*., 1953).

Raw and processed soyabean possesses the following ANFs: protease inhibitors 45-60 mg/g and 4-8 mg/g; glycinin 150-200 mg/g and 40-70 mg/g; lectins 50-200 mg/g and 50-200 mg/g; β-conglycin 50-100 mg/g and 10-40 mg/g; phytic acid 0.6% and 0.6%; saponins 0.5% and 0.6%. (Van Eys *et al*., 2004)

2.3.1.1 Protease inhibitors

According to Daniel e*t al*. (2011), soyabean nutritive value is limited primarily by trypsin and chymotrypsin inhibitors, pectins. Trypsin inhibitors are the most important inhibitors - the Bowman-Birk inhibitors and the Kunitz inhibitors (Winiarska–Mieczan, 2007). In animal, it has decreased performance, lowered nitrogen retention and increased metabolic nitrogen excretion. Liener (1994) reported that protease inhibitors are active against chymotrypsin and trypsin. Also, it has been proven that eliminating the Bowman-Birk inhibitors from soyabeans is more difficult (Livingstone *et al.*, 2007). Therefore, trypsin inhibitor free soyabean varieties should be developed in a commercial quantity though they are sensitive to denaturation by thermal treatment.

Most soyabean products used globally in the production of livestock feeds are treated with heat so as to inactivate any ANFs related with adminstering raw soyabeans. The presence of this inhibitors do have adverse effect in the digestion and utilization of proteins which could result in stunted growth of the animal. Also, the presence of trypsin inhibitors in raw soyabeans do results in reduced proteins digestibility by inhibiting activities of trypsin and chymotrypsin produced by pancreas (Winiarska–Mieczan, 2007). Roasted or any other heated processes could minimizing the activity of these inhibitor in soyabean products (Ari *et al*., 2012). Though heating is necessary to reduce soyabean ANFs (heat-labile), excess heat will reduce lysine concentration, lysine to crude protein

ratio, crude protein digestibility and amino acid due to Maillard reactions (Song *et al.,* 2010). Consequently, in achieving the maximum nutritional value of soyabean products, adequate heating or other methods of reducing trypsin inhibitor concentration is essential, however overheating could decrease digestibility of amino acid. Elimination of antitrypsin activity (above 90%) in soyabean and its product is achievable through heat processing. Different species of animals react differently to trypsin inhibitors in feeds. Chickens and goslings do react to the presence of trypsin inhibitors in diets than calves and piglets (Livingstone *et al.*, 2007).

There are novel variety of soyabean in which the level of trypsin inhibitors were decreased to 10mg/kg of seeds (Kulasek *et al*., 1995). Better growth rate were observed when broiler chickens was fed soyabean meal in which trypsin inhibitors level had been reduced. Also, heat processing of the seeds further increased improved growth as a result of decreased trypsin inhibitor activity, inactivation of lectins and immunogenic proteins. Goebel and Stein (2011) reported that trypsin inhibitor concentrations could be reduced in soyabean products used in feeding swine through genetic selection in plant breeding in order to detect novel low-trypsin inhibitor varieties.

2.3.1.2 Lectins

The proteins that are bind to carbohydrates are called Lectins (hemaglutinins). Feeding animals with raw soyabean can increase mortality rate and decrease growth rate. The lectins level in soyabean could vary from 37 to 323 HU /mg of protein (Kakade *et al*., 1972). Soyabean meal content of lectins with carbohydrates ranges from 0.2 to 3.1g/kg and they are mostly agglutinating lectins. Fasina *et al*. (2003) ascertain that the effect of lectins in soyabean could be diminished after autoclaving. Van Eys *et al*. (2004) reported that lectins are present at residual levels in soyabean meal because they are heat sensitive. Heat treatment inactivation in soyabean meal ANFs are less effective for antigen than for lectins or trypsin inhibitors (Van Eys *et al*., 2004). The soyabean lectins level could be calculated by measuring the hemagglutination activity.

2.3.1.3 Phytates

Phytic acid chelates magnesium, calcium, iron, zinc, phosphorus and potassium making them not to be available to non-ruminant animals. Availability of these minerals, such as calcium, phosphorus and zinc reduced due to the presence of loads of phytates in feeds. Protein availability and enzymes activity (trypsin, pepsin and amylase), amino acids, starch and energy are also decreased by phytates (Ravindran *et al*., 2000). Low appetite by fish as well as their growth are been influenced by phytate (Shan and Davis, 1994). Liener (2000) stated that about two-thirds of the phosphorus in soyabean is bound as phytate and unless freed, it will not be available to animals. Phytic acid is present in most soyabean and its products contain phytic acid at the level 1-1.5g/100g of dry matter.

2.3.1.4 Tannins

Tannins are complex plant compounds that are frequently bitter, they are naturally-occurring plant polyphenols which merge with proteins and other polymers such as hemicellulose, cellulose and pectin, to form steady complexes (Mangan, 1988). Egounlety and Aworh (2003) noted that tannins content were higher in the hulls more than the whole soyabean (2. 31 x 1.52mg catechin equivalent/g). Tannin content can be reduced by 54.6% when soyabean is soaked for 12-14 hours, while no tannin content was observed in cooked, fermented and dehulled soyabean (Livingstone *et al.*, 2007). On the contrary, tannins may have considerable benefit in ruminant animal and offer fractional protection against predators in plant. In simple-stomached animals, such as *C. gariepinus*, tannins are undesirable in diet, due to decrease in growth rate and protein digestibility (Mangan, 1988).

2.3.2 Soyabean processing

Soyabean processing is beneficial and essential to eliminate or remove unwanted constituents, thereby improving palatability and releasing of encapsulated nutrients for the benefit of the animals. However, various treating methods changes the composition of soyabean meal compared with soyabean grains. Such processing methods includes mechanically extracted soyabean cake, thermal treated full-fat soyabeans, dehulled solvent extracted soyabean meal and solvent extracted soyabean meal. They are the

generally used soyabean meal in aquaculture feed production (Ari *et al.,* 2013). Although other processing methods used in reducing ANFs of soyabean products exists but cost effective and efficient method is thermal processing (Pusztai *et al*., 1996).

The common techniques used involved combination of cooking, extraction and fermentation. Thermal treatment has been reported to be efficient in decreasing trypsin inhibitor activity below biological threshold levels, as described by short-term animal bioassay. Edible-grade soy protein products of residual trypsin inhibitor activity is about 5-20% of the activity present in raw soyabeans (Rackis *et al.*, 1986).

The amount of heat needed to inactivate trypsin inhibitors and other hemagglutinins in raw soyabeans and it depend solely on time of exposure to heat and high temperatures for a shorter time period; it is as effective as lower temperatures for a prolong times (Leeson and Summers, 2005). Mechanical processing method using hydraulic or screw presses were been used in the 1930's to process soyabean which pressed out oil from heated or cooked soyabeans, while solvent-extraction processing methods, which removes more oil from the soybea6n were adopted in late 1940's and early 1950's.

2.3.2.1 Solvent extracted soyabean

Solvent extracted processing method utilises a fat Solvent, usually hexane to remove oil from soyabean seeds were soaked and washed with clean solvent to reduce the oil content. After the removing the oil, the by product is heated with steam to volatilize the solvent and then roasted to stop the growth of inhibiting proteins. The by-product or meal is then grinded to a desire uniform size. The hulls, removed at the onset of the processing could be added to the meal to increase the fibre or lower protein product.

Solvent extracted soyabean meal has been extensively utilized in livestock and aquaculture sector (Ari *et al.,* 2013, Akpoilih *et al*., 2017). Several researchers have used it for partial or total replacement of fishmeal in fish diet. Supplementation of solvent extracted soyabean meal with fishmeal was documented by Fagbenro and Davies (2001), the authors noted that supplemetation of solvent extracted soyabean meal with fishmeal up to 50% without supplementation with amino acid improved feed conversion ratio, growth rate and that soyabean were acceptable as a substitute for up to 75% in *C. gariepinus* diets if combined with 5g/kg of methionine. Akpoilih *et al*. (2017) noted that

roasted and standard solvent-extracted soyabean meal supplemented with amino acids showed improved nutrient digestibility and growth performance, which could be as a result of ANFs been deactivated.

2.3.2.2 Roasted soyabean meal

Roasting method of processing soyabean meal had been illustrated in some studies (Ari *et al*., 2012; Musa *et al*., 2016). The processing method involves cleaning and sorting of the grains, and subjected to heating with continuous stirring to allow even distribution of heat at 100 ^{o}C for 15 minutes. After which it was allowed to cool before grinding (Ari *et al*., 2012). Heat treatment of soyabean is recognized to be efficacious in improving the nutritional values of soyabean meal and decreasing the ANFs (Mridula *et al.*, 2008). High quality soyabean meal with optimum nutritional value needs suitable roasting conditions including temperature, moisture content, duration of heating and shear force (Musa *et al*., 2016).

Use of roasted soyabean meal as partial or total replacement for fish meal in livestock and fish (Adeniji, 2008; Harlıoğlu, 2012) production has been documented. They noted partial substitution of roasted soyabean meal up to 66.7% gave good growth performance of *Clarias gariepinus* compared with other processed soyabean diet. Musa *et al*. (2016) further recommended that partially or total substitution of soyabean meal in animal protein diet provided the essential amino acid (methionine and lysine) are supplemented to improved feed so as to maximize profit. Ajani *et al*. (2016) established that soyabean meal could partly replace fishmeal up to 46.7% and replaced fishmeal completely when methionine is supplemented in *Orechromis niloticus* diet.

Researches reported roasted soyabean meal to be a good candidate that could be supplemented with dietary enzyme in poultry and fish diet (Akpoilih *et al*., 2016; 2017). Also, it is a good source of energy and fatty acids. Evaluation of solvent extracted and roasted soyabean meal is shown in Table 1

Table 1 Comparative nutrient composition of soyabean meal

Parameters (%)	Solvent extracted soybean meal	Roasted soyabean meal
Dry matter	90	90

Crude protein	48	38
Digestible energy (MJ/kg)	14.9	19.5
Crude fibre	4.2	5.5
Available lysine	2.63	2.11
Available threonine	1.58	1.30
Available methionine	0.60	0.52
Available isoleusine	0.61	1.58
Available tryptophan	0.59	0.43
Fat	2.5	19.0
Available phosphorus	0.24	0.19
Calcium	0.30	0.23

Source: Poultry Feeding Standard, 2005

2.4 Amino acid requirement of *Clarias gariepinus*

Protein is the most costly constituent in aquaculture feeds. Fish don't really requires dietary protein, but a relatively stable quantity of dietary amino acids, therefore determining amino acid requirements of fish is essential (Wilson *et al*., 2015). Protein and amino acid requirements of some cultured fish species (common carp, salmon, trout, gold fish, Siberian sturgeon, tilapia, Channel catfish) have been determined (Ogunji *et al.*, 2005; NRC, 2011;Wilson *et al*., 2015) while that of channel catfish has been presumed from that of African catfish (Fagbenro *et al.*, 1999). The quantifiable amino acid requirements of fish have been established by supplementing amino acids at graded levels and method of daily incrementation with an amino acid in diet in order to cause a dose response curve (Wilson *et al*., 2015). The optimum amino acid (lysine and methionine) requirement were observed for growth response using 'break point' analysis (Fagbenro *et al.*, 1998; 1999). Studies have also reported the relationships between each of the essential amino acid profile in relation with lysine using an ideal protein concept which have been established as a base to formulate high quality fish diets (Kaushik and Seiliez, 2010; NRC, 2011). The benefit of the concept is that it could be adjusted to

different situations, if the amino acids is not altered irrespective of the growth stage (Kaushik and Seiliez, 2010).

A critical analysis and assessment of the various methods earlier mentioned revealed the association among protein and amino acid (Tibbets *et al*., 2000; Ogunji *et al*., 2005) which are: (1) fish requires a balanced combination of essential amino acids and do not require true protein like other animals ; (2) the fish requirement for essential amino acids contained in dietary protein depend solely on its dietary requirement for protein; (3) in so far as synthesis of dispensable amino acids requires expenditure of energy, feeding dietary proteins that most nearly meets the needs of fish for both essential and non-essential amino acids will result in the most efficient growth by the fish; (4) gross dietary protein requirement has a significant effect on the amino acid composition of the diet and (5) the concept of balance amino acids is essential to protein requirement.

Furthermore, supplementation of dietary amino acid in fish diet which has been a common practice as fish meal and other animal protein are substituted with plant protein sources in formulated fish feed have been reported(Yuan *et al*., 2011; Furuya *et al*., 2012). The most limiting amino acids, when fish meal is partially or totally substituted by plant protein sources in fish diets are lysine and methionine (Mai *et al*., 2006; Ajani *et al*., 2016).

Furuya *et al*. (2012) observed improvements in Final weight, nutrient utilisation and digestibility in the broiler chicken when fed soyabean based diet supplemented with combined methionine and lysine. The improvement has been attributed to the fact that suitable amount of essential amino acids is required for protein synthesis resulted from balanced nutrient combination. Cheng and Hardy (2003) observed that lysine and methionine supplementation could reduce the total dietary protein requirement for trout from 42% crude protein to 37%. Yamamoto *et al*. (2005) revealed that all essential amino acids supplementation to a low-protein (35%) diet could improve crude protein diet to 50% in a 35% crude protein diet and protein retention efficiency in trout to 35% in a 45%. Chang *et al*. (2003) stated lysine supplementation in soyabean based diets in Rainbow trout improved lysine levels and crude protein, and decreased ether extract in

whole body of rainbow trout. Supplementation of lysine in the diet further reduced dietary protein from 46% to 43% in plant based diet.

Chu *et al*. (2014) stated that methionine supplementation in plant protein based diet fed to juvenile Chinese sucker improved growth performance and feed utilisation. The findings concluded that juvenile Chinese sucker optimum requirement was assessed to be 32g/kg. The earlier requirement corroborate Fagbenro *et al*. (1999) that projected the optimum methionine requirement of *Clarias gariepinus* to be 32g/kg using breaking point analysis. Fagbenro *et al*. (1998) further estimated the optimum lysine requirement of *Clarias gariepinus* to be 57g/kg. Findings on the combine requirement and impact of methionine and lysine in *C. gariepinus* diet is yet to be properly documented. Information on this requirement will be beneficial in formulating balanced diets for highly intensive production of *C. gariepinus*.

2.5 Exogenous enzymes in fish feed

Exogenous enzymes are known to increase nutritional worth of livestock feed and reduce environmental pollution (Bedford, 1995). Castillo and Gatlin, (2015) reported that, dietary enzymes application as additives increased nutrient digestibility of plant-based diet in poultry and swine. Also, its application has helped to decrease ANFs effects of Non Starch Polysaccharides (NSP) and phytic acid, improving the utilisation of phosphorus and carbohydrates.

Exogenous enzymes supplementation in fish diets containing plant based feedstuffs was first researched in to by Carter *et al*. (1994). They researched on supplementation of an enzyme mix (acid protease, alkaline protease, trypsin, amylase, cellulase and amyloglucosidase) in soyabean meal based diet (33% by weight) fed to Atlantic salmon (*Salmo salar*). It was noted that performance and feed efficiency ratio were significantly improved with the addition of the enzyme in fish diet. The result observed suggested that supplementary enzymes in plant based protein sources have the potential to increase utilization of nutrients in the diet.

Addition of exogenous phytase in fish diets resulted in improved phytate phosphorus, other trace elements and protein utilisation, also, phosphorus discharge into water decreased (Kumar *et al*., 2012). Therefore, in fish feed formulations, supplementation of

phytase as an additive has been considered to be cost-effective and environmental friendly. Furthermore, supplementation of phytase significantly improved growth and feed conversion in tilapia, yellow catfish, striped bass, Atlantic salmon and African Catfish (Zhu *et al*., 2014; Akpoilih *et al* ., 2017). Microbial phytase application into fish and shrimp feed has been documented to increase nitrogen bio-availability and phytate-bound phosphorus, decreasing P and N discharges into the aquatic environment (Akpoilih *et al*., 2017)

In addition, several experiments with fish have been performed with diets supplemented with pancreatic enzymes such as proteases (Drew *et al*., 2005; Lin *et al*., 2007; Castillo and Gatlin, 2015). Which observed improved feed efficiency, growth and protein digestibility by exogenous enzymes supplementation in plant protein diets to fish. Recently, an increased focus has been into research on NSP enzymes in fish feeds. These have been studied and utilized in swine and poultry industry for some time (Khattak *et al*., 2006). NSP-enzymes include glucanases, pentosanases, cellulosases and xylanases. These enzymes hydrolyse NSP to products available for bacteria as prebiotics or for the fish as digestible nutrients (Sinha *et al*., 2011). Supplementations of these enzymes have also revealed to increased protein utilisation and growth in fish (Castillo and Gatlin, 2015).

2.6 Exogenous protease

The utilization of proteases in livestock in improving protein digestibility has been broadly studied in poultry (Ghazi *et al.,* 2002) and pigs (Jianjun *et al*., 2015).There are scanty work on the supplementation of enzyme in fish diets (Drew *et al*., 2005; Zhong and Zhou, 2005). Recent research findings recommends that enzymes that are beneficial to the livestock could enhance animal's digestibility. Though the efficiency of supplementation of enzyme in livestock feed in increasing protein digestibility is established (Drew *et al*., 2005; Zhong and Zhou, 2005), but the effect of supplementing protease in *C. gariepinus* diet on growth performance, feed utilization and the secretion of endogenous enzymes is scanty (Zhong and Zhou 2005).

Ghazi *et al.* (2002) reported that exogenous protease supplementation combined with phytase, xylanase or amylase in an enzyme cocktail has the potential to reduce ANFs

(protease inhibitors, lectins, and antigenic proteins) present in soyabean meal. Bacillus or Aspergillus spp are the major source of commercially available protease. Aspergillus proteases are active at neutral to acidic pH and Bacillus proteases are active at neutral to alkaline pH (Ghazi *et al*., 2002). Odetallah *et al*. (2005) found that supplemental protease had no influence on body weight of broilers fed diets formulated to 18% crude protein, but those increases were not similar to diet with 21% crude protein. Odetallah *et al*. (2005) ascertained that supplementation of keratinase in corn and soyabean meal diets formulated to 17.78% CP and 95% of the amino acid requirements according to NRC, increased body weigh gain and feed conversion ratio when compared with diets deprived of keratinase supplementation. These increaments in body weigh gain were observed even up to maturity, though enzyme suppl ementation was withdrawn at day 21.

Naela *et al*. (2017) observed improvement in the growth performance, feed utilisation and feed conversion ratio of *O. niloticus* when fed 28% and 26% crude protein supplemented with serine-protease enzyme (Ronozyme ProAct) compared with control diet. The improvement was ascribed to increase protein digestibility and amino acid availability by protease. The findings established that exogenous dietary supplementation of protease (Ronozyme ProAct) could be used safely and economically to improve protein digestibility and reduce protein content of *O. niloticus* diet while maintaining the growth performance parameters. Information on the supplementation of exogenous protease is still very scanty; there is urgent need for researcher to establish information in this area particularly in aquaculture.

2.7 Digestibility in fish

The most important aspects in the assessment of effectiveness of fish feed is the determination of its digestibility. Therefore, The nutritional assessment tools that could be used to deduce the nutrients availability levels in the plant based diets that can be turned into fresh is digestibility determination (Rostagno *et al.*, 2011). It is the major step in assessing the potential of an ingredient for use in the diet of aquaculture species. It is also determined the extent to which feed and its nutrient constituents are digested and utilized by the animal. Thus, digestibility studies have great importance in feed processing for use in aquaculture (Bertechini *et al*., 2009) and may be a potential indicator of nutrients availability for growth, maintenance and reproduction of the

animal, besides level of indigestible nutrients for the assessment of waste released by aquaculture (Angel *et al*., 2010). Cabanillas-Beltran *et al.* (2013) noted improvement in digestibility of white shrimp fed processed grains of legumes. Moreover, anti-nutritional factors in different ingredients can bind to proteins and amino acid, which may reduce their digestibility (Angel *et al*., 2010).

Chemical composition and the digestive characteristics of the species to which feed is fed determines the digestibility of a feed ingredient. Also, factors that are distinct to diet formulation, like feeding practices, manufacturing techniques and environmental conditions in the production system, also alter digestion of feed under practical culture conditions (Dalolio *et al.*, 2016). Furthermore, the digestibility coefficients evaluation of feedstuffs is a significant tool to ascertain the nutritional values to be employed in nutritionally-complex fish feeds formulation. Digestibility coefficients determination is mostly on fecal measurements, animal conditions and methods employed during digestibility trials (Pozza *et al.,* 2003).

Several methods of digestibility had been developed such as stripping of the intestine (Mangan, 1988), suction (Kakade *et al*., 1972), intestinal dissection (Wilson *et al.*, 1981) or marker technique. For feacal collection techniques, marker technique is the most frequently used, but overestimation of digestibility values could result from leaching of feed components and feaces fragmentation (Carter *et al*., 1994). Preventing nutrient leaching from the feaces requires removal of fish from the water and collecting feacal samples right from the distal intestinal region, such as the dissection technique (Wilson *et al.*, 1981). Markers are categorized as internal**,** when present in the diet (e.g. lignin), or as external**,** if they are included to the diet (e.g. chromic oxide) (Wilson *et al.*, 1981). They are utilized when feed intake and faecal output measurement is difficult. However, there are two common approaches to the use of the marker or any method. The approaches are the apparent and true digestibility

2.7.1 Apparent digestibility

Apparent digestibility is assessed by subtracting nutrients contained in the faeces from nutrients contained in the dietary intake. Therefore, lost of nutrients such as methane gas

or metabolic waste products excreted in the faeces are not considered (Sotolu and Faturoti, 2009). Apparent digestibility coefficients provides estimations of readly availability nutrient in diets, cost of formulated diets and to select ingredients that could enhance the nutritional value of diets. Yamamoto *et al*. (1998) noted that apparent digestibility determination methods could affect the value of the coefficients obtained. Thus, apparent digestibility coefficients range could occur for specified feed ingredient under diverse dietary, physiological and environmental conditions.

Several works had been conducted to establish the apparent nutrients digestibility (such as protein, lipid, dry matter, ash and energy) in fish. NRC (1993) documented that apparent digestibility values ranges from 75 to 95% for plant protein-rich feed ingredients. Also, apparent protein digestibility of feed ingredients for commonly cultured fish species such as *C. gariepinus*, rohu (*Labeo rohita*) and red drum (*Sciaenops ocellatus*) ranged from 65% to 92% in animal and plant protein sources (Lu *et al*., 2014). The apparent protein digestibility of soyabean meal have been reported to be above 90% for common carp, *C. gariepinus*, tilapia and silver perch species, with some species values above 95% (Odetallah *et al*., 2005; Wang *et al*., 2015). However, the quality of dietary protein solely depends on amino acid composition and its digestibility. The apparent amino acids digestibility coefficient has been reported high with least values of 81% for *C. gariepinus* and higher values ranging between 91 and 95% (Singh *et al*., 2011; Lawrence-Azua *et al.,* 2012). High protein and amino acid digestibility coefficients of soybean products for *C. gariepinus* is expected most especially in amino acid supplemented diets. However, the data of amino acid availability should be achieved for *C. gariepinus* for precise diet formlation on available amino acid basis. Although, reports on supplemental amino acid for carp has been established to improve quality of alternative protein sources (Nwanna *et al*., 2012). Lipid digestibility in soyabean based diets has been reported to range from 74 to 90%, while dry matter digestibility coefficients ranges from 75 to 90%. In soyabean meal, energy digestibility values is higher and ranges from 57 to 84% (Singh *et al*., 2011; Lawrence-Azua *et al.,* 2012). However, apparent digestibility measurement is less complex than true digestibility.

2.7.2 True digestibility

True digestibility is the correction of endogenous and microbial amount of nutrient that is really lost in the faeces. It could be estimated by the difference in the amounts of nutrient or amino acids in the diet and feaces, bearing in mind the endogenous losses of nutrient and amino acids that are deducted from the total amount of amino acids in feaces (Ribeiro *et al*. (2012). True amino acid digestibility helps in considering the role of endogenous amino acids and quantity of amino acids used by fish. Also, it results in better precision in formulation of rations for *C. gariepinus.* Results of true digestibility are relatively higher than that of apparent digestibility. This could be due to higher levels of digestive enzyme secretions and its inclusion in the feaces from the protein free diets (Pozza *et al*., 2003). Few literatures has reported true digestibility values in channel catfish (*Ictalurus punctatus*) (Wilson *et al.*, 1981). Also, Yamamoto *et al.* (1998) reported for rainbow trout (*Oncorhynchus mykiss*), red sea bream (*Pagrus major*) and common carp (*Cyprinus carpio*). Ribeiro *et al*. (2011; 2012) reported for *Oreochromis niloticus* using dissection and stripping techniques, respectively.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Effect of processing on chemical composition of soyabean cake and diets for *Clarias gariepinus*

3.1 Experimental site

Processing of soyabean meal and feeding trial was conducted at Aquaculture Research Laboratory, Aquatech College of Aquaculture, Fodacis, Ibadan, Nigeria. The college is situated in the tropical rain forest zone of Nigeria with latitude 7 º 36 N and longitude 3º 86 N with a mean altitude of 275 meters above sea level. Temperature range and the average relative humidity of the location were between 20-37 ^{o}C and 60%, repectively.

3.1.1 Sample collection

Test plant protein (raw soyabean grains) was purchased from Oja-Oba market in Ibadan. It was processed into cake, which was later used in the formulation of the diets.

3.1.2 Processing of samples

3.1.2.1 Roasting method

40kg of the grains were processed according to Johnson and Smith (2004). Soyabean grains were cleaned, sorted, placed in a pot and roasted for 15 minutes at 110 ^{0}C during which they were continually stirred. The roasted grains were milled with hammer grinder.

3.1.2.2 Solvent extracted processing method

Soyabean grain were flaked and 8kg were soaked in 6000ml of fat solvent (hexane) for 12 hours to extract oil. The oil content was later washed with solvent. The residual meal was further heated with steam to volatilize the remaining solvent. The meal was milled with hammer grinder to 0.2mm mesh size according to Fagbenro and Davies (2001).

3.2 Proximate analysis

This was conducted at the Central Nutrition Laboratory, Department of Animal Science, University of Ibadan. Samples were analysed in triplicate according to the official methods of analysis (AOAC, 2005).

3.2.1 Crude protein

The semi-micro Kjeldahl procedure, was used to determine the crude protein contents and it consisted of the following analysis distillation, digestion and titration (Lowry *et al.*, 1951). Concentrated H_2SO_4 was used to digest each of the sample. Digestion was accelerated by increasing the boiling point by adding Kjeldahl catalyst (a salt of copper). The mixture was make alkaline by adding 50 mL of 40 % NaOH solution. The digested samples were distilled into 25 mL boric acid for 5 minutes. The total nitrogen was represented by the amount of ammonia extracted and determined by titration with 0.47M HCl until a grey colour appeared. The crude protein contents of diets and *Clarias gariepinus* were calculated as follows:

$$\% \text{ Nitrogen} = \text{value of } \frac{HCL \times 0.1 \times 0.014}{\text{Weight of sample}} \times 100$$

% of Crude Protein = % Nitrogen × Conversion factor

(Conversion factor for *C. gariepinus* and diet is 6.25 & 5.90, respectively).

3.2.2 Crude fibre

Petroleum ether using Soxhlet extractor was used to defat the sample (Roe, 1955). Two gram of sample defatted was measured in to 600 mL beaker and 100 mL trichloroacetic acid was added for digestion. The mixture was boiled and refluxed for 40 minutes. The filtered concentrate was washed six times with hot distilled water and once with methylated spirit. The residue was placed in porcelain crucible and ashed in a Gallenkamp muffle furnace at 60 ^{o}C for 5 hours. The ash was cooled in a desiccator and weighed. The percentage crude fibre content was calculated as follows:

$$\text{Crude fibre content \%} = \frac{W1}{W2} \times 100$$

Where W1 = Initial weight of sample (g)

W2 = final weight of sample (g)

3.2.3 Ash content

Two gram of diets and fish samples were placed into a porcelain crucible and transferred into the muffle furnace set at 550 ^{o}C and left for 4 hours. The crucible and its content were cooled to 100 ^{o}C, then to room temperature in a desiccator and weighed.

% Ash content = {weight of Ash / original weight of sample} x 100

3.2.4 Ether extract

Soxhlet extraction method was used to determine the fat content, diethyl ether (40–60 ^{o}C boiling points) as reagents was used. Three gram of sample were added to a thimble and placed in the extraction unit. Samples were boiled for 30 min in ether and rinsed for 2 hours and the extracted fat was completely collected in the extraction cups. After extraction the solvent was evaporated through a water bath and the extracted material weighed to determine difference of cups before and after extraction.

Calculation:

% of crude fat = (corrected weight of fat ÷ weight of sample) ×100

3.2.5 Moisture content and dry matter determination

Following Pearson (1973) method of moisture content and dry matter determination, two gram each were weighed into a previously weighed crucible. The crucible plus sample taken were then transferred into the oven set at 100 ^{o}C and dried to a constant weight for 24 hours. At the end of 24 hours, the crucible plus sample were removed from the oven and transferred to a desiccator, cooled for 10 minutes and weighed according to the following process.

Assume weight of empty crucible = W0

Weight of crucible + sample = W1

Weight of crucible + oven dried sample = W3

% Dry matter = {W3 – W0 / W1 – W0} x 100/1

% Moisture = {W1 – W3 / W1 – W0} x 100/1 or 100 - % dry matter.

3.2.6 Nitrogen Free Extract (NFE)

NFE was calculated as follows;

% NFE = 100 - % (Ash + Crude Fiber + Moisture + Ether extract + Crude protein).

3.3 Determination of Mineral element

3.3.1 Calcium, Potassium and Sodium determination

The ash of each sample was digested by adding 5 mL of 2 MHCL to the ash in the crucible and heated to dryness on a heating mantle. 5 mL of 2 MHCL was added again, heated to boil, and filtered through a Whatman No. 1 filter paper into a 100 mL volumetric flask. The filterate was made up to mark with distilled water stoppered and made ready for the reading of concentration of calcium, potassium and sodium on the Jenway Digital Flame Photometer(PFP7 Model) using the filter corresponding to each mineral element.

The concentration each element was calculated using the following formula: % Ca or % K or % Na = $\frac{\text{Meter Reading (MR) x Slope x Dilution factor}}{1000}$

3.3.2 Phosphorus determination (Spectrophotometric method)

The vanado-molybdate colorimetric or spectrophotometric method was used to determine Phosphorus. 2 MHCL solution was used to treat each of the ash samples as described for calcium determination above. Accurately 10 mL of the filterate solution was pipetted into 50 mL standard flask and 10 mL of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of phosphorus was obtained by taking the Optical Density (OD) or absorbance of the solution on a Spectronic 20 spectrophotometer or colorimeter at a wavelength of 470nm.

The percentage phosphorus was calculated from using the formula:

% Phosphorus = $\frac{\text{Absorbance} \times \text{slope} \times \text{dilution factor}}{10000}$

3.4 Anti-nutritional factors quantification in the processed Samples

3.4.1 Heamagglutinin (Lectin)

Procedure were carried out according to AOAC (2005). Accurately 0.20g of defatted sample was weighed into a screw cap centrifuge tube 10 mL of 0.1M phosphate and 10

mL of 0.85% Nacl was added. The mixture was shaken at room temperature or stirred on a UDY shaker. The suspension after 18hours was centrifuged at 1,500 rpm for 15 minutes. The supernatant obtained was transferred into a separate clean screw cap centrifuge tube by decantation. The heamagglutinin inhibitior activity was tested by preparing a set of heamagglutin in standard solutions by serial dilution % range 0 to 1.0 mL of the stock heamagglutinin.

Extract of sample were pipetted into a triplicate set of test tubes with each set for each level of heamagglutinin. Each sample and standard spoliation was treated with 0.9% satrain of sample as well as standard solutions would be read on a spectronic 21D spectrophotometer.

The heamagglutinin activity was calculated using the formula:

HU/mg = 98.56 (Absorbance of sample – absorb blank).

3.4.2 Tannin

Procedure were carried out according to AOAC (2005). Precisely 0.20g of sample was measured into a 50 mL beaker 20 mL of 50% methanol was added and covered with parafilm and placed in a water bath at 77-80 ^{o}C for one hour. To ensure a uniform mixing, it was shaken thoroughly. The extract was quantitatively filtered using a double layered Whatman No 41 filter paper into a 100 mL volumetric flask, 20 mL water added, 2.5 mL folin-Denis reagent and 10mL of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allow to stand for 20 mins. The bluish –green colour will developed at the end of range 0-10ppm were treated similarly as 1 mL sample above. The absorbance of the tannic acid standard solutions as well as samples were read after colour development on a spectronic 21D spectrophotometer at a wavelength of 760 nm. % tannin was calculated using the formula.

$$\%\text{Tannin} = \frac{\text{absorbance of sample} \times \text{average gradient factor} \times \text{Dilution factor}}{\text{Weight of Sample} \times 10{,}000}$$

3.4.3 Phytate determination

Procedure were carried out according to Wheeler and Ferrel (1971). Precisely 2g of each sample was weighed into 250 mL conical flask. 100 mLs of 2% Hcl was added to soak

each sample in the conical flask for 3 hours. This was filtered through a double layer of hardened filter paper. 50 mL of each filterate was placed in 0.50 mL conical flask and 107 mL distilled water was added in each case to give proper acidity. 10 mL of 0.3% ammonium thiocyanate solution was added into each solution as indicated. This was titrated with standard iron (III) chloride solution which contained 0.00195g IRON per mL. The end point was slightly brownish-yellow which persisted for 5 minutes. The % phytic acid was calculated using the formula:

$$\% \text{ Phytic Acid} = \frac{\text{Titre value x 0.00195 x 1.19 x 100 x 3.55}}{\text{Weight of Sample}}$$

3.4.4 Determination of Trypsin Inhibitor Activity (TIA)

3.4.4.1 Casein digestion method

Procedure were carried out according to Reddy *et al*. (1982). Accurately 0.2g of defatted ground sample was weighed into a centrifuge tube. 10 mL of 0.1M Phosphate buffer added and shaken on a shaker at room temperature for 1hour. The suspension was centrifuged at 5000rpm in a centrifuge for 5min.The content was later filtered through a Whatman No. 42 filter paper into a 250 mL conical flask 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the filterate were pipette into a set of triplicate set of test-tubes (one set for each level of extract). The final volume is adjusted to 2 mL by the addition of 0.1M phosphate buffer. These test-tubes were arranged into a water bath maintained at 37 ^{0}C. A blank was prepared by adding 6 mL of 5% TCA solution to one set of triplicate tubes. Precisely 2 mL of 2% casein solution was added to all the tubes which is previously kept at 37 ^{0}C to incubate for 20 min.The reaction of casein was stopped by the addition of 6 mL of 5% TCA solution and this was allowed to proceed for 1hour at room temperature .The mixture was later filtered at room temperature through a Whatman No 42 filter paper into 100 mL conical flask.0.2, 0.4, 0.6, 0.8, and 1.0mL of stock trypsin solution were also pipetted into a triplicate set of test-tubes (one set for each level of trypsin) as above and treated similarly as sample to the point of filteration.

The absorbance of the filterates of both samples and standard trypsin solution were read on a spectrophotometer at a wavelength of 280 nm. The actual absorbance of sample was

the difference between absorbance of stock trypsin filterate and that of sample filterate. The absorbance of blank was also read. One trypsin inhibitor unit (TIU) was arbitrarily defined as an increase of 0.01 absorbance units at 280 nm in 20 min per 10 mL of the reaction mixture under the conditions mentioned herein.

Trypsin Inhibitor Unit for each sample was calculated using the formula:

$$\frac{\text{Change in absorbance of sample extract}}{0.01 \times \text{mg protein in sample}}$$

3.4.5 Chymotrypsin inhibitor activity

Procedure were carried out according to Reddy *et al*. (1982). Accurrately 0.2g of defatted sample was weighed into a screw cap centrifuge tube 10 mL of 0.1M borate buffer was added and contents was shaken at room temperature for 1 hour on the shaker. The suspension obtained as centrifuged for 5min at 3000rpm and filter through Whatman No 42 filter paper. One set of each level of enzyme was prepared by pipetting 0.2, 0.4, 0.6, 0.8 and 1.0 mL stock chymotrypsin into a triplicate set of test tubes. The final volume of each tube was adjusted to 1.0 mL with 0.01mL HCL containing 0.08M $CaCl_2$ in 0.01M HCL. 1 mL 0.1M borate buffer was added to all the tubes and set in a water bath maintain at 37 ^{o}C 6 mL of TCA reagent was added to onset of the triplicate tubes to serve as a blank for the other two sets. Precisely 2 mL casein solution was then added to all the tubes which has been previously brought to 37 ^{o}C and allowed to remain at 37 ^{o}C for 10 minutes. The reaction was stopped by adding 6 mL TCA reagent to the experimental tubes and mixture was homogenize and incubated for 30 minutes at room temperature. The suspension was filtered through a Whatman SS0 42 filter paper and absorbance of the filterate read on a spectronic 21D Digital spectrophotometer at a wavelength of 275 nm.

Chymotrypsin Inhibitor Unit /mg = 92.48 (absorbance of sample-absorbance of blank)

3.5 Amino acid analysis of the processed sample

Accurately 0.1mol/litre Standard solutions of different amino acids (alanine, leucine, aspartic acid, isoleucine, lysine, glycine, methionine, threonine, glutamic acid, cysteine, valine, tryptophan, phenylalanine, ornithine, tyrosine, histidine, serine, proline, asparate, pyrrolysine); Acetate buffer at pH 5.5, Methylcellosolve (ethyleneglycolmonomethyl), 50% ethanol (V/V), hydrindantin, ninhydrin reagent (which was prepared by dissolving 0.8g of ninhydrin and 0.12g of hydindantin in 30 mL of methyl cellosolve and 10 mL of acetate buffer prepare fresh and store in a brown bottle) and 6 MHCl.

3.5.1 Preparation of sample by hydrolysis

Accurately 1g of well ground sample was weighed into a stoppered 250 mL conical flask, 100 mL of 6 MHCl was added to the sample stoppered and heated in an oven or incubated for 16hours to hydrolyse the sample. The mixture obtained was filtered through a double layered Whatman No 42 Filter paper into another 250 mL conical flask and stoppered. The hydrolysate obtained was stored at -4 ^{0}C prior to analysis.

3.5.2 Determination

Accurately 2 mL of above hydrolysate was pipetted into a 30 mL test tube. 10 mL of buffered ninhydrin reagent added, heated in a boiling water bath for 15 min, cool to room temperature and 3 mL of 50% Ethanol added immediately. 0-5 µg/mL working standard amino acids were prepared from each standard solution of amino acids 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 absorbances or transmittance of sample buffered heated hydrolysate and working standards were measured at the wavelength of colour developed by each amino acids.

$$\%\text{amino acid (any one)} = \frac{\text{Absorbance of sample X Gradient factor X Dilution Factor}}{10{,}000}$$

3.6 Performance and amino acid digestibility by *Clarias gariepinus* fed of soyabean based diet supplemented with lysine and methionine

3.6.0 Research methodology

3.6.1 Experimental procedures

Iso-nitrogenous basal diet for each of the processed soyabean with varying inclusion levels of supplemental lysine and methionine to formulate 12 dietary treatments as follows:

Roasted soyabean meal (g/100g)

- RS1 (Control) = 0 lysine + 0 methionine
- RS2 = 0 lysine + 1 methionine
- RS3 = 0.25 lysine + 0.75 methionine
- RS4 = 0.5 lysine +0.5 methionine
- RS5 = 0.75 lysine + 0.25 methionine
- RS6 = 1 lysine + 0 methionine

Solvent extracted soyabean meal (g/100g)

- SS1 (Control) = 0 lysine + 0 methionine
- SS2 = 0 lysine+ 1 methionine
- SS3 = 0.25 lysine + 0.75 methionine
- SS4 = 0.5 lysine +0.5 methionine
- SS5 = 0.75 lysine + 0.25 methionine
- SS6 = 1 lysine + 0 methionine

The diets were formulated to 40% crude protein (Faturoti *et al*., 1986) and endogenous losses during digestion was corrected with formulated protein free diet. Gross composition of experimental diet are shown in Table 2. *Clarias gariepinus* juveniles (n=720) aged two months weighing 19.70±0.20g were acquired from Aquatech college of aquaculture, fodacis, Ibadan. Fish were acclimatised under laboratory condition for two weeks and the fed 2mm Durante diets. Water temperature was kept at optimum range of 25 and 32 ^{0}C and oxygen supplied to avoid stress, the fish were left for 2-3 days unfed before the onset of experiment. Twenty fishes were stocked per experimental rectangular plastic aquarium with the dimension of 50cm x 34cm x 27cm of 40 litres capacity of water used in triplicates for each treatment. They were fed to satiation twice daily (0800 and 1500 hours) at 3% body weight. The weight of the fish and feeding rate were measured and adjusted every two weeks, respectively. Water quality was monitored every other day in static water system. Indigestible marker, chromium oxide was included in the feed at 0.5% (Guggenbuhl *et al*., 2012) to measure the nutrient digestibility.

