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Original Article

Effect of replacing fish meal by full fat soybean meal on growth performance, feed utilization and gastrointestinal enzymes in diets for African catfish *Clarias gariepinus*

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(With 1 figure)

Abstract

Study aimed to evaluate the effect of replacing fish meal with different levels of full fat soybean meal (FFSBM) on growth and digestive enzyme activities in the stomach, Liver and intestine for *Clarias gariepinus*. Four diets (D1, D2, D3 and D4) were formulated with 0, 15, 20 and 20 g 100^{-1} protein + DL-methionine by alternating FFSBM with fish meal. The growth of *C. gariepinus* was found to be significantly decreased when FFSBM replacement increased. Final body weight was 89.69, 79.70, 70.82 and 68.29 g for fish fed on D1, D2, D3 and D4, respectively, with significant differences between treatments. Specific growth rate (SGR) ranged between 3.11 to 2.78%. Proteolytic activity was higher only with alkaline pHs, whereas only very low activity was shown with acidic. Results of liver showed approximately similar results at acid and alkaline. In contrast, higher proteolytic activity in the stomach was observed at acid pHs 3.0 and 4.0 µg tyrosine^{-1} minute^{-1} mg^{-1} protein) whereas lower values were observed at neutral pH 7.0 g tyrosine^{-1} minute^{-1} mg^{-1} protein for catfish fed on the experimental diets. Moreover, trypsin activity was higher for the stomach, followed by the intestine and liver. However, higher amount of amylase observed in the liver than intestine and stomach.

Keywords: hepato-pancreas, amino acids, enzyme activities.

Efeito da substituição de farinha de peixe por farelo de soja integral no desempenho de crescimento, utilização de ração e enzimas gastrointestinais em dietas para bagre africano *Clarias gariepinus*

Resumo

Objetivou-se avaliar o efeito da substituição da farinha de peixe por diferentes níveis de farelo de soja integral (FSI) sobre o crescimento e atividades de enzimas digestivas no estômago, fígado e intestino de *Clarias gariepinus*. Quatro dietas (D1, D2, D3 e D4) foram formuladas com 0, 15, 20 e 20 g 100^{-1} proteína + DL-metionina, alternando FSI com farinha de peixe. O crescimento de *C. gariepinus* foi significativamente reduzido quando aumentou a substituição de farinha de peixe por FSI . O peso corporal final foi de 89,69, 79,70, 70,82 e 68,29 g para peixes alimentados com D1, D2, D3 e D4, respectivamente, com diferenças significativas entre os tratamentos. Taxa de crescimento específico (TCE) variou entre 3,11 a 2,78%. A atividade proteolítica foi maior apenas com pHs alcalinos, enquanto que atividades muito baixas foram somente mostradas com ácido. Os resultados no fígado mostraram valores aproximadamente semelhantes tanto em meio ácido quanto alcalino. Em contraste, observou-se maior atividade proteolítica no estômago em pH ácido (3,0 e 4,0 g de tirosina^{-1} minuto^{-1} mg^{-1} de proteína), ao passo que valores mais baixos foram observados em pH neutro (7,0 g de tirosina^{-1} minuto^{-1} mg^{-1} de proteína) para os bagres que se alimentaram com as dietas experimentais. Além disso, a atividade da tripsina foi maior para o estômago, seguida pelo intestino e pelo fígado. No entanto, observou-se maior quantidade de amilase no fígado,intestino e estômago.

Palavras-chave: hepatopâncreas, aminoácidos, atividades enzimáticas.

1. Introduction

Clarias gariepinus is an important species in fish culture sector related to its fertilization, high growth rate, tolerate of high stocking density, resistance to the common diseases and can able to accept a wide different of feed (Huisman and Richter, 1987). Many investigations have been conducted to evaluate the appropriateness of the

