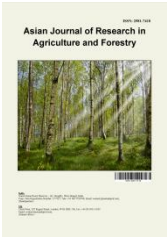

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Apparent and True Digestibility in *Clarias gariepinus,* Burchell, 1822 FED Soyabean Meal Based Diets Supplemented with Protease

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Soyabean meal (SBM) could be a suitable replacement for the expensive fishmeal but for its low digestibility in fish. However, protease supplementation could improve digestibility of SBM based diets. Therefore, apparent and true digestibility in *Clarias gariepinus* fed SBM based diets

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supplemented with protease were investigated. Six diets containing varied inclusion levels of protease (ppm) in solvent extracted soybean-based diets (SESBD) were formulated; Control (without protease), SS_{100} (100), SS_{200} (200), SS_{300} (300), SS_{400} (400), SS_{500} (500). The *Clarias gariepinus* (n=720) weighing 12.00±0.10g were fed to satiation with the diets for 12 weeks. Each treatment was in triplicate. Protease supplementation significantly (P<0.05) influenced the weight gain of *C. gariepinus* with the higher value in fish fed SS400 (44.63±3.13) and least value in control diet (32.03±0.65). FCR had a significantly (P<0.05) least value in *C. gariepinus* fed SS400 (1.62±0.18) but similar (P>0.05) to *C. gariepinus* diet SS300 (1.88±0.11). Supplemental protease in soyabean-based diet improved (P<0.05) apparent protein digestibility of *C. gariepinus* on diet SS400 (87.38±1.10) but similar (P>0.05) to *C. gariepinus* on diet SS200 (87.29±0.97). Also, true lysine digestibility was enhanced with protease supplementation in soybean-based diet with the higher value in diet SS200 (95.90±0.07). Optimal FCR occurred SESBD of 350ppm dietary inclusion (R^2= 0.8147) of protease. The findings suggest that, protease supplementation in solvent extracted soyabean based diet could improve growth performance and amino acid digestibility in *C. gariepinus* at 350ppm inclusion level.

Keywords: Fish; enzymes; plant proteins; growth performance; digestibility.

1. INTRODUCTION

The digestibility efficiency and nutrient utilization in the body of animals depends on feed quality and growth of fish which is all dependent on presence of either endogenous or exogenous enzymes [1]. Introduction of exogenous enzymes in the livestock industry has helped to improve the nutritive value of animal feed by reducing manure'snutrient, which have high environmental benefits in areas with concentrated production. Several researchers have documented the environmental advantages of using exogenous enzymes such as xylanase, phytase and protease in animal diets [2,3] in either pig or poultry diets. They have been established to increase the digestibility of poorly digested intakes than those properly digested diets [4]. Whereas exogenous protease supplementation in livestock diet has been shown as a beneficial approach to enhanced nutritional value of soyabean meal [3].

Although dietary exogenous protease is still at infant stage in aquaculture sector but several works has 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 combinationsof enzymes on broilers performance [5,6,7]. 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., [8] reported that dietary proteases inclusion in maize-soya-based diets increasedthe digestibility of protein by improving protein hydrolysis and inactivating anti-nutrients such as trypsin inhibitors [9]. Similar studies on fish could probably enhance feed efficiency in cultured fish). There is therefore the need for aquaculturist to explore the possibility of fully augmenting the maximum use of the nutrients contained in plant protein supplemented with dietary protease.Therefore, there is need to assess performance and digestibility of *Clarias gariepinus* fedsolvent extracted soyabean based diets supplemented with dietary protease.

2. MATERIALS AND METHODS

2.1 Feed Ingredients and Diets Preparation

The feeding trial was conducted at the Research Laboratory, Aquatech College of Aquaculture, Fodacis, Ibadan, Nigeria. Six isonitrogenous and isocaloric diets were formulated with varying levels of lysine and methionineas shown in Table 1. The dietary protein level was 40% crude protein reported for the optimum growth of *C. gariepinus* [10]. The ingredients were thoroughly mixed together and each diet mixture was pelleted at 60^{o}C, using 2mm pellet die to form noodle-like strands, which were manually crumbled into a suitable size for the juveniles. The pellets were sundried, packed into labeled transparent bag and stored in a cool dry place to prevent fungal growth. The six dietary treatments supplemented withprotease (ppm/kg) in solvent extracted soyabean based diets (SESBD) were: SS_1 (without supplemental protease), SS_{100} (100), SS_{200} (200), SS_{300} (300), SS_{400} (400), SS_{500} (500).