3.6.2 Feeding experiment

The feacal and the uneaten feed were siphoned out of each tanks prior to changing of water; fresh water replaced, while water quality parameters were analysed. The analysed were done at the Department of Veterinary Medicine, University of Ibadan.

After the feeding experiment, 150 fish were randomly selected and starved of feed for 48 hours. Protein free diet was introduced to the fish and they were fed twice daily (0800 and 1500) for two days. All of the fishes were sacrificed and were opened laterally to remove the content of the distal intestinal segment at 6 cm from the anus (between the ileal-rectal valve and the anus) and placed on a petri dish. Using surgical scissors, the intestine was opened longitudinally and the feacal was removed with a spatula and placed in a different petri dish (Ribeiro *et al*., 2011). The digesta of fish in each experimental unit, consisting of 150 fish, was pooled into a single sample. The feacal samples were oven dried at 60 ^{0}C to stop nitrogen degradation.

TABLE 2: Gross composition of roasted and solvent extracted processed soyabean based diets fed to fish.

Ingredient (g/100g)	Control	RS2	RS3	RS4	RS5	RS6	Control	SS2	SS3	SS4	SS5	SS6	Protein-free diet
Soyabean meal	81.6	81.6	81.6	81.6	81.6	81.6	70.0	70.0	70.0	70.0	70.0	70.0	
Yellow maize	14.4	14.4	14.4	14.4	14.4	14.4	26.0	26.0	26.0	26.0	26.0	26.0	
Corn starch	-	-	-	-	-	-	-	-	-	-	-	-	850
Cellulose	-	-	-	-	-	-	-	-	-	-	-	-	80
Glucose													50
*Vit/min premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Soyabean oil	1	1	1	1	1	1	1	1	1	1	1	1	1
Calcium carbonate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chromic Oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	**0**	**0**	**0.25**	**0.5**	**0.75**	**1**	**0**	**0**	**0.25**	**0.5**	**0.75**	**1**	**-**
Methionine	**0**	**1**	**0.75**	**0.5**	**0.25**	**0**	**0**	**1**	**0.75**	**0.5**	**0.25**	**0**	**-**
Total (%)	99	100	100	100	100	100	99	100	100	100	100	100	100

*1kg of premix contains Vitamin A-22,000I.U; Vitamin D3-5,000I.U, Vitamin E-300mg; Vitamin k3-10mg; Vitamin B1-20mg; Vitamin B2-25mg; Vitamin C-300mg; Niacin-120mg; Calcium Pantothenate-60mg; Vitamin B6-10mg; Vitamin B12-0.05mg; Folic Acid-5mg; Biotin-1mg; Choline Chloride-500mg; Inositol-50mg; Manganese-30mg; Iron-35mg; Zinc-45mg; Copper-3mg; Iodine-5mg; Cobalt-2mg; Lysine-85mg; selenium-0.15mg; Anti-Oxidant-80mg; Methionine-100mg

3.7 Growth Parameter of *Clarias gariepinus*

The following growth parameters were monitored in the course of the studies according to Falayi, 2009.

3.7.1 Final weight (FW)

FW = Final weight – Initial weight

3.7.2 Percentage Final weight

$$PFW = \frac{\text{Weight}}{\text{Initial}} \times 100$$

3.7.3 Specific Growth Rate (SGR) (%)

$$SGR = \frac{\text{Log}_c W_2 - \text{Log}_c W_1}{T_2 - T_I} \times \frac{100}{1}$$

Where W_1 = Initial weight of fish (gm), W_2 = Final weight of fish (gm), T_2 = Time T_2,

T_1 = Time

3.7.4 Feed Intake (FI)

This was obtained by summing up the amount of feed taken per week for each of the treatments for the 12 weeks duration of the experimental period.

3.7.5 Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Feed Consumed}}{\text{Final weight}}$$

3.7.6 Gross Efficiency of Feed Conversion (GEFC)

$$GEFC = \frac{1}{FCR} \times 100$$

3.7.7 Protein Intake (P1)

$$P1 = \frac{\text{Total feed consumed x percentage protein}}{100}$$

3.7.8 Protein Efficiency Ratio (PER)

$$PER = \frac{\text{Net Final weight}}{\text{Protein intake}}$$

3.7.9 Gross Protein Retention (GPR)

$$GPR = \frac{\text{Final crude protein of fish – initial protein of fish}}{\text{Dry protein fed}}$$

3.7.10 Nitrogen Retention Efficiency (NRE)

$$NRE = \frac{\text{(Mean final weight×final body nitrogen)-(initial mean weight×initial body nitrogen)}}{\text{Nitrogen consumed}}$$

3.7.11 Survival Rate (SR)

The mortalities during the experimental period were monitored and recorded. Survival rate (%) was calculate as follows:

$$\frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

3.8 Proximate analysis of feed, fish and feacal

The proximate determination for feed, fish and feacal samples is as earlier described in 3.2.

3.9 Haematology

This was undertaken at the Haematology Laboratory, Department of Veterinary Medicine, University of Ibadan. Blood was aseptically drawn from fish for haematology and serum biochemical analyses. Needle and syringe of 5 mL was inserted 4cm – 5cm from the genital opening, fish sample from each treatment was wiped with dry tissue paper to avoid infection. The needle was inserted to the vertebral column of the fish at a right angle, during the penetration, the needle was aspirated gently. Blood was drawn through gentle aspiration until about 2 mL of blood was obtained.

The needle was thereafter removed gently while blood obtained was transferred into heperarinised plastic bottles. The blood were thereafter mixed gently so as to prevent clotting. Serum was acquired from the blood samples by centrifugation and then drawn into $2cm^3$ plastic syringe. This was transferred into a universal bottle in refrigerator for subsequent biochemical analysis. The standard heamocytometre was used in both erythrocyte and leucocyte counts according to the methods of Blaxhall and Daisley (1973) using modified haem's dilution fluid. The collected blood was introduced into an improved Neubaeur Counting Chamber (Neubauer improved bright line Marienfield, Germany 0.100 mm, 0.0025 mm^2) and the cells were counted under the microscope at 100 x objective.

3.9.1 Erythrocyte (RBC) and Leucocyte (WBC) counts

Red and White blood cell counts were done using Newbauer heamocytometer as described by Natt and Herrick (1952) and Kaplow (1955). Red blood cell count was carried out by diluting 1:200 of blood sample with Dacie fluid (99 mL of 3% aqueous solution of sodium citrate and 1mL of 40% formaldehyde). White blood cell count was determined by diluting 1:200 of blood sample with 3% aqueous solution of acetic acid and gentian violet added to the mixture later. The mixture was allowed to settle for 2 minutes, mounted on microscope and counted.

3.9.2 Haematocrit (PCV)

Pre-heparinised sample bottles was filled with 2 mL of blood from the individual fish sample and sealed immediately with plasticine. The sample bottles were centrifuged for 5 minutes using a micro haematocrit centrifuge. The PCV was then read using the haematocrit ready (Blaxhall and Daisley, 1973)

3.9.3 Haemoglobin concentration (Hb)

About 0.02 mL of well mixed blood was added to 4mL of Drabkins solution (Potassium ferricyanide, 200mg Potassium cyanide and 50mg Potassium dihydrogen phosphate) as described by Dacie and Lewis (1976). The entire mixture was made up to 1 litre with distilled water and pH adjusted to neutral (pH=7.0). The mixture was later allowed to stay

for about 10 minutes and the haemoglobin read photometrically by comparing with a cyanomethaemoglobin standard at 625 nm.

3.9.4 Mean Corpuscular Volume (MCV)

MCV (fl) was estimated using the model as described by Feldman *et al*. (2000):

$$\text{MCV} = \frac{\text{Hematocrit (in mL per 100 mL blood)}}{\text{Number of red blood cells per 100 mL blood}} \times 10$$

3.9.5 Mean Corpuscular Haemoglobin (MCH)

The MCH (pg) was estimated using the model as described by Stoskopf (1992)

$$\text{MCH} = \frac{\text{Haemoglobin (g/100 mL)}}{\text{Number of red blood cells (millions/L of blood)}} \times 100$$

3.9.6 Mean Corpuscular Haemoglobin Concentration (MCHC)

The MCHC (g/dL) was estimated using the model as described by Stoskopf (1992)

$$\text{MCHC} = \frac{\text{Haemoglobin concentration}}{\text{Packed cell volume}} \times 100$$

3.10 Serum biochemical analyses

Blood samples for biochemical analysis were centrifuged for 5 minutes at 3000 rpm with Hawsley Minor Bench Centrifuge (P spectra, Centromix no 231254 CD7000549, Spain). The blood was stored at -4 ^{o}C and analysis was conducted at Veterinary Clinical Pathology Laboratory of Veterinary Pathology Department, University of Ibadan for total protein, albumin, aspartate amino transaminase, globulin, alkaline phosphatase, alanine amino transaminase, creatinine and blood urea nitrogen while albumin:globulin was calculated. (Henry *et al.,* 1974)

3.11 Amino acid analysis

The amino acid determination of fish and feacal samples was as earlier described in 3.5.

3.12 Water quality parameter

3.12.1 Dissolved Oxygen (DO) mg/L

This was measured with a combined digital YSI Model 57, VWL Company, New Jersey (USA).

3.12.2 Temperature

Temperature was measured using combined digital YST meter.

3.12.3 pH

pH was determined by using pH meter (Melter Teldo-320 model UK). Further pH determination was made by colorimetry method in which 5 drops of prepared chemical in the test kit is added to 10 mL of sampled water. The attendant colour change was compared to a colour chart provided by the manufacturer which was eventually determined the pH range.

3.13 Digestibility studies

3.13.1 Collection of feacal

After 84 days of feeding and monitoring the growth parameter, Indigestible marker, chromium oxide was included in the feed at 0.5% in order to measure the digestibility of the feed. The digestibility experiment was set up using a completely randomised design. The fish were fed to satiation 0900 and 1700 hours. Daily feed supplied were recorded while portions not consumed were siphoned out 30 minutes after each feeding to prevent excess feed from contaminating the feaces. Feacal wastes were siphoned four hours after each feeding using 2 mm diameter hose. The siphoned feaces were strained using filter papers. The feaces were pooled for each treatment, oven dried between 85 and 105 ^{0}C and stored for analysis Fagbenro *et al.* (2003).

3.13.2 Determination of digestibility indices

Chromic oxide in diets and feaces were analysed as described (Furukawa and Tsukahara 1966). Samples were digested with concentrated nitric acid and oxidation of chromic oxide with 70% prechloric acid. 50 mg of the samples was poured in a kjedahl flask. 5mL of concentrated nitric acid was added to the flask and the mixture was gently boiled for 20 minutes. The boiled sample was cooled and 3 mL of 70% perchloric acid was added to the flask. The resultant mixture was gently heated for another 10 minutes until the solution changed from green to orange to ensure complete oxidation. The oxidised solution was then put inside a 100 mL volumetric flask and diluted to 100 mL with distilled water. The absorbance of the solution was determined by means of a spectrophotometer at 350 nm. Percentage chromic oxide content in sample was calculated as follows:

$$\text{Weight of chromic oxide in sample} = \text{absorbance} - \frac{0.0032}{0.2089}$$

Chromic oxide (%) = (weight of chromic oxide ÷ weight of the sample) × 100

3.13.3 Apparent Nutrient Digestibility Coefficient % (ANDC)

$$100 - 100 \times \frac{[(\%\ Cr_2O_3 \text{ in diet}) \times (\text{nutrient in feaces})]}{[(\%\ Cr_2O_3 \text{ in feaces}) \quad (\text{nutrient in diet})]}$$

3.13.4 True Nutrient Digestibility Coefficient % (TNDC)

$$100 \times \frac{[\ \%\text{ nutrient in diet} - (\%\text{ nutrient in feaces} - \text{MF nutrient diet})]}{\%\ Cr_2O_3 \text{ in diet} \quad (\%Cr_2O_3 \text{ in feaces} \quad \%\ Cr_2O_3 \text{ in diet})}$$

$$\frac{\%\text{ nutrient in diet}}{\%\ Cr_2O_3 \text{ in diet}}$$

3.13.5 Apparent Amino Acid Digestibility Coefficient % (AAADC)

$$100 - 100 \times \frac{[(\%\ Cr_2O_3 \text{ in diet}) \times (\%\text{ amino acid in feaces})]}{[(\%\ Cr_2O_3 \text{ in feaces}) \quad (\%\text{ amino acid in diet})]}$$

3.13.6 True Amino Acid Digestibility Coefficient % (TAADC)

$$100 \times \frac{[\ \%\text{ amino acid in diet} - (\%\text{ amino acid in feaces} - \text{MF amino acid diet})]}{\%\ Cr_2O_3 \text{ in diet} \quad (\%Cr_2O_3 \text{ in feaces} \quad \%\ Cr_2O_3 \text{ in diet})}$$

$$\frac{\%\text{ amino acid in diet}}{\%\ Cr_2O_3 \text{ in diet}}$$

MFAA = metabolic feacal amino acid (it was determined by feeding protein free diet)

Growth performance and amino acid digestibility by *Clarias gariepinus* fed soyabean based diet supplemented with dietary protease

3.14 Research methodology

3.14.1 Experimental procedures

Iso-nitrogenous basal diets for each of the processed soyabean (roasted and solvent extracted soyabean based diets) were supplemented with varying inclusion levels of protease at 0, 100, 200, 300, 400 and 500 ppm (Iwaniuk *et al.,* 2011) to formulate 12 dietary treatments as follows:

Basal diets obtained from preliminary study = Control,

Control + 100 ppm,

Control + 200 ppm,

Control + 300 ppm,

Control + 400 ppm,

Control + 500 ppm

The diets were formulated to 40% crude protein level (Faturoti *et al*., 1986) and endogenous losses during digestion was corrected with formulated protein free diet. Gross composition of experimental diet are shown in Table 3. *Clarias gariepinus* juveniles (n=720) aged two months weighing 19.70±0.20g were acquired from Aquatech college of aquaculture, fodacis, Ibadan. Fish were acclimatised under laboratory condition for two weeks and the fed 2mm Durante diets. Water temperature was kept at optimum range of 25 and 32 ^{0}C and oxygen supplied to avoid stress, the fish were left for 2-3 days unfed before the onset of experiment. Twenty fishes were stocked per experimental rectangular plastic aquarium with the dimension of 50cm x 34cm x 27cm of 40 litres capacity of water used in triplicates for each treatment. They were fed to satiation twice daily (0800 and 1500 hours) at 3% body weight. The weight of the fish and feeding rate were measured and adjusted every two weeks, respectively. Water quality was monitored every other day in static water system. Indigestible marker, chromium oxide was included in the feed at 0.5% (Guggenbuhl *et al*., 2012) to measure the nutrient digestibility.

Table 3: Gross composition of roasted and solvent extracted soyabean based diets supplemented with varying inclusion levels of protease.

Ingredient (g/100g)	Control	RS100	RS200	RS300	RS400	RS500	Control	SS100	SS200	SS300	SS400	SS500	Protein-free diet
Soyabean meal	81.6	81.6	81.6	81.6	81.6	81.6	70.0	70.0	70.0	70.0	70.0	70.0	-
Yellow maize	14.4	14.4	14.4	14.4	14.4	14.4	26.0	26.0	26.0	26.0	26.0	26.0	-
Corn starch	-	-	-	-	-	-	-	-	-	-	-	-	85.0
Cellulose	-	-	-	-	-	-	-	-	-	-	-	-	8.0
Glucose	-	-	-	-	-	-	-	-	-	-	-	-	5.0
*Vit/min premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	0.25
Soyabean oil	1	1	1	1	1	1	1	1	1	1	1	1	1
$CaCO_3$	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chromic Oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	-
Methionine	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	-
Protease (ppm/kg)	0	100	200	300	400	500	0	100	200	300	400	500	
Total (g)	100	100	100	100	100	100	100	100	100	100	100	100	100

*1kg of premix contains Vitamin A-22,000I.U; Vitamin D3-5,000I.U, Vitamin E-300mg; Vitamin k3-10mg; Vitamin B1-20mg; Vitamin B2-25mg; Vitamin C-300mg; Niacin-120mg; Calcium Pantothenate-60mg; Vitamin B6-10mg; Vitamin B12-0.05mg; Folic Acid-5mg; Biotin-1mg; Choline Chloride-500mg; Inositol-50mg; Manganese-30mg; Iron-35mg; Zinc-45mg; Copper-3mg; Iodine-5mg; Cobalt-2mg; Lysine-85mg; selenium-0.15mg; Anti-Oxidant-80mg; Methionine-100mg

3.15 Growth parameter of *Clarias gariepinus*

The growth parameter of *Clarias gariepinus* was determined as earlier described in 3.7.

3.16 Proximate analysis of feed, fish and feacal

The proximate determination of feed, fish and feacal was determined as earlier described in 3.2.

3.17 Haematology analysis

The haematology determination was determined as earlier described in 3.9.

3.18 Serum biochemistry analysis

The serum biochemistry determination was determined as earlier described in 3.10.

3.19 Amino acid analysis

The amino acid determination was determined as earlier is as described in 3.5.

3.20 Water quality analysis

Water quality analysis was determined as earlier described in 3.12.

3.21 Digestibility study

Digestibility study was determined as earlier described in 3.13.

3.22 Statistical analysis

Data were subjected to descriptive statistics, regression and analyses of variance (SAS, 2005). Means were separated using new Duncan Multiple range test of the same software at $\alpha_{0.05.}$ The regression model is shown below:

Regression model

Where:

$y = \beta_o+\beta_1 x+\beta_2 x^2+\varepsilon$

y = parameters analysed or examined (Final weight, crude protein)

x = varying inclusion of amino acid or protease.

CHAPTER 4

4.0 RESULTS

4.1 Experiment One: Effect of processing on chemical composition of soyabean meal and diets for *Clarias gariepinus*

4.1.1 Proximate composition of soyabean meal

Proximate composition of experimental test ingredients is shown in Table 4. Roasting and solvent extraction significantly improved ($P<0.05$) the proximate compositions of raw soybean. Processing significantly ($P<0.05$) increased the dry matter of solvent extracted and roasted soybean (92.29±0.04 and 92.19±0.01) compare with raw soybean (90.07±0.20). Crude protein was higher in solvent extracted soybean (58.45±2.50%) and closely followed by roasted soybean (52.50±2.78). Also, processing significantly reduced ($P<0.05$) ether extract in solvent extracted soybean meal (8.09±0.02) compared with the higher value in raw soybean (19.20±0.10). Lower crude fibre (3.10±0.10) was in solvent extracted soybean meal compared with ($P<0.05$) raw soybean (5.50±0.10). Raw soybean had a significantly ($P<0.05$) higher Nitrogen Free Extract (NFE) value (24.19±0.23) than the processed soybean. Solvent extracted soybean meal had lower ($P<0.05$) gross energy (kcal/g) content of 3.74±0.04 than roasted (4.00±0.20) and raw soybean (4.13±0.03). However, processing had no significant influenced ($P>0.05$) on mineral composition of soybean samples.

4.1.2 Amino acid composition of the experimental ingredients

The amino acid composition of test ingredients is shown in Table 5. Processing significantly ($P<0.05$) altered amino acid content of raw soyabean. Methionine (0.50±0.00), methionine+cysteine (0.97±0.01) and lysine (2.32±0.01) were lower ($P<0.05$) in roasted soyabean meal compare to raw soyabean. Solvent extracted soyabean meal had higher methionine (0.61±0.01), methionine+cysteine (1.28±0.03) and lysine (2.77±0.05) than roasted soyabean meal. Raw soyabean contained lower threonine (1.50±0.01), tryptophan (0.53±0.00), isoleucine (1.78±0.02), leusine (2.99±0.04), valine (1.84±0.02), histidine (1.05±0.02), phenylalanine (2.03±0.02) than ($P<0.05$) solvent extracted SBM. Arginine was higher ($P<0.05$) in solvent extracted soyabean (3.45±0.06) than raw (3.04±0.04) and roasted soyabean (3.06±0.03). Processing significantly influenced non-essential amino acid with the higher values of 2.03±0.03 (glycine), 2.37±0.05 (serine), 2.37±0.04 (proline), 2.03±0.04 (alanine), 5.37±0.13 (aspartic acid), 8.36±0.16 (glutamine) and 0.64±0.01 (cysteine) in solvent extracted soyabean meal compared with lower values of 1.66±0.01 (glycine), 2.01±0.02 (serine), 2.05±0.02 (proline), 1.66±0.01 (alanine), 4.54±0.05 (aspartic acid), 7.29±0.09 (glutamine) and 0.47±0.01 (cysteine) in the raw soyabean.

Table 4: Nutrient composition of test ingredients

Parameter (%)	Raw soybean	Roasted soybean meal	Solvent extracted soybean meal
Dry matter	90.07±0.20[a]	92.19±0.01[b]	92.29±0.04[c]
Crude Protein	46.01±0.06[a]	52.50±2.78[b]	58.45±2.50[c]
Ash	5.10±0.30[b]	3.70±0.20[a]	8.10±0.10[c]
Ether Extract	19.20±0.10[c]	18.50±0.40[b]	8.09±0.02[a]
Crude Fibre	5.50±0.10[c]	3.50±0.10[b]	3.10±0.10[a]
NFE	24.19±0.23[c]	21.80±0.15[a]	22.26±0.33[b]
Gross energy (kcal/g)	4.13±0.03[b]	4.00±0.20[b]	3.74±0.04[a]
Calcium	0.25±0.03	0.24±0.02	0.23±0.03
Phosphorus	0.36±0.03	0.35±0.02	0.35±0.03
Potassium	0.49±0.05	0.48±0.02	0.49±0.01
Sodium	0.03±0.00	0.03±0.00	0.04±0.00

Means with same letter in row are not significantly different (P>0.05)

Table 5: Amino acid composition of the test ingredient

Parameter	Raw soyabean	Roasted soyabean meal	Solvent extracted soyabean meal
Essential Amino Acid			
Methionine	0.52±0.00[b]	0.50±0.00[a]	0.61±0.01[c]
Methionine+Cysteine	1.09±0.01[b]	0.97±0.01[a]	1.28±0.03[c]
Lysine	2.46±0.02[b]	2.32±0.01[a]	2.77±0.05[c]
Threonine	1.50±0.01[a]	1.54±0.01[b]	1.80±0.04[c]
Tryptophan	0.53±0.00	ND	0.61±0.01
Isoleusine	1.78±0.02[a]	1.87±0.01[b]	2.18±0.05[c]
Leusine	2.99±0.04[a]	3.17±0.02[b]	3.64±0.09[c]
Valine	1.84±0.02[a]	1.95±0.01[b]	2.26±0.05[c]
Histidine	1.05±0.02[a]	1.09±0.01[b]	1.23±0.03[c]
Phenylalanine	2.03±0.02[a]	2.15±0.02[b]	2.45±0.06[c]
Non-Essential Amino Acid			
Glycine	1.66±0.01[a]	1.73±0.01[b]	2.03±0.03[c]
Serine	2.01±0.02[a]	2.05±0.02[b]	2.37±0.05[c]
Proline	2.05±0.02[a]	2.07±0.06[b]	2.37±0.04[c]
Alanine	1.66±0.01[a]	1.74±0.01[b]	2.03±0.04[c]
Aspartic Acid	4.54±0.05[a]	4.80±0.04[b]	5.37±0.13[c]
Glutamine	7.29±0.09[a]	7.56±0.08[b]	8.36±0.16[c]
Cysteine	0.58±0.01[b]	0.47±0.01[a]	0.64±0.01[c]
Arginine	3.04±0.04[a]	3.06±0.03[a]	3.45±0.06[b]

abc Means with same letter in row are not significantly different (P>0.05)

ND: Not Detected

4.1.3 Anti-nutritional composition of the test ingredients

The anti-nutritional compositions of the processed and raw soyabean grain are shown in Table 6. Processing soyabean using roasting and solvent extraction methods significantly reduced anti-nutrient contents (trypsin inhibitor, cyanogens, phytates and tannins) while chymotrypsin inhibitor and heamagglutinnins were not detected. Trypsin inhibitor (0.13±0.03), cyanogens (0.20±0.01), phytates (41.33±3.21) and tannins (57.00±2.65) content were significantly lowered in solvent extracted soyabean meal and roasted soyabean (0.16±0.02, 0.26±0.04, 65.00±5.00 and 123.33±5.77) compared to raw soyabean (0.20±0.01, 0.33±0.03, 83.33±2.89 and 187.33±2.52).

Table 6: Effect of processing on the anti-nutritional factors of tests soyabean meal

Parameter (mg/100g)	Soyabean Grain	Roasted Soyabean Meal	Solvent Extracted Soyabean Meal
Trypsin inhibitor	0.20 ± 0.01^{b}	0.16 ± 0.02^{ab}	0.13 ± 0.03^{a}
Chymotrypsin inhibitor	ND	ND	ND
Cyanogens	0.33 ± 0.03^{b}	0.26 ± 0.04^{ab}	0.20 ± 0.01^{a}
Phytates	83.33 ± 2.89^{c}	65.00 ± 5.00^{b}	41.33 ± 3.21^{a}
Tannins	187.33 ± 2.52^{c}	123.33 ± 5.77^{b}	57.00 ± 2.65^{a}
Heamagglutinnins	ND	ND	ND

Means with same letter in row are not significantly different (P>0.05)

ND: Not Detected

4.1.4 Nutrient composition of roasted soyabean based experimental diets

The chemical composition of roasted soyabean based diets is shown in Table 7. Crude protein was higher (P>0.05) in diet RS6 (41.53±0.60) and least in RS2 (39.98±0.46). In diet RS3 (6.45±0.70), ash was higher (P<0.05) and least value of 5.00±0.14 was in RS4. Also, ether extract was significantly higher (P<0.05) in diet RS6 (7.15±0.07) and least in RS3 (6.50±1.14). Similar pattern was noted in crude fibre and least value of 3.60±0.14 was in RS2.

Gross energy content (kcal/g) of the diet was higher (P>0.05) in RS6 diet (4.13±0.00) and least in RS5 (4.01±0.00). Calcium and phosphorus were higher (P<0.05) in RS2 (1.86±0.00 and 0.83± 0.00) and the least value (1.28±0.00 and 0.57± 0.00) in diet RS4. Also, sodium was higher in diet RS2 (0.38±0.00) while the least value (0.27±0.00) was in diet RS6 (P<0.05).

4.1.5 Nutrient composition of solvent extracted soyabean based experimental diets

The chemical composition of solvent extracted soyabean based diets is shown in Table 8. Higher (P>0.05) crude protein of 41.59±0.62 was in control diet and least in SS4 (40.75±0.43). Significantly higher value of 9.30±0.14 (SS3) was in ash content and least value in SS5 (7.20±0.14). Also, higher Ether extract level was observed in diet SS5 (7.05±0.14) and the least value of 6.35±0.07 obtained in SS2 (P<0.05). Crude fibre was higher (P<0.05) in diet SS5 (6.70±0.14) and least in SS6 (5.00±0.14). No significant difference (P>0.05) was observed in dry matter with values ranging from 92.31±0.15 (SS4) to 92.09±0.68 (SS5). Energy value ranged from 4.11±0.01 (SS4) to 3.98±0.00 (SS6).

The mineral composition also varied significantly (P<0.05). Higher calcium and phosphorus were in control diets (1.58±0.00 and 0.66±0.00) while least values were in diet SS4 (1.36±0.00) and SS5 (0.59±0.00), respectively. Likewise, higher potassium was obtained in SS2 (0.84±0.00) and least in SS6 (0.78±0.00). No significant differences between SS4 and SS5 were detectedfor sodium.

Table 7: Nutrient composition of roasted soyabean based test diets

Ingredient (%)	Control	Diet (RS)				
		2	3	4	5	6
Crude protein	40.90±0.28	39.98±0.46	40.96±1.27	40.88±1.24	40.681.10	41.53±0.60
Ash	5.68±0.04^{bc}	5.30±0.14^{ab}	6.45±0.70^{b}	5.00±0.14^{a}	5.90±0.28^{cd}	6.15±0.70^{d}
Ether extract	6.60±0.14^{a}	6.80±1.14^{a}	6.50±1.14^{a}	6.95±0.14^{a}	6.60±0.14^{a}	7.15±0.07^{b}
Crude fibre	4.05±0.21^{bc}	3.60±0.14^{a}	4.75±0.70^{ab}	4.15±0.70^{c}	4.75±0.21^{ab}	7.90±0.14^{abc}
Dry Matter	92.19±0.17	91.36±0.33	92.07±0.04	92.88±0.39	92.67±0.16	92.24±0.08
Gross energy (kcal/g)	4.12±0.00	4.02±0.00	4.03±0.00	4.11±0.00	4.01±0.00	4.13±0.00
Calcium	1.29±0.00^{b}	1.86±0.00^{e}	1.77±0.00^{d}	1.28±0.00^{a}	1.63±0.00^{c}	1.29±0.00^{ab}
Phosphorus	0.59±0.00^{b}	0.83±0.00^{e}	0.81±0.00^{d}	0.57±0.00^{a}	0.75±0.00^{c}	0.60±0.00^{b}
Potassium	0.82±0.00^{a}	0.97±0.00^{d}	0.97±0.00^{d}	0.82±0.00^{b}	0.95±0.00^{c}	0.81±0.00^{ab}
Sodium	0.28±0.00^{c}	0.38±0.00^{e}	0.38±0.00^{e}	0.27±0.00^{b}	0.36±0.00^{d}	0.27±0.00^{a}

Means with same letter in row are not significantly different (P>0.05)

Table 8: Nutrient composition of solvent extracted soyabean based test diets

		Diet (SS)				
Parameter (%)	Control	2	3	4	5	6
Crude protein	41.59±0.62	40.98±0.32	41.08±0.67	40.75±0.43	40.83±1.02	40.78±0.81
Ash	9.05±0.35^{b}	7.95±0.07^{a}	9.30±0.14^{a}	8.15±0.07^{a}	7.20±0.14^{b}	7.80±0.14^{b}
Ether extract	6.75±0.07^{bc}	6.35±0.07^{a}	6.55±0.21^{ab}	6.55±0.07^{ab}	7.05±0.14^{c}	6.70±0.14^{bc}
Crude fibre	5.85±0.21^{ab}	5.15±0.07^{bc}	6.05±0.21^{abc}	5.25±0.07^{c}	6.70±0.14^{a}	5.00±0.14^{abc}
Dry matter	92.29±0.37	92.25±0.08	92.09±0.68	92.31±0.15	92.09±0.19	92.11±0.05
Gross energy (kcal/g)	3.99±0.00	4.06±0.06	3.98±0.00	4.11±0.01	4.02±0.01	3.98±0.00
Calcium	1.58±0.00^{e}	1.35±0.00^{a}	1.55±0.00^{d}	1.36±0.00^{a}	1.37±0.00^{b}	1.55±0.00^{c}
Phosphorus	0.66±0.00^{e}	0.59±0.00^{a}	0.63±0.00^{d}	0.60±0.00^{b}	0.59±0.00^{a}	0.62±0.00^{c}
Potassium	0.80±0.00^{b}	0.84±0.00^{d}	0.79±0.00^{a}	0.84±0.00^{c}	0.83±0.00^{c}	0.78±0.00^{a}
Sodium	0.27±0.00^{b}	0.29±0.00^{c}	0.26±0.00^{a}	0.29±0.00^{d}	0.29±0.00^{cd}	0.25±0.00^{a}

Means with same letter in row are not significantly different (P>0.05)

4.2 PERFORMANCE AND AMINO ACID DIGESTIBILITY OF SOYABEAN BASED DIET SUPPLEMENTED WITH VARYING INCLUSION OF AMINO ACID IN *C. GARIEPINUS*

4.2.1 Growth performance and feed utilisation by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Growth performance and feed utilisation of *C.gariepinus* fed roasted soyabean based diet supplemented with varying inclusion of dietary amino acid is shown in Table 9. Lysine and methionine supplementation in roasted soyabean based diet fed to fish had no significant influence (P>0.05) on final weight, feed conversion ratio, feed intake, specific growth rate and protein intake. The values of these parameter ranged from 32.60±3.59 (RS6) to 36.37±4.59 (RS5); 3.03±0.67 (RS5) to 3.76±0.46 (control); 10.25±0.27 (RS6) to 11.47±0.63 (RS3); 0.68±0.02 (RS6) to 0.76±0.04 (RS3) and 0.34±0.04 (control) to 0.40±0.08 (RS6), respectively. Gross protein retention of fish fed diet RS3 (1.01±0.04) was not significantly difference (P>0.05) from that of RS5 diet. Nitrogen retention efficiency increased with amino acid supplementation and least values was in diet control (33.78±4.11). The survival rate of fish fed diet RS5 (85.60±0.60) was significantly higher than those fed control, RS2 and RS6, however, fish fed RS3 and RS4 were intermidate to them. The relationship between lysine and methionine inclusion and weight, feed conversion ratio and gross protein retention of *C. gariepinus* are represented by the regression equations 1, 2, 3, 4, 5 and 6, respectively.

For Final weigh gain

$y = -0.1709x^2 + 1.8928x + 30.547$……. $R^2 = 0.8001$……1 (Lysine)
$y = -0.5298x^2 + 3.6396x + 29.877$……. $R^2 = 0.8821$……2 (Methionine)

Feed conversion ratio

$y = 0.0914x^2 - 0.6463x + 4.212$…….. $R^2 = 0.8232$……3 (Lysine)

$y = 0.0293x^2 - 0.3439x + 4.096$…….. $R^2 = 0.9724$……4 (Methionine)

Gross protein retention

$y = -0.0087x2 + 0.0981x + 0.611$…… $R^2 = 0.2397$……5 (Lysine)
$y = -0.0302x2 + 0.2024x + 0.571$…… $R^2 = 0.3184$……6 (Methionine)

From the graphs (Figures 1, 2 and 3) it could be depicted that 0.6 and 0.4 g/100g inclusion of lysine and methionine was observed at the equations for it optimum inclusion in soyabean based diet.