plant and animal feedstuffs as substitutes for fishmeal in the diets of African catfish. Conventionally, fish meal has been considered to be the major source of protein because of its high contents of essential nutrients, balanced in amino acid profile, good growth performances and the fact that it is acceptable for most aquatic animals (Sun, et al., 2015). The problem, however, is that the protein source represents the highest cost element in feed for aquaculture and there is therefore an incentive to seek cheaper alternatives. Furthermore, the development of alternative protein sources, including plant proteins, means that fish meal can at least be partially substituted with more economical products (Francis, et al., 2001), like meal of poultry by product (Abdel-Warith, et al., 2001; Fagbenro and Davies, 2001; Goda, et al., 2007), waste of shrimp head (Nwanna et al., 2004) soybean meal (Imorou Toko, et al., 2008), rice husks meal (Zaid and Ganiyat, 2009), and grasshopper meal (Alegbeleye et al., 2012; Olaleye, 2015). Some investigations have been reported that partially substitute fish meal with soybean meal effects the performance of a few fish species, such as for Japanese seabass *Lateolabrax japonicus* (Zhang, et al., 2014), juvenile tench *Tinca tinca* L. (Garcia, et al., 2015), gilthead sea bream *Sparus aurata* L. (Kokou, et al., 2012), rainbow trout *Oncorhynchus mykiss* (Harlioğlu, 2011) and Nile tilapia *Oreochromis niloticus* (Abdel-Warith et al., 2013). Some Anti-nutritional factors ANFs suppress the activities of specific enzymes, such as proteinase and amylase; also, many protein components, such as haemagglutinins and lectins, can react in specific ways with certain carbohydrates (Hendricks, 2002). Protease enzymes such as trypsin and chymotrypsin are very important for the digestibility operations in digestive system of fish. These digestive enzymes, like others, are active into the proximal intestine. The hydrolysis of protein intake from the diets which occurs by these enzymes that active in the proximal intestine, serve to break down the protein to simple molecules that can then be absorbed throughout the intestine and used in the metabolic process (Lovatto et al., 2017). While some studies have focused their attention on the influence of different substitute plant proteins on fish growth and feed utilization, few have looked at how diet changes the activities of digestive enzymes) Xu et al. (2012) and Zhao et al. (2016).

This study aimed to investigate the potential use of partially substitution of fishmeal by full fat soybean meal in diets for the *C. gariepinus* by examine the subsequent effects on growth performance, feed utilization and the digestive enzyme activities in the stomach, hepato-pancreas and intestine, given that these are still poorly understood, especially in respect to *C. gariepinus*.

2. Material and Methods

2.1. Experimental fish

A total of 160 *C. gariepinus* with average weight 7.82±2.02 g) were distributed into eight fiberglass tanks containing 100 L of water, and were suspended over a 1000 litre bio-filter. Water entered each tank via a spray bar after filtration and an aerator was placed in the center of the tank. Partial water changes amounted approximately 20% of the systems volume per week. Filters of the systems were cleaned daily to avoid the buildup of nitrate levels in the water. 20 fish have been distributed in each tank with replicate. Treatments of water temperature were monitored at 28±1°C by controlled heaters (Atman, AT-300W), the values of pH maintained at 7.1-8.0, ammonia (NH3) (0.07-0.20 mg L^{-1}), nitrite (NO2) (0.15-0.35 mg L^{-1}), nitrate (NO3) (4.35-5.77 mg L^{-1}) and dissolved oxygen (5.3-6.7 mg L^{-1}) were all monitored twice a week to ensure that they remained at acceptable levels. Other twenty fish were euthanized using buffered MS222 (50 mg/l) then kept frozen at -20°C to estimate the initial chemical analysis of carcass composition. At the end of the experiment, five fish of each treatment were dissected and sample of stomach, liver and intestine were removed and kept frozen at -80°C for enzymes assays, other five fish of each group were used for final carcass composition.

2.2. Diet formulation

Experimental diets were prepared in various levels of full fat soybean (FFSBM) substituted by fishmeal. Table 1 shows the formulation and chemical analysis of experimental diets, and Table 2 observed the diets contents of essential amino acids expressed as percentage of protein. Experimental diets were prepared to replace the fishmeal protein with full-fat soybean with ratio 0, 15, 20 and 20 g 100^{-1} g protein +1% DL-methionine for D1, 2, 3 and 4, respectively.

2.3. Experimental procedure

Weight of fish have been done every two weeks and fed 3.5% by hand twice a day of their body weight six days a week for the first half of the period (6 weeks), reduced to 3% for the second half (6 weeks), giving an average of 3.25%. The experiment was held for 12-week and the feed intake was regulated according to the increase of biomass. Five fish from each group were collected at the end of the experimental period, then dissected and sample of stomach, liver and intestine were removed and kept frozen at -80 °C for enzymes assays, other five fish from each treatment were euthanized and frozen at -20 °C to determine final chemical composition.

2.4. Proximate composition

Chemical analyses of fish carcass and diets for moisture, lipid, protein, and ash were estimated using AOAC (1995) methods and gross energy were calculated according to Hepher et al. (1983).