2.2 Fish and Experimental Procedure

The *C. gariepinus* juveniles (n=360) pieces aged two months weighing 12.06±0.85g were purchased from a reliable Fish Farm in Ibadan, Nigeria. The feeding trial was conducted at the Research Laboratory, Aquatech College of Aquaculture, Ibadan, Nigeria using 18 plastic tanks with the dimension measuring 60 cm × 45 cm × 30 cm. Each tank was supplied with water up to 80% capacity which was replaced every two days to maintain relatively uniform physico-chemical parameters and prevent fouling from feed residues. There were six dietary treatments, each was in triplicate and each replicate comprised 20 fish. The fish were weighed and randomly distributed into experimental tanks after they have been acclimatized for 14 days. The fish were fed to satiation throughout the 84days (12 weeks) duration of the experiment at week 9, total faecal collection was carried out for 21 days (3 weeks). During the faecal collection, the faeces was siphoned twice daily (07:00 and 16:00hrs). Faeces were immediately oven dried at 55 ^{o}C and stored at -20 ^{o}C until analysed chemically.

2.3 Chemical Composition

Table 1. Chemical composition of the diets and faeces were determined according to AOAC [11]

Ingredient % (g/100g DM)	Control	SS_{100}	SS_{200}	SS_{300}	SS_{400}	SS_{500}	Protein-free diet
Soyabean meal	70	70	70	70	70	70	
Yellow maize	26	26	26	26	26	26	
Corn starch	-	-	-	-	-	-	85
Cellulose	-	-	-	-	-	-	8
Glucose							5
*Vit/min premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Soyabean oil	1	1	1	1	1	1	1
Calcium carbonate	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
Chromic Oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0	0.6	0.6	0.6	0.6	1	-
Methionine	0	0.4	0.4	0.4	0.4	0	-
Protease (ppm/kg)	0	100	200	300	400	500	
Total (%)	99	100	100	100	100	100	100
Calculated crude protein (%)	40	40	40	40	40	40	0

2.4 Growth Studies

Calculations of the growth performance and nutrient utilization data were according to Falayi [12].
Final weight (FW) = Final weight – Initial weight

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed Consumed}}{\text{Final weight}}$$

$$\text{Gross Efficiency of Feed Conversion (GEFC)} = \frac{1}{\text{FCR}} \times 100$$

$$\text{Protein Intake (P1)} = \frac{\text{Total feed consumed} \times \text{percentage protein}}{100}$$

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.

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Net Final weight}}{\text{Protein intake}}$$

$$\text{Specific Growth Rate (SGR) (\%)} = \frac{\text{Logc W2} - \text{LogcW1}}{\text{T2} - \text{TI}} \times \frac{100}{1}$$

Where W_1 = Initial weight of fish (gm), W_2 = Final weight of fish (gm), T_2 = Time T_2, T_1 = Time

$$\text{Gross Protein Retention (GPR)} = \frac{\text{Final crude protein of fish} - \text{initial protein of fish}}{\text{Dry protein fed}}$$

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

Survival rate (SR%)was calculate as follows:

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

2.5 Chemical Composition

Feed ingredients, diets and the whole body were analysed chemically according to the official methods of analysis as described by AOAC [11]. All determinations were in triplicate.