Table 9: Growth performance and feed utilisation by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

		Diet (RS)				
Parameter	Control	2	3	4	5	6
IW (g)	19.70±0.42	19.70±0.20	19.50±0.20	19.63±0.12	19.90±0.20	19.53±0.25
FW (g)	32.75±2.90	35.40±2.90	35.30±4.98	35.03±3.16	36.37±4.59	32.60±3.59
MFW(g)	13.05±2.47	15.7±3.00	15.80±4.85	15.40±3.59	16.47±4.50	13.07±3.59
PFW (%)	66.80±11.14	79.75±15.80	80.93±24.50	78.4±2±15.74	82.67±22.27	66.91±18.46
FCR	3.76±0.46	3.13±0.55	3.37±1.14	3.19±0.56	3.03±0.67	3.54±0.78
GEFC	67.44±1.30	73.56±4.82	71.06±9.37	73.23±7.34	75.87±3.70	73.28±6.41
PI	11.20±0.78	11.10±0.23	11.47±0.63	11.07±0.86	11.04±1.01	10.25±0.27
FI	0.75±0.05	0.74±0.02	0.76±0.04	0.74±0.07	0.74±0.06	0.68±0.02
PER	10.92±0.97	11.80±0.97	11.76±1.66	11.68±1.05	12.12±1.53	10.87±1.20
SGR	0.34±0.04	0.39±0.06	0.39±0.09	0.39±0.06	0.40±0.08	0.34±0.07
GPR	0.74 ± 0.03^{b}	0.64 ± 0.01^{a}	1.01 ± 0.04^{d}	0.71 ± 0.03^{b}	0.97 ± 0.04^{d}	0.86 ± 0.03^{c}
NRE	33.78 ± 4.11^{a}	34.12 ± 3.71^{a}	47.41 ± 7.82^{b}	36.27 ± 4.57^{ab}	47.12 ± 8.86^{b}	37.52 ± 5.23^{ab}
SR %	70.00 ± 2.00^{a}	71.00 ± 1.00^{a}	82.27 ± 0.31^{b}	82.20 ± 0.10^{b}	85.60 ± 0.60^{c}	69.40 ± 0.40^{a}

Means with same letter in row are not significantly different (P>0.05)

IW = Initial Weight, FW= Final weight, TFI= Total Feed Intake, MFW= Mean Final weight, PFW= Percentage Final weight, FCR= Feed Conversion Ratio, GEFC= Gross Efficiency Feed Conversion, PI= Protein Intake, FI= Feed Intake, PER= Protein Efficiency Ratio, SGR= Specific Growth Rate, GPR= Gross Protein Retention, NRE= Nitrogen Retention Efficiency, SR= Survival Rate

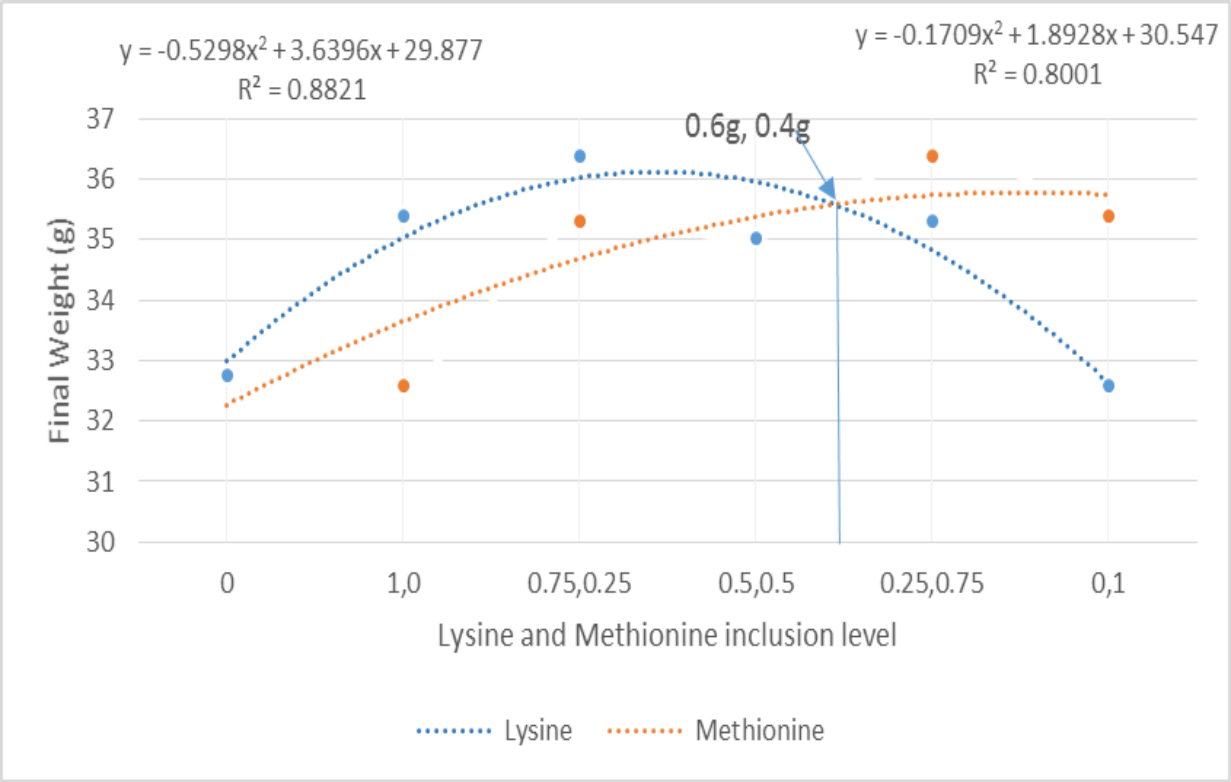

Figure 1: Relationship between dietary supplement of lysine and methionine in a roasted soyabean based diet and Final weight of *Clarias gariepinus*

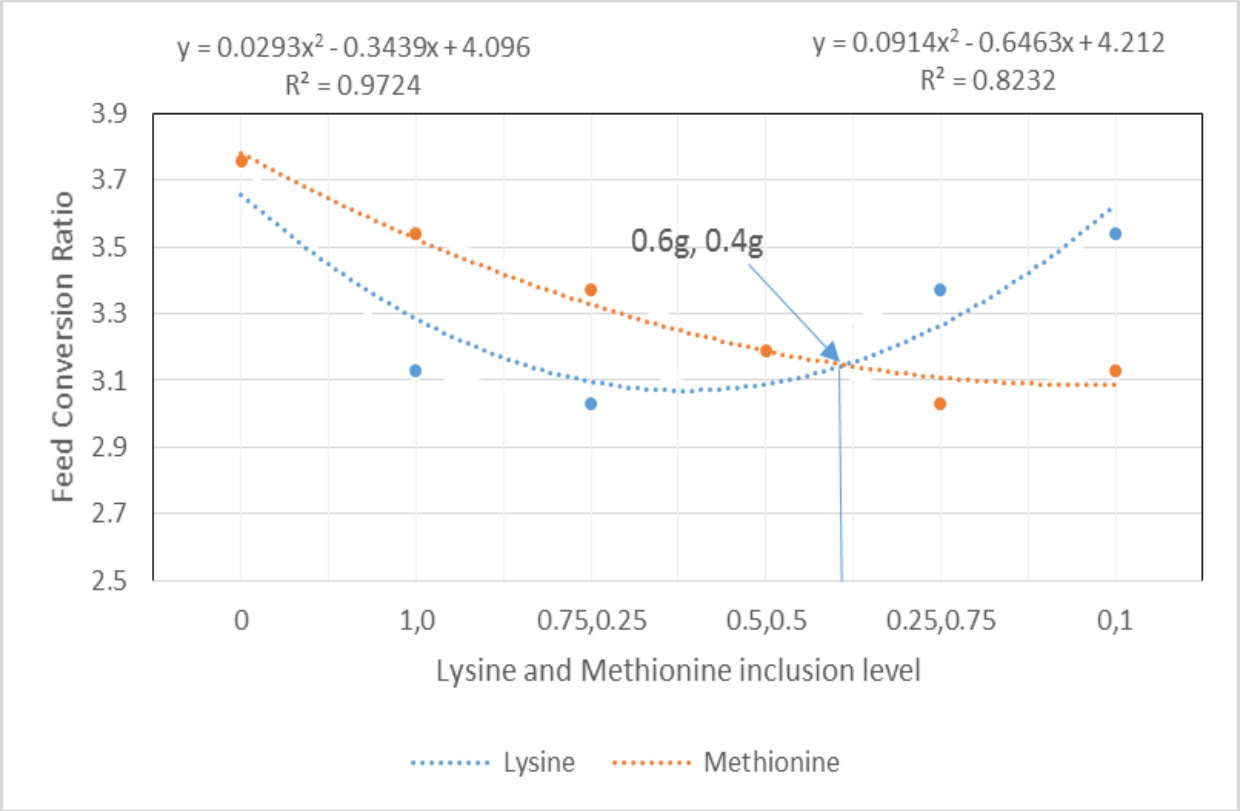

Figure 2: Relationship between dietary supplement of lysine and methionine in a roasted soyabean based diet and feed conversion ratio of *Clarias gariepinus*

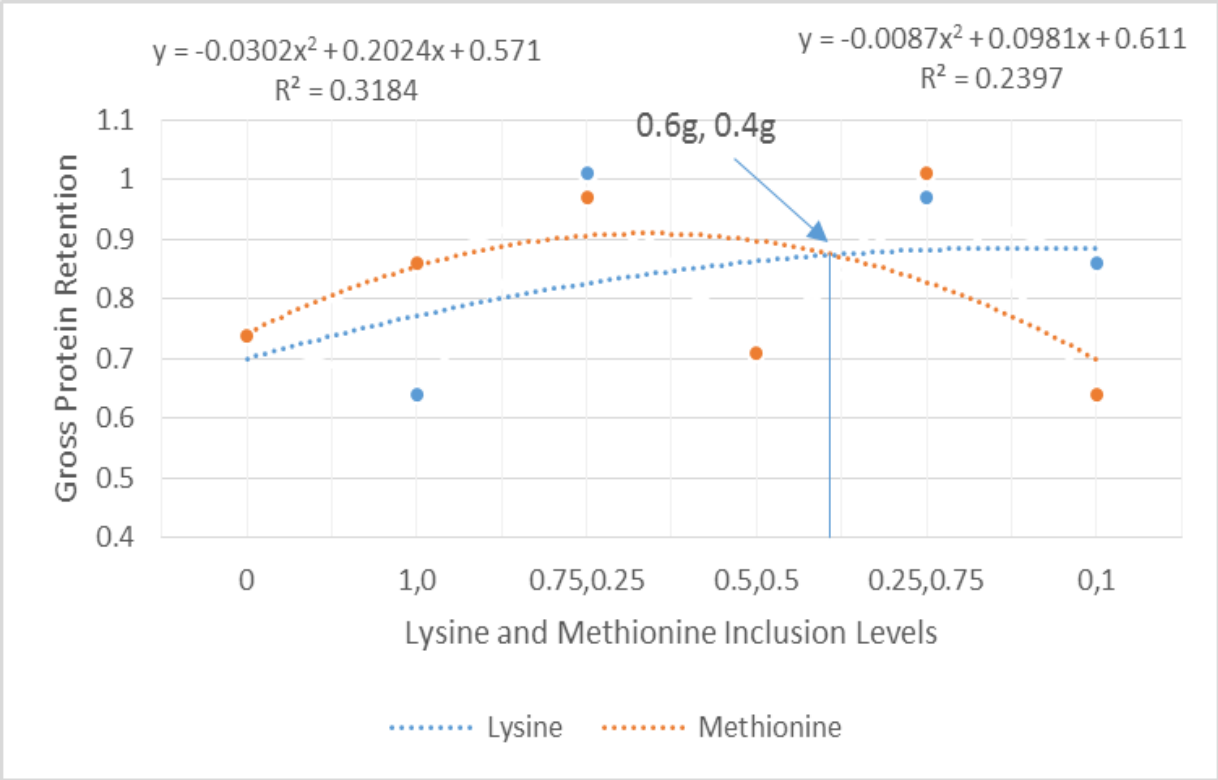

Figure 3: Relationship between dietary supplement of lysine and methionine in a roasted soyabean based diet and gross protein retention of *Clarias gariepinus*

4.2.2 Growth performance and feed utilisation by *C. gariepinus* fed solvent extracted soyabean based diet supplemented with amino acid

Growth performance of *C. gariepinus* fed solvent extracted soyabean based diet supplemented with varying inclusion of dietary amino acid is shown in Table 10. Supplemental amino acid in solvent extracted soyabean based diet significantly ($P<0.05$) influenced final weight with higher value in control (43.35±4.03) but this was not significant from those fed diets SS3 and SS5, however, these were superior to other diets. Mean Final weight and percentage Final weight significantly ($P<0.05$) decreased with amino acid supplementation with the higher value in control diet (25.50±4.53g and 143.3±29.33g). Supplementation of amino acid had no significant effect ($P>0.05$) on FCR and GEFC values of the dietary treatment. Values of FCR and GEFC ranged from 2.27±0.32 (SS3) to 3.12±0.31 (SS2) and 66.97±7.03 (SS5) to 77.97±8.93 (SS3), repectively. Protein intake and feed intake of fish fed RS6 diet were significantly lower than fish fed control diet, however, fish fed diet SS2, SS3, SS4 and SS5 were intermidiete to them. Protein efficiency ratio of control diet significantly higher than those of fish fed diet SS2, SS4 and SS6. However, specific growth rate differ significantly among diets except fish fed diet SS4 and SS5 that were similar ($P>0.05$). Higher ($P<0.05$) GPR and NRE values were in *C.gariepinus* fed diet SS5 (0.79±0.02) and control (49.49±6.19) and least value diet SS2 (0.66±0.02 and 35.15±1.42), respectively. Survival rate differ significantly ($P<0.05$) among treatment. The relationship between lysine and methionine inclusion and Final weight, weight, feed conversion ratio and gross protein retention of *C. gariepinus* are represented by the regression equations 7, 8, 9, 10, 11 and 12, respectively.

Final weight

$y = 0.26x^2 - 2.9237x + 44.148$……. $R^2 = 0.406$..……7 (Lysine)

$y = 0.4121x^2 - 3.6641x + 44.432$……. $R^2 = 0.2889$……8 (Methionine)

Feed conversion ratio

$y = -0.0093x^2 + 0.1436x + 2.38$ ……. $R^2 = 0.1852$……9 (Lysine)

$y = -0.02x^2 + 0.1957x + 2.36$……. $R^2 = 0.1153$……10 (Methionine)

Gross protein retention

$y = -0.0005x2 + 0.024x + 0.669$……. $R^2 = 0.6138$……11 (Lysine)

$y = -0.0155x2 + 0.097x + 0.641$…….. $R^2 = 0.9712$……12(Methionine)

From the graphs (Figures 4, 5 and 6) it could be depicted that 0.6 and 0.4g/100g inclusion of lysine and methionine was observed at the equations for it optimum inclusion in solvent extracted soyabean based diet.

Table 10: Growth performance and feed utilisation of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

		Diet (SS)				
Parameter	Control	2	3	4	5	6
IW(g)	17.85±0.49	18.03±0.38	17.50±0.10	17.77±0.32	17.50±0.00	17.80±0.17
FFW (g)	43.35±4.03^{b}	34.77±1.17^{a}	40.80±1.74^{ab}	36.13±1.27^{a}	36.70±5.11^{ab}	35.40±4.99^{a}
MFW(g)	25.50±4.53^{b}	16.73±1.05^{a}	23.30±1.82^{ab}	18.36±0.97^{a}	19.20±5.11^{ab}	17.60±4.85^{a}
PFW (%)	143.26±29.33^{b}	92.81±5.79^{a}	133.18±11.03^{ab}	103.34±3.92^{a}	109.71±29.21^{ab}	98.74±26.53^{a}
FCR	2.35±0.15	3.12±0.31	2.27±0.32	2.88±0.26	2.95±0.60	2.88±0.62
GEFC	72.96±1.52	67.25±4.32	77.97±8.93	68.66±4.85	66.97±7.03	72.53±7.51
PI	13.73±1.56^{b}	11.95±0.67^{ab}	12.15±1.05^{ab}	12.17±0.47^{ab}	12.65±1.14^{ab}	11.24±0.72^{a}
FI	0.92±0.10^{b}	0.80±0.04^{ab}	0.81±0.07^{ab}	0.81±0.03^{ab}	0.84±0.08^{ab}	0.75±0.05^{a}
PER	14.45±1.34^{b}	11.59 ±0.39^{a}	13.60±0.58^{ab}	12.04±0.42^{a}	12.23±1.70^{ab}	11.80±1.66^{a}
SGR	0.59±0.08^{c}	0.44±0.02^{a}	0.57±0.03^{bc}	0.47±0.01^{abc}	0.49±0.09^{abc}	0.46±0.09^{ab}
GPR	0.72±0.02^{b}	0.66±0.02^{a}	0.75±0.04^{bc}	0.77±0.03^{cd}	0.79±0.02^{d}	0.78±0.03^{cd}
NRE	49.49±6.19^{b}	35.15±1.42^{a}	47.64±2.10^{b}	41.27±1.66^{ab}	43.17±7.76^{ab}	40.74±7.38^{ab}
SR %	87.70±0.20^{d}	87.70±0.20^{d}	82.20±0.20^{b}	85.60±0.20^{c}	88.90±0.10^{e}	77.80±0.20^{a}

Means with same letter in row are not significantly different (P>0.05)

IW = Initial Weight, FW= Final weight, TFI= Total Feed Intake, MFW= Mean Final weight, PFW= Percentage Final weight, FCR= Feed Conversion Ratio, GEFC= Gross Efficiency Feed Conversion, PI= Protein Intake, FI= Feed Intake, PER= Protein Efficiency Ratio, SGR= Specific Growth Rate, GPR= Gross Protein Retention, NRE= Nitrogen Retention Efficiency, SR= Survival Rate.

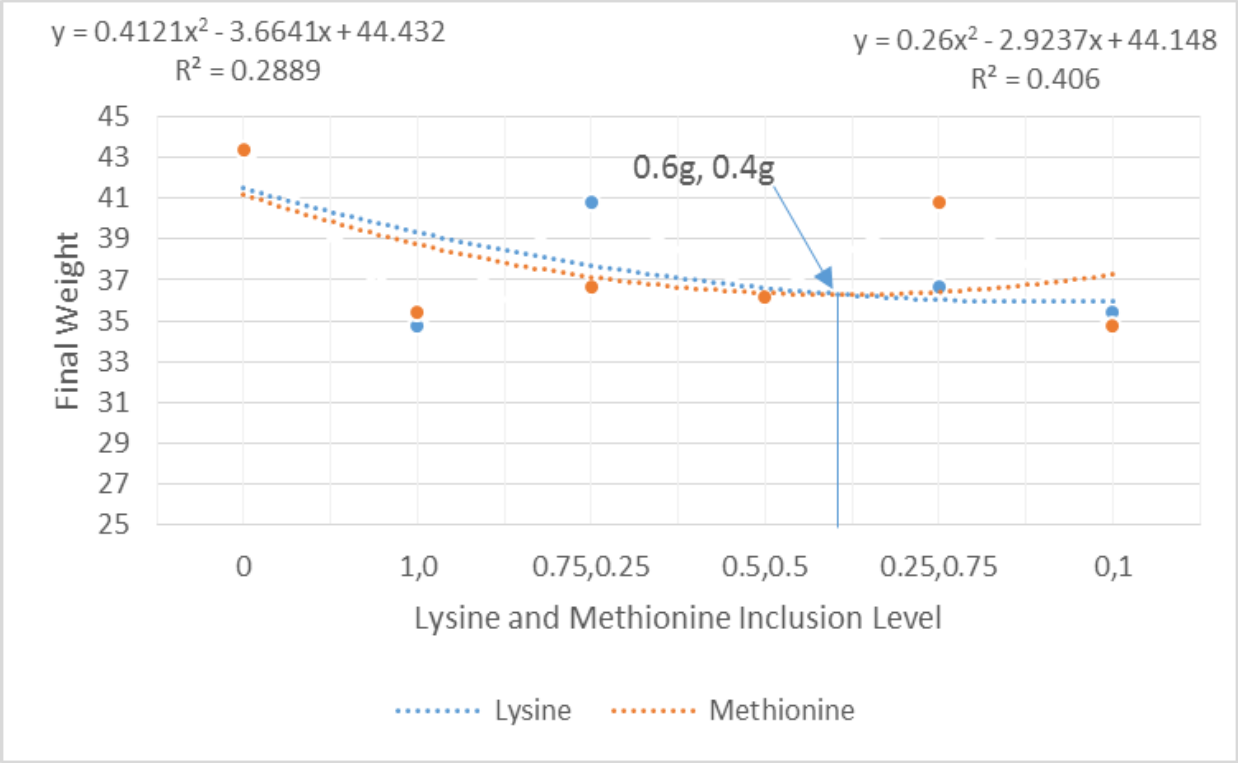

Figure 4: Relationship between dietary supplement of lysine and methionine in a solvent extracted soyabean based diet and Final weight of *Clarias gariepinus*.

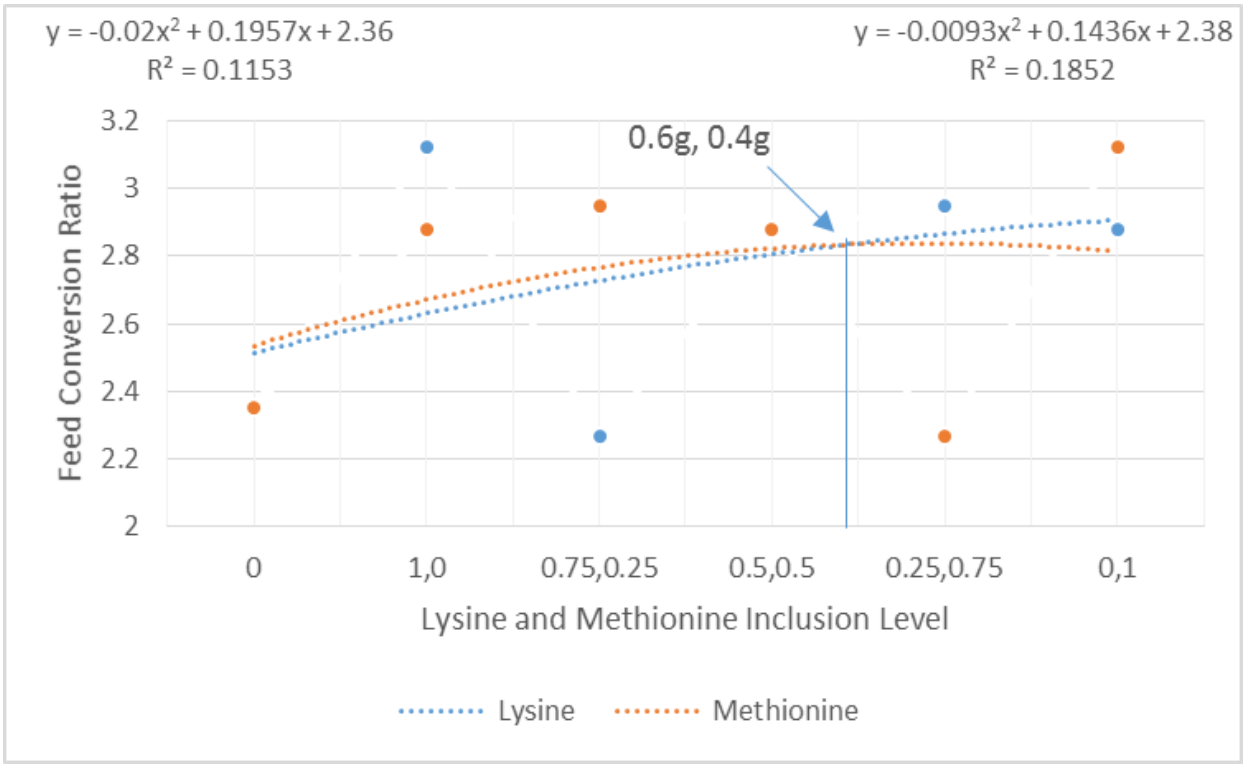

Figure 5: Relationship between dietary supplement of lysine and methionine in a solvent extracted soyabean based diet and feed conversion ratio of *Clarias gariepinus*

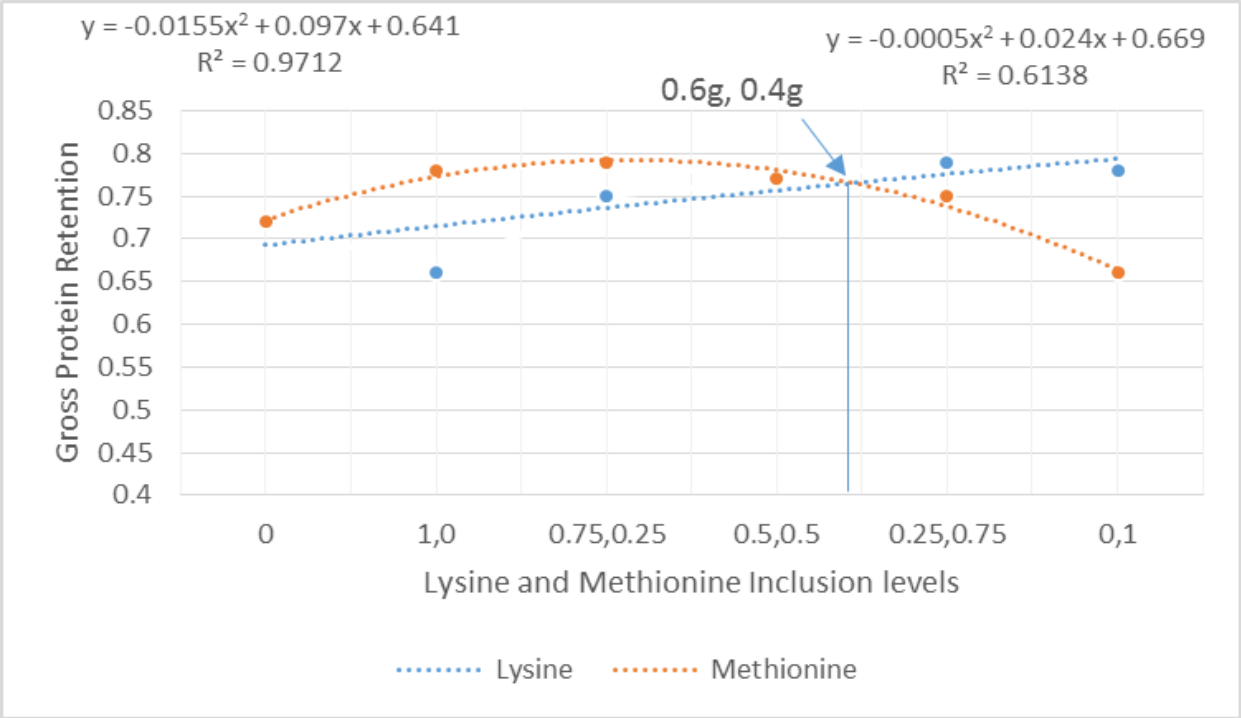

Figure 6: Relationship between dietary supplement of lysine and methionine in a solvent extracted soyabean based diet and gross protein retention of *Clarias gariepinus*.

4.2.3 Haematological parameters of *C. gariepinus* fed roasted soyabean based diets supplementaled with amino acid

Haematological parameters of *C.gariepinus* fed roasted soyabean based diet supplemented with varying inclusion of dietary amino acid is shown in Table 11. Significantly (P<0.05) higher PCV (%) and hemoglobin volume were observed in fish fed diet RS4 and RS5 while RS2, RS3 and RS6 did not differ significantly. Red blood cell ($x10^{12}$/L) value ranged from 1.43±0.05 (control) to 2.75±0.92 (RS5). However, white blood cell ($x10^{9}$/L) differ significantly among diets. MCV (fl), MCH (pg) and MCHC (g/dL) values ranged from 105.35±31.76 (RS5) to 146.76±8.93 (control); 33.11±10.29 (RS5) to 47.30±0.87 (control) and 31.17±0.14 (RS2) to 33.16±1.82 (RS4).

Supplementation of amino acid in roasted soyabean based diet significantly (P<0.05) reduced platelet ($x10^{9}$/L) value and platelet value of fish fed control diet was significantly higher than other treatment. Also, a significantly higher lymphocytes (%) values was observed in RS5, RS6 and control diet. Moreso, heterocytes (%) count was higher (P<0.05) in RS3 (39.00±4.58) and least value of 27.00±2.00 in RS5. Lymphocytes: heterocytes ratio values varies significantly between the treatments. Supplemental amino acid had no influences (P>0.05) on Monocytes (%), Eosinophils (%) and basophils (%).

Table 11: Haematological parameters of *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

		Diet (RS)				
Parameter	Control	2	3	4	5	6
PCV (%)	21.00±1.73^{a}	24.33±2.52^{ab}	24.00±1.00^{ab}	26.00±1.73^{b}	27.00±2.65^{b}	24.67±2.08^{ab}
HB(g/dL)	6.77±0.38^{a}	7.77±0.80^{ab}	7.73±0.70^{ab}	8.60±0.10^{b}	8.47±0.76^{b}	8.00±1.00^{ab}
RBC(x10^{12}/L)	1.43±0.05	2.19±0.88	1.64±0.10	2.28±0.98	2.75±0.92	2.23±0.93
WBC(x10^{9}/L)	12.82±0.33^{a}	15.43±0.98^{abc}	18.13±0.49^{c}	16.05±0.59^{bc}	15.00±2.96^{ab}	15.42±1.38^{abc}
MCV (fl)	146.76±8.93	119.35±30.41	146.49±5.54	124.96±37.46	105.35±31.76	120.04±33.54
MCH (pg)	47.30±1.58	38.11±9.78	47.14±3.06	41.87±14.25	33.11±10.29	38.72±10.60
MCHC(g/dL)	32.27±0.87	31.17±0.14	32.18±1.61	33.16±1.82	31.38±0.93	32.36±1.67
Platelet (x 10^{9}/L)	32.83±6.48^{b}	19.03±9.46^{a}	15.30±3.54^{a}	12.00±2.80^{a}	15.13±3.53^{a}	12.60±3.80^{a}
Lym (%)	63.33±3.21^{b}	56.33±4.16^{a}	53.33±3.79^{a}	59.00±5.00^{a}	65.33±2.08^{b}	63.67±3.51^{b}
Het (%)	28.67±2.52^{a}	37.33±3.51^{bc}	39.00±4.58^{c}	33.33±2.52^{abc}	27.00±2.00^{a}	30.67±5.86^{ab}
Lym:Het Ratio	0.46±0.06^{a}	0.67±0.11^{bc}	0.74±0.14^{c}	0.57±0.10^{abc}	0.41±0.05^{a}	0.49±0.11^{ab}
Mono (%)	3.00±1.00	2.67±2.08	2.33±1.15	3.67±0.58	3.67±1.15	3.33±1.53
Eos (%)	4.67±1.53	3.67±0.58	4.33±2.52	3.67±1.53	3.67±1.53	2.00±3.46
Baso (%)	0.33±0.58	0.00±0.00	0.33±0.58	0.33±0.58	0.33±0.58	0.33±0.58

Means with same letter in row are not significantly different (P>0.05)

PCV= Packed Cell Volume, HB= Hemoglobin, RBC= Red Blood Cell, WBC= White Blood Cell, MCV= Mean Cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, Lym= Lymphocytes, Het= Heterocytes, Mono= Monocytes, Eos = Eosinophils, Baso = Basophils.

4.2.4 Haematological parameters of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Haematological parameters of *C.gariepinus* fed solvent extracted soyabean based diet supplemented with dietary amino acid is shown in Table 12. Amino acid supplementation had no significantly (P>0.05) influenced on Packed cell volume (%), hemoglobin (g/dL) and red blood cell ($x10^{12}$/L). There values ranged from 22.67±3.79 (control) to 26.33±1.53 (SS4), 7.57±1.44 (control) to 8.77±0.67 (SS5) and 1.51±0.04 (SS3) to 2.74±0.65 (SS4), respectively. White blood cell ($x10^{9}$/L) was significantly higher (P<0.05) in SS4 (17.48±0.60) and least value in SS3 (13.78±1.26) while fish fed diet SS2, SS5, SS6 and control were intermediate to them. MCV (fl) and MCH (pg) of fish fed diet SS4 and SS5 were not significantly different from each other, but these were all significantly lower than other diets. MCHC (g/dL) values were not significantly (P<0.05) different among the treatment and ranged from 34.15±1.06 (SS5) to 32.60±0.64 (SS2).

Platelet ($x10^{9}$/L) significantly ranged from SS2 (10.83±0.97) to SS4 (17.53±1.29), however, fish fed diet SS5 and SS6 were similar to SS4 diet. Lymphocytes (%) value significantly decreased with amino acid supplementation in *C. gariepinus* diet. Moreover, heterocytes (%) value significantly (P<0.05) increased with amino acid supplementation with higher value in fish fed diet SS4 (30.67±4.04) but differ significantly from fish fed diet SS2, SS3, SS5 and SS6. Supplementation of amino acid influenced (P<0.05) lymphocytes: heterocytes ratio with higher value of 0.50±0.10 in SS4 and control diet (0.34±0.08) had the least value while, fish fed diet SS2, SS3, SS5 and SS6 were similar to each other. The values observed in Monocytes (%), Eosinophils (%) and Basophiles (%) ranged from 2.33±0.58 (SS3) to 3.67±0.58 (SS5), 2.33±1.15 (SS6) to 4.33±2.08 (SS2) and 0.00±0.00 (SS2) to 0.67±0.58 (SS3) (P>0.05), respectively.

Table 12: Haematological parameters of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

		Diet (SS)				
Parameter	Control	2	3	4	5	6
PCV (%)	22.67±3.79	25.67±0.58	23.33±0.58	26.33±1.53	25.67±1.53	24.67±1.53
HB(g/dL)	7.57±1.44	8.37±0.12	7.90±0.61	8.63±0.71	8.77±0.67	8.30±0.78
RBC($x10^{12}$/L)	2.01±1.05	2.17±0.15	1.50±0.04	2.74±0.65	2.58±0.92	2.08±0.41
WBC($x10^{9}$/L)	$14.82±2.47^{ab}$	$16.27±0.38^{ab}$	$13.78±1.26^{a}$	$17.48±0.60^{b}$	$15.78±2.93^{ab}$	$14.6±1.98^{ab}$
MCV (fl)	$125.59±36.44^{ab}$	$118.39±6.32^{ab}$	$154.32±7.88^{b}$	$98.64±16.18^{a}$	$106.68±31.36^{a}$	$120.79±18.40^{ab}$
MCH (pg)	$41.68±11.55^{ab}$	$38.60±2.19^{ab}$	$52.29±5.21^{b}$	$32.24±4.77^{a}$	$36.33±10.40^{a}$	$40.46±4.75^{ab}$
MCHC(g/dL)	33.30±0.79	32.60±0.64	33.84±2.27	32.75±0.79	34.15±1.06	33.61±1.52
Platelet (x 10^{9}/L)	$12.53±2.64^{ab}$	$10.83±0.97^{a}$	$14.40±0.46^{bc}$	$17.53±1.29^{d}$	$15.63±0.91^{cd}$	$16.90±1.45^{cd}$
Lym (%)	$69.33±5.13^{b}$	$65.67±4.61^{ab}$	$63.33±1.52^{ab}$	$61.67±4.51^{a}$	$67.00±1.73^{ab}$	$66.67±4.04^{ab}$
Het (%)	$23.33±3.51^{a}$	$27.00±5.29^{ab}$	$30.00±2.00^{ab}$	$30.67±4.04^{b}$	$26.33±3.21^{ab}$	$27.67±3.06^{ab}$
Lym:Het Ratio (%)	$0.34±0.08^{a}$	$0.41±0.10^{ab}$	$0.48±0.05^{ab}$	$0.50±0.10^{b}$	$0.39±0.06^{ab}$	$0.42±0.07^{ab}$
Mono (%)	3.00±1.00	3.00±1.00	2.33±0.58	3.33±0.58	3.67±0.58	3.00±0.00
Eos (%)	4.00±1.00	4.33±2.08	3.67±0.58	4.00±1.00	3.00±1.00	2.33±1.15
Baso (%)	0.33±0.58	0.00±0.00	0.67±0.58	0.33±0.58	0.00±0.00	0.33±0.58

Means with same letter in row are not significantly different (P>0.05)

PCV= Packed Cell Volume, HB= Hemoglobin, RBC= Red Blood Cell, WBC= White Blood Cell, MCV= Mean Cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, Lym= Lymphocytes, Het= Heterocytes, Mono= Monocytes, Eos = Eosinophils, Baso = Basophils.

4.2.5 Serum biochemical indices of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of amino acid

Serum biochemical indices of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 13. Supplemental amino acid had no influence (P>0.05) on total protein (g/L), Globulin (g/L), Albumin (g/L), A-G ratio and AST (IU/L). There values ranged from 6.60±0.52 (RS6) to 7.87±0.55 (RS4), 1.67±0.15 (RS6) to 2.60±0.53 (RS4), 4.80±0.10 (control) to 5.27±0.06 (RS4), 0.30±0.00 (RS6) to 0.47±0.12 (RS4) and 182.67±5.13 (RS3) to 197.67±21.39 (RS4), respectively. Alanine Transaminase (IU/L) and Blood Urea Nitrogen (µmol/L) were significantly higher (P<0.05) in fish fed diet RS2, RS4, RS5 and RS6 while least value was in *C. gariepinus* fed control diet (19.33±5.03). Also, Alkaline Phosphatase (IU/L) was not significantly (P<0.05) different among the fish fed diet RS3, RS5 and RS6, however, these were significantly superior than fish fed RS2 diet. Furthermore, Creatinine (µmol/L) significantly increased with the supplementation of amino acid. Higher value was observed in diet RS2 (0.77±0.56) and least value in control diet (0.53±0.06).

4.2.6 Serum biochemical indices of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of amino acid

Serum biochemical indices of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 14. Supplemental amino acid had no effect (P>0.05) on total protein (%), Albumin (g/L), A-G ratio, Aspartate Transaminase (IU/L), Alanine Transaminase (IU/L) and Alkaline Phosphatase (IU/L). There values ranged from 6.50±0.50 (Control) to 7.70±1.32 (SS5), 1.63±0.49 (control) to 2.53±0.21 (SS5), 0.47±0.06 (SS5) to 0.27±0.27 (Control), 181.33±3.21 (SS6) to 206.00±37.04 (SS2), 23.00±2.00 (SS2) to 31.00±1.73 (SS3) and 336.67±48.81 (SS6) to 266.66±3.51 (SS2), respectively. However, Globulin (g/L) was not significantly (P<0.05) different between the fish fed diet SS5 and SS6 but significantly higher than other treatments. Increased Blood Urea Nitrogen (µmol/L) values were observed in diet SS5 (10.00±0.20) with *C.gariepinus* fed varying inclusion of Lysine and Methionine compare to the Control diet (8.60±0.40) with the least value. Supplementation of amino acid significantly increased Creatinine (µmol/L) values with higher value in fish fed diet SS3 and SS5 and least in control diet (0.57±0.06).

Table 13: Serum biochemical indices of *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

		Diet (RS)				
Parameter	Control	2	3	4	5	6
Total protein(g/L)	6.67±0.29	7.33±1.26	6.83±0.76	7.87±0.55	7.07±0.50	6.60±0.52
Albumin(g/L)	1.87±0.38	2.43±0.90	1.77±0.40	2.60±0.53	2.13±0.67	1.67±0.15
Globulin(g/L)	4.80±0.10	4.90±0.36	5.07±0.40	5.27±0.06	4.93±0.25	4.93±0.38
A-G ratio	0.33±0.06	0.47±0.15	0.30±0.10	0.47±0.12	0.37±0.15	0.30±0.00
AST (IU/L)	185.33±3.06	189.33±4.04	182.67±5.13	197.67±21.39	188.33±7.57	186.00±5.29
ALT (IU/L)	19.33±5.03^{a}	32.33±3.21^{b}	21.33±2.31^{a}	24.33±7.51^{ab}	26.67±3.79^{ab}	30.67±4.04^{b}
ALP (IU/L)	208.67±29.96^{ab}	144.67±18.50^{a}	289.33±57.74^{b}	231.67±121.33^{ab}	289.67±24.21^{b}	291.67±13.80^{b}
BUN(µmol/L)	8.57±0.74^{a}	9.40±1.11^{ab}	8.80±0.62^{a}	10.47±0.70^{b}	9.27±0.50^{ab}	9.10±0.66^{ab}
Creatinine (µmol/L)	0.53±0.06^{a}	0.77±0.56^{c}	0.60±0.10^{ab}	0.70±0.10^{bc}	0.60±0.10^{ab}	0.67±0.06^{abc}

Means with same letter in row are not significantly different (P>0.05)

A-G Ratio = Albumin-Globulin Ratio, AST = Aspartate Transaminase, ALT = Alanine Transaminase, ALP = Alkaline Phosphatase, BUN = Blood Urea Nitrogen.

Table 14: Serum biochemical indices of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

		Diet (SS)				
Parameter	Control	2	3	4	5	6
Total protein (g/L)	6.50±0.50	6.83±0.76	6.80±0.20	7.00±1.32	7.70±0.17	7.43±0.40
Albumin (g/L)	1.63±0.49	2.00±0.87	2.10±0.40	1.97±1.24	2.53±0.21	2.33±0.21
Globulin (g/L)	4.86±0.21^{ab}	4.83±0.21^{ab}	4.70±0.20^{a}	5.03±0.21^{ab}	5.17±0.06^{b}	5.10±0.20^{b}
A-G ratio	0.27±0.27	0.40±0.17	0.43±0.15	0.43±0.23	0.47±0.06	0.40±0.00
AST (IU/L)	188.67±2.52	206.00±37.04	184.00±4.00	187.00±7.21	190.00±3.00	181.33±3.21
ALT (IU/L)	29.00±7.55	23.00±2.00	31.00±1.73	24.00±7.94	31.00±3.61	23.67±7.77
ALP (IU/L)	309.00±43.51	266.66±3.51	336.00±13.75	270.33±11.02	326.67±76.14	336.67±48.81
BUN (µmol/L)	8.60±0.40^{a}	8.67±0.83^{a}	8.90±0.56^{ab}	8.87±0.90^{ab}	10.00±0.20^{b}	9.37±0.85^{ab}
Creatinine(µmol/L)	0.57±0.06^{a}	0.73±0.06^{bc}	0.76±0.06^{c}	0.73±0.06^{bc}	0.77±0.06^{c}	0.63±0.06^{ab}

Means with same letter in row are not significantly different (P>0.05)

A-G Ratio- Albumin-Globulin Ratio, AST = Aspartate Transaminase, ALT = Alanine Transaminase, ALP = Alkaline Phosphatase, BUN = Blood Urea Nitrogen.

4.2.7 Proximate composition of *C. gariepinus* whole body fed roasted soyabean based diets supplemented with amino acid

Proximate composition of *C. gariepinus* fed roasted soyabean based diet supplemented with dietary amino acid is shown in Table 15. Crude protein of *C. gariepinus* whole body fed diet RS5 (70.03±1.60) and RS3 (68.39±1.46) were not significantly different from each other, though, they were significantly higher than other diets. Ash content significantly differ among diets and the value ranged from RS6 (3.53±0.22) to RS5 (5.65±0.21). Supplementation of amino acid significantly (P>0.05) increased the ether extract values except for fish fed diet RS5 with the least value. Furthermore, Crude Fibre was significantly higher (P<0.05) in the Initial whole fish (0.04±0.01) that other diets. However, dry matter of the experimental fish varied significantly among diets with the higher value in RS2 (28.31±1.17) and least in initial whole fish (22.52±0.96). The regression equations of lysine and methionine inclusion on relationship between roasted soyabeans based diet and whole body crude protein as presented in equations 13 - 14 and shown in figures 7 respectively.

$y = -2.038x^2 + 21.401x + 12.103$…… $R^2 = 0.7633$…… 13 (Lysine)

$y = -2.9485x2 + 27.319x + 6.64$…… $R^2 = 0.7943$……14 (Methionine)

From the graphs (Figures 7) it could be depicted that 0.6 and 0.4 g/100g inclusion of lysine and methionine was observed at the equations for it optimum inclusion in roasted soyabean based diet.