2.5. Estimation of growth and nutrients efficiency

$$\text{Specific growth rate}(\text{SGR }\%) = \left[\text{Ln FBW}(\text{g}) - \text{Ln IBW}(\text{g})\right]/\text{feeding days} \times 100 \quad (1)$$

Whereas: FBW final body weight, IBW initial body weight

Table 1. Composition and proximate analysis of the control and test diets (g/100 g dry weight) fed to *Clarias gariepinus*.

Ingredients	Experimental diets			
	D1(0 g/100 g protein)	D2(15 g/100 g protein)	D3(20 g/100 g protein)	D4(20 g/100 g Protein + AAs)
Fish meal[1]	43.00	23.00	16.00	16.00
Full-fat soybean meal[2]		41.00	57.00	57.00
Wheat meal[3]	32.00	20.00	17.50	16.50
Corn oil[4]	8.77	2.30		
Cod liver oil[5]	0.70	2.90	2.50	2.50
Vitamin premix[6]	2.00	2.00	2.00	2.00
Mineral premix[7]	1.00	1.00	1.00	1.00
DL-Methionine				1.00
Binder[8]	2.00	2.00	2.00	2.00
∝-Cellulose[9]	10.03	4.80		
Proximate composition (% as fed)				
Moisture	4.23	3.25	3.85	4.05
Protein	36.55	35.73	35.51	36.42
Lipid	14.33	15.18	14.79	14.56
Ash	7.41	7.29	8.28	8.22
Gross energy ($MJkg^{-1}$)[10]	20.78	20.97	20.77	20.37

[1]Fish meal LT94, Trouw Aquaculture (Nutreco company); [2]Full fat soybean, Central Soya Michigan, USA; [3]Wheat meal, Kalpro S™ . Orsan, Paris, France; [4]Mazola- pure corn oil; [5]Fish oil- seven pure cod liver oil; [6]Vitamin premix, Trouw Aquaculture (Nutreco company); [7]Mineral premix, Trouw Aquaculture (Nutreco company); [8]Carboxymethyl Cellulose (CMC); [9]Sigma Chemical Co., Poole, Dorset; [10]Gross energy (GE) was calculated according to Hepher et al. (1983) using the equivalent factors of 5.65, 9.45 and 4.2; kcal/g for CP, EE, and NFE, respectively

Table 2. The amino acid composition of the test diets is expressed as a percent of protein fed to *Clarias gariepinus* and their requirements.

Amino acids	Experimental diets				
	D1	D2	D3	D4	*C. gariepinus* Req*
Arginine	5.99	5.39	5.33	5.76	1.20
Histidine	2.52	2.23	2.25	2.65	0.42
Isoleucine	4.01	3.79	3.55	3.65	0.73
Leucine	6.99	6.70	6.19	6.56	0.98
Lysine	6.03	4.83	4.62	5.30	1.43
Methionine + Cysteine	2.51	2.15	1.78	3.75	0.64
Phenylalanine	3.90	4.29	4.00	4.19	ND
Phenylalanine + Tyrosine	6.33	7.30	6.74	7.03	1.40
Threonine	4.36	3.79	3.38	3.84	0.56
Tryptophan	ND	ND	ND	ND	ND

*Amino acid requirement according to NRC (1993); ND (not detected).

$$\text{Feed conversion ratio (FCR)} = \text{FI (g)} / \text{BWG (g)} \quad (2)$$

Whereas: FI feed intake and BWG body weight gain

$$\text{Protein efficiency Ratio (PER)} = \text{BWG (g)} / \text{PI protein intake (g)} \quad (3)$$

Whereas: BWG body weight gain and PI protein intake

$$\text{Apparent net protein utilization (ANPU \%)} = (\%\,\text{FBP} \times \text{FBW}) - (\%\,\text{IBP} \times \text{IBW}) / \text{TPI (g)} \times 100 \quad (4)$$

Whereas: FBP final body protein, FBW final body weight, IBP initial body protein, IBP initial body weight and TPI total protein intake

2.6. Determination of amino acids

The amino acid contents of the diets were determined following acid hydrolysis method of McCullagh et al. (2006). Amino acids were assayed using a Kontron Chromakon 500 automatic amino acid analyzer [column 250 × 4.6 mm, cation ion-exchange resin material (AS70)] and the procedures were done as described in our previous studies Abdel-Warith et al. (2014). Table 2 showed the amino acids composition expressed as % of protein in the diets.