2.6 Amino Acid Analysis

Amino acid samples of oven dried whole body were treated with performic acid at 0°C to oxidize methionie and cystine to methionine sulphone and cysteic acid prior to the hydrolysis. The samples were prepared by 6 N HCL hydrolysis for 24 h at 110°C. After which the samples were vaporised in sodium citrate buffer (0.2 mol.l Na^+, pH 2.2) and the mixture was equalized to a 50 mL volume. The amino acids in the hydrolysate were determined by an AA analyser (Biochrom 30. 30 plus, Biochrom Ltd, Cambridge, UK)

2.7 Statistical Analysis

The experiment is a completely randomized design. Data were subjected to one-way ANOVA followed by Duncan Multiple Range test was used to compare differences among individual means and polynomial regression. All statistics were performed using SPSS 20.0 (SPSS, Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Results

Chemical composition of solvent extracted soyabean based experimental diets supplemented dietary protease is shown in Table 2. 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 washigher (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 SS400and 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. 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 was 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).

Growth performance and nutrient utilisation by*C. gariepinus* fed solvent extracted soyabean based diet supplemented with varying inclusion of dietary protease is shown Table 3. Protease supplementation significantly (P<0.05) influenced*C. gariepinus* fed SS400(44.63±3.13) and the least value in the control diet (32.03±0.65). MWG and WG values of 32.53±3.30 and 269.16±31.23 were higher (P<0.05) in *C. gariepinus* on diet SS400 and least values of 20.10±0.60 and 168.42±4.34 were in control diet, respectively. Supplementation of protease in soyabean based diet significantly influence (P<0.05) FCR with the least value in *C.gariepinus* fed SS400 (1.62±0.18)but similar (P>0.05) to *C. gariepinus* diet SS300 (1.88±0.11). Similar trend was also observed in GEFC values with the higher value in diet SS400 (85.44±7.48) but similar (P>0.05) to *C. gariepinus* fed diet SS300 (76.34±2.03). Also, supplemental protease 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, least (P<0.05) values were noted in *C. gariepinus* fed control diet for PER, SGR, NRE and survival rate level andhigher in diet SS400 (14.88±1.04), SS400 (0.81±0.05), SS400(62.15±5.03) and SS300 (93.41±0.17), respectively. Finally, GPR had higher value in *C. gariepinus* fed control diet (0.84±0.01) and least value of 0.67±0.01 observed in diet SS300 (P<0.05).

Table 2. Chemical composition of solvent extracted soyabean based experimental diets supplemented with protease

	Solvent Extracted (SS)					
Parameter (%)	**Control**	**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}
Energy	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 different superscripts on the same row are significantly different (P<0.05)

The linear regression of protease activity and graded levels of protease as shown in figure 1 was positive and strong after 84 days feeding trial as shown in equation 1

$$y = 87.497x + 1619 \quad R^2 = 0.9932 \quad (1)$$

The relationship between protease inclusion and FCR of *C. gariepinus* are presented by regression equations 2 and shown in Figs 2.

$$y = 4E\text{-}06x^2 - 0.0027x + 1.7796 \quad R^2 = 0.8147 \quad (2)$$

Table 3. Growth performance and nutrient utilisation by*C. gariepinus* fed solvent extracted soyabean based diets supplementesd with protease

	Solvent Extracted (SS)					
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
FW	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}
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 different superscripts on the same row are significantly different (P<0.05)
IW = Initial Weight, WG= Final Weight Gain, TFI= Total Feed Intake, 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

Apparent nutrient digestibility by*C.gariepinus* fed solvent extracted soyabean based diet supplemented with varying inclusion of protease is shown in Table 4. Supplemental protease influenced (P<0.05) digestion by *C. gariepinus* on diet SS400 (87.38±1.10) but similar (P>0.05) to *C. gariepinus* on diet SS200 (87.29±0.97). Ash content was least (P<0.05) value inSS100 and higher in diet SS400. However, Ether extract was higher (P<0.05) inSS200(94.17±0.39) and was closely followed (P>0.05) by *C. gariepinus* on diet SS400 (93.75±0.26) as compared with the

least value in diet SS100. Ether extract had higher(P<0.05) value in diet SS200(77.25±1.86) but similar (P>0.05) to *C. gariepinus* on with diet SS400 (75.10±0.87).Furthermore, dry matter was higher(P<0.05) in *C. gariepinus* fed diet SS200 and least in diet SS300.

True nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of protease is shown in Table 5. Dry matter of *C. gariepi*nus fed diet SS200 increased significantly (P<0.05) that other treatments. Supplemental protease increase (P<0.05) crude protein digestion 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 and crude fibre had the least values in*C. gariepinus* on diet SS100(4.45±5.53 and 57.94±3.80) and higher in diet SS400 (54.03±3.32) and SS200 (77.64±0.81), respectively. Ether extract ranged from 91.02±1.76 (control) to 96.11±65.21 (SS500).

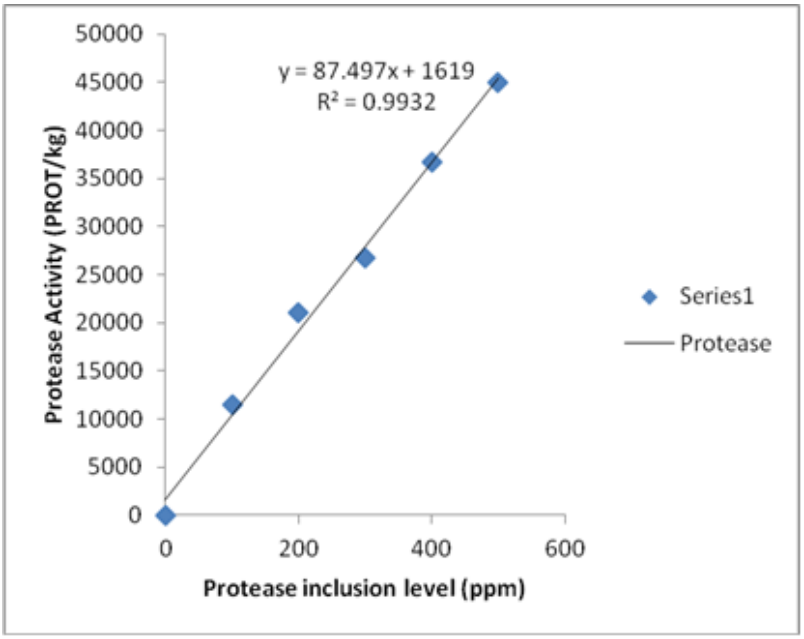

Fig. 1. Relationship between protease inclusion level (ppm) and solvent extracted soyabean based-diet protease activity fed to *C. gariepinus*

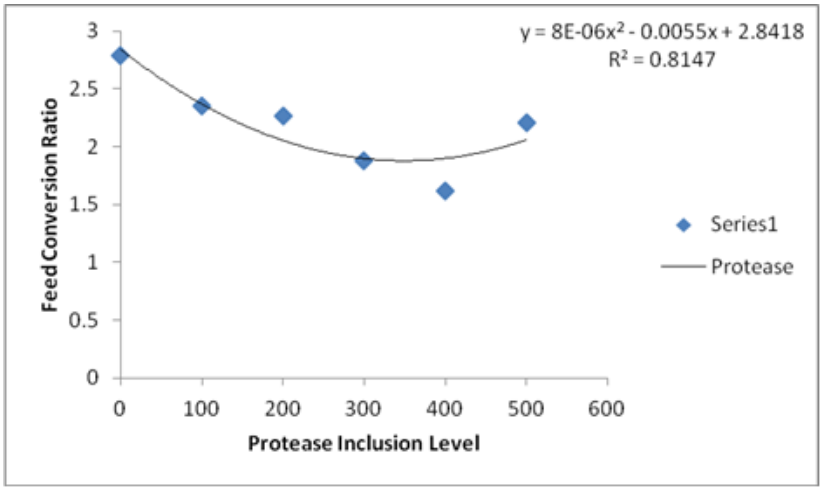

Fig. 2. Relationship between dietary supplemental protease of a solvent extracted soyabean based diet and feed conversion ratio of *Clarias gariepinus*

Table 4. Apparent nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

	Solvent Extracted (SS)					
Parameter	**Control**	**100**	**200**	**300**	**400**	**500**
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}
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}

Means with different superscripts on the same row are significantly different (P<0.05)

Table 5. True nutrient digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

	Solvent Extracted (SS)					
Parameter	**Control**	**100**	**200**	**300**	**400**	**500**
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}
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}

Means with different superscripts on the same row are significantly different (P<0.05)

Apparent amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of protease is shown in Table 6.*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 indiet SS300 with the higher values in diet SS200 (70.29±0.58), SS100 (86.97±0.11) and SS500 (80.84±0.16), respectively.

True amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with varying inclusion of protease is shown in Table 7. 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 werein *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 was significantly influenced (P<0.05) in diet SS500 (93.73±0.07)and SS200 (77.10±0.49) while it was 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 6. Apparent amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

		Solvent Extracted (SS)				
Parameter	**Control**	**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 different superscripts on the same row are significantly different (P<0.05)

Table 7. True amino acid digestibility of *C. gariepinus* fed solvent extracted soyabean based diets supplemented with protease

		Solvent Extracted (SS)				
Parameter	**Control**	**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}

		Solvent Extracted (SS)				
Parameter	**Control**	**100**	**200**	**300**	**400**	**500**
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 different superscripts on the same row are significantly different (P<0.05)

3.2 Discussion

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 nutrient utilization [5,6,8,13]. Present study revealed that growth performance and nutrient utilization of *C. gariepinus* on soyabean based diet supplemented with protease were different from control diet with higher values for WG, PWG and least values of FCR observed in Diet SS400. Contrary to this, Adeoye et al. [16] 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. [14] reported that supplemental protease influenced growth performance of tilapia fed least crude protein diet related to higher crude protein diet. Naela et al. [15] 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. Theimprovement 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. gariepinus* on soyabean based with supplemental protease agrees with the research verdicts of [16,17,18,19]. They all observed enhancement in weight gain and FCR with supplemental protease in broiler chicken's 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. [18] 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 SS400. Singh et al. [20] 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 (RonozymeProAct) in soyabean based diets could be used securely and economically to increase growth performance and nutrient utilization when fed *C. gariepinus* at 400ppm /kg. This is evidence in figure 2 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 350ppm/kg of diet.

Supplemental protease enhanced digestion of protein in the fed diet with the higher values observed in diet SS400 (87.38±1.10) in roasted and solvent soyabean based diets, respectively. Li et al. [19] reported similar improvement using serine protease to improve broiler performance and increases protein digestion. Similar reports had been documented in poultry [9], Pigs [21] and Cattle [9]. 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 nutrient utilization of fish feed diet. Carter et al. [22] 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 [23]. Zhong and

Zhou [24] reported significant positive effect of multienzyme on nutrient digestibility of tilapia and crucian carp fingerlings. Prabjeet et al. [25] 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 [18,26]. 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. [27] 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. 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.

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 95.33±0.17 (control) and 95.74±0.07 (SS200), respectively for roasted soyabean based diets. 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. [28] that assess 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 [29,30].

Angel et al. [29] reported improvement in WG 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 was highly digestible when soyabean based diets were supplemented with protease and it supports the improvement noted in WG and FCR. Increased digestibility and performance improvement observed can only arise when improvement in amino acid digestibility is 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 compositions depend on ingredient [31].

Furthermore, the true amino acid digestibility of *C. gariepinus* fed 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. [32] reported protease inclusion in fish diet to improved coefficient of true digestibility of essential amino acid of roasted soyabean meal. Bertechini et al. [33] stated better true amino acid digestibility of soyabean meal and corn with or without monocomponent protease supplementation. Angel et al. [29] also reported improved true amino acid of broiler chicken with supplemental monocomponent protease. Also, this digestibility study suggests that supplemental protease in soyabean based diets is a better substitute to the diet formulation for *C. gariepinus*.

4. CONCLUSION

Significant improvement with protease supplementation in solvent extracted soyabean based diet was recorded for the growth performance, apparent and true digestibility when fed to *C. gariepinus*. The optimal FCR in the regression analysis occurred in solvent extracted soyabean based diet at 350ppm dietary inclusion (R^2= 0.8147) of protease.Therefore, the inclusion of protease in solvent extracted soyabean based diet for *C. gariepinus*at 350ppm could improve growth performance and digestibility significantly and recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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