Table 15: Proximate composition of *C. gariepinus* whole body fed roasted soyabean based supplemented with amino acid

Parameter	Initial	Control	Diet (RS)				
			2	3	4	5	6
Crude protein	24.50±0.00^{a}	59.01±1.22^{c}	55.01±0.23^{b}	68.39±1.46^{e}	57.95±1.39^{c}	70.03±1.60^{e}	63.75±1.11^{d}
Ash content	3.80±0.18^{ab}	3.93±0.13^{bc}	3.73±0.10^{ab}	5.68±0.31^{d}	4.16±0.19^{c}	5.65±0.21^{d}	3.53±0.22^{a}
Ether extract	7.15±0.21^{a}	8.10±0.24^{bc}	8.33±0.17^{c}	8.35±0.21^{c}	8.88±0.25^{d}	7.90±0.36^{b}	8.03±0.25^{bc}
Crude fibre	0.04±0.01^{b}	0.03±0.01^{ab}	0.03±0.01^{a}	0.03±0.01^{ab}	0.04±0.01^{ab}	0.03±0.01^{ab}	0.03±0.01^{ab}
Dry matter	22.52±0.96^{a}	25.56±0.50^{b}	28.31±1.17^{e}	27.49±0.41^{de}	26.14±0.71^{bc}	27.18±0.24^{cd}	26.92±0.31^{cd}

Means with same letter in row are not significantly different (P>0.05)

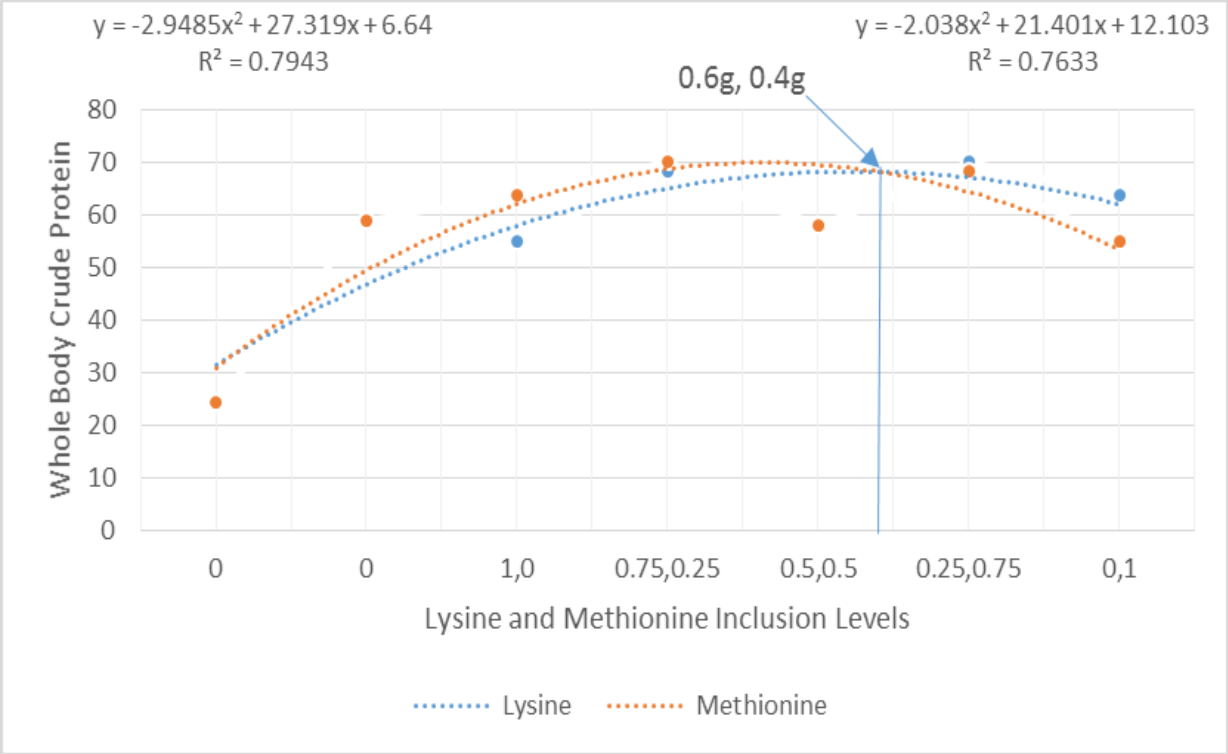

Figure 7: Relationship between dietary supplement of lysine and methionine in a roasted soyabean based diet and Whole body crude protein of *Clarias gariepinus*

4.2.8 Proximate composition of *C. gariepinus* whole body fed solvent extracted soyabean based diets supplemented with amino acid

Proximate composition of *C. gariepinus* fed solvent extracted soyabean based diet supplemented with dietary amino acid is shown Table 16. Significantly higher ($P<0.05$) crude protein was in SS5 (61.71±0.62) but similar to *C. gariepinus* in diet SS4 and SS6. Ash was significantly higher ($P<0.05$) in diet SS3 (6.73±0.36) while, fish fed diet SS4 and SS5 were not significantly different from each other. However, these were significantly higher than fish fed control diet, SS2 and SS6. Amino acid supplementation in *C. gariepinus* diets improved the ether extract except for *C. gariepinus* on diet SS5 that was related ($P>0.05$) to control diet. Crude fibre value ranged from 0.02±0.01 (control) to 0.04±0.02 (SS2). Dry matter values varies significantly ($P>0.05$) with the higher value in diet SS5 (76.54±0.60) and least in initial (22.52±0.96) whole body not fed experiemental diet.

The regression equations of lysine and methionine inclusion on relationship between roasted soyabeans based diet and whole body crude protein as presented in equations 15 - 16 and shown in figures 8, respectively.

$y = -1.883x^2 + 19.329x + 14.64$ …… $R^2 = 0.7641$…… 15 (Lysine)

$y = -2.4606x^2 + 23.084x + 11.17$…… $R^2 = 0.7876$…… 16 (Methionine)

From the graphs (Figures 8) it could be depicted that 0.6 and 0.4 g/100g inclusion of lysine and methionine was observed at the equations for it optimum inclusion in solvent extracted soyabean based diet.

Table 16: Proximate composition of *C. gariepinus* whole body fed solvent extracted soyabean based diets supplemented with amino acid

Parameter	Initial	Control	Diet (SS) 2	3	4	5	6
Crude protein	29.50±0.00^{a}	58.26±0.99^{c}	55.73±0.75^{b}	59.36±1.52^{cd}	60.18±1.13^{de}	61.15±0.62^{e}	60.90±1.01^{de}
Ash content	3.80±0.18^{a}	4.73±0.39^{b}	4.65±0.13^{b}	6.73±0.36^{d}	5.33±0.17^{c}	5.53±0.17^{c}	4.60±0.18^{b}
Ether extract	7.15±0.21^{a}	8.13±0.33^{b}	8.35±0.21^{bc}	8.58±0.17^{cd}	8.63±0.22^{cd}	8.10±0.18^{b}	8.78±0.21^{d}
Crude fibre	0.04±0.01	0.02±0.01	0.03±0.01	0.03±0.01	0.04±0.02	0.03±0.01	0.03±0.01
Dry matter	22.52±0.96^{a}	74.96±0.82^{cd}	75.86±0.42^{de}	74.19±0.19^{bc}	75.76±0.42^{de}	76.54±0.60^{e}	73.34±0.73^{b}

Means with same letter in row are not significantly different (P>0.05)

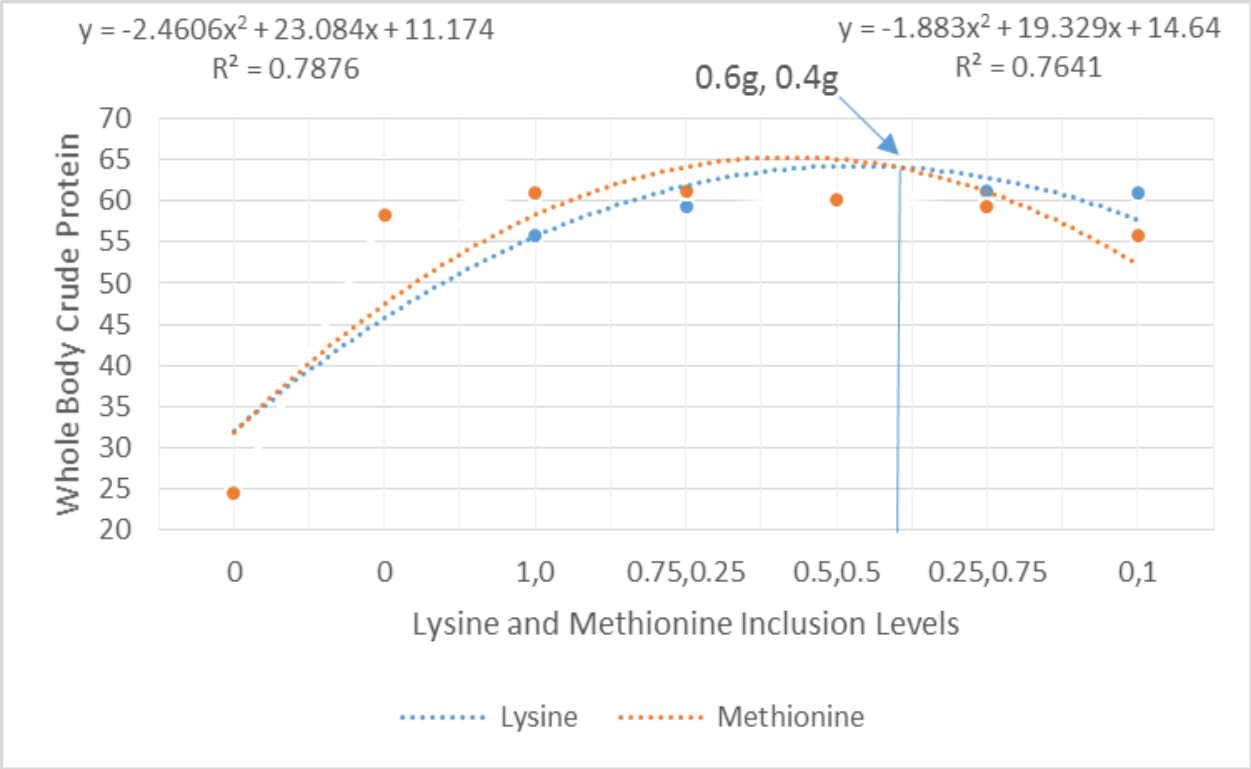

Figure 8: Relationship between dietary supplement of lysine and methionine in a solvent extracted soyabean based diet and whole body crude protein of *Clarias gariepinus*

4.2.9 Amino acid composition of *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Amino acid composition of *C.gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown Table 17. Methionine content were significantly (P<0.05) influenced with amino acid supplementation with the higher value in RS4 (2.42±0.04) and least value of RS2 (2.12±0.02). Lysine had higher (P<0.05) value of 10.69±0.01 (RS5) while the least value of 6.94±0.04 in control diet. Also, significantly (P<0.05) higher value of 3.02 ±0.02 (control) was in threonine and fish fed diet RS3 and RS4 were similar. Tryptophan had higher (P<0.05) value in *C.gariepinus* fed diet RS3 (2.60±0.10) than other treatments.

Isoleusine and leusine had higher (P<0.05) values of 8.93±0.03 and 8.84±0.02 in *C.gariepinus* fed RS2 and least values of 1.22±0.02 and 1.19±0.01 were in RS1. Significantly difference (P<0.05) values was obtained in Valine, Histidine, and Phenyalalnine with the least value in RS5 (2.25±0.05), RS6 (3.20±0.20), RS2 (1.87±0.02) and the higher value in RS4 (2.94±0.10), control (8.13±0.03), RS6 (2.73±0.03), respectively. The values obtained in Glycine, Alanine, Glutamic, Cysteine and Arginine the least value obtained in *C.gariepinus* fed diet RS6 (3.32±0.02, 3.20±0.20, 3.12±0.10, 1.30±0.10, 2.73±0.03) and the higher values were obtained in control diet (8.68±0.02, 9.04±0.04, 8.63±0.03, 3.65±0.05 7.29±0.01), respectively.

Table 17: Amino acid composition of *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

		Diet (RS)				
Parameter	Control	2	3	4	5	6
Essential Amino Acid						
Methionine	2.30±0.10^{cd}	2.12±0.02^{a}	2.24±0.04^{bc}	2.42±0.02^{e}	2.18±0.02^{ab}	2.35±0.05^{de}
Lysine	6.94±0.04^{a}	7.91±0.01^{b}	9.28±0.02^{d}	9.89±0.01^{e}	10.69±0.01^{f}	8.52±0.02^{c}
Threonine	3.02±0.02^{e}	2.57±0.03^{d}	2.23±0.03^{b}	2.18±0.02^{b}	2.40±0.10^{c}	2.05±0.05^{a}
Tryptophan	2.41±0.10^{c}	1.91±0.01^{a}	2.60±0.10^{d}	2.44±0.04^{c}	2.18±0.02^{b}	3.01±0.01^{e}
Isoleusine	1.22±0.02^{a}	8.93±0.03^{f}	7.42±0.02^{d}	7.06±0.03^{c}	7.92±0.02^{e}	3.64±0.02^{b}
Leusine	1.19±0.01^{a}	8.84±0.02^{e}	7.34±0.02^{cd}	6.98±0.02^{c}	7.57±0.50^{d}	3.76±0.02^{b}
Valine	2.38±0.02^{b}	2.26±0.02^{a}	2.30±0.10^{ab}	2.94±0.10^{d}	2.25±0.05^{a}	2.51±0.01^{c}
Histidine	8.13±0.03^{f}	7.47±0.03^{e}	6.00±0.20^{c}	5.71±0.01^{b}	6.69±0.01^{d}	3.20±0.20^{a}
Phenyalanine	2.25±0.05^{e}	1.87±0.02^{a}	2.17±0.03^{d}	2.07±0.01^{c}	1.97±0.03^{b}	2.73±0.03^{e}
Arginine	7.29±0.01^{f}	6.62±0.02^{e}	5.42±0.02^{c}	5.07±0.07^{b}	5.85±0.05^{d}	2.73±0.03^{a}
Non-Essential Amino Acid						
Glycine	8.68±0.02^{f}	7.78±0.02^{e}	6.34±0.04^{c}	6.08±0.02^{b}	6.98±0.02^{d}	3.32±0.02^{a}
Serine	2.73±0.03^{d}	2.11±0.01^{a}	2.38±0.01^{b}	2.52±0.02^{c}	2.40±0.10^{b}	2.49±0.01^{c}
Proline	2.32±0.02^{a}	2.31±0.01^{a}	2.29±0.01^{a}	2.96±0.04^{c}	3.01±0.01^{d}	2.48±0.02^{b}
Alanine	9.04±0.04^{f}	7.85±0.05^{e}	6.45±0.05^{c}	6.25±0.05^{b}	6.91±0.01^{d}	3.20±0.20^{a}
Aspartic	6.09±0.03^{e}	4.83±0.03^{d}	1.85±0.05^{b}	1.38±0.02^{a}	3.26±0.01^{c}	6.280.02^{f}
Glutamic	8.63±0.03^{f}	7.61±0.01^{e}	6.31±0.01^{c}	5.96±0.04^{b}	6.76±0.06^{d}	3.12±0.02^{a}
Cysteine	3.65±0.05^{e}	3.18±0.02^{d}	2.67±0.02^{b}	2.56±0.06^{b}	2.80±0.10^{c}	1.30±0.10^{a}
Ornithine	2.64±0.04^{d}	2.30±0.20^{c}	1.99±0.01^{b}	1.82±0.02^{a}	2.09±0.03^{b}	1.97±0.03^{ab}
Pyrrolysine	1.94±0.04^{a}	2.46±0.04^{c}	1.97±0.03^{a}	2.16±0.04^{b}	2.17±0.02^{b}	1.90±0.10^{a}
Tyrosine	4.00±0.10^{e}	3.87±0.02^{d}	3.98±0.02^{e}	2.74±0.04^{a}	3.61±0.01^{c}	3.23±0.03^{b}

Means with same letter in row are not significantly different (P>0.05)

4.2.10 Amino acid composition of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Amino acid composition of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 18. Supplemental amino acid significantly improved (P<0.05) *C. gariepinus* whole body methionine in *C. gariepinus* fed diet SS5 (2.75±0.04) and least in control and SS3. Significantly higher (P<0.05) value of lysine was in *C. gariepinus* on SS4 (9.83±0.03). For threonine, higher value of 3.01±0.01 were in *C. gariepinus* fed diet SS5 and least value in diet SS4 (P<0.05). Furthermore, Least (P<0.05) values for tryptophan, isoleusine, leusine, valine and histidine in *C. gariepinus* fed control diet were 1.96±0.03, 1.25±0.05, 1.21±0.01, 2.17±0.02 and 1.52±0.02 while the higher values were in diet SS6 (3.48±0.02), SS3 (9.58±0.02), SS3 (9.48±0.02), SS4 (2.65±0.05) and SS5 (8.08±0.02), respectively. Phenyalanine had higher (P<0.05) value in control diet (2.88±0.08).

Furthermore, supplementation of amino acid significantly (P<0.05) improved Glycine, Proline, Alanine, Glutamic, Cysteine and arginine with the higher values in diet SS5 (8.73±0.03), SS4 (2.60±0.10), SS5 (9.24±0.04), SS5 (8.74±0.04), SS5 (3.72±0.02) and SS5 (7.28±0.02) while the control diet (1.37±0.02, 2.07±0.01, 0.91±0.01, 1.14±0.04, 0.39±0.01 and 1.13±0.03) had the least values, respectively. The value recorded for serine fed control diet (2.96±0.08) had the higher value and least value of 1.96 ±0.04 (SS4). For Aspartic acid, *C. gariepinus* fed diet SS4 (1.69±0.01) had the least value while diet SS2 (7.85±0.05) had the higher value (P<0.05). Tyrosine, Ornithine and Pyrrolysine had a significantly higher values in *C. gariepinus* fed diet SS5 (4.00±0.02, 2.66±0.01 and 2.50±0.20) while the least values of 2.77±0.03 (SS3), 2.18±0.02 (SS2) and 2.21±0.01 (SS4).

Table 18: Amino acid composition of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

		Diet (SS)				
Parameter	Control	2	3	4	5	6
Essential Amino Acid						
Methionine	1.96±0.05^{a}	2.35±0.07^{d}	1.97±0.02^{a}	2.27±0.03^{c}	2.75±0.04^{e}	2.19±0.01^{b}
Lysine	6.76±0.04^{a}	7.81±0.01^{c}	8.29±0.01^{d}	9.83±0.03^{f}	8.83±0.03^{e}	6.87±0.03^{b}
Threonine	2.55±0.05^{c}	2.14±0.02^{b}	2.20±0.10^{b}	1.97±0.03^{a}	3.02±0.02^{e}	2.93±0.03^{d}
Tryptophan	1.96±0.03^{a}	3.01±0.01^{e}	2.23±0.01^{b}	2.70±0.10^{c}	2.87±0.02^{d}	3.48±0.02^{f}
Isoleusine	1.25±0.05^{a}	4.70±0.10^{d}	9.58±0.02^{f}	6.13±0.03^{e}	1.37±0.03^{b}	4.61±0.01^{c}
Leusine	1.21±0.01^{a}	4.51±0.01^{c}	9.48±0.02^{e}	6.06±0.03^{d}	1.34±0.04^{b}	4.48±0.02^{c}
Valine	2.17±0.02^{a}	2.57±0.02^{d}	2.19±0.01^{a}	2.65±0.05^{e}	2.26±0.04^{b}	2.45±0.05^{c}
Histidine	1.52±0.02^{a}	3.94±0.04^{b}	7.48±0.02^{d}	5.64±0.04^{c}	8.08±0.02^{e}	3.94±0.01^{b}
Phenyalanine	2.88±0.08^{e}	1.95±0.05^{b}	1.82±0.02^{a}	2.39±0.01^{c}	2.32±0.02^{c}	2.64±0.02^{d}
Arginine	1.13±0.03^{a}	3.39±0.03^{b}	3.79±0.01^{d}	4.75±0.05^{e}	7.28±0.02^{f}	3.49±0.01^{c}
Non-Essential Amino Acid						
Glycine	1.37±0.02^{a}	4.09±0.01^{c}	8.09±0.01^{e}	5.59±0.01^{d}	8.73±0.03^{f}	3.99±0.01^{b}
Serine	2.96±0.08^{d}	2.11±0.01^{b}	2.36±0.02^{c}	1.96±0.04^{a}	2.30±0.10^{c}	2.27±0.02^{c}
Proline	2.07±0.01^{a}	2.40±0.10^{bc}	2.31±0.01^{b}	2.60±0.10^{d}	2.15±0.05^{a}	2.49±0.01^{c}
Alanine	0.91±0.01^{a}	4.06±0.03^{c}	8.44±0.04^{e}	5.26±0.06^{d}	9.24±0.04^{f}	3.93±0.03^{b}
Aspartic	3.06±0.02^{b}	7.85±0.05^{e}	3.42±0.02^{c}	1.69±0.01^{a}	5.93±0.03^{d}	7.85±0.05^{e}
Glutamic	1.14±0.04^{a}	3.98±0.02^{c}	8.07±0.02^{e}	5.37±0.01^{d}	8.74±0.04^{f}	3.86±0.03^{b}
Cysteine	0.39±0.01^{a}	1.59±0.01^{b}	3.41±0.01^{d}	2.18±0.02^{c}	3.72±0.02^{e}	1.60±0.10^{b}
Tyrosine	3.40±0.10^{d}	3.11±0.01^{b}	2.77±0.03^{a}	3.31±0.01^{c}	4.00±0.02^{f}	3.86±0.04^{e}
Ornithine	2.35±0.05^{b}	2.18±0.02^{a}	2.44±0.04^{c}	2.30±0.10^{b}	2.66±0.01^{d}	2.17±0.02^{a}
Pyrrolysine	2.23±0.03^{ab}	2.32±0.02^{ab}	2.39±0.01^{bc}	2.21±0.01^{a}	2.50±0.20^{c}	2.25±0.05^{ab}

Means with same letter in row are not significantly different (P>0.05)

4.2.11. Apparent nutrient digestibility by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Apparent nutrient digestibility of *C. gariepinus* fed roasted soyabean based diet supplemented with varying inclusion of dietary amino acid is shown Table 19. Amino acid supplementation significantly increased ($P<0.05$) crude protein digestibility values with higher value in fish fed RS5 (95.32±0.25) diet but fish fed diet RS2, RS4 and RS6 were similar to each other. Also, supplementation of amino acid significantly ($P<0.05$) reduced ash digestibility with least value in diet RS4 (51.59±3.72) and RS6 (50.70±1.88). Ether Extract digestibility of *C. gariepinus* fed diet RS2, RS4, RS5 and RS6 were not significantly ($P<0.05$) different from each other but these differ significantly from fish fed diet RS3. Varying inclusion of dietary amino acid significantly increased in crude fibre digestibility with the higher value in fish fed diet RS5 (84.13±0.81). The Dry matter digestibility value was not significantly ($P<0.05$) different between fish fed diet RS2 and RS5, though, these were significantly higher than other treatments. Furthermore, energy digestibility significantly ($P>0.05$) varied from RS4 (79.87±0.03) to RS5 (84.75±0.02).

4.2.12. Apparent nutrient digestibility by *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Apparent nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diet supplemented with varying inclusion of amino acid is shown in Table 20. Amino acid supplementation significantly ($P<0.05$) improved crude protein digestibility in *C. gariepinus* fed diet SS5 (96.34±0.31) compared to other diets. However, ash digestibility significantly reduced with amino acid supplementation except for fish fed diet SS5 that was significantly higher than other treatments. Ether extract digestibility of fish fed diet SS5, control and SS6 were not differ significantly but higher than fish fed diet SS2, SS3 and SS4. Also, crude fibre digestibility value was significantly higher in *C.gariepinus* fed diet SS5 (90.34±2.27) while, fish fed diet control, SS3 and SS6 were not significantly different from each other. The Dry matter and energy digestibility values varied significantly ($P>0.05$) among the diets with the higher value observed in *C. gariepinus* fed diet SS5 (80.78±0.05 and 89.04±0.00), respectively.

Table 19: Apparent nutrient digestibility by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Parameter	Control	Diet (RS)				
		2	3	4	5	6
Crude protein	91.04±0.11[a]	94.51±0.03[c]	92.29±0.33[b]	94.69±0.07[c]	95.32±0.25[d]	94.65±0.02[c]
Ash	72.26±6.78[c]	61.51±0.67[b]	61.07±1.74[b]	51.59±3.72[a]	64.43±3.60[bc]	50.74±1.88[a]
Ether Extract	94.21±0.07[a]	96.19±0.30[b]	94.07±0.02[a]	96.40±0.23[b]	96.62±0.16[b]	96.29±0.01[b]
Crude fibre	79.52±2.25[a]	83.88±0.59[b]	77.75±0.94[a]	83.57±0.11[b]	84.13±0.81[c]	83.86±0.57[b]
Dry Matter	66.71±0.09[b]	73.94±0.11[d]	65.18±0.01[a]	71.69±0.11[c]	73.75±0.07[d]	71.54±0.05[c]
Energy	80.28±0.04[b]	85.24±0.04[e]	79.87±0.03[a]	83.91±0.03[c]	84.75±0.02[d]	83.95±0.02[c]

Means with same letter in row are not significantly different (P>0.05)

Table 20: Apparent nutrient digestibility by *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

		Diet (SS)				
Parameter	Control	2	3	4	5	6
Crude Protein	94.29±0.04^{c}	91.49±0.07^{b}	94.23±0.11^{c}	90.27±0.14^{a}	96.34±0.31^{e}	95.95±0.02^{d}
Ash	69.58±0.19^{e}	51.62±0.70^{b}	55.70±0.36^{c}	44.51±2.03^{a}	72.54±0.10^{f}	65.89±1.39^{d}
Ether Extract	96.45±0.12^{b}	93.70±0.01^{a}	93.54±2.20^{a}	93.56±0.23^{a}	97.22±0.29^{b}	96.12±0.05^{b}
Crude Fibre	84.94±1.06^{c}	67.53±0.35^{a}	82.61±1.28^{c}	73.59±0.14^{b}	90.34±2.27^{d}	83.96±0.61^{c}
Dry Matter	74.41±0.09^{e}	49.71±0.05^{a}	69.00±0.22^{c}	51.41±0.02^{b}	80.78±0.05^{f}	71.56±0.03^{d}
Energy	85.56±0.03^{e}	68.99±0.01^{a}	82.12±0.30^{c}	71.56±0.04^{b}	89.04±0.00^{f}	82.73±0.04^{d}

Means with same letter in row are not significantly different (P>0.05)

4.2.13 True nutrient digestibility by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

True nutrient digestibility of *C.gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 21. Supplementation of amino acid significantly increased crude protein digestibility with the highest value in fish fed diet RS5 (95.42±0.25) but fish fed diet RS2, RS4 and RS6 were not significantly different among each other. Ash digestibility values significantly decreased with amino acid supplementation, however, fish fed control diet and RS5 were significantly ($P>0.05$) higher than other diets. A significantly higher ether extract digestibility values were observed in fish fed diet RS4, RS5 and RS6 than those fed diet RS2, RS3 and control. Crude fibre digestibility values were no significantly ($P<0.05$) different among the fish fed diet RS2, RS4, RS5 and RS6 but significantly higher than those on control and RS3 diets. Similarly, supplementation of amino acid significantly increased dry matter digestibility.

4.2.14 True nutrient digestibility by *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

True nutrient digestibility of *C.gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 22. Crude protein digestibility values varied significantly between the diets with the higher value in *C. gariepinus* fed diet SS5 (96.51±0.31). Amino acid supplementation significantly decreased ash digestibility value except for fish fed diet SS5 (72.94±0.13) that was significantly ($P>0.05$) higher than other treatments. Ether Extract digestibility values were significantly lower in fish fed diet SS2 (93.77±0.29), SS3 (93.67±2.17) and SS4 (93.67±0.23) than those on SS5, SS6 and control. Crude fibre and dry matter digestibility values differs among diets with the higher values observed in fish fed diet SS5 (90.72±0.38 and 98.60±0.01), respectively.

Table 21: True nutrient digestibility by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

		Diet (RS)				
Parameter	Control	2	3	4	5	6
Crude Protein	91.15±0.10^{a}	94.62±0.03^{c}	92.39±0.33^{b}	94.79±0.07^{c}	95.42±0.25^{d}	94.76±0.02^{c}
Ash	72.86±6.82^{c}	62.16±0.66^{b}	61.69±1.70^{b}	52.30±3.66^{a}	65.01±3.59^{bc}	51.28±1.85^{a}
Ether Extract	94.32±0.07^{a}	96.29±0.29^{b}	94.19±0.03^{a}	96.50±0.22^{bc}	96.73±0.16^{c}	96.39±0.00^{bc}
Crude Fibre	79.91±2.18^{a}	84.34±0.51^{b}	78.19±0.87^{a}	83.95±0.15^{b}	84.56±0.83^{b}	84.27±0.60^{b}
Dry Matter	94.89±0.03^{a}	97.58±0.02^{d}	96.60±0.03^{b}	97.90±0.01^{e}	97.65±0.00^{d}	96.84±0.06^{c}

Means with same letter in row are not significantly different (P>0.05)

Table 22: True nutrient digestibility by *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

		Diet (SS)				
Parameter	Control	2	3	4	5	6
Crude Protein	94.39±0.05^{c}	91.58±0.07^{b}	94.33±0.11^{c}	90.37±0.14^{a}	96.51±0.31^{e}	96.05±0.03^{d}
Ash	69.93±0.19^{e}	51.96±0.68^{b}	56.09±0.35^{c}	44.90±2.05^{a}	72.94±0.13^{f}	66.25±1.37^{d}
Ether Extract	96.56±0.12^{b}	93.77±0.29^{a}	93.67±2.17^{a}	93.67±0.23^{a}	97.34±0.01^{b}	96.23±0.04^{b}
Crude Fibre	85.36±0.97^{c}	67.98±2.19^{a}	83.00±1.30^{c}	73.96±0.18^{b}	90.72±0.38^{d}	84.36±0.64^{c}
Dry Matter	97.39±0.01^{e}	91.44±0.06^{a}	97.26±0.01^{d}	94.71±0.05^{b}	98.60±0.01^{f}	95.58±0.03^{c}

Means with same letter in row are not significantly different (P>0.05)

4.2.15 Apparent amino acid digestibility by *C.gariepinus* fed roasted soyabean based diets supplemented with amino acid

The apparent amino acid digestibility of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 23. Significantly higher value of 97.49±0.47 was obtained in *C.gariepinus* fed diet RS5 for methionine but similar (P>0.05) to *C. gariepinus* on diet RS2 (92.45±0.25) and RS3 (92.86±0.40) while least value was in diet RS4 (92.44±0.80). Lysine values revealed that *C. gariepinus* fed diet RS2 (90.70±0.26) had the least while the higher value of 92.66±0.41 was in diet RS4 but similar (P>0.05) to diet RS6 (92.60±0.66). For Threonine, *C. gariepinus* fed diet RS6 (91.99±1.13) was not difference (P>0.05) to *C. gariepinus* on diet RS1 but lower (P<0.05) compared to other diet on amino acid supplementation. Also, *C. gariepinus* on diet RS5 (88.18±0.35) had the least (P<0.05) value for tryptophan. Significantly difference (P<0.05) was in isoleusine with higher value in diet RS2 (77.81±0.22) and least in diet RS5 (67.37±0.15). Leusine, Histidine and Phenyalanine had a higher (P<0.05) values in *C.gariepinus* on RS2 and least values in *C. gariepinus* fed diet RS3.

Furthermore, supplementation of amino acid significantly influence (P<0.05) digestibility of glycine on diet RS5 (80.38±2.34) but similar (P>0.05) to *C. gariepinus* on diet RS2 (79.55±0.11) and RS6 (79.12±0.36). Also, serine has the higher (P<0.05) value of 97.61±0.62 in *C. gariepinus* fed control diet and least in diet RS6 (91.93±0.46). The values obtained in proline indicated that *C. gariepinus* fed diet RS4 (96.78±0.55) had higher (P<0.05) value but closely followed by control diet (95.96±0.76) while the least value was in diet RS3 (93.40±1.26). However similar trend was observed in Alanine level with the least value in RS3 (76.26±0.88) and higher value was in RS5 (85.08±0.11). *C. gariepinus* fed diet RS6 had a higher (P<0.05) value of 86.55±0.01 in Aspartic acid with the least value in diet RS2 (77.40±0.95). Least (P<0.05) value were in Cysteine, Arginine, Pyrrolysine and Tyrosine levels from the control diet (71.91±0.12, 63.89±1.07, 90.50±0.12, 77.90±0.52), repectively but similar (P>0.05) to *C.gariepinus* on diet RS3 (87.81±4.48) for Tyrosine.

Table 23: Apparent amino acid digestibility by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Parameter	Control	Diet (RS) 2	3	4	5	6
Essential Amino Acid						
Methionine	95.43±2.22^{c}	92.45±0.25^{a}	92.86±0.40^{ab}	92.44±0.80^{a}	97.49±0.47^{d}	94.45±0.38^{bc}
Lysine	91.63±0.54^{b}	90.70±0.26^{a}	91.40±0.29^{ab}	92.66±0.41^{c}	91.57±0.15^{b}	92.60±0.66^{c}
Threonine	92.57±0.22^{a}	96.28±0.44^{b}	96.17±0.87^{b}	96.11±0.55^{b}	95.45±0.05^{b}	91.99±1.13^{a}
Tryptophan	92.49±1.62^{c}	96.85±0.28^{d}	91.12±0.38^{b}	90.27±0.60^{b}	88.18±0.35^{a}	97.56±0.13^{d}
Isoleusine	74.25±0.68^{b}	77.81±0.22^{c}	67.38±3.67^{a}	75.79±0.52^{bc}	67.37±0.15^{a}	77.71±0.44^{c}
Leusine	73.26±0.44^{b}	78.77±0.64^{c}	69.85±0.14^{a}	76.59±0.16^{c}	82.56±0.19^{d}	69.45±3.86^{a}
Valine	91.76±0.77^{bc}	92.68±0.37^{c}	94.75±0.55^{d}	95.08±0.43^{d}	91.29±0.48^{b}	88.55±1.07^{a}
Histidine	70.30±0.39^{b}	80.74±0.11^{d}	66.08±1.41^{a}	79.37±0.48^{c}	80.09±0.45^{cd}	91.49±0.42^{e}
Phenyalanine	86.10±2.56^{ab}	92.27±0.33^{d}	84.60±2.34^{a}	92.99±0.10^{d}	90.39±0.07^{cd}	88.07±0.21^{bc}
Arginine	63.89±1.07^{a}	68.38±0.29^{c}	65.91±0.82^{b}	81.05±1.15^{e}	70.70±1.57^{d}	84.52±0.12^{f}
Non-Essential Amino Acid						
Glycine	69.59±0.97^{b}	79.55±0.11^{d}	61.71±1.38^{a}	76.38±0.20^{c}	80.38±2.34^{d}	79.12±0.36^{d}
Serine	97.61±0.62^{d}	95.93±0.35^{bc}	96.71±0.23^{cd}	92.24±0.77^{a}	95.16±0.49^{b}	91.93±0.46^{a}
Proline	95.96±0.76^{b}	93.40±1.26^{a}	92.56±1.06^{a}	96.78±0.55^{b}	95.31±0.66^{b}	92.75±0.38^{a}
Alanine	77.19±0.42^{ab}	79.87±0.67^{c}	76.26±0.88^{a}	77.83±0.28^{b}	85.08±0.11^{d}	84.84±0.61^{d}
Aspartic	81.77±0.44^{c}	77.40±0.95^{a}	82.24±0.38^{c}	79.87±1.08^{b}	84.34±1.06^{d}	86.55±0.01^{e}
Glutamic	77.89±0.22^{b}	77.49±0.14^{b}	74.62±0.69^{a}	81.26±0.21^{d}	79.44±0.51^{c}	85.15±0.71^{e}
Cysteine	71.91±1.32^{a}	88.66±0.03^{b}	89.45±0.51^{b}	82.44±1.14^{b}	85.91±0.08^{b}	82.58±0.17^{b}
Pyrrolysine	90.50±0.12^{a}	96.71±0.41^{b}	97.54±0.29^{b}	96.30±0.18^{b}	98.06±0.34^{b}	95.75±2.86^{b}
Tyrosine	77.90±0.52^{a}	85.04±0.65^{b}	79.81±4.48^{a}	87.32±1.03^{b}	87.25±2.32^{b}	86.65±1.32^{b}

Means with same letter in row are not significantly different (P>0.05)

4.2.16 Apparent amino acid digestibility by *C.gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Apparent amino acid digestibility of *C.gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 24. Methionine level revealed that *C. gariepinus* on treatment SS2 had higher (P<0.05) value of 96.96±0.13 and was closely followed (P>0.05) by diet SS5 (96.86±3.68) and SS6 (96.55±1.65). Lysine digestibility was higher (P<0.05) in *C. gariepinus* on SS5 (95.75±0.26) and least value of 96.08±1.05 was in diet SS4. Threonine and valine was least (P<0.05) digested when fed diet SS3 (92.48±0.85 and 89.66±0.58) with the higher values in diet SS5 (98.18±0.31and 96.20±0.75). Also, significantly (P<0.05) least digested values for Tryptophan and Isoleusine were in *C. gariepinus* fed SS5 (85.14±0.69 and SS4 (64.41±0.27) and higher were in diet SS3 (97.83±0.67) and SS2 (86.55±0.53), respectively. Leusine had the higher digestible value of 92.97±0.84 in diet SS2 and least value of 69.66±2.39 was in diet SS2. Histidine and Phenyalanine had higher (P<0.05) values in *C.gariepinus* on diet SS2 (88.40±0.53 and 96.96±0.18) and least in *C. gariepinus* on diet SS4 (66.83±0.58) and SS5 (86.16±0.67), respectively.

Apparent Digestibility values for Glycine revealed that *C. gariepinus* fed SS1 (93.01±0.06) was influenced (P<0.05) compared diets on amino acid supplementation. Significantly (P<0.05) least digested values for Serine, Proline, Glutamic, Cysteine, Arginine and Tyrosine were observed in *C. gariepinus* fed diet SS4 (93.06±1.54, 87.74±0.39, 64.37±0.04, 70.92±0.90, 55.38±0.66, and 72.22±1.89) than other treatments. Furthermore, Alanine and Aspartic acid had the least (P<0.05) value of 67.25±0.12 and 71.33±0.35 in *C. gariepinus* fed SS5 and higher values were observed in diet SS2. Finally, higher (P<0.05) value of Pyrrolysine was in diet SS2 (95.25±0.16) and least in diet SS5 (85.27±3.34).

Table 24: Apparent amino acid digestibility by *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

		Diet (SS)				
Parameter	Control	2	3	4	5	6
Essential Amino Acid						
Methionine	85.09±0.36^{a}	96.96±0.13^{c}	92.33±2.10^{b}	92.18±0.15^{b}	96.86±3.68^{c}	96.55±1.65^{c}
Lysine	94.01±0.21^{c}	86.21±0.17^{a}	94.06±0.25^{c}	86.08±1.05^{a}	95.75±0.26^{d}	92.80±0.14^{b}
Threonine	97.04±0.21^{c}	97.67±0.19^{cd}	92.48±0.85^{a}	96.09±0.71^{b}	92.69±0.41^{a}	98.18±0.31^{d}
Tryptophan	94.88±0.25^{d}	92.74±0.38^{c}	97.83±0.67^{e}	90.12±1.07^{b}	85.14±0.69^{a}	92.45±1.38^{c}
Isoleusine	83.67±0.11^{e}	86.55±0.53^{f}	78.42±0.51^{d}	64.41±0.27^{a}	67.85±0.79^{b}	74.93±2.62^{c}
Leusine	90.38±0.02^{e}	92.97±0.84^{f}	78.11±0.39^{c}	69.66±2.39^{a}	72.96±0.24^{b}	83.44±0.26^{d}
Valine	96.87±0.51^{de}	97.88±1.08^{e}	89.66±0.88^{a}	92.55±0.57^{b}	96.20±0.75^{d}	94.70±0.78^{c}
Histidine	84.37±0.83^{c}	88.40±0.53^{e}	86.21±0.30^{d}	66.83±0.58^{a}	75.87±0.83^{b}	83.85±0.02^{c}
Phenyalanine	92.46±0.30^{c}	96.96±0.18^{d}	89.80±1.11^{b}	89.44±0.61^{b}	86.16±0.67^{a}	92.99±0.23^{c}
Arginine	88.74±0.26^{e}	91.96±0.27^{f}	87.40±0.29^{d}	55.38±0.66^{a}	74.55±65^{b}	86.18±0.37^{c}
Non-Essential Amino Acid						
Glycine	93.01±0.06^{f}	90.71±0.13^{e}	73.36±0.93^{a}	76.08±0.11^{b}	78.00±0.45^{c}	83.03±0.06^{d}
Serine	95.77±0.23^{c}	98.24±0.12^{d}	94.74±0.31^{bc}	93.06±1.54^{a}	94.06±0.51^{ab}	97.44±0.10^{d}
Proline	95.35±0.61^{d}	93.66±0.06^{c}	94.03±0.21^{cd}	87.74±0.39^{a}	94.91±0.33^{cd}	91.36±1.89^{b}
Alanine	90.43±0.19^{e}	93.88±0.26^{f}	79.73±0.04^{c}	75.62±1.03^{b}	67.25±0.12^{a}	83.00±0.87^{d}
Aspartic	84.55±0.20^{d}	90.33±0.82^{e}	82.37±0.62^{c}	75.18±1.19^{b}	71.33±0.35^{a}	83.43±0.30^{cd}
Glutamic	85.09±0.22^{e}	89.72±0.58^{f}	77.34±0.06^{c}	64.37±0.04^{a}	67.20±0.64^{b}	82.98±0.34^{d}
Cysteine	91.56±0.34^{c}	96.95±0.49^{d}	84.90±0.36^{b}	70.92±0.90^{a}	84.17±0.89^{b}	90.49±0.36^{c}
Pyrrolysine	94.55±26^{c}	97.65±0.25^{d}	93.96±0.23^{bc}	91.78±1.26^{b}	85.27±3.34^{a}	96.13±0.45^{cd}
Tyrosine	96.82±0.21^{d}	95.25±0.16^{d}	82.75±0.80^{b}	72.22±1.89^{a}	81.08±1.96^{b}	86.94±0.90^{c}

Means with same letter in row are not significantly different (P>0.05)

4.2.17 True amino acid digestibility by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

True amino acid digestibility of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 25. Methionine level showed higher ($P<0.05$) value in *C.gariepinus* fed diet RS5 (97.83±0.46) and least in *C.gariepinus* on diet RS2 (92.84±0.25) and RS4 (92.81±0.80). Lysine had a higher ($P<0.05$) value of 92.88±0.41 in *C. gariepinus* fed diet RS4 but similar ($P>0.05$) to *C.gariepinus* on diet RS6 (92.82±0.66) while the least value was in diet RS2 (90.88±0.26). Significantly ($P<0.05$) lower values for Threonine, Leusine and Valine digestibility from *C. gariepinus* fed diet RS6 (92.82±0.66, 69.86±3.86 and 88.80±1.04) were observed but differed significantly from other treatment. Furthermore, Tryptophan and Isoleusine had the least ($P<0.05$) value in diet RS5 (88.64±0.34 and 67.51±0.15) while the higher values were in diet RS2. Histidine was higher ($P<0.05$) in *C. gariepinus* on RS6 (92.46±0.41) and least in RS3. Higher ($P<0.05$) values in Phenyalanine was in fish on diet RS4 (93.24±0.08) but similar ($P>0.05$) to *C. gariepinus* fed diet RS2 (92.49±0.30) and varied from other treatment.