2.7. Determination of enzymes

Proteolytic activity of enzymes were expressed as the amount of tyrosine (µg) digested by 100 µl of enzyme

solution /minute/mg protein at acid, natural and alkaline pHs at 37 °C and determined using the casein hydrolysis according to the method of Kunitz (1947) as modified by Walter (1984). Trypsin activity was expressed as the amount of tyrosine (µg) liberated by 0.5 ml of enzyme extract per minutes /mg protein at 37 °C and measured in the test tubes using benzoyl-Arg-*p*-nitroanilide (BAPNA) as a substrate according to Erlanger et al. (1961). Amylase activity were expressed as the amount of maltose liberated by 50 µl of enzyme extract /minute/ml at 37 °C then assayed by the starch hydrolysis method According to Tietz (1970). However, the lipase activity was expressed as the amount of fatty acids neutralized by 0.05 NaOH liberated by 1 ml enzyme solution minute at 37°C, and determined by the aid of a Sigma diagnostic test-kit.

2.8. Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) technique. The means were separated by Fisher's LSD test and compared using Duncan's Multiple Range Test, as described by Snedecor and Cochran (1989). The significant differences level was defined at $P < 0.05$.

3. Results

3.1. Growth performance

Results of growth performance and feed utilization for the *C. gariepinus* fed the experimental diets are displayed in Table 3. Growth performance (mean final weight, weight gain and specific growth rate (SGR) Equation 1 decreased significantly as increasing proportions of FFSBM were included in the diets (from D2 to D4). Amino acid supplementation had no effect on the parameters when compared with an unsupplemented diet3. The results obtained for final weight were 89.69, 79.70, 70.82 and 68.29 g for catfish fed the experimental diets, with significant differences between treatments (Table 3). The results for SGR were 3.11, 2.98, 2.82 and 2.78 for fish fed D1, D2, D3 and D4 respectively.

3.2. Feed consumption and feed utilization

Clarias gariepinus well accepted to the control diet whereas, the palatability for other test diets containing partial replacement with FFSBM were decline. Average of feed intake ranged between 0.85 and 0.72 g fish^{-1} day^{-1}. The presence of FFSBM in the diet had a noticeable effect on feed intake (Table 3). The results of feed intake showed significant difference ($P<0.05$) between fish fed D1 and D2 when compared with D3 and D4 which containing high levels of FFSBM even with adding methionine to D4. The results obtained of Feed Conversion Ratio (FCR) Equation 2 showed significant differences ($P<0.05$) among fish fed on control diet (0.82) and the other groups fed on diets containing different levels FFSBM especially D4, However, D1, D2 and D3 are presented similar FCR, also, in the same way of D2, D3 and D4.with FCRs of 0.87 for D2, 0.91 for D3 and 0.93 for D4 (including amino acid supplementation) respectively, The protein efficiency ratio (PER) Equation 3 was also showed significant differences ($P<0.05$) between D1 and D4; However, D2, D3 and D4 are presented no significant differences between treatments. Superior PER (3.36) was obtained for fish fed control diet while fish fed different inclusion levels of FFSBM observed PERs of 3.24, 3.12 and 2.97. Apparent net protein utilization (ANPU %) Equation 4 also supported this trend with significant differences ($P<0.05$) between the control and D4. however, no significant differences between D2, D3, and D4. The ANPU for catfish fed a control diet was 54.48%, whereas the lowest value was observed for fish fed 20g 100 g^{-1} protein of FFSBM (D4) with amino acid supplementation, at 48.6% (Table 3).

3.3. Fish body composition

Table 4 shows the chemical of carcass composition for fish fed the experimental diets. Carcass composition of final body of fish showed slightly significant differences as a result of experimental diet. There were few differences in the moisture content, whereas, the protein and lipid content and ash content showed only slight differences (*P>0.05*) between treatments Table 4.

3.4. Enzyme activities

3.4.1. Proteolytic activity

The total activity of proteolytic enzymes used the (sum of pHs 1.5, 3, 4, 7, 8.5, 9, and 10) showed higher activity in the intestine than the activity of this enzyme in the stomach and liver which ranged

Table 3. Weight increase, feed consumption, nutritive utilization of feed and protein for *Clarias gariepinus* (mean ± SD).