Significantly lower ($P<0.05$) values were obtained in Glycine, Proline, Alanine, Glutamic and Tyrosine from *C. gariepinus* fed diet RS3 (62.85±1.37, 92.55±1.06, 76.33±0.81, 75.80±0.68 and 80.42±4.45) and higher in diet RS5 (81.23±2.25), RS4 (96.92±0.55), RS5 (85.16±0.11), RS6 (86.30±0.70), RS4 (87.82±1.01), respectively. Significantly higher ($P<0.05$) value of 97.87±0.62 was obtained from Serine level when fed control diet and least value of 92.26±0.46 was recorded in diet RS6. Aspartic acid, Cysteine, Arginine and Pyrrolysine were higher ($P<0.05$) values in *C.gariepinus* on RS6 (86.82±0.01), RS3 (89.69±0.50), RS6 (84.91±0.11) and RS5 (98.25±0.33), respectively but differed significantly from other treatments.

Table 25: True amino acid digestibility by *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Parameter	Control	Diet (RS) 2	3	4	5	6
Essential Amino Acid						
Methionine	95.83±2.23^{c}	92.84±0.25^{a}	93.29±0.39^{ab}	92.81±0.80^{a}	97.83±0.46^{d}	94.82±0.38^{bc}
Lysine	91.81±0.54^{b}	90.88±0.26^{a}	91.58±0.29^{ab}	92.88±0.41^{c}	91.78±0.15^{b}	92.82±0.66^{c}
Threonine	92.86±0.22^{a}	96.58±0.44^{b}	96.51±0.83^{b}	96.50±0.54^{b}	95.87±0.05^{b}	92.34±1.12^{a}
Tryptophan	92.92±1.61^{c}	97.25±0.29^{d}	91.38±0.38^{b}	90.74±0.60^{b}	88.64±0.34^{a}	97.82±1.04^{d}
Isoleusine	74.35±0.68^{b}	77.93±0.22^{c}	67.50±3.66^{a}	75.89±0.52^{bc}	67.51±0.15^{a}	77.82±0.44^{c}
Leusine	73.63±0.43^{b}	79.17±0.64^{c}	70.27±0.13^{a}	76.87±0.16^{c}	82.87±0.19^{d}	69.86±3.86^{a}
Valine	92.01±0.77^{bc}	92.93±0.38^{c}	95.00±0.56^{d}	95.30±0.42^{d}	91.55±0.48^{b}	88.80±1.04^{a}
Histidine	71.57±0.38^{b}	81.76±0.12^{d}	67.30±1.38^{a}	80.20±0.46^{c}	81.12±0.46^{cd}	92.46±0.41^{e}
Phenyalanine	86.36±2.57^{ab}	92.49±0.30^{d}	84.85±2.30^{a}	93.24±0.08^{d}	90.61±0.09^{cd}	88.30±0.20^{bc}
Non-Essential Amino Acid						
Glycine	70.48±0.96^{b}	80.30±0.10^{d}	62.85±1.37^{a}	77.02±0.19^{c}	81.23±2.25^{d}	79.91±0.35^{d}
Serine	97.87±0.62^{d}	96.23±0.34^{bc}	97.07±0.23^{cd}	92.55±0.77^{a}	95.41±0.48^{b}	92.26±0.46^{a}
Proline	96.16±0.77^{b}	93.61±1.25^{a}	92.76±1.06^{a}	96.92±0.55^{b}	95.53±0.65^{b}	92.94±0.37^{a}
Alanine	77.28±0.42^{ab}	79.97±0.67^{c}	76.33±0.81^{a}	77.92±0.28^{b}	85.16±0.11^{d}	84.93±0.60^{d}
Aspartic	82.04±044^{c}	77.71±0.94^{a}	82.53±0.38^{c}	80.16±1.08^{b}	84.63±1.07^{d}	86.82±0.01^{d}
Glutamic	78.95±0.22^{b}	78.29±0.15^{b}	75.80±0.68^{a}	82.07±0.20^{d}	80.59±0.50^{c}	86.30±0.70^{e}
Cysteine	72.15±11.32^{a}	88.88±0.02^{b}	89.69±0.50^{b}	82.66±1.13^{b}	86.14±0.08^{b}	82.82±0.16^{b}
Arginine	64.31±1.07^{a}	68.82±0.29^{c}	66.29±0.81^{b}	81.43±1.16^{e}	71.08±1.55^{d}	84.91±0.11^{f}
Pyrrolysine	90.69±0.12^{a}	96.95±0.40^{b}	97.73±0.29^{b}	96.49±0.18^{b}	98.25±0.33^{b}	95.94±2.86^{b}
Tyrosine	78.48±0.51^{a}	85.79±0.64^{b}	80.42±4.45^{a}	87.82±1.01^{b}	87.77±2.32^{b}	87.19±1.32^{b}

Means with same letter in row are not significantly different (P>0.05)

4.2.18 True amino acid digestibility by *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

True amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 26. *C. gariepinus* fed supplemental amino acid improved (P<0.05) methionine digestibility with higher value in diet SS5 (97.25±3.69) and least in control diet. Similar trend was also observed in *C. gariepinus* on diet SS5 (95.91±0.26) and least value of 86.24±1.04 was in diet SS4. Higher value was recorded for Threonine from *C. gariepinu*s fed diet SS6 (98.53±0.32) but similar (P>0.05) to *C.gariepinus* on diet SS2 (98.02±0.20). Tryptophan had a higher (P<0.05) value in *C. gariepinus* on control diet (95.23±0.24) and least in diet SS5 (85.51±0.68). Isoleusine, leusine and histidine had least (P<0.05) values in fish on diet SS4 (64.48±0.27, 69.90±2.39 and 67.53±0.58) and higher values of 86.63±0.91, 93.21±0.84 and 89.01±0.54 were in diet SS2, respectively. Additionally, supplementation of amino acid altered (P<0.05) valine and phzenyalanine in *C.gariepinus* on diet SS2 (98.10±1.07 and 97.13±0.17) and least in SS3 (89.93±0.87) and SS2 (97.13±0.17), respectively.

Also, Glycine was higher in *C. gari*epinus fed control diet (93.39±0.06) and the least value in diet SS3 (73.69±0.93) (P<0.05). Significantly least (P<0.05) values for serine, proline, glutamic, cysteine, arginine and tyrosine were observed in *C. gariepinus* fed SS4 (93.37±1.48, 87.95±0.39, 65.18±0.04, 71.18±0.89, 55.67±0.66 and 72.89±1.87) but serine value was similar in *C. gariepinus* on diet SS5 (94.32±0.51) and differed significantly with other treatments. However, alanine, aspartic acid and pyrrolysine had least values in diet SS5 with the higher values of 93.96±0.26, 90.58±0.82 and 97.84±0.25 in diet SS2.

Table 26: True amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Parameter	Control	Diet (SS) 2	3	4	5	6
Essential Amino Acid						
Methionine	85.51±0.34^{a}	97.32±0.13^{c}	92.70±2.09^{b}	92.62±0.19^{b}	97.25±3.69^{c}	96.90±1.66^{c}
Lysine	94.20±0.21^{c}	86.37±0.17^{a}	94.22±0.25^{c}	86.24±1.04^{a}	95.91±0.26^{d}	92.97±0.14^{b}
Threonine	97.33±0.21^{c}	98.02±0.20^{cd}	92.85±0.84^{a}	96.41±0.69^{b}	92.99±0.42^{a}	98.53±0.32^{d}
Tryptophan	95.23±0.24^{d}	93.17±0.38^{c}	98.07±0.67^{e}	90.43±1.07^{b}	85.51±0.68^{a}	92.87±1.38^{c}
Isoleusine	83.75±0.11^{e}	86.63±0.91^{f}	78.49±0.51^{d}	64.48±0.27^{a}	67.92±0.79^{b}	75.02±2.62^{c}
Leusine	90.57±0.02^{e}	93.21±0.84^{f}	78.31±0.39^{c}	69.90±2.39^{a}	73.19±0.24^{b}	83.64±0.26^{d}
Valine	97.07±0.51^{de}	98.10±1.07^{e}	89.93±0.87^{a}	92.79±0.58^{b}	96.48±0.75^{d}	94.88±0.78^{c}
Histidine	85.22±0.79^{c}	89.01±0.54^{e}	86.56±0.29^{d}	67.53±0.58^{a}	76.67±0.82^{b}	84.72±0.02^{c}
Phenyalanine	92.66±0.30^{c}	97.13±0.17^{d}	90.00±1.11^{b}	89.61±0.61^{b}	86.33±0.66^{a}	93.18±0.22^{c}
Arginine	89.04±0.26^{e}	92.19±0.26^{f}	87.65±0.29^{d}	55.67±0.66^{a}	74.80±0.64^{b}	86.45±0.37^{c}
Non-Essential Amino Acid						
Glycine	93.39±0.06^{f}	91.16±0.13^{e}	73.69±0.93^{a}	76.57±0.12^{b}	78.50±0.45^{c}	83.51±0.06^{d}
Serine	96.06±0.23^{c}	98.54±0.12^{d}	95.01±0.30^{bc}	93.37±1.48^{a}	94.32±0.51^{ab}	97.66±0.11^{d}
Proline	95.57±0.60^{d}	93.84±0.06^{c}	94.21±0.21^{cd}	87.95±0.39^{a}	95.09±0.34^{cd}	91.52±1.89^{b}
Alanine	90.50±0.18^{e}	93.96±0.26^{f}	79.80±0.04^{c}	75.68±1.03^{b}	67.31±0.12^{a}	83.05±0.87^{d}
Aspartic	84.84±0.19^{d}	90.58±0.82^{e}	82.65±0.62^{c}	75.44±1.17^{b}	71.61±0.35^{a}	83.68±0.30^{cd}
Glutamic	85.93±0.22^{e}	90.30±0.56^{f}	77.83±0.05^{c}	65.18±0.04^{a}	68.27±0.60^{b}	83.72±0.34^{d}
Cysteine	91.74±0.34^{c}	97.20±0.49^{d}	85.15±0.35^{b}	71.18±0.89^{a}	84.42±0.89^{b}	90.73±0.36^{c}
Pyrrolysine	94.73±0.26^{c}	97.84±0.25^{d}	94.15±0.23^{bc}	91.98±1.25^{b}	85.49±3.33^{a}	96.32±0.45^{cd}
Tyrosine	97.16±0.20^{d}	95.68±0.17^{d}	83.27±0.78^{b}	72.89±1.87^{a}	81.58±1.96^{b}	87.37±0.90^{c}

Means with same letter in row are not significantly different (P>0.05)

4.2.19 Water quality parameter of *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Water quality parameters of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 27. Water quality parameter showed that the hydrogen ion concentration pH was recorded within the range of 6.02±0.00 to 6.39±0.02 while Nitrate was within the range of 0.30±0.02 to 1.75±0.01. Also, the Dissolve Oxygen (DO) was within the range of 0.70±0.01 to 12.50±0.12 throughout the 84 days experiment. Ammonia was also within the range of 0.06±0.01 to 0.36±0.00. Carbon dioxide was within the range of 0.19±0.02 to 0.37±0.11. Also, temperature of the water during the 84 days experiment ranges within 24.90±0.11 to 26.10±0.45.

4.2.20 Water quality parameter of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Water quality parameters of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary amino acid is shown in Table 28. Water quality parameter showed that pH, Nitrate, Ammonia, Dissolved Oxygen, Carbon dioxide and Temperature values were within the range of 6.02±0.00 to 6.53±0.11, 0.39±0.03 to 1.75±0.00, 0.08±0.01 to 0.36±0.00, 0.70±0.01 to 12.50±0.03, 0.19±0.01 to 0.45±0.06 and 25.40±0.75 to 25.80±0.50 throughout the 84 days experiment respectively.

Table 27: Water quality parameter of *C. gariepinus* fed roasted soyabean based diets supplemented with amino acid

Parameter	Fresh sample	Control	Diet (RS) 2	3	4	5	6
pH	6.02±0.00	6.31±0.02	6.39±0.02	6.37±0.05	6.31±0.02	6.36±0.12	6.25±0.03
Nitrate (mg/g)	1.75±0.01	0.45±0.01	0.46±0.01	0.30±0.02	0.54±0.01	0.50±0.01	0.44±0.01
Ammonia (mg/g)	0.36±0.00	0.09±0.00	0.10±0.01	0.06±0.01	0.11±0.01	0.11±0.03	0.09±0.00
Dissolve Oxygen(mg/g)	0.70±0.01	11.50±0.45	12.00±0.20	12.50±0.12	11.50±0.22	12.00±0.08	12.50±0.11
Carbon dioxide (ppm)	0.19±0.02	0.28±0.23	0.33±0.34	0.31±0.12	0.35±0.24	0.31±0.22	0.37±0,11
Temperature (^{o}C)	25.60±0.03	25.00±0.00	25.90±0.05	26.10±0.45	25.50±0.23	24.90±0.11	25.20±0.12

Table 28: Water quality parameter of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with amino acid

Parameter	Fresh sample	Control	Diet (SS) 2	3	4	5	6
pH	6.02±0.00	6.35±0.21	6.49±0.02	6.45±0.23	6.41±0.06	6.53±0.09	6.53±0.11
Nitrate (mg/g)	1.75±0.00	0.39±0.03	0.49±0.03	0.41±0.04	0.47±0.02	0.50±0.04	0.43±0.03
Ammonia (mg/g)	0.36±0.00	0.08±0.01	0.10±0.02	0.09±0.01	0.10±0.01	0.10±0.02	0.09±0.01
Dissolve Oxygen(mg/g)	0.70±0.01	6.00±0.04	10.50±0.04	11.00±0.03	11.00±0.04	12.50±0.03	11.00±0.07
Carbon dioxide (ppm)	0.19±0.01	0.33±0.03	0.45±0.05	0.45±0.06	0.30±0.04	0.41±0.04	0.34±0.04
Temperature (^{o}C)	25.40±0.01	26.30±0.23	25.80±0.50	25.40±0.75	25.20±0.20	25.50±0.22	25.70±0.12

4.3: PERFORMANCE AND AMINO ACID DIGESTIBILITY BY *C. gariepinus* FED SOYABEAN BASED DIETS SUPPLEMENTED WITH PROTEASE

4.3.1 Nutrient composition of roasted extracted soyabean based experimental diets supplemented with protease

Chemical composition of roasted soyabean based experimental diets supplemented with dietary protease is shown in Table 29. Supplementation of protease had no influence ($P>0.05$) on roasted soyabean based diet with the higher crude protein value in experimental diet RS500 and lower in RS200. Ash content had higher ($P<0.05$) value in diet RS300 and least value in diet RS100. Ether extract of experimental diet RS200 (6.60±0.14) was significantly higher than the least values in diet RS400 (7.00±0.14). For crude fibre, higher ($P<0.05$) value was in control diet (3.30±0.14) and diet RS200 had the least value. Also, dry matter had higher ($P<0.05$) in experimental diet RS300 (93.15±0.08) and least in diet RS200 (92.17±0.31). However, grosss energy level was higher in diet RS200 (4.10±0.00) and least value in control diet. Potassium, sodium, Calcium and phosphorus were higher in RS500 (0.70±0.00, 0.24±0.00, to 0.90±0.00 and 0.49±0.00) and least values in Control diet (0.67±0.00, 21±0.00, 0.86±0.00 and 0.46±0.00), respectively.

Table 29: Nutrient composition of roasted soyabean based experimental diets supplemented with protease

Parameter (%)	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Crude Protein	41.35±0.21	39.96±0.09	39.28±0.17	40.55±0.49	39.55±0.28	41.40±0.28
Ash	5.30±0.28^{ab}	4.80±0.14^{a}	5.15±0.21^{ab}	5.65±0.21^{b}	4.75±0.21^{a}	5.05±0.21^{a}
Ether Extract	6.99±0.12^{b}	6.85±0.07^{ab}	6.60±0.14^{a}	6.92±0.17^{ab}	7.00±0.14^{b}	7.00±0.14^{b}
Crude Fibre	3.30±0.14^{b}	3.00±0.14^{ab}	2.80±0.14^{a}	2.95±0.21^{ab}	2.90±0.14^{ab}	3.00±0.14^{ab}
Dry Matter	92.99±0.18	92.43±0.20	92.17±0.31	93.15±0.08	92.66±0.04	92.35±0.23
Gross energy (kcal/g)	4.00±0.00	4.09±0.00	4.10±0.00	4.07±0.00	4.08±0.00	4.08±0.00
Potassium	0.67±0.00^{a}	0.68±0.00^{b}	0.68±0.00^{c}	0.69±0.00^{d}	0.69±0.00^{e}	0.70±0.00^{e}
Sodium	0.21±0.00^{a}	0.22±0.00^{ab}	0.22±0.00^{b}	0.23±0.00^{c}	0.23±0.00^{d}	0.24±0.00^{e}
Calcium	0.86±0.00^{a}	0.87±0.00^{b}	0.87±0.00^{c}	0.88±0.00^{c}	0.89±0.00^{d}	0.90±0.00^{e}
Phosphorus	0.46±0.00^{a}	0.46±0.00^{ab}	0.47±0.00^{b}	0.48±0.00^{c}	0.48±0.00^{cd}	0.49±0.00^{d}

Means with same letter in row are not significantly different (P>0.05)

4.3.2 Nutrient composition of solvent extracted soyabean based experimental diets supplemented with protease

Chemical composition of solvent extracted soyabean based experimental diets supplemented dietary protease is shown in Table 30. Supplemental protease in solvent extracted soyabean based diet had no effect ($P>0.05$) on crude protein with the value ranged from 41.56±0.18 (SS500) to SS100 (39.85±0.29). Ash content was higher ($P<0.05$) in diet SS400 (6.90±0.28) and least in diet SS300 (6.00±0.28). Ether extract had higher ($P<0.05$) value of 7.10±0.14 in diet SS400 and least value was in SS100 (6.55±0.21). Crude fibre values were similar ($P>0.05$) in the diets. Also, Dry matter level was higher in diet SS400 (92.95±0.19) and least in Control diet. Gross energy was higher ($P<0.05$) in diet SS500 than least value in Control diet (4.07±0.03). The values recorded for potassium were significantly difference with the values ranging from 0.78±0.00 (Control) to 0.81±0.00 (SS500). No effect ($P>0.05$) was noted in Sodium and values ranged from 0.31±0.00 to 0.64±0.00. Significant difference were observed in Calcium and phosphorus values with the higher value in diet SS500 (1.24±0.00 and 0.54±0.00) and the least value in control diet (0.98±0.00 and 0.50±0.00).

Table 30: Nutrient composition of solvent extracted soyabean based experimental diets supplemented with protease

Parameter (%)	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Crude Protein	40.90±0.28	39.85±0.29	40.68±0.72	41.21±0.23	41.51±0.30	41.56±0.18
Ash	6.70±0.28^{bc}	6.20±0.14^{ab}	6.65±0.35^{abc}	6.00±0.28^{a}	6.90±0.28^{c}	6.60±0.14^{abc}
Ether Extract	6.70±0.28^{ab}	6.55±0.21^{a}	6.75±0.21^{ab}	6.65±0.21^{ab}	7.10±0.14^{b}	6.98±0.11^{ab}
Crude Fibre	3.00±0.14	2.95±0.21	3.05±0.21	3.20±0.14	3.15±0.07	3.05±0.21
Dry Matter	91.98±0.13^{a}	92.75±0.37^{bc}	92.79±0.13^{bc}	92.22±0.40^{ab}	92.95±0.19^{c}	92.38±0.11^{abc}
Gross energy (kcal/g)	4.07±0.03	4.12±0.00	4.14±0.00	4.15±0.00	4.16±0.00	4.17±0.00
Potassium	0.78±0.00^{a}	0.79±0.00^{b}	0.80±0.00^{c}	0.80±0.00^{cd}	0.80±0.00^{de}	0.81±0.00^{e}
Sodium	0.64±0.49	0.31±0.00	0.31±0.00	0.32±0.00	0.33±0.00	0.33±0.00
Calcium	0.98±0.00^{a}	1.00±0.00^{a}	1.04±0.00^{b}	1.11±0.00^{c}	1.19±0.00^{d}	1.24±0.01^{e}
Phosphorus	0.50±0.00^{a}	0.52±0.00^{b}	0.52±0.00^{c}	0.53±0.00^{cd}	0.53±0.00^{d}	0.54±0.00^{e}

Means with same letter in row are not significantly different (P>0.05)

4.3.3 Growth performance and feed utilisation by *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Growth performance and feed utilisation by *C. gariepinus* fed roasted soyabean based diet supplemented with varying inclusion of dietary protease is shown in Table 31. Weight gain values was significantly (P>0.05) higher in *C. gariepinus* fed diet RS400 (46.90±9.25) than those on diet RS100 and control, however, fish fed diet RS200, RS300 and RS500 were intermediate to them. Similar pattern as observed in WG were noted for MWG, PWG, PER and SGR. Feed conversion ratio differ significantly (P>0.05) among treatment with the least value observed in *C. gariepinus* on RS400 (1.42±0.28). The values observed in GEFC revealed that *C. gariepinus* fed diet RS400 (97.46±13.49) was significantly (P<0.05) influenced with protease supplementation than other treatments.

Furthermore, no significant (P<0.05) differences were noted in PI and FI with the values ranging from 2.87±0.17 (RS400) to 3.36±0.34 (RS500) and 0.19±0.02 (RS400) to 0.55±0.02 (RS500), respectively. Supplementation of protease in roasted soyabean based diet significantly (P>0.05) improved GPR and NRE in *C. gariepinus* fed diet RS400. Survival rate were significantly higher in *C. gariepinus* fed diet RS200 and RS400 than other diets.

The linear regression of protease activity and graded levels of protease as shown in figure 9 was positive and strong after 84 days feeding trial as shown in equation 17

$y = 77.343x - 306.95$……... $R^2 = 0.9929$…… 17

The relationship between protease inclusion and FW, FCR of *C. gariepinus* are presented by regression equations 18 and 19 and shown in figures 10 and 11, respectively.

$y = -1E\text{-}05x^2 + 0.0309x + 31.267$……. $R^2 = 0.6679$……18

$y = 2E\text{-}06x^2 - 0.0017x + 1.6282$…….. $R^2 = 0.6384$…… 19

From the graphs (Figures 11 - 12) it could be depicted that 400ppm/kg inclusion of protease was observed at the equations for it optimum inclusion in roasted soyabean based diet.

Table 31: Growth performance and feed utilisation by *C. gariepinus* fed roasted soyabean based diets supplemented with protease

		Protease inclusion level (ppm)				
Parameter	Control	100	200	300	400	500
IW	11.87±0.15	12.20±0.10	12.10±0.20	12.00±0.10	11.87±0.21	12.17±0.12
WG	32.87±6.20^{a}	31.23±0.36^{a}	38.70±8.02^{ab}	36.40±2.88^{ab}	46.90±9.25^{b}	41.57±6.83^{ab}
MWG	21.00±6.35^{a}	19.03±3.31^{a}	26.60±7.86^{ab}	24.40±2.79^{ab}	35.03±9.35^{b}	29.40±6.78^{ab}
PWG	177.44±55.83^{a}	155.95±26.73^{a}	219.31±61.52^{ab}	203.22±21.54^{ab}	295.74±82.26^{b}	241.51±55.03^{ab}
FCR	2.50±0.78^{bc}	2.75±0.59^{c}	1.88±0.29^{ab}	2.10±0.04^{abc}	1.42±0.28^{a}	1.85±0.29^{abc}
GEFC	66.41±11.56^{a}	61.57±10.72^{a}	79.45±7.00^{a}	71.18±3.00^{a}	97.46±13.49^{b}	73.96±4.96^{a}
PI	2.98±0.38	3.07±0.26	2.90±0.35	3.08±0.39	2.87±0.17	3.36±0.34
FI	0.20±0.03	0.20±0.02	0.19±0.02	0.21±0.03	0.19±0.01	0.55±0.02
PER	10.96±2.07^{a}	10.41±1.12^{a}	12.90±2.67^{ab}	12.13±0.96^{ab}	15.63±3.08^{b}	13.85±2.28^{ab}
SGR	0.62±0.13^{a}	0.58±0.06^{a}	0.71±0.12^{ab}	0.69±0.04^{ab}	0.84±0.12^{b}	0.76±0.11^{ab}
GPR	0.78±0.02^{b}	0.73±0.01^{a}	0.84±0.01^{c}	0.81±0.01^{c}	0.91±0.01^{d}	0.77±0.02^{b}
NRE	40.96±9.09^{a}	36.83±5.00^{a}	52.22±12.52^{ab}	47.75±4.47^{a}	53.63±14.93^{b}	53.63±10.21^{ab}
SURVIVAL RATE %	80.00±10.00^{ab}	78.40±0.10^{a}	91.20±0.20^{c}	80.00±5.00^{ab}	89.00±1.00^{bc}	75.00±5.00^{a}
Protease activity (PROT/Kg)	-	7013	13860	24180	32090	37030

Means with same letter in row are not significantly different (P>0.05)

IW = Initial Weight, WG= Weight gain, MWG= Mean weight gain, PWG= Percentage weight gain, FCR= Feed Conversion Ratio, GEFC= Gross Efficiency Feed Conversion, PI= Protein Intake, FI= Feed Intake, PER= Protein Efficiency Ratio, SGR= Specific Growth Rate, GPR= Gross Protein Retention, NRE= Nitrogen Retention Efficiency, SR= Survival Rate

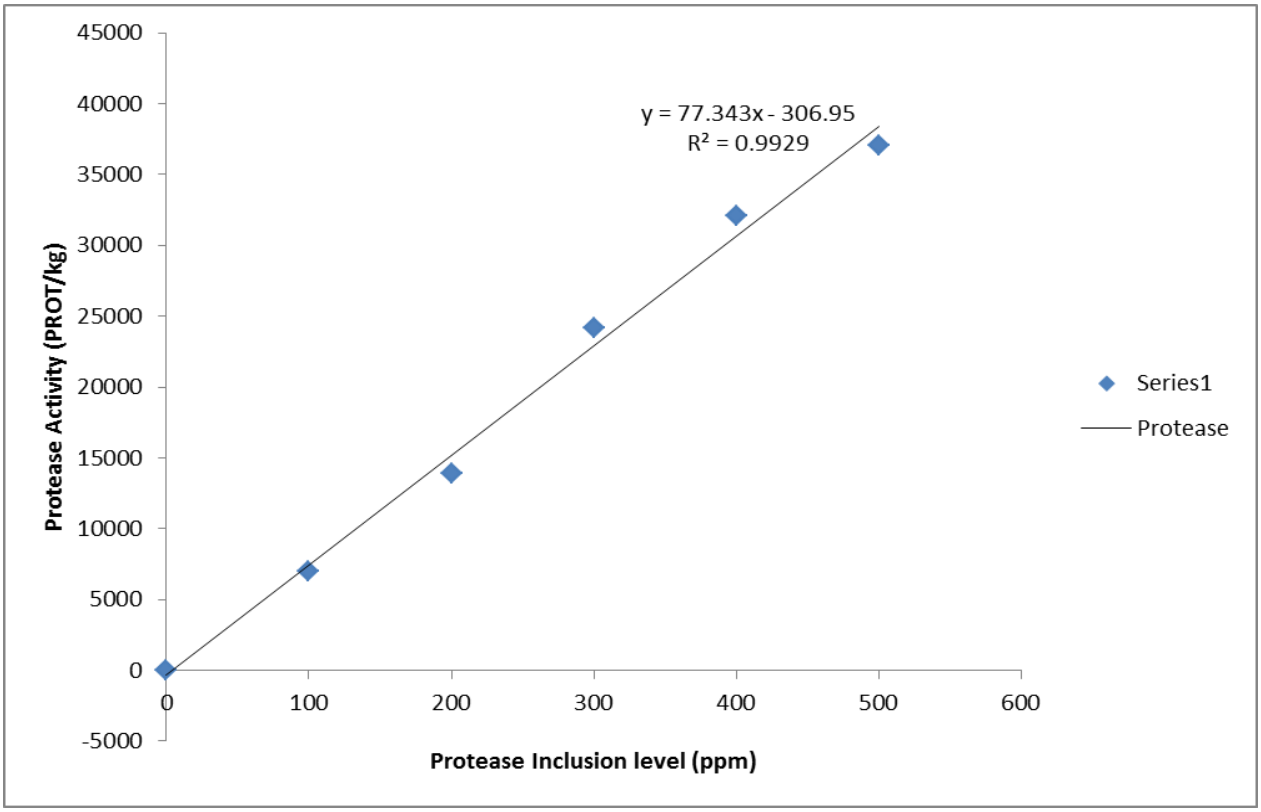

Figure 9: Relationship between protease inclusion level (ppm) and roasted soyabean based-diet protease activity fed to *C. gariepinus*.

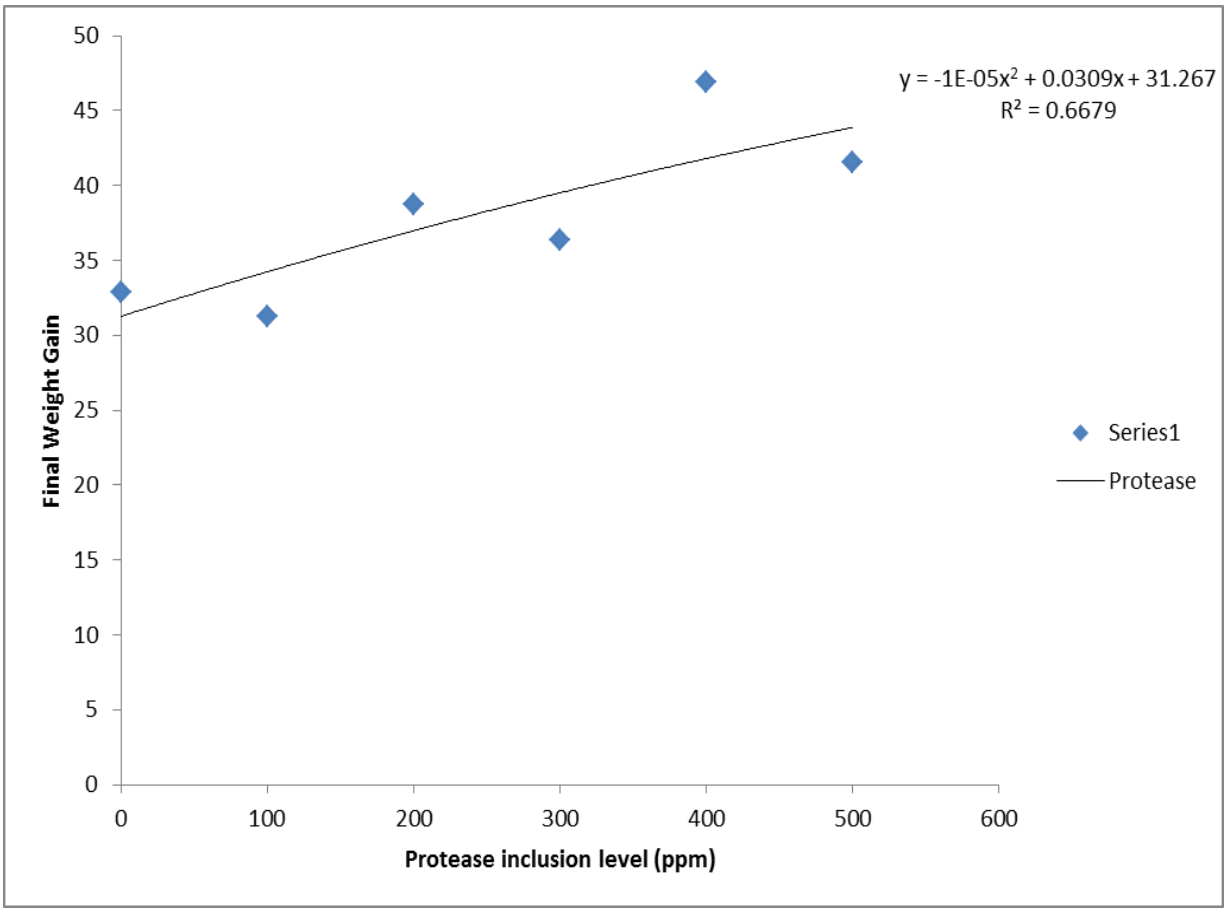

Figure 10: Relationship between dietary supplement of protease in a roasted soyabean based diet and Final weight of *Clarias gariepinus*

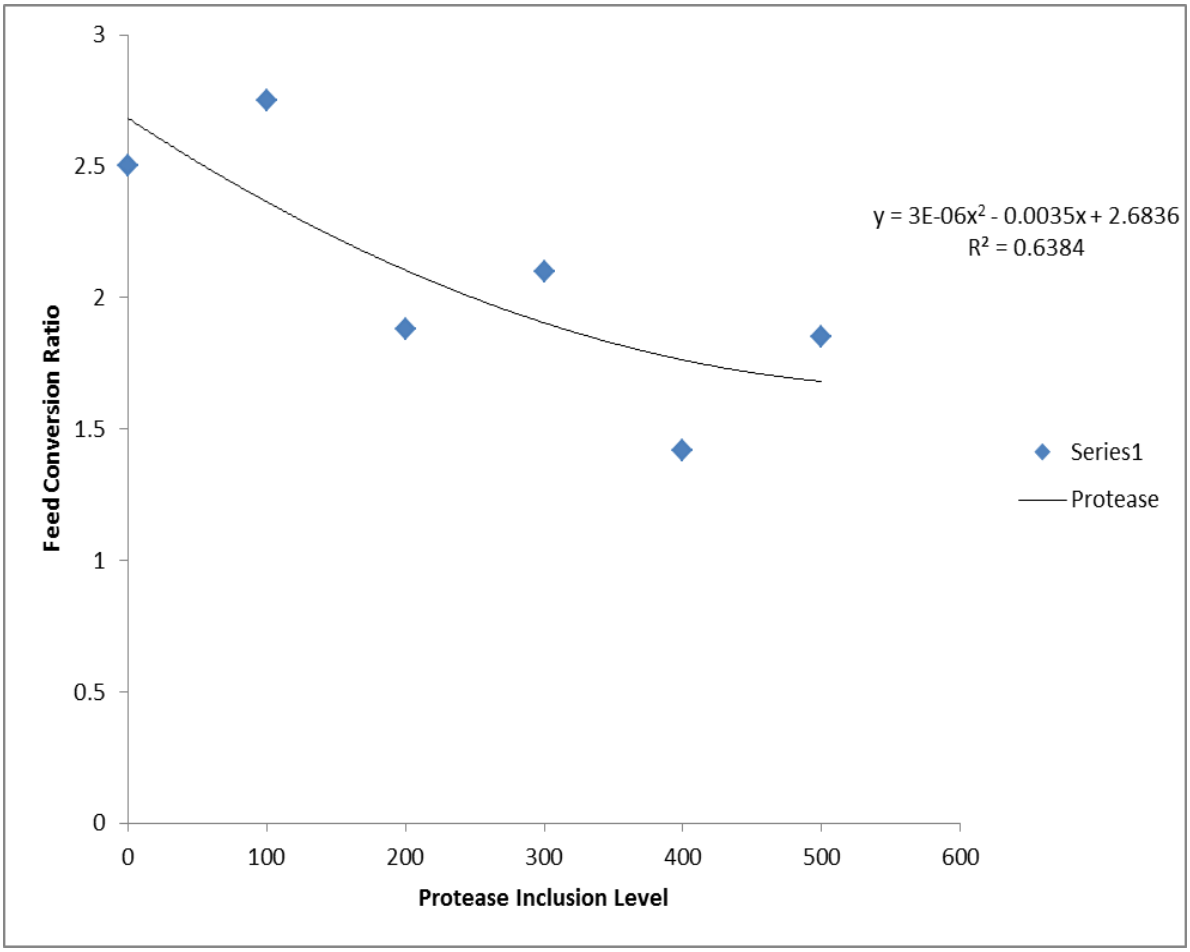

Figure 11: Relationship between dietary supplement of protease in a roasted soyabean based diet and feed conversion ratio of *Clarias gariepinus*

4.3.4 Growth performance and feed utilisation by *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Growth performance and feed utilisation by *C. gariepinus* fed solvent extracted soyabean based diet supplemented with varying inclusion of dietary protease is shown Table 32. Protease supplementation significantly (P<0.05) increased WG, MWG and PWG values with the higher values of 44.63±3.13, 32.53±3.30 and 269.16±31.23 in *C. gariepinus* fed diet SS400, respectively. Supplementation of protease in soyabean based diet significantly (P>0.05) reduced FCR values with the least value in *C. garieinus* fed SS400 (1.62±0.18) but similar (P>0.05) to *C. gariepinus* diet SS300 (1.88±0.11). Similar pattern was observed in GEFC values. Also, protease supplementation has on effect (P>0.05) on PI and FI with the values ranged from 3.14±0.14 (SS400) to 3.39±0.44 (SS500) and 0.21±0.01 (SS400) to 0.23±0.03 (SS500), respectively. Furthermore, PER, SGR and NRE values significantly (P>0.05) increased with protease supplementation with the highest values observed in fish fed diet SS400. The GPR differ among diets. Survival rate of the fish fed diet SS100, SS200, SS300, SS400 were not significantly (P<0.05) different but these were significantly higher than those on diet SS500 and control.

The linear regression of protease activity and graded levels of protease as shown in figure 12 was positive and strong after 84 days feeding trial as shown in equation 20

$y = 87.497x + 1619$…… $R^2 = 0.9932$……. 20

The relationship between protease inclusion and FW, FCR of *C. gariepinus* are presented by regression equations 21 and 22 and shown in figures 13 and 14, respectively.

$y = -7E\text{-}05x^2 + 0.051x + 31.098$…… $R^2 = 0.7387$……. 21

$y = 4E\text{-}06x^2 - 0.0027x + 1.7796$…… $R^2 = 0.8147$……. 22

From the graphs (Figures 13 - 14) it could be depicted that 400ppm/kg inclusion of protease was observed at the equations for it optimum inclusion in solvent extracted soyabean based diet.

Table 32: Growth performance and feed utilisation by *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

		Protease inclusion level (ppm)				
Parameter	Control	100	200	300	400	500
IW	11.93±0.06	12.07±0.21	11.93±0.15	12.00±0.10	12.10±0.17	12.03±0.21
WG	32.03±0.65^{a}	35.03±1.24^{ab}	36.57±0.86^{bc}	40.13±3.33^{c}	44.63±3.13^{d}	37.73±2.75^{bc}
MWG	20.10±0.60^{a}	22.97±1.19^{ab}	24.63±0.71^{bc}	28.13±3.36^{c}	32.53±3.30^{d}	25.70±2.60^{bc}
PWG	168.42±4.34^{a}	190.35±10.02^{ab}	206.40±3.29^{bc}	234.51±28.61^{cd}	269.16±31.23^{d}	213.44±19.05^{bc}
FCR	2.79±0.01^{c}	2.35±0.21^{b}	2.27±0.08^{b}	1.88±0.11^{a}	1.62±0.18^{a}	2.21±0.32^{b}
GEFC	57.13±0.45^{a}	65.24±5.76^{ab}	65.39±2.02^{ab}	76.342.03^{cd}	85.44±7.48^{d}	67.52±8.90^{bc}
PI	3.36±0.09	3.23±0.23	3.36±0.03	3.15±0.24	3.14±0.14	3.39±0.44
FI	0.22±0.01	0.22±0.02	0.22±0.00	0.21±0.02	0.21±0.01	0.23±0.03
PER	10.68±0.22^{a}	11.60±0.41^{ab}	12.19±0.29^{bc}	13.38±1.11^{c}	14.88±1.04^{d}	12.58±0.92^{ab}
SGR	0.62±0.01^{a}	0.66±0.02^{ab}	0.69±0.01^{bc}	0.75±0.05^{bc}	0.81±0.05^{d}	0.71±0.04^{bc}
GPR	0.84±0.01^{d}	0.82±0.00^{d}	0.67±0.01^{a}	0.74±0.01^{c}	0.86±0.01^{e}	0.70±0.00^{a}
NRE	41.58±1.08^{a}	45.77±1.98^{ab}	42.82±1.28^{a}	50.29±4.97^{b}	62.15±5.03^{c}	45.26±3.83^{ab}
SR %	77.80±0.10^{a}	88.90±0.10^{b}	88.90±0.10^{b}	93.41±0.17^{b}	91.11±0.01^{b}	80.00±10.00^{a}
Protease Activity (PROT/Kg)	0	11460	21100	26740	36660	45000

Means with same letter in row are not significantly different (P>0.05)

IW = Initial Weight, WG= Weight gain, TFI= Total Feed Intake, MWG= Mean weight gain, PW= Percentage weight gain, FCR= Feed Conversion Ratio, GEFC= Gross Efficiency Feed Conversion, PI= Protein Intake, FI= Feed Intake, PER= Protein Efficiency Ratio, SGR= Specific Growth Rate, GPR= Gross Protein Retention, NRE= Nitrogen Retention Efficiency, SR= Survival Rate

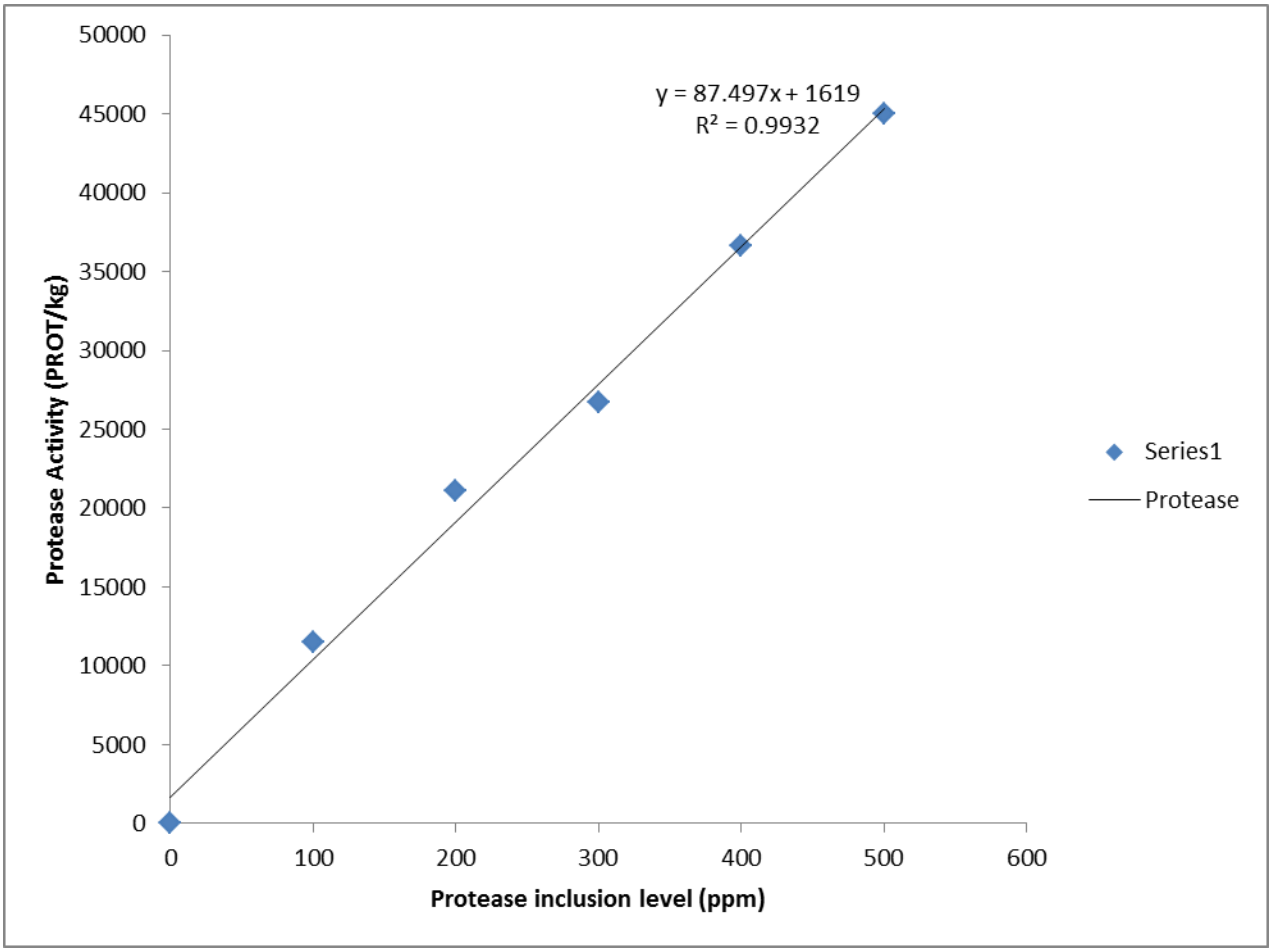

Figure 12: **Relationship between protease inclusion level (ppm) and solvent extracted soyabean based-diet protease activity fed to *C. gariepinus*.**

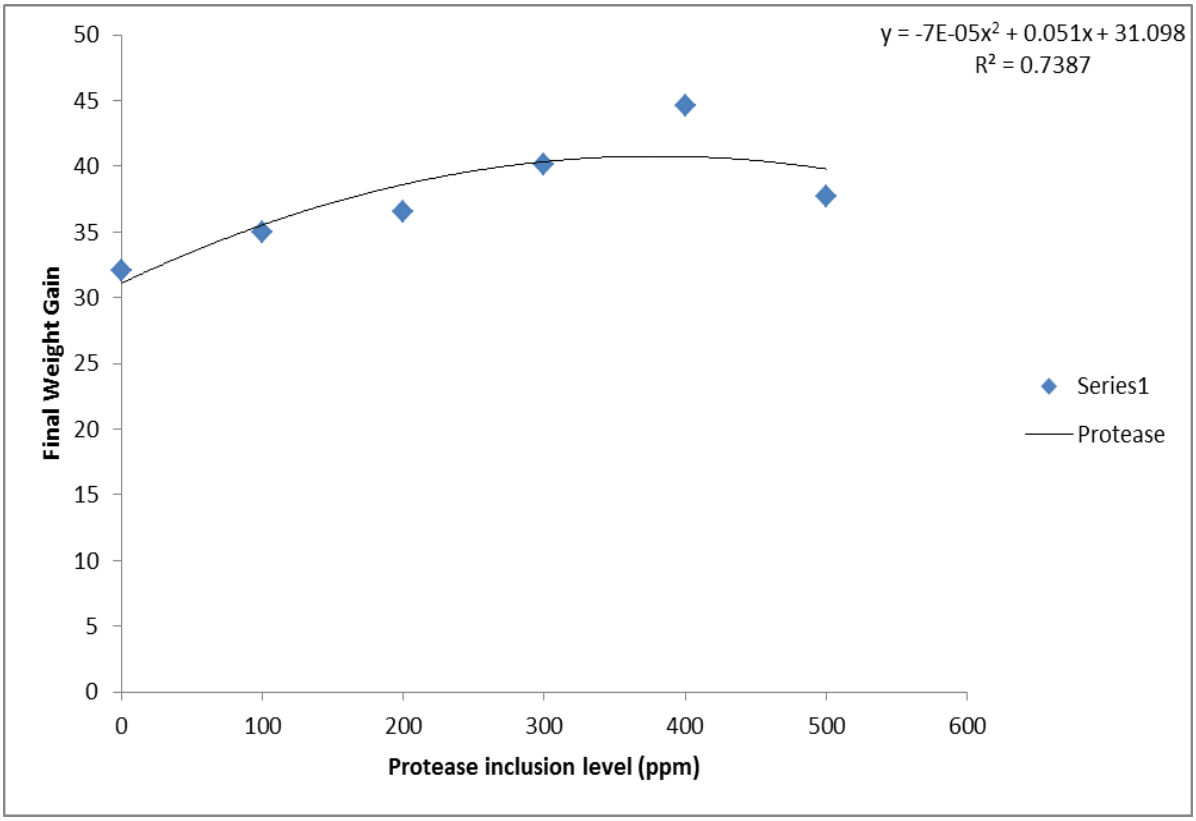

Figure 13: Relationship between dietary supplement of protease in a solvent extracted soyabean based diet and Final weight of *Clarias gariepinus*

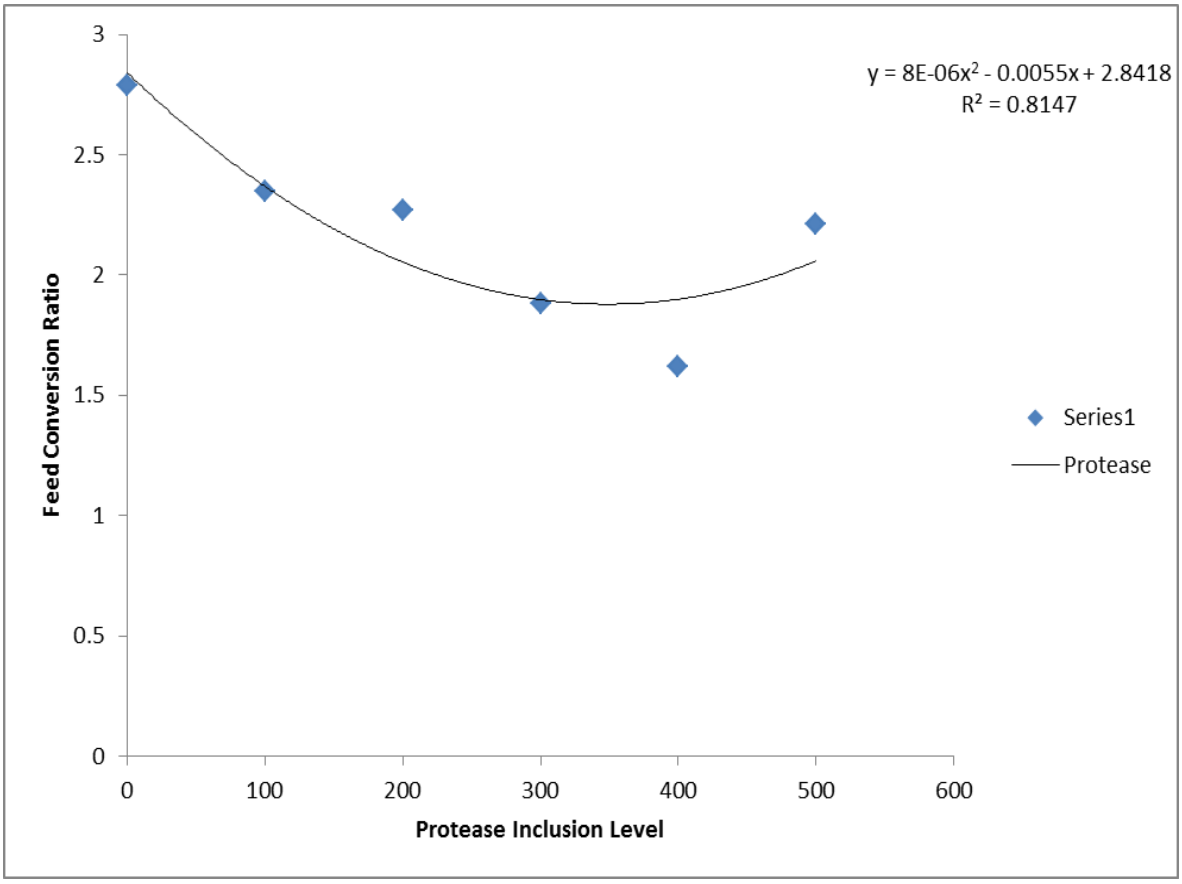

Figure 14: Relationship between dietary supplement of protease in a solvent extracted soyabean based diet and feed conversion ratio of *Clarias gariepinus*

4.3.5 Haematological parameter of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Haematological parameter of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary protease is shown in Table 33. Packed cell volume (%), hemoglobin (g/dL), red blood cell ($x10^{12}$/L), white blood cell ($x10^{9}$/L), platelet ($x10^{9}$/L) and basophils (%) were unaffected (P>0.05) with supplementation of protease in diets. Packed cell volume (%) ranged from 23.50±0.71 (RS300) to control (27.00±1.00), hemoglobin (g/dL) from 7.50±1.56 (RS500) to 9.00±0.36 (Control), red blood cell ($x10^{12}$/L) from 1.87±0.06 (RS300) to 3.37±0.46 (control), white blood cell ($x10^{9}$/L) from 12.62±3.57 (RS500) to 16.00±0.89 (RS100), platelet ($x10^{9}$/L) from 16.60±2.80 (control) to 25.33±8.41 (RS400) and basophils (%) from 0.00±0.00 (control) to 0.50±0.71 (RS500). Protease supplementation significantly (P<0.05) increased MCV (fl) and MCH (pg) values, however, no significant difference were observed with protease supplemented diets. MCHC (g/dL) value was significantly (P<0.05) higher in *C. gariepinus* on treatment RS100 (34.10±0.74) and least in RS200 (31.64±1.20) but fish fed control, RS300, RS400 and RS500 were intermediate. Supplementation of protease significantly reduced lymphocytes (%) values while, no significant differences was observed between the supplemented diets. On the contrary, Heterocytes (%) and lymphocytes: heterocytes ratio values increased with protease supplementation. Lymphocytes: heterocytes ratio values of fish fed diet RS400 and control did not differ. Monocytes (%) values were not significantly different from fish fed diet RS100, RS200, RS300 and RS400, though, significantly higher than fish fed control and RS500. However, Eosinophils (%) values was significantly higher in fish fed diet RS500 with protease supplementation compared with other diets,

Table 33: Haematological parameters of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

		Protease inclusion level (ppm)				
Parameter	Control	100	200	300	400	500
PCV (%)	27.00±1.00	24.33±0.58	25.33±4.04	23.50±0.71	26.00±2.00	23.50±4.95
HB(g/dL)	9.00±0.36	8.30±0.36	8.00±1.14	7.55±0.35	8.37±0.32	7.50±1.56
RBC($x10^{12}$/L)	3.37±0.46	1.91±0.64	2.76±0.99	1.87±0.06	2.64±0.85	2.54±0.99
WBC($x10^{9}$/L)	11.93±3.17	16.00±0.89	13.05±2.51	14.55±1.77	15.20±1.25	12.62±3.57
MCV (fl)	81.07±9.58^{a}	135.50±35.47^{b}	96.94±21.31^{ab}	126.01±0.51^{ab}	104.35±28.29^{ab}	96.02±17.94^{ab}
MCH (pg)	27.01±3.14^{a}	46.05±11.35^{b}	30.77±7.32^{ab}	40.47±0.52^{ab}	33.88±10.41^{ab}	30.66±5.83^{ab}
MCHC(g/dL)	33.35±1.51^{ab}	34.10±0.74^{b}	31.64±1.20^{a}	32.12±0.54^{ab}	32.24±1.37^{ab}	31.93±0.11^{ab}
Platelet (x 10^{9}/L)	16.60±2.80	21.17±5.06	17.83±3.54	25.50±8.06	25.33±8.41	28.85±16.90
Lym (%)	71.33±5.03^{b}	57.33±10.41^{a}	53.00±2.65^{a}	57.50±4.95^{a}	62.33±3.51^{ab}	57.00±5.66^{a}
Het (%)	21.33±4.16^{a}	35.00±9.85^{b}	40.67±1.15^{b}	35.00±2.83^{b}	29.67±1.15^{ab}	34.50±4.95^{b}
Lym:Het Ratio	0.30±0.08^{a}	0.64±0.27^{b}	0.77±0.06^{b}	0.62±0.11^{b}	0.48±0.05^{a}	0.62±0.15^{b}
Mono (%)	2.33±0.58^{a}	3.33±1.15^{ab}	4.33±1.15^{b}	4.00±0.00^{b}	3.67±1.53^{ab}	1.50±0.71^{a}
Eos (%)	5.00±1.00^{ab}	4.00±1.00^{ab}	2.00±2.65^{a}	3.00±1.41^{a}	4.33±1.53^{ab}	6.50±0.71^{b}
Baso (%)	0.00±0.00	0.33±0.58	0.00±0.00	0.50±0.71	0.00±0.00	0.50±0.71

Means with same letter in row are not significantly different (P>0.05)

PCV= Packed Cell Volume, HB= Hemoglobin, RBC= Red Blood Cell, WBC= White Blood Cell, MCV= Mean Cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, Lym= Lymphocytes, Het= Heterocytes, Mono= Monocytes, Eos = Eosinophils, Baso = Basophils

4.3.6 Haematological parameters of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Haematological parameters of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary protease is shown in Table 34. PCV (%) values significantly reduced with protease supplementation while fish fed diet SS200, SS300, SS400 were not significantly different from each other. Hemoglobin (g/dL) values of fish fed control, SS200, SS300 and SS400 diets were not significantly different from each other though these were intermediate to fish fed diet SS100 and SS500. RBC ($x10^{12}$/L) ranged from 2.42±1.01 (SS500) to 3.70±0.22 (Control), WBC ($x10^{9}$/L) from 11.92±2.32 (SS500) to 14.68±4.35 (SS200), MCH (pg) from 27.17±3.45 (control) to 104.78±23.62 (SS300) and MCHC (g/dL) from 32.07±0.92 (control) to 34.08±1.08 (SS400). Significant different ($P<0.05$) were observed in platelet ($x10^{9}$/L) values of *C. gariepinus* fed diet SS400 (18.00±5.72) compared to *C. gariepinus* fed other diets. Lymphocytes (%), heterocytes (%) and lymphocytes: heterocytes ratio values decreased significantly in fish fed protease supplemental diets. No effect ($P>0.05$) were observed in Monocytes (%), Eosinophils (%) and Basophils (%) values with the values ranged from 2.33±1.53 (SS200) to 3.67±1.53 (SS500), 3.00±2.00 (Control) to 4.67±0.58 (SS300) and 0.00±0.00 (SS400) to 0.67±0.58 (SS200), respectively.

Table 34: Haematological parameters of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
PCV (%)	31.33±4.04^{b}	31.00±3.61^{b}	25.67±4.04^{ab}	26.00±4.00^{ab}	28.33±2.08^{ab}	22.67±4.62^{a}
HB(g/dL)	10.07±1.51^{ab}	10.50±1.05^{b}	8.43±1.50^{ab}	8.50±1.61^{ab}	9.67±0.95^{ab}	7.43±1.53^{a}
RBC(x10^{12}/L)	3.70±0.22	3.56±0.10	2.81±0.88	2.61±0.88	3.14±0.35	2.42±1.01
WBC(x10^{9}/L)	14.12±3.51	13.62±3.73	14.68±4.35	12.53±2.48	13.42±3.08	11.92±2.32
MCV (fl)	84.54±8.48	87.10±9.12	94.75±17.88	104.78±23.62	90.63±4.72	98.70±18.13
MCH (pg)	27.17±3.45	29.52±2.86	30.96±4.99	34.14±7.78	30.89±2.00	32.36±5.94
MCHC(g/dL)	32.07±0.92	33.93±1.35	32.78±0.84	32.61±1.78	34.08±1.08	32.79±0.26
Platelet (x 10^{9}/L)	13.03±1.12^{ab}	13.27±0.64^{ab}	15.33±2.91^{ab}	14.53±1.37^{ab}	18.00±5.72^{b}	11.87±1.63^{a}
Lym (%)	50.67±4.16^{a}	59.00±3.46^{b}	65.00±5.00^{bc}	60.00±5.57^{bc}	68.00±4.00^{c}	63.00±4.00^{bc}
Het (%)	42.00±4.58^{a}	33.67±3.51^{b}	28.33±3.21^{b}	32.00±5.29^{b}	25.67±3.79^{b}	28.67±4.73^{b}
Lym:Het Ratio (%)	0.84±0.16^{a}	0.57±0.09^{b}	0.44±0.08^{b}	0.54±0.14^{b}	0.38±0.08^{b}	0.46±0.11^{b}
Mono (%)	3.33±0.58	2.67±0.58	2.33±2.31	3.00±1.00	2.33±1.53	3.67±1.53
Eos (%)	3.00±2.00	4.33±2.52	3.67±2.08	4.67±0.58	4.00±2.00	4.33±2.08
Baso (%)	0.33±0.58	0.33±0.58	0.67±0.58	0.33±0.58	0.00±0.00	0.33±0.58

Means with same letter in row are not significantly different (P>0.05)

PCV= Packed Cell Volume, HB= Hemoglobin, RBC= Red Blood Cell, WBC= White Blood Cell, MCV= Mean Cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, Lym= Lymphocytes, Het= Heterocytes, Mono= Monocytes, Eos = Eosinophils, Baso = Basophils

4.3.7 Serum biochemical indices of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Serum biochemical indices of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary protease is shown in Table 35. Total protein (%), albumin (g/L), globulin (g/L), AST (IU/L), and creatinine (µmol/L) were unaffected by protease supplementation in the diets. Total protein (%) ranged from 6.50±1.25 (RS100) to 7.20±0.20 (RS400), albumin (g/L) from 1.50±0.20 (RS200) to 2.33±0.29 (RS400), globulin (g/L) from 4.57±0.55 (RS100) to 5.07±0.17 (control), AST (IU/L) from 204.00±1.73 (Control) to 239.00±46.67 (RS300) and creatinine (µmol/L) from 0.53±0.06 (RS500) to 0.63±0.12 (Control). A-G ratio had a significantly higher ($P<0.05$) value in *C. gariepinus* on diet RS400 (0.50±0.10) but similar to *C. gariepinus* on diet RS100 (0.40±0.17), RS300 (0.40±0.00) and RS500 (0.50±0.10). ALT (IU/L) had a significantly higher value in *C. gariepinus* fed diet RS400 (34.67±13.32) but similar ($P>0.05$) to *C. gariepinus* on diet RS500 (28.00±4.36) and RS200 (22.67±2.52). Furthermore, ALP (IU/L) had higher ($P<0.05$) value in *C. gariepinus* on diet RS200 (309.33±33.02) and least in diet RS100 (120.33±28.57). BUN (µmol/L) values varies significantly among the treatment.

4.3.8 Serum biochemical indices of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Serum biochemical indices of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary protease is shown in Table 36. Supplemental protease had no effect ($P>0.05$) on serum biochemical parameters monitored in this phase except for ALT. Total protein (%), albumin (g/L), globulin (g/L), A-G ratio and AST (IU/L) ranged from 6.13±1.33 (control) to 7.27±0.32 (SS100), 1.33± 0.31(SS400) to 2.23±0.40 (SS100), 4.60±0.53 (SS200) to 5.13±0.06 (SS500), 0.23±0.06 (SS500) to 0.43±0.23 (SS200) and 185.67±18.48 (SS300) to 219.67±40.46 (control). ALT (IU/L) had least ($P<0.05$) value in *C. gariepinus* fed Control (20.00±1.00) and increase from fish fed diet SS200 to SS400. Meanwhile, ALP (IU/L), BUN (µmol/L), Creatinine (µmol/L) values ranged from 203.33±172.10 (SS100) to 307.67±98.76 (SS500), 43.13±54.45 (SS300) to 10.33±0.31 (SS500) and 0.57±0.06 (SS100) to 0.60±0.10 (SS500).

Table 35: Serum biochemical indices of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Total protein(g/L)	6.57±0.25	6.50±1.25	6.13±0.15	6.65±0.78	7.20±0.20^{a}	6.67±0.42
Albumin(g/L)	1.50±0.17	1.93±0.83	1.50±0.20	2.05±0.35	2.33±0.29	1.73±0.42
Globulin(g/L)	5.07±0.15	4.57±0.55	4.63±0.15	4.60±0.42	4.80±0.26	4.93±0.23
A-G ratio	0.23±0.06^{a}	0.40±0.17^{ab}	0.27±0.06^{a}	0.40±0.00^{ab}	0.50±0.10^{b}	0.30±0.10^{ab}
AST (IU/L)	204.00±1.73	221.33±28.31	205.33±3.06	239.00±46.67	219.33±15.70	198.67±19.86
ALT (IU/L)	22.00±3.46^{a}	20.33±0.58^{a}	22.67±2.52^{ab}	21.50±2.12^{a}	34.67±13.32^{b}	28.00±4.36^{ab}
ALP (IU/L)	169.33±57.47^{ab}	120.33±28.57^{a}	309.33±33.02^{b}	126.50±23.33^{a}	307.67±90.52^{b}	168.00±117.78^{ab}
BUN(µmol/L)	10.50±0.50^{ab}	10.97±0.29^{abc}	10.23±0.40^{a}	11.35±0.64^{bcd}	12.27±0.25^{d}	11.73±0.76^{cd}
Creatinine(µmol/L)	0.63±0.12	0.57±0.12	0.57±0.06	0.60±0.14	0.60±0.00	0.53±0.06

Means with same letter in row are not significantly different (P>0.05)

A-G Ratio- Albumin-Globulin Ratio, AST = Aspartate Transaminase, ALT = Alanine Transaminase, ALP = Alkaline Phosphatase, BUN = Blood Urea Nitrogen.

Table 36: Serum biochemical indices of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Total protein(g/L)	6.13±1.33	7.27±0.32	6.60±0.53	6.67±0.61	6.20±0.53	6.63±0.30
Albumin(g/L)	1.50±0.66	2.23±0.40	2.00±0.69	1.77±0.21	1.33±0.31	1.50±0.30
Globulin(g/L)	4.63±0.74	5.03±0.12	4.60±0.53	4.83±0.38	4.87±0.31	5.13±0.06
A-G ratio	0.30±0.10	0.40±0.10	0.43±0.23	0.33±0.06	0.27±0.06	0.23±0.06
AST (IU/L)	219.67±40.46	200.67±17.62	215.33±24.58	185.67±18.48	193.33±14.05	218.00±27.71
ALT (IU/L)	20.00±1.00^{a}	20.00±1.00^{a}	24.67±2.52^{ab}	24.67±3.21^{ab}	26.33±3.06^{b}	25.33±5.51^{ab}
ALP (IU/L)	208.67±99.76	203.33±172.10	261.67±124.28	248.67±130.29	207.33±84.88	307.67±98.76
BUN(µmol/L)	10.63±0.35	11.30±0.46	11.10±1.14	43.13±54.45	10.57±0.67	10.33±0.31
Creatinine(µmol/L)	0.60±0.10	0.57±0.06	0.60±0.10	0.60±0.10	0.60±0.10	0.60±0.10

Means with same letter in row are not significantly different (P>0.05)

A-G Ratio- Albumin-Globulin Ratio, AST = Aspartate Transaminase, ALT = Alanine Transaminase, ALP = Alkaline Phosphatase, BUN = Blood Urea Nitrogen.

4.3.9 Proximate composition of *C. gariepinus* whole body fed roasted soyabean based diets supplemented with protease

Proximate composition of *C. gariepinus* whole body fed roasted soyabean based diets supplemented with varying inclusion of protease is shown in Table 37. Protease supplementation significantly improved crude protein with the highest value in *C. gariepinus* fed diet RS400 and varied significantly from other treatment. Ash content of the whole body were higher in diet RS200 (5.05±0.21) but similar to fish fed diet RS400. Meanwhile, similar trend were observed in Ether extract with higher value noted in whole body of *C. gariepinus* on diet RS300 (8.45±0.21) but similar ($P>0.05$) to fish on diet SS100, SS400 and SS500. No Significant effect in crude fibre with values ranged from 0.02±0.01 (RS200) to 0.04±0.01 (RS100). Moisture content differ significantly among diets. True protein of the *C. gariepinus* whole body was higher in diet RS400 (61.19±0.42).

4.3.10. Proximate composition of *C. gariepinus* whole body fed solvent extracted soyabean based diets supplemented with protease

Proximate composition of *C. gariepinus* whole body fed solvent extracted soyabean based diets supplemented with varying inclusion of protease is shown in Table 38. Supplemental protease influenced ($P<0.05$) crude protein of *C. gariepinus* whole body fed diet SS400 (63.70±0.49). Ash content of fish fed diets SS100 and SS400 were not significantly different ($P<0.05$) from each other, but these were significantly higher than the ash content recorded for other diets. However, supplemental protease had no effect ($P>0.05$) on ether extract and crude fibre with values ranged from 8.30±0.14 (SS100) to 8.65±0.17 (SS300) and 0.02±0.01 (SS400) to 0.04±0.01 (SS200), respectively. For moisture content, *C. gariepinus* fed diet SS100 and SS300 but superior to fish fed diet SS400. True protein values varied significantly among the diets, however, fish fed diet SS400 had highest value recorded.

Table 37: Proximate composition of *C. gariepinus* whole body fed roasted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Crude protein	60.57±0.70^{b}	58.68±0.20^{a}	63.20±0.57^{c}	62.20±0.35^{c}	65.89±0.45^{d}	60.25±0.92^{b}
Ash content	4.14±0.09^{a}	4.35±0.21^{ab}	5.05±0.21^{c}	4.05±0.21^{a}	4.75±0.21^{bc}	4.30±0.14^{ab}
Ether extract	8.00±0.14^{ab}	8.40±0.14^{bc}	7.90±0.14^{a}	8.45±0.21^{c}	8.25±0.21^{abc}	8.10±0.14^{abc}
Crude fibre	0.02±0.01	0.04±0.01	0.02±0.01	0.02±0.00	0.03±0.01	0.02±0.01
Moisture	75.93±0.45^{d}	74.80±0.19^{c}	74.63±0.11^{bc}	76.20±0.14^{d}	73.97±0.31^{ab}	73.72±0.17^{a}
True Protein	56.25±0.65^{b}	54.50±0.19^{a}	58.70±0.53^{c}	57.76±0.33^{c}	61.19±0.42^{d}	55.95±0.86^{b}

Means with same letter in row are not significantly different (P>0.05)

Table 38: Proximate composition of *C. gariepinus* whole body fed Solvent extracted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Crude protein	62.91±0.28^{d}	62.42±0.16^{d}	56.47±0.28^{a}	58.95±0.21^{c}	63.70±0.49^{e}	57.39±0.00^{b}
Ash content	3.75±0.21^{a}	5.20±0.14^{c}	4.65±0.21^{b}	4.75±0.21^{bc}	5.20±0.14^{c}	4.95±0.21^{bc}
Ether extract	8.65±0.07	8.30±0.14	8.55±0.21	8.65±0.21	8.40±0.14	8.60±0.14
Crude fibre	0.03±0.01	0.03±0.01	0.04±0.01	0.03±0.01	0.02±0.01	0.03±0.01
Moisture	73.97±0.13^{a}	75.35±0.08^{d}	73.73±0.33^{a}	74.93±0.30^{cd}	74.66±0.04^{bc}	74.21±0.39^{ab}
True Protein	58.43±0.26^{d}	57.97±0.15^{d}	52.44±0.25^{a}	54.75±0.19^{c}	59.16±0.46^{e}	53.30±0.00^{b}

Means with same letter in row are not significantly different (P>0.05)

4.3.11 Amino acid composition of *C. gariepinus* whole body fed roasted soyabean based diets supplemented with protease

Amino acid composition of *C. gariepinus* whole body fed roasted soyabean based diets supplemented with varying inclusion of protease is shown in Table 39. Methionine value was higher (P<0.05) in *C. gariepinus* on diet RS300 (2.60±0.10) and was closely followed by diet RS400 (2.21±0.01). Protease supplementation increased (P<0.05) lysine value in *C. gariepinus* on diet RS100 (9.06±0.06) and least in diet RS300 (7.79±0.01). Threonine and Tryptophan values varied significantly among the diets Also, Isoleusine and Leusine significantly increased with protease supplementation. Valine and Histidine had least (P<0.05) values in *C. gariepinus* fed diet RS500 and higher in diet RS200 and Control diet (2.90±0.10 and 8.24±0.04), respectively. Phenyalanine values were higher (P<0.05) in *C. gariepinus* on diet RS100 and RS500 and least value in Control diet.

However, Glycine, Alanine, Glutamic, Cysteine and Arginine had least (P<0.05) values in *C. gariepinus* on diet RS500 (3.20±0.10, 3.31±0.01, 3.38±0.01, 1.63±0.03 and 3.36±0.02) and higher in control diet. Higher (P<0.05) values for Serine and Proline were observed in *C. gariepinus* fed diet RS300 (4.10±0.10 and 2.61±0.01). Also, significantly difference (P<0.05) were observed in Aspartic acid, Ornithine, Pyrrolysine and Tyrosine with the higher values in RS500 (6.51±0.01), control (2.27±0.03), RS500 (3.11±0.01) and RS500 (3.45±0.05) and least values in RS300 (1.70±0.20), RS200 (1.93±0.03), control (2.41±0.01) and RS400 (2.59±0.01).

Table 39: Amino acid composition of *C. gariepinus* whole body fed roasted soyabean based diets supplemented with protease

		Protease inclusion level (ppm)				
Parameter	Control	100	200	300	400	500
Essential Amino Acid						
Methionine	1.99±0.01^{b}	1.96±0.02^{ab}	1.89±0.01^{a}	2.60±0.10^{d}	2.21±0.01^{c}	2.02±0.02^{b}
Lysine	7.89±0.03^{b}	9.06±0.06^{e}	8.63±0.03^{d}	7.79±0.01^{a}	8.34±0.04^{c}	7.89±0.01^{b}
Threonine	1.93±0.03^{b}	1.99±0.01^{b}	1.86±0.03^{a}	2.11±0.01^{c}	1.85±0.05^{a}	1.95±0.05^{b}
Tryptophan	2.81±0.01^{d}	2.53±0.03^{c}	2.06±0.03^{a}	2.33±0.01^{bc}	2.44±0.26^{c}	2.160.02^{ab}
Isoleusine	1.41±0.01^{a}	9.11±0.01^{f}	7.60±0.10^{c}	7.11±0.01^{b}	8.11±0.02^{d}	8.55±0.05^{e}
Leusine	1.36±0.03^{a}	8.59±0.01^{f}	7.05±0.05^{d}	6.77±0.03^{c}	8.47±0.03^{e}	3.92±0.02^{b}
Valine	2.64±0.04^{cd}	2.60±0.10^{c}	2.90±0.10^{e}	2.73±0.03^{d}	2.32±0.02^{b}	2.07±0.02^{a}
Histidine	8.24±0.04^{f}	7.63±0.03^{e}	6.08±0.04^{c}	5.92±0.02^{b}	6.83±0.03^{d}	3.40±0.20^{a}
Phenyalanine	1.91±0.01^{a}	2.95±0.05^{d}	2.80±0.10^{c}	2.48±0.02^{b}	2.70±0.10^{c}	2.950.05^{d}
Arginine	8.10±0.10^{d}	4.30±1.65^{ab}	5.70±0.30^{c}	5.29±0.01^{bc}	5.30±0.10^{bc}	3.36±0.02^{a}
Non-Essential Amino Acid						
Glycine	8.84±0.04^{f}	7.97±0.03^{e}	6.51±0.01^{c}	6.10±0.10^{b}	6.70±0.10^{d}	3.20±0.10^{a}
Serine	2.90±0.10^{b}	2.48±0.02^{a}	2.82±0.02^{c}	4.10±0.10^{e}	3.50±0.10^{d}	3.16±0.06^{c}
Proline	2.07±0.07^{b}	1.96±0.04^{a}	2.03±0.03^{ab}	2.61±0.01^{e}	2.52±0.02^{d}	2.24±0.04^{c}
Alanine	7.99±0.01^{f}	7.59±0.01^{e}	6.75±0.05^{c}	6.00±0.20^{b}	7.05±0.05^{d}	3.31±0.01^{a}
Aspartic	6.26±0.06^{e}	5.00±0.20^{d}	3.01±0.01^{b}	1.70±0.20^{a}	3.53±0.03^{c}	6.51±0.01^{f}
Glutamic	9.11±0.01^{f}	8.09±0.01^{e}	6.53±0.03^{c}	5.70±0.10^{b}	6.91±0.01^{d}	3.38±0.20^{a}
Cysteine	3.47±0.03^{d}	3.30±0.10^{c}	2.60±0.10^{b}	2.63±0.03^{b}	2.68±0.02^{b}	1.63±0.03^{a}
Ornithine	2.27±0.03^{d}	1.98±0.02^{b}	1.93±0.03^{a}	1.99±0.01^{b}	1.99±0.03^{b}	2.21±0.01^{c}
Pyrrolysine	2.41±0.01^{a}	2.83±0.03^{c}	2.76±0.06^{bc}	2.70±0.10^{b}	3.09±0.01^{d}	3.11±0.01^{d}
Tyrosine	3.16±0.02^{d}	3.06±0.06^{c}	3.35±0.05^{e}	2.88±0.01^{b}	2.59±0.01^{a}	3.45±0.05^{f}

Means with same letter in row are not significantly different (P>0.05)

4.3.12 Amino acid composition of *C. gariepinus* whole body fed solvent extracted soyabean based diets supplemented with protease

Amino acid composition of *C. gariepinus* whole body fed solvent extracted soyabean based diets supplemented with varying inclusion of protease is shown in Table 40. Protease supplementation significantly influenced ($P<0.05$) methionine with higher value in *C. gariepinus* on diet SS500 (2.27±0.03) and least in diet SS300 (1.82±0.02) but similar ($P>0.05$) to SS100 (1.83±0.02). Diet SS200 (8.15±0.15) was influenced ($P<0.05$) with supplemental protease in lysine and least in diet SS500 (3.21±0.01). Threonine had higher ($P<0.05$) value in *C. gariepinus* on diet SS200 (2.21±0.01) and least value in diet SS300 (1.85±0.05). Higher ($P<0.05$) tryptophan values was in diet SS200 (2.48±0.02) with the least value in SS500 (1.32±0.02). Isoleusine, leusine and valine had higher ($P<0.05$) values in *C. gariepinus* on diet SS300 (6.21±0.01, 6.21±0.01 and 2.81±0.01) than other diets with protease supplementation. Higher ($P<0.05$) phenyalanine was in diet SS300 (2.88±0.02) and the least value of 2.33±0.03 was in diet SS500. Histidine and arginine of *C. gariepinus* on diet SS400 had higher ($P<0.05$) value.

Additionally, least ($P<0.05$) values of glycine, proline, alanine, glutamic, and cysteine were observed in *C. gariepinus* fed control diet with the higher values in diet SS400 (9.03±0.03), SS100 (2.68±0.02), SS400 (8.62±0.02), SS400 (8.75±0.05) and SS400 (4.03±0.03), respectively. Serine level had the higher value in diet SS500 (3.459±0.01) with least in diet SS100 (2.39±0.01). Higher ($P<0.05$) value of Aspartic acid level was noted in *C. gariepinus* fed diet SS100 (7.91±0.01) and least value in diet SS300 (2.18±0.02). Ornithine, Pyrrolysine and Tyrosine were significantly higher in *C. gariepinus* fed diet SS300 (2.12±0.02), SS500 (3.45±0.05) and SS400 (3.21±0.01) while the values were in diet SS500 (0.40±0.10), SS400 (2.42±0.02) and SS500 (1.64±0.04, respectively.