	D1	D2	D3	D4
Mean initial weight (g)	7.85±2.31	7.83±1.76	7.88±1.99	7.80±2.02
Mean final weight (g)	88.69±4.27^{b}	79.7±1.09^{b}	70.82±1.42^{a}	68.29±3.54^{a}
Mean weight gain (g)	80.86±4.25^{b}	71.87±1.03^{b}	62.94±1.34^{a}	60.49±3.54^{a}
Mean daily feed Intake(gfish^{-1} d)	0.85±0.01^{b}	0.80±0.01^{b}	0.73±0.03^{a}	0.72±0.01^{a}
SGR (%)[1]	3.11±0.06^{c}	2.98±0.01^{b}	2.82±0.01^{a}	2.78±0.07^{a}
FCR[2]	0.82±0.05^{a}	0.87±0.02^{ab}	0.91±0.02^{ab}	0.93±0.06^{b}
PER[3]	3.36±0.13^{b}	3.24±0.09^{ab}	3.12±0.07^{ab}	2.97±0.18^{a}
ANPU (%)[4]	54.48±1.01^{b}	51.88±1.53^{ab}	52.63±1.23^{ab}	48.60±2.89^{a}

Values in the same row with different letters indicate significant difference ($P<0.05$); [1]SGR: [Ln final body weight (g) - Ln initial body weight (g)]/feeding days ×100; [2]FCR: feed intake (g)/body weight gain (g); [3]PER: body weight gain (g)/protein intake (g); [4]ANPU (%) = (% final body protein × final body weight) - (% initial body protein × initial body weight) / total protein ntake (g) × 100.

between 5.68 to 2.98 µg tyrosine^{-1} minute^{-1} mg^{-1} protein. The mean of the activities of proteolytic between *C. gariepinus* fed on all diets did not observe any significant difference ($p > 0.05$) in the intestine. However, the results obtained of proteolytic activities in the stomach showed a significant differences (P<0.05) between fish fed control diets when compared to other fish fed D2, D3 and D4 that containing high amount of FFSBM (Table 5). Proteolytic activity in the liver was less than the activity of the stomach, however, and also the mean of proteolytic activity of sum of pHs µg tyrosine^{-1} minute^{-1} mg^{-1} protein showed a decreased when replacement FFSBM increased for all organs for *C. gariepinus* fed the experimental diets.

Proteolytic Activities of enzyme for the intestine were higher with alkaline pHs, whereas only very low amounts of activity were observed with acidic pHs (Figure 1). For liver, the proteolytic activity recorded showed similar results at acid and alkaline pHs. In contrary, the proteolytic activity in the stomach was higher at acid pHs (3.0 and 4.0 µg tyrosine^{-1} minute^{-1} mg^{-1} protein) whereas lower amounts were recorded at neutral pH 7.0 (Figure 1) for catfish fed experimental diets.

3.4.2. Trypsin activity

Trypsin activities were also higher in the stomach, followed by intestine and liver. While there were no significant differences ($p > 0.05$) between the treatments in respect to the liver and stomach, there was significantly higher trypsin activity in the intestines 2.75 of fish fed the control basic diet (Table 5) when compared with *C. gariepinus* fed D2, D3 and D4 which resulted 2.31, 2.07 and 1.71 respectively.

3.4.3. Amylase activity

Amylase activity showed the highest values in the liver compared to the intestine and stomach (Table 5). Higher amount of amylase was observed in the hepatic tissue of catfish fed the control diet, 4.49 µg maltose^{-1} minute^{-1}ml^{-1} followed by that of *C. gariepinus* fed on D2 (2.94). *C. gariepinus* fed diets 3 and 4 showed lower values of amylase activity. Only a little value of amylase activities were revealed in the stomach and intestine Table 5.

Table 4. Body composition of *Clarias gariepinus* fed graded levels of FFSB (g100^{-1} g wet weight) of whole fish experiment diets (mean ± SD).

	Initial fish	D1	D2	D3	D4
Moisture	76.45	72.24±1.26^{a}	72.71±1.81^{a}	72.97±1.19^{a}	73.81±1.39^{a}
Protein	12.01	16.01±0.27^{ab}	15.82±0.48^{a}	16.55±0.55^{b}	16.11±0.49^{ab}
Lipid	7.40	9.75±1.05^{bc}	10.24±0.96^{c}	9.24±0.55^{b}	7.99±0.33^{a}
Ash	2.46	3.27±0.10^{b}	2.96±0.15^{a}	3.04±0.19^{a}	2.96±0.10^{a}

Values in the same row with different letters indicate significant difference (P<0.05).