Table 40: Amino acid composition of *C. gariepinus* whole body fed solvent extracted soyabean based diets supplemented with protease

		Protease inclusion level (ppm)				
Parameter	Control	100	200	300	400	500
Essential Amino Acid						
Methionine	1.92±0.02^{b}	1.83±0.03^{a}	2.01±0.01^{c}	1.82±0.02^{a}	1.92±0.04^{b}	2.27±0.03^{d}
Lysine	7.57±0.03^{d}	7.92±0.02^{e}	8.15±0.05^{f}	7.51±0.01^{c}	6.81±0.01^{b}	3.21±0.01^{a}
Threonine	1.96±0.01^{b}	1.94±0.04^{b}	2.21±0.01^{c}	1.85±0.05^{a}	1.91±0.01^{b}	1.85±0.02^{a}
Tryptophan	2.27±0.03^{d}	2.06±0.06^{bc}	2.48±0.02^{e}	2.11±0.01^{c}	2.00±0.10^{b}	1.32±0.02^{a}
Isoleusine	1.41±0.01^{a}	4.82±0.02^{c}	9.70±0.10^{e}	6.21±0.01^{d}	1.50±0.10^{a}	4.40±0.40^{b}
Leusine	1.41±0.01^{a}	4.90±0.01^{b}	9.68±0.02^{e}	6.21±0.01^{d}	1.50±0.10^{a}	4.70±0.10^{c}
Valine	2.02±0.02^{a}	2.60±0.10^{c}	2.43±0.01^{b}	2.81±0.01^{d}	2.78±0.02^{d}	2.37±0.03^{b}
Histidine	1.73±0.03^{a}	4.12±0.02^{c}	7.61±0.01^{e}	5.84±0.02^{d}	8.24±0.04^{f}	4.05±0.05^{b}
Phenyalanine	2.88±0.02^{c}	2.61±0.01^{b}	2.35±0.05^{a}	2.88±0.02^{c}	2.65±0.05^{b}	2.33±0.03^{a}
Arginine	1.70±0.10^{a}	3.23±0.03^{b}	4.00±0.20^{c}	4.04±0.59^{c}	7.50±0.10^{d}	3.74±0.04^{c}
Non-Essential Amino Acid						
Glycine	1.58±0.02^{a}	4.11±0.01^{b}	8.20±0.10^{d}	5.71±0.01^{c}	9.03±0.03^{e}	4.14±0.04^{b}
Serine	2.99±0.01^{d}	2.39±0.01^{a}	3.59±0.01^{e}	2.73±0.03^{c}	2.56±0.04^{b}	3.59±0.01^{e}
Proline	2.07±0.02^{a}	2.68±0.02^{e}	2.52±0.02^{d}	2.11±0.01^{b}	2.41±0.01^{c}	2.52±0.02^{d}
Alanine	1.16±0.02^{a}	3.96±0.01^{c}	8.29±0.01^{e}	5.35±0.05^{d}	8.62±0.02^{f}	3.68±0.02^{b}
Aspartic	3.10±0.10^{b}	7.91±0.01^{f}	3.63±0.01^{c}	2.18±0.02^{a}	6.06±0.04^{d}	7.50±0.01^{e}
Glutamic	1.40±0.10^{a}	4.16±0.02^{c}	8.11±0.01^{e}	5.71±0.01^{d}	8.75±0.05^{f}	4.07±0.01^{b}
Cysteine	0.51±0.01^{a}	1.72±0.02^{b}	3.61±0.01^{d}	2.26±0.02^{c}	4.03±0.03^{e}	1.71±0.01^{c}
Ornithine	1.84±0.04^{b}	1.92±0.02^{b}	2.03±0.03^{c}	2.12±0.02^{c}	1.85±0.05^{b}	0.40±0.10^{a}
Pyrrolysine	2.76±0.06^{b}	2.78±0.02^{b}	3.20±0.20^{c}	3.18±0.02^{c}	2.42±0.02^{a}	3.45±0.05^{d}
Tyrosine	2.60±0.10^{b}	2.70±0.10^{b}	2.69±0.01^{b}	2.91±0.01^{c}	3.21±0.01^{d}	1.64±0.04^{a}

Means with same letter in row are not significantly different (P>0.05)

4.3.13. Apparent nutrient digestibility by *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Apparent nutrient digestibility by *C.gariepinus* fed roasted soyabean based diet supplemented with varying inclusion of protease is shown in Table 41. Protease supplementation significantly increased ($P<0.05$) crude protein digestibility and *C. gariepinus* fed diet RS300 (87.12±0.50) was significantly higher than fish fed diet RS400 and RS500. However, these were siginficantly higher than those on control, RS100 and RS200. Ash digestibility was significantly higher ($P<0.05$) in fish on diet RS300 (46.95±1.14) than other diets. Ether extract digestibility values was not significantly different between fish fed diet RS300 (92.29±0.36) and RS400 (91.59±0.06) though, these were significantly higher than other diets. Crude fibre of *C. gariepinus* on diet RS300 (75.67±1.35) had higher ($P<0.05$) value than other treatments. However, dry matter digestibility differ among diets

4.3.14. Apparent nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Apparent nutrient digestibility by *C.gariepinus* fed solvent extracted soyabean based diet supplemented with varying inclusion of protease is shown in Table 42. Crude protein digestibility values were not significantly different between the fish fed diet SS200 (87.29±0.97) and SS400 (87.38±1.10) but these were better than other diets. Ash digestibility was significantly higher in fish fed diet SS400. However, Ether extract digestibility values were significantly higher in fish fed diet SS200 (94.17±0.39) and SS400 (93.75±0.26) than other treatments. Crude fibre and dry matter digestibility follows similar pattern with ether extract digestibility.

Table 41: Apparent nutrient digestibilty of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Crude protein	77.93±0.72^{a}	77.97±2.22^{a}	76.23±0.55^{a}	87.12±0.50^{c}	82.88±1.15^{b}	82.77±0.80^{b}
Ash content	26.19±2.01^{c}	5.62±4.69^{a}	23.19±11.00^{b}	46.95±1.14^{d}	23.72±0.04^{c}	13.96±0.45^{bc}
Ether extract	88.83±0.51^{b}	89.38±0.22^{b}	86.75±0.52^{a}	92.29±0.36^{d}	91.59±0.06^{cd}	90.73±0.38^{c}
Crude fibre	63.50±2.11^{b}	62.79±0.99^{b}	57.82±1.40^{a}	75.67±1.35^{d}	64.35±1.05^{b}	67.99±1.51^{c}
Dry Matter	93.58±0.53^{b}	90.56±0.77^{a}	90.96±0.84^{a}	95.83±0.11^{c}	93.34±0.13^{b}	94.47±0.14^{b}

Means with same letter in row are not significantly different (P>0.05)

Table 42: Apparent nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

	Protease inclusion level (ppm)					
Parameter	Control	100	200	300	400	500
Crude protein	79.80±0.38^{b}	77.42±1.01^{a}	87.29±0.97^{d}	81.24±0.75^{bc}	87.38±1.10^{d}	83.08±0.74^{c}
Ash content	23.02±0.63^{c}	4.91±5.51^{a}	38.59±4.98^{d}	5.49±2.94^{b}	53.62±3.31^{e}	30.67±1.16^{cd}
Ether extract	91.78±0.54^{b}	89.13±0.65^{a}	94.17±0.39^{c}	91.03±0.63^{b}	93.75±0.26^{c}	92.08±0.06^{b}
Crude fibre	63.42±1.29^{b}	57.53±3.87^{a}	77.25±1.86^{c}	63.55±1.33^{b}	75.10±0.87^{c}	65.55±2.54^{b}
Dry Matter	94.40±0.34^{bc}	93.86±0.21^{b}	95.47±0.38^{c}	92.53±0.78^{a}	95.40±0.29^{c}	93.96±0.40^{b}

Means with same letter in row are not significantly different (P>0.05)

4.3.15 True nutrient digestibilty of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

True nutrient digestibility of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of protease is shown in Table 43. Protease supplementeation improved (P<0.05) crude protein digestibility in *C. gariepinus* on diet RS300 than other treatments observed. Higher ash digestibility value was in fish on diet RS300 (47.44±1.18) and the value was not similar (P<0.05) to diet RS400 (24.31±0.02). No significant difference was observed in ether extract with the values ranged from 88.05±1.76 (RS200) to 95.55±64.42 (RS500). Also, Crude fibre level was higher (P<0.05) in *C. gariepinus* on diet RS300 (76.08±1.27) and least value in diet RS200 (58.25±1.33). Dry matter digestibility value was higher in *C. gariepinus* fed diet RS300 (96.00±0.10) than those fed diet RS400 and RS500.

4.3.16 True nutrient digestibilty of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

True nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of protease is shown in Table 44. Supplementation of protease increase (P<0.05) crude protein digestibility in *C. gariepinus* fed diet SS200 (87.51±0.97) but similar (P>0.05) to *C. gariepinus* on diet SS400 (87.40±1.10). Ash digestibility values was significantly better in fish fed diet SS400 (54.03±3.32) than other treatments. Ether extract ranged from 91.02±1.76 (control) to 96.11±65.21 (SS500). Crude fibre and dry matter digestibility values were not significantly different between fish fed diet SS200 and SS400, but these were significantly higher than other diets

Table 43: True nutrient digestibilty of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Crude protein	78.05±0.72^{a}	78.10±2.21^{a}	76.37±0.55^{a}	87.25±0.50^{c}	83.01±1.15^{b}	82.89±0.80^{b}
Ash content	26.72±2.06^{c}	6.21±4.64^{b}	22.65±11.01^{a}	47.44±1.18^{d}	24.31±0.02^{c}	14.52±0.40^{bc}
Ether extract	88.05±1.76	90.16±0.73	88.61±1.97	91.46±1.68	91.99±0.37	95.55±64.42
Crude fibre	63.86±2.14^{b}	63.19±1.02^{b}	58.25±1.33^{a}	76.08±1.27^{d}	64.76±1.08^{b}	68.39±1.44^{c}
Dry Matter	93.75±0.52^{b}	90.73±0.76^{a}	91.14±0.82^{a}	96.00±0.10^{c}	93.52±0.14^{b}	94.64±0.15^{b}

Means with same letter in row are not significantly different (P>0.05)

Table 44: True nutrient digestibilty of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Crude protein	79.93±0.38^{b}	77.56±1.01^{a}	87.51±0.97^{d}	81.37±0.75^{bc}	87.40±1.10^{d}	83.20±0.74^{c}
Ash content	23.44±0.59^{c}	4.45±5.53^{a}	39.02±4.98^{d}	5.96±2.89^{b}	54.03±3.32^{e}	31.09±1.13^{cd}
Ether extract	91.02±1.76	91.52±2.58	92.37±3.08	92.90±1.86	92.70±1.88	96.11±65.21
Crude fibre	63.82±1.31^{b}	57.94±3.80^{a}	77.64±1.88^{c}	63.92±1.35^{b}	75.49±0.81^{c}	65.95±2.46^{b}
Dry Matter	94.57±0.35^{bc}	94.03±0.20^{b}	95.64±0.40^{c}	92.71±0.78^{a}	95.57±0.27^{c}	94.13±0.42^{b}

Means with same letter in row are not significantly different (P>0.05)

4.3.17. Apparent amino acid digestibilty of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Apparent amino acid digestibilty of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of protease is shown in Table 45. Protease supplementation increased (P<0.05) methionine digestibility in *C. gariepinus* on diet RS200 (93.15±0.04) than other treatments. Lysine also had higher (P<0.05) value in RS100 (96.00±0.04) but not similar (P<0.05) to *C. gariepinus* on diet RS400 (94.57±0.03). Threonine, leusine, valine and histidine had the least (P<0.05) in diet RS200 (90.95±0.27, 38.81±1.29, 81.80±0.16 and 29.24±0.43) and higher values were in diet RS100 (97.52±0.01), RS300 (65.55±0.58), RS500 (96.09±0.21), RS300 (71.16±1.23), respectively. Tryptophan had the significantly higher (P<0.05) value in diet RS400 (88.92±0.38) and least value in diet RS100 (79.50±0.48). Isoleusine and phenyalanine were significantly increased (P<0.05) in diet RS300 (64.98±0.20) and RS500 (88.42±0.53) and least in diet RS400 (35.25±1.27) and control diet (78.20±0.37).

Glycine and Alanine were significantly (P<0.05) least in *C. gariepinus* on diet RS200 (42.06±3.22 and 52.96±0.82) while higher values were observed in diet RS300 (62.60±0.20) and RS500 (77.64±0.35). Also, least (P<0.05) values of Serine, Aspartic acid, Glutamic and Arginine were observed in *C. gariepinus* fed diet RS100 (82.95±0.52, 45.33±0.26, 48.25±0.21 and 18.62±5.45) and higher values werein diet RS500. Likewise, Proline, cysteine, pyrrolysine and tyrosine were higher (P<0.05) in diet RS100, RS200, RS300 and RS500 and least values in diet RS200 (88.35±0.43), Control (60.57±0.25), RS400 (98.16±0.06), RS400 (62.90±0.06), respectively.

Table 45: Apparent amino acid digestibilty of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Essential Amino Acid						
Methionine	82.33±0.15^{b}	86.10±0.13^{c}	93.15±0.04^{f}	89.49±0.28^{e}	87.06±0.36^{d}	77.32±0.20^{a}
Lysine	93.58±0.04^{c}	96.00±0.04^{e}	93.25±0.05^{b}	92.51±0.09^{a}	94.57±0.03^{d}	93.54±0.11^{c}
Threonine	96.93±0.06^{e}	97.52±0.01^{f}	90.95±0.27^{a}	96.63±0.04^{d}	95.25±0.06^{c}	94.36±0.06^{b}
Tryptophan	87.37±0.19^{d}	79.50±0.48^{a}	82.44±0.13^{b}	86.17±0.09^{c}	88.92±0.38^{e}	86.41±0.32^{c}
Isoleusine	53.01±0.29^{d}	46.65±0.68^{c}	41.55±0.70^{b}	64.98±0.20^{f}	35.25±1.27^{a}	55.94±0.27^{e}
Leusine	52.97±0.29^{c}	47.66±3.49^{b}	38.81±1.29^{a}	65.55±0.58^{e}	62.56±0.61^{d}	39.60±1.27^{a}
Valine	91.03±0.07^{b}	93.23±0.03^{c}	81.80±0.16^{a}	93.59±0.03^{d}	94.76±0.31^{e}	96.09±0.21^{f}
Histidine	47.66±0.32^{b}	47.69±2.09^{b}	29.14±0.43^{a}	71.16±1.23^{d}	57.34±0.63^{c}	84.60±0.27^{e}
Phenyalanine	78.20±0.37^{a}	84.06±0.17^{c}	82.37±0.54^{b}	87.27±0.17^{e}	85.91±0.21^{d}	88.42±0.53^{f}
Arginine	40.08±0.67^{c}	18.62±5.45^{a}	34.88±0.37^{b}	80.20±0.41^{e}	36.24±0.39^{bc}	72.10±0.51^{d}
Non-Essential Amino Acid						
Glycine	42.22±1.75^{a}	49.32±3.62^{b}	42.06±3.22^{a}	62.60±0.20^{c}	61.23±0.22^{c}	59.52±1.04^{c}
Serine	83.68±0.24^{b}	82.95±0.52^{a}	86.59±0.71^{d}	89.65±0.25^{e}	84.48±0.09^{c}	91.40±0.05^{f}
Proline	90.63±0.30^{c}	94.56±0.15^{e}	88.35±0.43^{b}	91.77±0.33^{cd}	93.10±1.96^{de}	89.67±0.12^{a}
Alanine	58.37±2.98^{b}	53.97±1.64^{a}	52.96±0.82^{a}	69.96±0.68^{d}	65.73±0.22^{c}	77.64±0.35^{e}
Aspartic	67.65±0.41^{c}	45.33±0.26^{a}	60.07±0.38^{b}	67.47±0.16^{c}	67.69±0.15^{c}	74.94±0.53^{d}
Glutamic	64.51±0.26^{d}	48.25±0.21^{a}	62.16±-.14^{c}	70.93±0.10^{e}	56.61±0.36^{b}	72.64±0.23^{f}
Cysteine	60.57±0.25^{a}	72.47±0.15^{d}	79.15±0.13^{f}	75.63±1.36^{e}	70.61±0.18^{c}	63.18±0.26^{b}
Pyrrolysine	92.71±0.14^{e}	91.70±0.08^{d}	89.62±0.10^{b}	95.62±0.09^{f}	89.16±0.06^{a}	90.51±0.04^{c}
Tyrosine	71.14±1.07^{b}	75.65±0.20^{d}	71.08±0.30^{b}	74.15±0.56^{c}	62.90±0.06^{a}	80.44±0.11^{e}

Means with same letter in row are not significantly different (P>0.05)

4.3.18. Apparent amino acid digestibilty of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Apparent amino acid digestibilty of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of protease is shown in Table 46. *C. gariepinus* on Control diet (95.33±0.17) had higher (P<0.05) value in methionine and least in diet SS100 (83.81±0.13). Protease supplementation significantly increased lysine digestibility in *C. garipinus* on diet SS200 (95.74±0.0) and least value in diet SS100 (87.55±0.09). Threonine and valine increased (P<0.05) with supplemental protease in *C. gariepinus* fed diet SS200 (96.25±0.02) and SS100 (95.84±0.23). Furthermore, tryptophan, isoleusine and phenyalanine had siginificantly (P<0.05) least values observed in diet SS500 (84.29±0.29, 47.41±0.58 and 88.13±0.06) and higher values in diet SS400 (92.71±0.19), SS200 (73.00±0.97) and SS200 (94.07±0.06), respectively. Leusine and histidine had least (P<0.05) values in *C. gariepinus* fed diet SS300 and higher values were in diet SS100 (78.82±0.09) and SS200 (81.66±0.13), respectively.

Also, protease supplementation reduced (P<0.05) the digestion of glycine, serine and pyrrolysine with least values in diet SS100 (64.86±0.69, 78.20±0.75 and 85.27±0.14) with higher values in diet control (83.20±0.10), SS200 (92.77±0.07) and SS400 (95.50±0.02), respectively. Proline and Aspartic acid had the least (P<0.05) values in *C. gariepinus* fed Control diet (79.14±0.19 and 62.56±0.50) and higher in diet SS500 (93.61±0.07) and SS200 (76.83±0.49), respectively. Higher value of 79.59±0.11 was in *C. gariepinus* on control treatment in Alanine and least in diet SS200 (60.64±0.33). Finally, Glutamic, Cysteine and Tyrosine had least (P<0.05) values in diet SS300 with the higher values in diet SS200 (70.29±0.58), SS100 (86.97±0.11) and SS500 (80.84±0.16), respectively.

Table 46: Apparent amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm) 100	200	300	400	500
Essential Amino Acid						
Methionine	95.33±0.17^{f}	83.81±0.13^{a}	90.56±0.41^{b}	92.59±0.14^{d}	91.64±0.08^{c}	94.63±0.05^{e}
Lysine	92.39±0.05^{d}	87.55±0.09^{a}	95.74±0.07^{f}	89.61±0.13^{b}	92.82±0.02^{e}	89.92±0.16^{c}
Threonine	93.58±0.04^{a}	94.84±0.94^{bc}	96.25±0.02^{d}	94.23±0.03^{ab}	95.36±0.04^{c}	94.49±0.13^{b}
Tryptophan	86.58±0.55^{c}	89.14±0.05^{d}	92.49±0.03^{e}	85.21±0.09^{b}	92.71±0.19^{e}	84.29±0.29^{a}
Isoleusine	62.63±0.34^{c}	52.82±0.23^{b}	73.00±0.97^{d}	51.34±2.12^{b}	72.41±0.22^{d}	47.41±0.58^{a}
Leusine	78.83±0.07^{e}	78.82±0.09^{e}	73.02±0.19^{c}	60.40±0.49^{a}	77.65±0.34^{d}	66.89±0.23^{b}
Valine	90.17±0.07^{a}	95.84±0.23^{d}	95.58±0.30^{d}	93.99±0.06^{c}	92.37±0.12^{b}	93.38±0.55^{b}
Histidine	62.41±0.36^{b}	61.64±0.65^{b}	81.66±0.13^{e}	56.18±0.52^{a}	79.87±0.15^{d}	69.54±0.89^{c}
Phenyalanine	88.73±0.17^{b}	93.56±0.15^{e}	94.07±0.06^{f}	89.92±0.19^{c}	92.45±0.04^{d}	88.13±0.06^{a}
Arginine	71.34±0.42^{b}	74.65±0.09^{d}	83.46±0.34^{f}	44.11±1.05^{a}	77.17±0.19^{e}	72.74±0.69^{c}
Non-Essential Amino Acid						
Glycine	83.20±0.10^{e}	64.86±0.69^{a}	67.94±0.15^{b}	69.54±0.22^{c}	80.46±0.20^{d}	67.63±0.12^{c}
Serine	89.85±0.07^{b}	78.20±0.75^{a}	92.77±0.07^{e}	90.49±0.09^{c}	91.69±0.09^{d}	91.96±0.08^{d}
Proline	79.14±0.19^{a}	92.75±2.56^{bc}	91.03±0.16^{b}	92.46±0.06^{bc}	92.74±0.18^{bc}	93.61±0.07^{c}
Alanine	79.59±0.11^{f}	78.19±0.15^{b}	60.64±0.33^{a}	64.91±0.18^{c}	71.92±0.42^{d}	62.11±0.36^{b}
Aspartic	62.56±0.50^{a}	66.58±0.38^{c}	76.83±0.49^{f}	67.65±0.27^{d}	75.10±0.11^{e}	65.90±0.14^{b}
Glutamic	62.16±0.14^{bc}	58.40±4.66^{ab}	70.29±0.58^{c}	49.98±1.85^{a}	69.92±0.37^{bc}	67.33±0.52^{bc}
Cysteine	79.35±0.21^{b}	86.97±0.11^{c}	80.67±0.12^{b}	59.73±2.89^{a}	86.32±0.33^{c}	81.44±0.33^{b}
Pyrrolysine	87.38±0.31^{b}	85.27±0.14^{a}	93.68±0.07^{d}	89.98±0.02^{c}	95.50±0.02^{e}	93.43±0.04^{d}
Tyrosine	68.82±0.55^{c}	56.81±1.15^{b}	79.40±0.79^{e}	46.93±0.95^{a}	72.13±1.64^{d}	80.84±0.16^{e}

Means with same letter in row are not significantly different (P>0.05)

4.3.19 True amino acid digestibility of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

True amino acid digestibility of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of protease is shown in Table 47. Protease supplementation in roasted soyabean based diet showed that methionine had higher (P<0.05) value in *C. gariepinus* on diet RS200 (93.52±0.13) with the lease value in diet RS500 (77.87±0.20). Lysine had lower (P<0.05) value of 92.70±0.08 in *C.gariepinus* fed diet RS300 with the higher value (96.16±0.04) in diet RS100. Threonine, leusine and valine had a significantly (P<0.05) least values obtained in *C. gariepinus* on diet RS200 with the higher values observed in diet RS100 (97.79±0.01), RS300 (65.84±0.58) and RS500 (96.31±0.20), respectively. Furthermore, tryptophan and histidine had lower (P<0.05) values observed in diet RS100 (79.91±0.47 and 48.25±2.07) with the higher values in diet RS400 and RS500 respectively. Higher (P<0.05) value of isoleusine was in *C. gariepinus* fed diet RS300 (65.08±0.58) with least value of 35.36±1.27 in diet RS400. Phenyalanine was least (P<0.05) in control diet (78.48±0.36) with the higher value in diet RS500 (P<0.05).

Glycine and alanine had leas (P<0.05) values in *C. gariepinus* on diet RS200 (42.34±3.21 and 53.04±0.81) with the higher values observed in diet RS300 (62.86±0.19 and 70.06±0.68). Serine, Aspartic acid and Glutamic had least values in *C. gariepinus* fed RS100 (83.26±0.52, 45.64±0.26 and 48.60±0.21) while the higher values were observed in diet RS500, RS500 and RS500, respectively. Also, Proline and Cysteine had the higher values in *C. gariepinus* fed diet RS100 (94.68±0.14) and RS200 (79.40±0.13) with the least values in diet RS500 (86.87±0.12) and Control diet (60.81±0.25), respectively (P<0.05). Least (P<0.05) values of Pyrrolysine and Tyrosine were in *C. gariepinus* fed diet RS400 (89.38±0.06 and 63.61±0.40) while the higher values were in diet RS300 (95.80±0.09) and RS500 (80.80±0.11).

Table 47: True amino acid digestibilty of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Essential Amino Acid						
Methionine	82.85±0.14^{b}	86.68±0.13^{c}	93.52±0.04^{f}	90.04±0.26^{e}	87.64±0.35^{d}	77.87±0.20^{a}
Lysine	93.74±0.04^{c}	96.16±0.04^{e}	93.41±0.05^{b}	92.70±0.08^{a}	94.73±0.03^{d}	93.70±010^{c}
Threonine	97.22±0.06^{e}	97.79±0.01^{f}	91.29±0.26^{a}	96.89±0.04^{d}	95.50±0.06^{c}	94.65±0.06^{b}
Tryptophan	87.77±0.19^{d}	79.91±0.47^{a}	82.94±0.13^{b}	86.53±0.09^{c}	89.28±0.37^{e}	86.82±0.31^{c}
Isoleusine	53.12±0.29^{d}	46.76±0.68^{c}	41.65±0.70^{b}	65.08±0.19^{f}	35.36±1.27^{a}	56.05±0.27^{e}
Leusine	53.33±0.29^{c}	48.05±3.47^{b}	39.22±1.28^{a}	65.84±0.58^{e}	62.88±0.61^{d}	40.01±1.26^{a}
Valine	91.33±0.07^{b}	93.42±0.03^{c}	82.13±0.15^{a}	93.79±0.03^{d}	94.99±0.30^{e}	96.31±0.20^{f}
Histidine	48.07±0.32^{b}	48.25±2.07^{a}	29.54±0.43^{d}	71.45±1.21^{d}	57.68±0.62^{c}	84.91±0.27^{e}
Phenyalanine	78.48±0.36^{a}	84.24±0.17^{c}	82.57±0.53^{b}	87.42±0.17^{e}	86.07±0.21^{d}	88.57±0.51^{f}
Arginine	40.43±0.67^{c}	19.05±5.42^{a}	35.23±0.37^{b}	80.53±0.40^{e}	36.63±0.39^{bc}	72.49±0.51^{d}
Non-Essential Amino Acid						
Glycine	42.51±1.74^{a}	49.67±3.60^{b}	42.34±3.21^{a}	62.86±0.19^{c}	61.51±0.22^{c}	59.78±1.03^{c}
Serine	83.92±0.23^{a}	83.26±0.52^{a}	86.86±0.69^{c}	89.89±0.24^{d}	84.76±0.09^{b}	91.68±0.05^{e}
Proline	90.82±0.30^{c}	94.68±0.14^{e}	88.52±0.43^{b}	91.95±0.32^{cd}	93.24±1.92^{de}	86.87±0.12^{a}
Alanine	58.46±2.97^{b}	54.07±1.64^{a}	53.04±0.81^{a}	70.06±0.68^{d}	65.81±0.22^{c}	77.71±0.35^{e}
Aspartic	67.92±0.41^{c}	45.64±0.26^{a}	60.38±0.38^{b}	67.80±0.16^{c}	67.99±0.15^{c}	75.19±0.53^{d}
Glutamic	64.83±0.25^{d}	48.60±0.21^{a}	62.49±0.14^{c}	71.23±0.10^{e}	56.97±0.35^{b}	73.00±0.22^{f}
Cysteine	60.81±0.25^{a}	72.68±0.15^{d}	79.40±0.13^{f}	75.74±1.35^{e}	70.84±0.18^{c}	63.44±0.25^{b}
Pyrrolysine	92.91±0.14^{e}	91.90±0.08^{d}	89.81±0.10^{b}	95.80±0.09^{f}	89.38±0.06^{a}	90.47±0.04^{c}
Tyrosine	71.62±1.05^{b}	76.19±0.20^{d}	71.74±0.29^{b}	74.86±0.55^{c}	63.61±0.40^{a}	80.80±0.11^{e}

Means with same letter in row are not significantly different (P>0.05)

4.3.20 True amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

True amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of protease is shown in Table 48. Methionine had higher ($P<0.05$) values in *C. gariepinus* on control diet (95.79±0.16) while the least value was in diet SS100 (84.30±0.13). Least ($P<0.05$) values of lysine was in *C. gariepinus* fed diet SS100 (87.73±0.09) and the higher value in diet SS200 (95.90±0.07). Threonine and valine had the least ($P<0.05$) values in *C. gariepinus* fed control diet (93.90±0.03 and 90.42±0.06) with the higher values observed in diet SS200 (96.59±0.02) and SS100 (96.06±0.21), respectively. Least ($P<0.05$) values were in tryptophan, isoleusine and phenyalanine in *C. gariepinus* fed diet SS500 while the higher values were in diet SS400 (92.98±0.18), SS200 (73.06±0.96) and SS200 (94.23±0.06), respectively. Likewise, leusine and histidine were higher ($P<0.05$) in *C. gariepinus* fed diet SS100 (79.05±0.08) and SS200 (81.90±0.31) with the least values in diet SS300 (60.64±0.49) and SS500 (69.88±0.88), respectively.

Furthermore, glycine and serine had least ($P<0.05$) values in *C gariepinus* on diet SS100 (65.10±0.69 and 78.56±0.74) while the higher values were observed in control diet (83.35±0.10) and SS200 (93.01±0.07). In proline and aspartic acid, protease supplementation were significantly influenced ($P<0.05$) in diet SS500 (93.73±0.07) and SS200 (77.10±0.49) while it were least digested in control diet (79.34±0.19 and 62.84±0.49). Alanine had higher ($P<0.05$) value in control diet (79.65±0.11) with the least value obtained in diet SS200 (60.75±0.33). Significantly difference ($P<0.05$) least Glutamic, Cysteine and Tyrosine were in *C. gariepinus* on diet SS300 (50.30±1.84, 60.01±2.87 and 48.10±0.93) with the higher values recorded in diet SS200 (70.64±0.58), SS100 (87.29±0.11) and SS500 (81.37±0.15).

Table 48: True amino acid digestibilty of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Parameter	Control	Protease inclusion level (ppm)				
		100	200	300	400	500
Essential Amino Acid						
Methionine	95.79±0.16^{f}	84.30±0.13^{a}	91.10±0.39^{b}	92.99±0.13^{d}	92.21±0.07^{c}	94.94±0.05^{e}
Lysine	92.55±0.05^{d}	87.73±0.09^{a}	95.90±0.07^{f}	89.81±0.13^{b}	92.98±0.02^{e}	90.07±0.16^{c}
Threonine	93.90±0.03^{a}	95.05±0.90^{bc}	96.59±0.02^{d}	94.53±0.03^{ab}	95.63±0.04^{c}	94.76±0.12^{b}
Tryptophan	86.83±0.06^{c}	89.38±0.05^{d}	92.74±0.04^{e}	85.50±0.08^{b}	92.98±0.18^{e}	84.60±0.28^{a}
Isoleusine	62.71±0.34^{c}	52.91±0.23^{b}	73.06±0.96^{d}	51.42±2.12^{b}	72.48±0.22^{d}	47.51±0.58^{a}
Leusine	79.03±0.07^{e}	79.05±0.08^{e}	73.23±0.18^{c}	60.64±0.49^{a}	77.87±0.34^{d}	67.10±0.23^{b}
Valine	90.42±0.06^{a}	96.06±0.21^{d}	95.84±0.28^{d}	94.19±0.05^{c}	92.67±0.11^{b}	92.60±0.53^{b}
Histidine	62.73±0.36^{b}	61.93±0.65^{b}	81.90±0.13^{e}	56.46±0.52^{a}	80.13±0.15^{d}	69.88±0.88^{c}
Phenyalanine	88.90±0.16^{b}	93.72±0.15^{e}	94.23±0.06^{f}	90.08±0.18^{c}	92.60±0.03^{d}	88.29±0.06^{a}
Arginine	71.65±0.42^{b}	74.87±0.09^{d}	83.72±0.33^{f}	44.41±1.04^{a}	77.44±0.19^{e}	73.01±0.69^{c}
Non-Essential Amino Acid						
Glycine	83.35±0.10^{e}	65.10±0.69^{a}	68.16±0.15^{b}	69.72±0.22^{c}	80.63±0.20^{d}	67.81±0.12^{b}
Serine	90.17±0.06^{b}	78.56±0.74^{a}	93.01±0.07^{e}	90.73±0.09^{c}	91.96±0.09^{d}	92.20±0.07^{d}
Proline	79.34±0.19^{a}	92.83±2.54^{bc}	91.23±0.16^{b}	92.63±0.06^{bc}	92.87±0.17^{bc}	93.73±0.07^{c}
Alanine	79.65±0.11^{f}	78.28±0.15^{e}	60.75±0.33^{a}	64.78±0.18^{c}	71.99±0.41^{d}	62.17±0.36^{b}
Aspartic	62.84±0.49^{a}	66.83±0.38^{c}	77.10±0.49^{f}	67.92±0.27^{d}	75.39±0.11^{e}	66.16±0.14^{b}
Glutamic	62.49±0.14^{bc}	58.72±14.55^{ab}	70.64±0.58^{c}	50.30±1.84^{a}	70.28±0.37^{c}	67.61±0.51^{bc}
Cysteine	79.55±0.21^{b}	87.29±0.11^{c}	80.91±0.12^{b}	60.01±2.87^{a}	86.59±0.32^{c}	81.67±0.33^{b}
Pyrrolysine	87.58±0.30^{b}	85.47±0.14^{a}	93.89±0.06^{d}	90.23±0.06^{c}	95.70±0.02^{e}	93.66±0.04^{d}
Tyrosine	69.39±0.53^{c}	57.66±1.12^{b}	80.02±0.77^{e}	48.10±0.93^{a}	72.89±1.59^{d}	81.37±0.15^{e}

Means with same letter in row are not significantly different (P>0.05)

4.3.21 Water quality parameter of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

The result in Table 49 revealed the water quality of *C. gariepinus* fed roasted soyabean based diets supplemented with varying inclusion of dietary protease respectively. Water quality parameter in table 38 showed that the hydrogen ion concentration pH was recorded within the range of 6.48±0.57 to 7.02±0.02 while Nitrate and nitrite were within the range of 0.13±0.01 to 2.86±2.76 and 0.30±0.28 to 0.83±0.35. Also, the dissolve Oxygen was within the range of 2.25±0.21 to 3.15±0.35 throughout the 84 days experiment. Ammonia was also within the range of 0.04±0.01 to 0.28±0.13. Carbon dioxide values ranged from 0.19±0.01 to 0.32±0.05.

4.3.22 Water quality parameter of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

The result in Table 50 revealed the water quality of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of dietary protease. Water quality parameter showed that pH, Nitrate, Dissolved Oxygen, Ammonia and Nitrite values were within the range of 6.87±0.01 to 7.36±0.39, 0.13±0.01 to 1.44±0.11, 2.25±0.21 to 3.20±0.42, 0.04±0.01 to 0.30±0.03 and 0.03±0.30 to 0.86±0.07 throughout the 84 days experiment. Carbon dioxide value ranged from 0.19±0.01 to 0.40±0.12.

Table 49: Water quality parameter of *C. gariepinus* fed roasted soyabean based diets supplemented with protease

Parameter	Initial	Control	Protease inclusion level (ppm)				
			100	200	300	400	500
pH	7.02±0.02	6.48±0.57	6.80±0.15	6.82±0.01	6.85±0.03	6.90±0.01	6.85±0.04
Carbon dioxide (ppm)	0.19±0.01[a]	0.31±0.09[ab]	0.21±0.03[ab]	0.26±0.03[ab]	0.32±±0.05[b]	0.31±0.06[b]	0.24±0.02[ab]
Nitrate (mg/l)	0.13±0.01	0.81±0.98	0.97±1.05	1.01±0.58	0.92±0.88	2.86±2.76	1.29±1.29
Dissolve oxygen (mg/l)	2.25±0.21	3.15±0.35	3.10±0.42	3.15±0.78	2.90±0.57	3.10±0.42	3.05±0.50
Ammonia (mg/l)	0.04±0.01	0.18±0.19	0.20±0.21	0.21±0.12	0.19±0.18	0.28±0.13	0.23±0.27
Nitrite (mg/l)	0.30±0.28	0.51±0.55	0.58±0.62	0.63±0.30	0.56±0.51	0.83±0.35	0.69±0.75

Means with same letter in row are not significantly different (P>0.05)

Table 50: Water quality parameter of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

Parameter	Initial	Control	Protease inclusion level (ppm)				
			100	200	300	400	500
pH	7.02±0.21	7.04±0.33	7.36±0.39	7.12±0.03	7.05±0.01	7.05±0.01	6.87±0.01
Carbon dioxide (ppm)	0.19±0.01[a]	0.32±0.04[ab]	0.32±0.05[ab]	0.40±0.12[b]	0.24±0.04[a]	0.30±0.08[ab]	0.26±0.00[ab]
Nitrate (mg/l)	0.13±0.01	1.44±0.11	1.04±0.32	1.19±0.42	1.20±0.61	0.94±0.89	1.20±1.19
Dissolve oxygen (mg/l)	2.25±0.21	2.90±0.42	2.80±0.42	3.10±0.57	2.75±0.42	2.90±0.28	3.20±0.42
Ammonia (mg/l)	0.04±0.01	0.30±0.02	0.30±0.03	0.25±0.09	0.25±0.12	0.19±0.19	0.25±0.25
Nitrite (mg/l)	0.30±0.30	0.86±0.07	0.78±0.04	0.71±0.25	0.72±0.37	0.57±0.53	0.72±0.71

Means with same letter in row are not significantly different (P>0.05)

CHAPTER FIVE

5.0 Discussion

5.1 Effect of soyabean processing on chemical composition (Proximate, amino acid and anti-nutrient factors)

Chemical composition of processed soyabean meal is shown in Table 3 revealed that crude protein had higher ($P<0.05$) value in solvent extracted soyabean meal (58.45±2.50%) and followed by roasted soyabean meal with 52.50±2.78% value. Least ($P<0.05$) ether extract was in Solvent extracted soyabean meal and this means that it had the better nutrient composition than roasted soyabean meal. Also, thermal processing inactivates the antinutrient bonds and released the encapsulated nutrients thereby improving the nutritional quality of the meal. This finding conformed to those of Ari *et al*. (2012) that solvent extracted soyabean meal had a better nutritional value than roasted soyabean. Similar observations were made by Siulapwa and Mwambunga (2014) on nutritional value of differently processed soyabean seed.

Amino acid profile result indicated that amino acid of soyabean could be altered with processing methods. Roasted soyabean meal had the least methionine, lysine and tryptophan while the higher values were recorded in Solvent extracted soyabean meal as reported in Table 4. Higher values observed in solvent extracted soyabean could be due to dehulling and removal of fat content of soyabean which allowed the concentration of nutrient in the beans. The least lysine, methionine and tryptophan observed in roasted soyabean meal could be as a result of overheating which could result from Maillard reaction which is the reaction from reactive amino group of amino acids and carbonyl group of reducing sugar which could occurred due to poor protein quality. Furthermore, it could be as a result of nutritive values been impaired through the cross-linking of peptide bonds by acylation of free amino group (Lokuruka, 2011). Similar trend was observed by Anderson and Wolf (1995) and Siulapwa and Mwambunga (2014) that hypothesized

amino composition of soyabean meal to be similar to that of animal protein origin excluding sulphur amino acids (methionine and cystine) contents that are limited. Balloun (1980) and Ari *et al*. (2012) posited improved amino acid profile in solvent extracted soyabean meal than extruded soyabean meal. Amino acid result noted in this study were within the range of observed by NRC (1998), OECD (2001) and poultry feeding standards (2005).

Yasothai (2016), reported that most soyabean meal used for fish feeds are thermal treated so as to decrease anti-nutrient influences related with administering raw SBM. This is analogous to findings in this research, where the lower trypsin inhibitor activity, cyanogens, phytates and tannins were in solvent extracted soyabean meal followed by roasted SBM and higher levels in the raw SBM samples. Osman (2007) reported that heat treatment reduced the amount of trypsin inhibitor and phytate by 23.05% and 52.29%, respectively. Pele *et al.* (2016) also reported reduction in phytate, tannin and trypsin inhibitor in differently processed soyabeans samples. Ari *et al*. (2012) in their review similarly showed further reductions in trypsin inhibitor activity and phytate as a result of thermal processing.

5.2 Growth performance of *C. gariepinus* fed soyabean based diets and supplemental amino acids

FFW values were not significantly different among the diets but higher value was observed in fish fed diet RS5 (36.50±4.59). Furthermore, *C.gariepinus* on diet SS1 (43.40±4.03), SS3 (40.80±1.74) and SS5 (36.70±5.11) in FW were not significant ($P>0.05$) compared to other diets as shown in Table 9 and 10. These indicated that *C. gariepinus* fed experimental diets supplemented with amino acid were adequately supplemented and it also demonstrated amino acid as a potent feeding stimulant for cultured aquatic animals. Also, it has been good in reducing feed intake while improving the body weights of fish (Lu *et al*., 2014).

Several researches has stated an analogous improvement in fish performance when lysine and methionine were supplemented in fish diet (Alam *et al*., 2005 and Khan and Abidi 2011). They affirmed that supplemental lysine and methionine increased utilisation of

absorbed lysine and methionine for protein synthesis and it indicated that methionine and lysine are essential in the development of *C.gariepinus*. Also, Nwanna *et al*. (2012) observed better utilisation of dietary nutrient which is indicated by the improvement of feed efficiency when supplemental DL-methionine were fed to common carp. Lin *et al.* (2007) noted similar improvement in Final weight of *Myxocyprinus asiaticus* when the dietary lysine requirement study was conducted.

To assess feed utilisation and absorption which is the ability to convert feed to flesh is called Feed Conversion Ratio (FCR). The better FCR were observed in diet RS5 (3.03±0.67) and SS2 (2.27±0.32) as reported in table 9 and 10, respectively. This suggested that supplemental lysine and methionine improved feed efficiency and this means that lysine and methionine supplementation could reduce the quantity of feed needed for fish development which could reduce cost of production. These agreed with Yuan *et al.* (2011); Nwanna *et al*. (2012); and Wang *et al*. (2015) that stated least FCR when determining lysine and methionine requirement of *Myxocyprinus asiaticus*, *Cyprinus carpio* and *Pseudobagrus ussuriensis,* respectively.

PER and SGR results observed in solvent extracted soyabean meal agrees with Zhou *et al*. (2010) that observed reduced growth, feed utilisation and PER when determining lysine requirement for *Sparus macrocephalus*. It could be the negative effects of excessive or insufficient quantity of free methionine and lysine and poor palatablity. Wang *et al*., 2015 suggested that quantity of methionine supplemented in the diet were not sufficient to induced lethal effect. Roasted soyabean based diet PER and SGR results exhibited positive effect of sufficient amount of supplemental amino acid.

Relationship between roasted soyabean based diet and FW and FCR of *C. gariepinus* fed dietary amino acid was best expressed by optimum growth response curve and the regression model was predicted at 0.6g/100g lysine and 0.4g/100g for methionine. However, the relationship between solvent extracted soyabean based diet and FW and FCR of *C. gariepinus* fed dietary amino acid was best expressed by optimum growth response curve and regression model was estimated at 0.6g/100g lysine and 0.4g/100g methionine for both FW and FCR. This finding corroborated with several research

findings who ascertained that most aquatic organism required more dietary lysine than methionine for optimum performance. The values recorded species supplemented with lysine are African catfish (57g/kg, Fagbenro *et al.,* 1998); striped bass (49g/kg, Small and Soares, 2000); grouper (55.6g/kg, Luo *et al*., 2006); Japaneses seabass (60.7g/kg, Mai *et al*., 2006); gilthead seabream (50.4g/kg, Marcouli *et al*., 2006) and black seabream (86.4g/kg, Zhou *et al*., 2010). While methionine were African catfish (32g/kg, Fagbenro *et al.*, 1998); grouper (32.3g/kg, Luo *et al*., 2005); Jian carp (42.9/kg, Tang *et al*., 2009) and juvenile chinese sucker (40.05g/kg, Chu *et al*., 2014). It was observed that, there was a wide variation between the lysine and methionine observed in this study and other researcher findings. This Variation could be as a result of age, fish size, water temperature, stocking density, diet ingredients and research focus (Zhou *et al*., 2010; Chu *et al*., 2014).

5.3 Haematology of *C. gariepinus* fed soyabean based diets and supplemental amino acids

The essence of studying PCV is to detect anaemia condition of fishes (Blaxhall and Daisley, 1973). PCV or haematocrit value improved from control diet with the value of 21.00±1.73% (RS1) to final value of 27.00±2.26% (RS5) in roasted soyabean diet while solvent extracted soyabean values ranged from 22.67±3.79% to 25.67±1.53%. The results agreed with Korzhuev (1964) who reported 20% to 35 % for fish haematocrit, Ozovehe, (2013) reported PCV value ranged 24% to 32% for *C. gariepinus*. Dienye and Olumuji (2014) also stated 21.00 to 32.00% which agrees with Pietse *et al*. (1981) who reported 20 to 50%. Adesina *et al.* (2017) further reported 20 to 21% ranged.

Increased in the level of PCV were observed in the study as the level of lysine increasing and methionine was decreasing. The increase could be related to the fact that inclusion of lysine and methionine reduces the manifestation of anaemia in the experimental fishes, enhance *C.gariepinus* growth, provided an effective platform for blood oxygen transporter system and to ascertain improved utilisation of nutrients at all inclusion of the limiting dietary amino acids. Similar results were noted by Ruchimat *et al.* (1997) that hemoglobin and PCV were improved with dietary methionine supplementation in yellow

tail. Also, PCV values increased as dietary methionine inclusion increases in juvenile Cobia (Zhou *et al*., 2006). Furthermore, Ahmed, (2017) also reported significantly increase in PCV and haemoglobin values as dietary lysine increases in Indian Catfish.

Furthermore, supplemental dietary lysine and methionine improved the blood hemoglobin as reported in Table 10 and 11, respectively. These results corresponds with Fagbenro *et al*. (2003); Dienye and Olumuji (2014) and Ahmed (2017). Hemoglobin is the protein inside red blood cells that transports oxygen and an increased in Hemoglobin values indicates air breathing characteristics and increase in activity of *C. gariepinus* as documented by Adesina *et al*. (2017). Etim *et al*. (1999) stated that hemoglobin is critical to fish existence due to it vital role in carrying capacity and oxygen-binding of the blood. Adesina *et al*. (2017) also reported that hemoglobin values could be an indicator to diagnosed anaemia while the present study indicated that lysine and methionine could reduced risk of anaemia in the experimental fishes.

The inclusion of methionine and lysine level resulted in the increase in RBC value up to 2.75±0.92 (RS5) from 1.43±0.05 (control) and 2.74±0.65 (SS4) from 1.50±0.04 (SS3) as recorded in Table 10 and 11, respectively. Values increased from 2.11 to 2.93 were reported by Ajiboye (2009) in *synodontis nigrita*. Lawali *et al*., (2015) reported higher values for Malaysian snakehead (3.01±0.56); Owolabi (2011) for *synodontis membranacea* (3.81±1.49), while lower values was documented by Ayoola (2011). Adesina *et al*. (2017) informed that an increase in RBC coluld result from the improvement observed in the carrying capacity of fish blood as a result of the discharge of new RBCs from the erythropoietic tissue. Since RBC helps in the transportation of oxygen around the body and removes carbon dioxide and waste. Supplementation of dietary lysine and methionine could have improved the blood viscosity, transporting oxygen and nutrient to the body tissues and ensuring availability of nutrient needed for fish growth.

C. gariepinus white blood cell (WBC) counts improved significantly in roasted soyabean meal and solvent extracted soyabean meal supplemental with dietary amino acid and it agrees with Zhou *et al.* (2006). WBC perform a significant role in fighting infection and

reactions of living organism. Therefore, increasing and reducing counts of WBCs shows the response of immune system and normal physiological reactions under toxic condition. *C. gariepinus* fed diet SS3 (13.78±1.26) and SS6 (14.60±1.98) in table 11 had lower count compared to control and reduced number of lymphocytes could be the cause as observed by Adesina *et al*. (2017). Also, least values of WBC recorded could be attributed to the fish been open to various pollutants, and reduced amount of circulating lymphocytes and thrombocytes. Increase in the values in this study might be ascribed to increased stress response of the *C. gariepinus* which might result in reducing the immune response as observed in the survival rate of the fishes.

Also, Mean Corpuscular volume (MCV) values obtained from *C. gariepinus* fed roasted and solvent extracted soyabean based diet ranged from 105.35±31.76 (RS5) to 146.76±8.93 (control) and 98.64±16.18 (SS4) to 154.32±7.88 (SS3) respectively. The values obtained were lower than the control diets except for SS3 values. This findings were in line with Adesina, (2008) who ascertained the reduction in MCV to the shrinking of RBCs which could be due either hypoxia or balanced water condition or microcytic anaemia. While, the decrease in values observed was not in agreement with Adesina *et al*. (2017) who attributed it to the swelling of erythrocytes which could result macrocytic. Similar trend as observed in MCV for roasted and solvent extracted soyabean based die. MCH is being referred to average mass of hemoglobin per red blood cell in the blood. The reduction in the values could be attributed to reduced iron deficiency and microcytic anemia, which is a condition where RBC is abnormally small and carrying less hemoglobin. The result of this finding agreed with Dienye and Olumuji (2014) who fed Heteroclarias with Carica papaya leaf meal incorporated diet. Furthermore, Mean Cell Hemoglobin Concentration values (31.17±0.14 to 33.84±2.27) was within the ranged documented by Adesina *et al*. (2017), Dienye and Olumuji (2014). Significantly different values in platelet ranged from 12.00±2.80 (RS4) to 32.83±6.48 (control) and 10.83±0.97 (SS2) to 17.53±1.29 (SS4) for both roasted and solvent extracted soyabean based diet. The reduction in the values observed in *C. gariepinus* fed roasted soyabean based diet with supplemental dietary amino acid could be due to fish reaction to anti-metabolites presence in diets as supported by Fagbenro *et al.* (2010).

Significantly lower values were observed in lymphocytes in *C. gariepinus* fed roasted and solvent extracted soyabean based diet. The results does not agrees with Olasunkanmi (2011) who noted on difference among initial and final lymphocyte values in raw mucuna meals fed to *C. gariepinus*. Lymphocytes help to determine the immune responses and *C. gariepinus* ability to fight infection as it help to defense cells of the body. Though, values corresponded with Blaxhall and Daisley, (1973) recommended ranges, lysine and methionine inclusion in *C. gariepinus* diet had significant influence on immune system. Heterocytes values observed revealed an increase for both roasted and solvent extracted soyabean based diet. This is an indication that *C. gariepinus* fed soyabean based diet is at risk of bacterial or any pathogenic infection or as a result of stress (Lawali *et al.,* 2015).

5.4 Effect of supplemental amino acids on serum biochemical of *C. gariepinus* fed soyabean based diets

Supplemental lysine and methionine in soyabean based diets improved total protein values from 6.67±0.29 (control) to 7.87±0.55 (RS4) and 6.50±0.50 (control) to 7.70±0.17 (SS5), though no significant difference were observed amon the diets. The results agreed with Ogunwole *et al*., 2014; 2017 that attributed increased in total protein to adequate or balanced crude protein in the feed which resulted in enhancing digestibility and absorption of protein. Furthermore, supplemental amino acid in either roasted or solvent extracted soyabean based diet fed to *C. gariepinus* had higher albumin levels. This implies that addition of lysine and methionine in experimental diet had the potential to improved albumin level of *C. gariepinus*.

Significantly difference (P>0.05) were in Globulin levels of *C. gariepinus* on solvent extracted soyabean based diet. *C. gariepinus* on diet SS5 and SS6 had higher globulin value of 5.17±0.06 and 5.10±0.20, respectively. Globulin level showed an indicator of body defense mechanism (Kabir, 2013). The increased level of globulin obtained in RS4 and SS5 was an indication of improved immune response which could be ascribed to presence of adequate supplemental lysine and methionine in the diet. Similar globulin improvement had been reported by Ogunwole *et al*. (2017).

The increased observed in AST and ALT levels observed in roasted soyabean meal based diet suggested that experimental fish proficiently utilized lysine and methionine for metabolic purposes through blood serum enzymes. This finding confirmed the observation of Adesina (2008), Adesina *et al.* (2017) and Ogunwole *et al*. (2017). The higher AST and ALT levels observed in some *C. gariepinus* fed varying lysine and methionine inclusion might be related to stress resulting from high level of ANFs in soyabean based diet. Tiwari and Singh, (2004) noted that stress do increase aminotransferase levels in fish. Higher values observed in ALT, AST, and ALP activities in *C. gariepinus* are indicative of hepatic cellula damage directing their leakage into the bloodstream (Mousa and Khattab, 2003 and Adesina *et al*., 2017). Transaminases functions effectively in the liver, and the function as marker enzymes which activities can be identified in small amounts. Values of ALP in these study agreed with Ogunwole *et al*. (2014) who noted that it is an indication that the liver functioning well and incease in level of bone mineralization in broiler chicken.

Furthermore, higher values in Blood Urea Nitrogen (BUN) and creatinine when fed soyabean based diet supplemented with dietary amino acid. Elevated levels of BUN and creatinine indicated that, there was no renal damage or muscle wastage that might be ascribed to dietary inclusion of amino acid in soyabean based diet. This observation was corroborated by the report of Azza and Naela (2014) and Ogunwole *et al*. (2017). Therefore, the serum biochemistry indices noted suggested that supplemental lysine and methionine has no adverse influences on the physiological indices of *C. gariepinus* because serum protein were within the normal range.

5.5 Effect of supplemental amino acids on whole body of *C. gariepinus* fed soyabean based diets

For optimal growth of fish, balanced amino acid is necessary in the fish feed. Lu *et al*., (2014) reported amino acids to be significant regulators necessary for optimum performance of fish. The results from this findings showed that *C. gariepinus* responses positively to the varying inclusion of amino acids when fed soyabean based diet. Also, supplemental lysine and methionine in soyabean based diet significantly enhanced

protein deposition in whole body. It could be ascribed to the fact that poised amino acid profile stimulated protein synthesis in the muscle while supplemental amino acid levels above what is required for growth does not improve deposition due to increased rate of protein catabolism (Iyayi and Adeola, 2014). Nwanna *et al*. (2012) stated same as above for common carp (*Cyprinus carpio*) on DL-Methinone supplementation significantly improved protein content in muscle than those fed Methionine deficient diet. Zhou *et al*., (2010) stated similar results in black sea beam. Furthermore, values obtained for the ether extract increased from 7.15±0.21 to 8.88±0.25 and 7.15±0.21 to 8.78±0.21 for roasted and solvent extracted soyabean based diet, respectively. Protein synthesis rates could be promoted with balanced amino acid profile in the muscles and also reduces ether extract content in diet RS5 and SS5 significantly compared to other treatment. Lu *et al*. (2012) stated similar trend in coated amino acid supplemented with protein based diets. Also, Ribeiro *et al*. (2004) corroborate similar trends.

Figure 7 and 8 revealed the optimum level of lysine and methionine inclusion in roasted and solvent extracted soyabean based diet and the optimum crude protein of *Clarias gariepinus* whole body to be expected. It showed that the optimum level of lysine and methionine inclusion in roasted and solvent extracted soyabean based diet was 0.6g/100g and 0.4g/100g. The regression analysis did not agreed with the finding of Fagbenro *et al*. (1998) and Fagbenro *et al*., (1998; 1999) who established the requirement of dietary lysine and methionine for *C. gariepinus* was 5.7g/100g and 3.2g/100g respectively. Though, the diet fed to fish comprising gelatin and casein as source of intact protein supplementation in amino acid and this could have influenced the improvement observed in *C. gariepinus* performance.

5.6 Nutrient digestibility of *C. gariepinus* fed soyabean based diets supplemented with dietary Lysine and Methionine

Supplemental methionine and lysine in roasted and solvent extracted soyabean based diet improved crude protein digestion with the values increased from 91.04±0.11 (control) to 95.32±0.25 (RS5) and 90.27±0.14 (SSS4) to 96.34±0.31 (SSS5), respectively. The results observed corroborated with Nwanna *et al*., (2012) findings who detected that supplemental methionine and lysine significantly enhanced protein digestibility in

Cyprinus carpio and *Orechromis niloticus*, respectively. Also, Ribeiro *et al*. (2012) reported similar trend when determining the digestibility of feedstuffs used in tilapia feed. Improvement in protein digestibility suggested that supplemental methionine and lysine in soyabean based diet increased availability of amino acid for maintenance and protein retention as revealed by final weigh gain, nitrogen retention efficiency and whole body protein of the study. Though, the value of protein digestibility observed in this experiment were greater than what Nwanna *et al*. (2012) and Ribeiro *et al*. (2012) stated. This suggested that combining supplemental methionine and lysine in soyabean based diet is imperative in *C.gariepinus* diets.

Apparent digestibility of the ether extract, crude fibre and dry matter of *C. gariepinus* fed solvent extracted and roasted soyabean based diets supplemented with methionine and lysine suggested an increased digestibility though no particular trend were observed. This indicated that supplemental methionine and lysine in soyabean based diet had little influence on the experimental fish. Supplemental methionine and lysine in soyabean based diet fed to *C. gariepinus* revealed that it supplementation could improved the energy digestibility. This result was in agreement with Sotolu and Faturoti (2009) and Nwanna *et al.* (2012) who observed improved energy digestibility in *C. gariepinus* and common carp, respectively.

For the true nutrient digestibility of *C. gariepinus* fed roasted and solvent extracted soyabean based diet with supplemental amino acid had few literature reported, apart from studies of Yamamoto *et al*. (1998), with *Cyprinus carpio*, Ribeiro *et al* (2011; 2012) and Wilson *et a*l. 1981 with *Ictalurus punctatus*;. The values observed in true digestibility were higher than what was observed in apparent digestibility of this experiment because the nutrients in the feacal are intact and has not leached away as observed in apparent digestibility. Also, it revealed that study of true digestibility has the potential to correct endogenous losses that do occur in apparent digestibility monitoring. The study showed that true crude protein digestibility value were higher than those determined by Yamamoto *et al.* (1998) and Ribeiro *et al*. (2012). This increase observed could be as a result of supplemental amino acid in soyabean based diet were effectively utilized by the experimental fishes. Also, the dissection method of feacal collection used in the study

could have contributed to the increase observed in the study and it was corroborated by Ribeiro *et al.* (2012).

5.7 Amino Acid digestibility of *C. gariepinus* fed soyabean based diets supplemented with dietary Lysine and Methionine

The apparent amino acid digestibility of *C. gariepinus* fed roasted and solvent extracted soyabean based diets supplemented with amino acid as observed in table 22 and 23 respectively. The values observed from the soyabean based diets increased from 85.09±0.36 (SS1) to97.49±0.47 (RS5) for Methionine, lysine values increased from 86.08±1.05 (SS4) to 95.75±0.26 (SS5), Threonine values increased from 91.99±1.13 (RS6) to 98.18±0.31 (SS6) while tryptophan values increased from85.14±0.69 (SS5) to97.83±0.67 (RS6). The increased observed are coherent with Yamamoto *et al*., 1998 result, who perceived comparative values when several protein sources were fed to fingerlings, red sea beam, rainbow trout and common crap. However, Wilson *et al.* (1981) and Ribeiro *et al.* (2012) reported relatively lower values when common feedstuffs were fed to Channel catfish and tilapia, respectively. The high values noted in this study might be ascribed to the fact that thermal treating of soyabean meal released the encapsulated protein structure and inactivates the antinutritional factors which could have enhanced soyabean based diet with supplemetal lysine and methionine to be more digestible by proteolytic enzymes. Higher methionine content presented in the study could be as a result of the higher sulfur amino acids (mucin layer and pancreatic secretions) equated with other amino acids (Pozza *et al*., 2003).

Isoleusine and arginine presented the least apparent amino acid digestibility values among essential amino acid for all the studies and it ranged from 86.55±0.53 (SS2) to 64.41±0.27 (SS4) and 91.96±0.27 (SS2) to 55.38±0.66 (SS4), respectively. Similar trend were observed by Ribeiro *et al.* (2011) for soyabean meal. The lease values obtained could be due to the fact that isoluesine is a hydrophobic amino acid situated in protein, deterring the hydrolysis of its peptide bonds, which may explained its low digestibility.

Results observed in Table 24 and 25 showed the true digestibility of *C. gariepinus* fed roasted and solvent extracted soyabean based diets with supplemental amino acid, respectively. True amino acid digestibility helped to consider the role of endogenous amino acids, quantity of amino acids and values of true digestibility used by fish in more gv nbprecise, resulting in precise formulation of diets for *C. gariepinus*. Results observed showed a relatively small difference from the apparent digestibility. This could be due to higher levels of digestive enzyme secretions and its inclusion in the feaces from the protein free diets for *C.gariepinus* in this study. Similar trend were observed by Pozza *et al*. (2003); Ribeiro *et al*. (2011). The values obtained were within the ranged stated by Yamamoto *et al*. (1998) and Pozza *et al*. (2003). Amino acid digestibility values observed in this study revealed that supplemental methionine and lysine in soyabean based diet served as a stimulant which enhance the digestibility of the diet and feed utilization as observed in the growth parameter.

5.8 Growth performance and feed utilisation of *C. gariepinus* fed soyabean based diets supplemented with protease

Supplemental protease in fish diet has been hypothesized by authors that it might damage complex proteins in fish diets into functioning amino acids and peptides thus causing enhanced growth performance and feed utilization. Present study revealed that growth performance and feed utilization of *C. gariepinus* on soyabean based diet supplemented with protease were different from control diet with higher values for FFW, PFW and least values of FCR observed in Diet RS400 and SS400. Contrary to this, Adeoye *et al*. (2016) stated that supplemental protease had no significant influence on broiler chicken and tilapia growth performance when comparing the effect of various exogenous enzymes. Also, Dias *et al*. (2014) stated that supplemental protease influenced growth performance of tilapia fed least crude protein diet related to higher crude protein diet. Naela *et al*. (2017) noted improvement in performance, Feed Conversion Ratio and feed utilization of *O. niloticus* when fed different dietary crude protein (28% CP and 26% CP) supplemented with protease compared with control diet. The improvement noted in the study can be ascribed to increased digestibility of protein and availability of amino acid by protease. The improvement in performance and utilization of nutrient by *C.*

gariepunus on soyabean based with supplemental protease agrees with the research verdicts of Odetallah *et al*. (2005); Wang *et al.* (2015); Angel *et al*. (2011) and Li *et al*. (2015). They all observed enhancement in Final weight and FCR with supplemental protease in broiler chickens diet.

The improvement observed in this study could occur, resulting from useful effects of digestible protein been catalyzed by protease to meet up with the fish requirement for maintenance and growth. Angel *et al*. (2011) attributed the improvement in growth to the increased amino acid availability with the addition of protease could enhanced further growth and protein utilization. Also, improvement observed suggested that complete removal of fishmeal could be accomplished by supplemental protease and the reason could be that higher residual activity of supplemental protease increased the use of soyabean. Furthermore, protease supplementation in soyabean diet further improved the GPR and NRE with higher values in *C. gariepinus* on diet RS400 and SS400. Singh *et al*. (2011) reported similar improvement in GPR and NRE when supplemental papain was used a growth promoter in *Cyprinus carpio* diet. Protease in fish diet breaks the ANFs in soyabean based diet, making more protein available to fish which in turn resulted in better protein efficiency by the experimental fish.

This study established that supplementation of protease (Ronozyme ProAct) in soyabean based diets could be used securely and economically to increase growth performance and feed utilization when fed *C. gariepinus* at 400ppm /kg. This is evidence in figure 11, 12, 13 and 14 that help to predict the optimum level of supplemental protease in soyabean based diet fed to *C. gariepinus*. It revealed optimum level of supplemental protease in soyabean based diet was 400ppm/kg of diet.

5.9 Haematology of *C. gariepinus* fed soyabean based diets supplemented with protease

Hematology parameters are valuable for examining the health and physiological responses of fish to stress. The present experiment revealed that supplemental protease had positive influence on PCV, HB, RBC and WBC when fed roasted soyabean based

diet. While protease supplementation in solvent extracted soyabean based diets had a positive effect on PCV and HB. Though the results observed were lower than those in control diet, it could be ascribed to stress factor during the experiment resulting from loss of appetite. The lower PCV values could also be attributed to anemia resulting from shrinking of red blood cells. Lower lym: het ratio observed in solvent extracted soyabean based diets further ascertained the stress factor and the capability of *C.gariepinus* to combat infection. While Lymphocytes: Heterocytes ratio of roasted soyabean based diet value was higher than control diet. Though, no clear understanding of proven interaction between dietary protease and *C. gariepinus* on hematology status. Therefore, further study is essential to establish the mode of action between protease and hematology parameter.

5.10 Serum biochemical indices of *C. gariepinus* fed soyabean based diets supplemented with protease

Protease supplementation in roasted soyabean based diet fed to *C. gariepinus* had no influence on total protein, albumin, globulin, AST and creatinine. Though, significant differences were observed in A-G ratio, ALP, ALT and blood urea nitrogen. Also, supplemental protease in solvent soyabean based diet in this study had no adverse effect on serum biochemical indices except for ALT with the values ranged from 20.00±1.00 (control) to 26.33±3.06 (SS400). Mahmood *et al.* (2017) reported no substantial effect on serum biochemical indices of gibel carp fed low fishmeal extruded and pelleted diets supplemented with protease. Liu *et al*. (2016) stated positive effect of protease in broiler diet on AST and ALP. On the contrary, significant effect was observed in total protein and Albumin of broiler chicken fed roasted African yam bean seed meal with supplemental enzyme as noted by Lawrence-Azua *et al.* (2012). Raji *et al*. (2016) stated similar improvement in rabbit fed cocoa bean shell supplemented with enzyme.

However, the results obtained in this study for the dietary treatments were enhanced than control diet and fall within normal range as described by Cowey and Walton (1989). AST and ALT are significant aminotransfarases active in amino acid metabolism in the livers of teleost fishes (Cowey and Walton, 1989). Increased ALP and AST activities in fish

revealed increased synthesis of enzymes by the liver and possible leakage of enzymes across damaged plasma membrances (Yang and Chen, 2003). Improved serum biochemistry indices in this experiment might be as result of stronger innate immune response of fish as described by Bello and Nzeh, (2013). Though, effect of serum biochemical indices with supplemental protease fed to fish diets has been scanty. Also, improvement noted in the serum biochemical indices of *C. gariepinus* fed soyabean based diets with supplemental protease could be attributed to the fact that protease supplementation enhanced blood serum enzymes in *C. gariepinus* to efficiently utilized amino acid for metabolic purposes. Also, this study affirmed that supplementation of protease in soyabean based diet of *C. gariepinus* could maintain or enhance the health status of fish at 400ppm/kg of protease.

5.11 Effect of supplemental protease on whole body quality of *C. gariepinus* fed soyabean based diets

Whole body quality of *C. gariepinus* fed roasted and solvent soyabean based diet with supplemental protease showed a significant improvement with the higher crude protein observed in RS400 (65.89±0.45) and SS400 (63.70±0.49), respectively. Significant improvements observed in this study agrees with Mahmood *et al*. (2017) who stated protease and versazyme improved the carcass quality of broiler chicken. Yildrim and Turan (2010) reported significant improvement crude protein of *C. gariepinus* at 0.75g/kg of exogenous enzyme and no significant effect were revealed in moisture, ether extract and ash. Furthermore, whole body quality of Gilthead sea bream on supplemental enzymes diets improved crude protein (Deguara *et al*., 1999). Analogous improvement was noted by Lin *et al*. (2007) as exogenous enzyme was supplemented in diets of tilapia. The above mentioned improvements were in line with the findings of this study.

Contrary to the improvement observed, supplemental enzyme diets had no effect on whole body composition of tilapia (Soltan, 2009). Liu *et al*. (2016) reported that protease supplementation in pelleted diets of gibel carp had no impact on whole body composition. Farhangi and Carter, 2007 also report similar trend in rainbow trout. Freitas

et al. (2011) reported on effect of mono-component protease supplementation on carcass parameters of broiler.

The improvement in whole body quality observed can be ascribed to the fact that supplemental protease in soyabean based diets catalyse the protein deposition in fish. The reason for the increased in deposition was that exogenous protease enhanced the performance of endogenous protease which promoted protein synthesis rates in the muscle of *C. gariepinus*. However, contrary results observed in other research work could also be ascribed to the amino acid required by those species are above what is needed for growth and does not improve protein deposition.

5.12 Effect of nutrient digestibility on *C. gariepinus* fed soyabean based diet supplemented with protease

Supplemental protease enhanced digestion of protein in the fed diet with the higher values observed in Diet RS300 (87.12±0.50) and SS400 (87.38±1.10) in roasted and solvent soyabean based diets, respectively. Li *et al*. (2015) reported similar improvement using serine protease to improve broiler performance and increases protein digestion. Similar reports had been documented in poultry (Ghazi *et al.*, 2002), Pigs (Yin *et al*., 2001) and Cattle (Ghazi *et al.*, 2002). The above mentioned studies showed improvement in nutrient digestibility by supplemental protease in plant based diets while studies in fish had also shown positive influence on growth performance and feed utilization of fish feed diet. Carter *et al*. (1994) reported significant effect in nutrient digestibility of *Salmo salar* Juveniles when fed dietary pancreatic enzymes. Rainbow trout fed coextruded canola and pea supplemented with commercial protease improved it nutrient digestibility (Drew *et al*., 2005). Zhong and Zhou (2005) reported significant positive effect of multienzyme on nutrient digestibility of tilapia and crucian carp fingerlings. Prabjeet *et al*. (2011) reported that feed supplemented with papain had higher protein digestibility values when mixed with papain. This study reavealed that apparent nutrient digestibility parameters were enhanced by supplemental protease in soyabean based diet. Analogous results were observed with monocomponent protease (Angel *et al*., 2011; Freitas *et al*., 2011). This

indicated improvement of nutrients metabolism, greater degradation of anti-nutritional factors, and increasing metabolizable diets.

Furthermore, improvement observed in this study could be ascribed to protease assisting in hydrolyzing proteins in soyabean based diet and degradation of proteinaceous components present in ANFs such as trypsin inhibitor. Also, the activity of the exogenous protease could have stimulated secretion of endogenous protease which resulted in pronounced significant improvement observed in the study. Liu *et al*. (2016) attributed that supplemental protease in fish diet reduced muscle layer thickness, and improving nutrients digestibility and eventually improving fish growth. This recommended that protease concentration played an imperative role in *C. gariepinus* diets. Therefore, supplementation of protease in soyabean based diet is inevitable in enhancing nutrient digestibility and utilization.

The inclusion of protease in soyabean based diets resulted in increased true protein digestibility with values up to 87.25±0.50 (RS400) from 78.05±0.72 (control) and 87.40±1.10 (SS400) from 77.56±1.01 (SS100) as recorded in Table 42 and 43, respectively. The values observed in true digestibility were higher than what was observed in apparent digestibility of this study. This might be ascribed to nutrients in the feacal are intact and has not leached away as observed in apparent digestibility. Also, it revealed that study of true digestibility has the potential to correct endogenous losses that do occur in apparent digestibility. Furthermore, reports on true nutrient digestibility of fish species with supplemental protease had not been observed. The improvement in the true digestibility in this study could also be attributed to the facts observed in apparent nutrient digestibility. This study further established the efficacy of protease in *C. gariepinus* diets.

5.13 Effect of amino acid digestibility on *C. gariepinus* fed soyabean based diet supplemented with protease.

It was observed that protease supplementation enhanced digestion of amino acid parameter as observed in methionine and lysine values that had the higher values of

93.15±0.04 (RS200) and 96.00±0.04 (RS100), respectively for roasted soyabean based diets. While, solvent soyabean based diet had the higher values for methionine and lysine observed in control diet (95.33±0.17) and SS200 (95.74±0.07). In this study, supplemental protease in soyabean based diet significantly improved digestibility of all amino acid parameter except for the higher values that was observed in methionine and alanine content of solvent extracted diet. The improvement observed agreed with Dalolio *et al.* (2016) that assesses effect of dietary amino acids of full-fat soyabean with or without supplemental protease in diets of broilers. The study revealed that supplemental protease in diets of broiler formulated with roasted soyabean improved digestibility and availability of essential and non-essential amino acid. The present study had similar improvement when protease was supplemented in soyabean based diet fed to *C. gariepinus*. Also, several studies have ascertained similar improvement in broiler chicken and in fish (Angel *et al*., 2011; Ravindran *et al*., 2014).

Angel *et al*. (2011) reported improvement in FW and FCR with supplemental protease are expected to occur by improved amino acid availability that could promote growth and protein utilization. Similar to the result observed, amino acid were highly digestible when soyabean based diets were supplemented with protease and it supports the improvement noted in FW and FCR. Increased digestibility and performance improvement observed can only arise when improvement in amino acid digestibility are balanced with other diet-available amino acid and it could be used effectively for growth. The improvement in digestibility observed could have resulted from the peptide bond specificity which influenced the rate of protein hydrolysis by proteases and amino acid quantity discharged and that are available for absorption by *C. gariepinus*. Also, improvements in amino acid digestibility obtained from any dietary proteases depend on the ingredients used in formulating feed because amino acid composition depend on ingredient (Vieira *et al*., 2013)

Furthermore, the true amino acid digestibility of *C. gariepinus* fed roasted and solvent extracted soyabean based diets supplemented with protease observed was slightly higher than the apparent amino acid digestibility values. True amino acid digestibility helps to consider the role of endogenous amino acids, values of true digestibility and quantity of

amino acids used by fish. Also, it is precise and resulting in better precision in rations formulation for *C. gariepinus*. This could be due to higher levels of digestive enzyme secretions and its inclusion in the feaces from the protein free diets for *C.gariepinus* in this study. Supplementation of protease in soyabean based diets improved all parameter of true amino acid digestibility. Rostagno *et al.* (2011) reported protease inclusion in fish diet to improved coefficient of true digestibility of essential amino acid of roasted soyabean meal. Bertechini *et al*. (2009) stated better true amino acid digestibility of soyabean meal and corn with or without monocomponent protease supplementation. Angel *et al*. (2011) also reported improved true amino acid of broiler chicken with supplemental monocomponent protease. Also, this digestibility study suggest that supplemental protease in soyabean based diets is a better substitute to the diet formulation for *C. gariepinus*.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.0 CONCLUSION

This study established that processing of soyabean grains using roasted and solvent extraction methods significantly improved their crude protein. Processing of soyabean grain further reduced the oil content of the meal which was observed in Ether extract content. Also, solvent extracted soyabean meal had the higher crude protein and amino acid values. The anti-nutrition compositions shows that heat processing method of soyabean grain deactivates anti-nutritional factor while solvent extracted soyabean meal had least value and closely followed by roasted soyabean meal. The study revealed that soyabean processing enhanced the nutritional potential of both solvent extracted and roasted soyabean meal for feeding *Clarias gariepinus.* However, solvent extracted soyabean meal had the most promising nutritional potential judging from the proximate, amino acid and ANFs.

Growth performance and feed utilisation proved that roasted soyabean based diet supplemented with dietary amino acid in *C. gariepinus* fed RS5 diet had the better FW and FCR. However, better FW for solvent extracted soyabean based diet was in control diet compared to *C. gariepinus* on SS3 and SS5 diet. Also, least values for FCR and the higher survival were in SS3 diet. The findings established that supplemental methionine and lysine in soyabean based diet has potential to improve growth performance of *C. gariepinus* when fed soyabean based diet with supplementation of lysine and methionine while regression curves for roasted and solvent extracted predicted 0.6g/100g lysine and 0.4g/100g methionine to be better amino acid combination for *C. gariepinus* optimum growth when fed soyabean based-diet.

Haematological data established that *C. gariepinus* fed diet RS5 for roasted soyabean based diet had a better PCV, HB, RBC, WBC and Lym: Het ratio values while the least values were obtained in the control diet. Diet SS4 in solvent extracted soyabean based diet had a better PCV, HB, RBC, WBC and Lym: Het ratio. Roasted and solvent extracted soyabean based diet further ascertain that inclusion of lysine and methionine has the potential to increase nutritional quality of diet and health statues of *C. gariepinus* as observed in the blood profile. The serum biochemical indices of *C. gariepinus* fed roasted and solvent extracted soyabean based supplemented with amino acid after 84 days of feeding trial revealed that diet RS4 and SS5 had better combination for all the parameters observed. This is an indication that supplemental amino acid in soyabean based-diet could improve the health statues of *C. gariepinus*.

Inclusion of amino acids in roasted and solvent extracted soyabean based diet contributed to the improvement of whole body nutritional quality of *C. gariepinus* on diet RS5 and SS5. Regression curves further predicted 0.6g/100g lysine and 0.4g/100g methionine to be better. Similar tread in whole body amino acid data were observed. It showed that amino acid inclusion in the soyabean based diet improved amino acid quality.

Apparent nutrient digestibility of *C. gariepinus* fed roasted and solvent extracted soyabean based diets supplemented with amino acid showed that *C. gariepinus* on diet RS5 and SS5 had a better result. Also, similar trend were observed in true nutrient digestibility of *C. gariepinus* fed roasted and solvent extracted soyabean based supplemented with methionine and lysine. Apparent and true amino acid digestibility of *C. gariepinus* fed roasted and solvent extracted soyabean based diet supplemented with amino acid revealed an improvement in amino acid digestibility in *C. gariepinus* on RS5 and SS5. This indicated that lysine and methionine has the ability to improve digestibility of soyabean based diet fed to *C. gariepinus* supplemented with dietary amino acid. Also, the result has provided based line information that lysine and methionine could further improved amino acid digestibility of soyabean based diet supplemented with dietary amino acid.

Growth performance and nutrient utilisation of *C. gariepinus* fed roasted and solvent extracted soyabean based diet supplemented with protease revealed higher and better

final weight and FCR with 400ppm/kg supplementation, respectively. Regression curves predicted 400ppm/kg to be better protease inclusion level. It showed that inclusion of protease could further improved growth and nutrient utilisation in *C. gariepinus* when fed roasted and solvent extracted soyabean based diet.

Haematology indices observed in the study showed that control diet had the higher values observed in *C. gariepinus* fed roasted and solvent extracted soyabean based diets supplemented with protease. While *C. gariepinus* fed on diet RS400 and SS400 (400ppm/kg) had the better result among the diet supplemented with protease in roasted and solvent extracted soyabean based diet. Serum biochemical indices showed that roasted and solvent extracted soyabean based diet with supplemental protease has higher and better result observed in diet 400ppm/kg and 100ppm/kg respectively. This is an indication that supplemental protease in soyabean based diet improved health statues of *C. gariepinus* as observed in the haematology and serum biochemical.

The whole body quality of *C. gariepinus* fed roasted and solvent extracted soyabean based diet supplemented with protease revealed an improvement in crude protein and true protein content at 400ppm/kg. Also, varying inclusion of protease in roasted and extracted soyabean based diet fed to *C. garipinus* improved the amino acid profiles of the fish whole body as against the values observed in the control diet. This indicated that nutritional quality of *C. gariepinus* could be improved with protease supplementation when fed soyabean based diet.

This study established that protease supplementation in roasted and solvent extracted soyabean based diet could improve digestibility of the diets. It was observed that apparent and true nutrient digestibility. *C. gariepinus* fed diet RS300 and SS400 had the better result for roasted and solvent extracted soyabean based diet digestibility. Also, apparent and true amino acid digestibility observed ascertained that protease supplementation improved amino acid digestibility when fed soyabean based diet to *C. gariepinus*.

Recommendation

The findings in study one shows that processing method improved the nutritional value of soyabean grains while solvent extraction meal tended to be the best and recommended to fish farmers. Though roasted soyabean meal could be a good candidate for formulating fish feed but needed to be improved with the limiting amino acids.

Inclusion of 0.6g/100g lysine and 0.4g/100g methionine in *C. gariepinus* fed soyabean based diet can adequately improve growth and nutritional quality of *C. gariepinus* diet.

Further incorporation of protease in soyabean based diet fed to *C.gariepinus* at 400ppm/kg can further improved nutrient utilisation and nutritional quality.

A study should be conducted to compare fishmeal based diet with soyabean based diet of *C. gariepinus.*

A study should be carried out to further improved digestibility of soyabean based diet with other plant proteins in *C. gariepinus* diet.

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