Table 5. Total proteolytic, trypsin, amylase and lipase activities in intestine, liver and stomach of *Clarias gariepinus* fed control and test diets determined at 37°C (mean ± SD).

	Proteolytic activity (mean)	Proteolytic Sum of pHs* (µg tyrosine/ min^{-1} mg^{-1} protein)	Trypsin activity (µg tyrosine/ min^{-1} mg^{-1} protein)	Amylase activity (µg maltose/ ml^{-1} min^{-1})	Lipase activity (Sigma/Tietz/ unit/L)/min^{-1} ml^{-1}
Intestine					
D1	0.81±0.57^{a}	5.68	2.75±0.18^{b}	0.96±0.37^{b}	1.87±0.49^{b}
D2	0.47±0.25^{a}	3.26	2.39±0.66^{ab}	1.01±0.30^{b}	1.37±0.38^{ab}
D3	0.43±0.20^{a}	2.98	2.07±0.97^{ab}	0.67±0.19^{a}	1.14±0.25^{a}
D4	0.48±0.31^{a}	3.38	1.71±0.73^{a}	0.94±0.29^{b}	1.07±0.18^{a}
Liver					
D1	0.15±0.02^{a}	1.08	1.37±0.31^{a}	4.49±1.17^{c}	1.01±0.40^{a}
D2	0.22±0.03^{b}	1.54	1.42±0.39^{a}	2.94±0.98^{ab}	0.92±0.16^{a}
D3	0.20±0.03^{b}	1.41	1.33±0.29^{a}	2.46±0.86^{a}	1.12±0.31^{a}
D4	0.14±0.01^{a}	0.95	1.05±0.65^{a}	3.66±0.63^{bc}	0.97±0.43^{a}
Stomach					
D1	0.54±0.17^{b}	3.79	3.09±0.79^{a}	0.76±0.18^{ab}	1.10±0.55^{a}
D2	0.29±0.24^{a}	2.03	2.77±0.66^{a}	0.82±0.18^{b}	1.18±0.57^{a}
D3	0.31±0.13^{a}	2.17	2.27±0.43^{a}	0.65±0.14^{a}	1.21±0.45^{a}
D4	0.20±0.09^{a}	1.40	2.29±0.46^{a}	0.69±0.10^{ab}	1.11±1.13^{a}

*Total proteolytic activity was obtained as the sum of those determined at pH 1.5, 3, 4, 7, 8.5, 9, and 10; Values in the same column with different letters indicate significant difference (P<0.05).

3.4.4. Lipase activities

In addition, lipase activities supported this trend, with a significant difference among the groups in the intestine but not in the stomach and liver (Table 5). Although, results of lipase activities in the intestine were 1.87, 1.37, 1.14 and 1.07 for fish fed D1, D2, D3 and D4 respectively with significant differences (P<0.05).between D1 and D2 when compared with D3 and D4 therefore, data also, showed no significant different (*p> 0.05*) among all groups fed diets containing FFSBM Table 5.

4. Discussion

The results obtained in this study show that plant ingredients used as a protein sources for example full fat soybean can be effective when replaced less than 50% of the fishmeal protein (LT94) in the diets for *C. gariepinus*. Catfish growth was slightly inferior with a diet containing about 15g/100g protein D2 but highly significant inferior in catfish fed 20g/100g protein and 20g/100g of total protein of FFSBM with additional of DL-methionine compared with fishmeal as the only protein source. Also, catfish fed 20g/100g of total protein of FFSBM with supplementation of DL-methionine did not improve their growth performance when compared to the unsupplemented diet. These results are in agreement with data obtained by Fagbenro and Davies (2001) who found that high nutritional value of soybean flour possesses used as a protein source in African catfish diets, particular partial substitution (>50%) of the protein from fish meal source in the diet. They also showed that there was a decrease in growth, inferior in the utilization of protein efficiency.

The results in this study showed that FFSBM diets were able to replace 15 g 100 g^{-1} of total protein of fishmeal high quality protein in *C. gariepinus* diets however, the growth and utilization of feed reduced with diets D3 and D4. Santigosa et al. (2008) reported that substitution of fish meal by plant ingredients as a protein source caused a decline in growth in other two fish species, rainbow trout *Oncorhynchus mykiss* and sea bream *Sparus aurata*. We argue that additional of amino acids may be inefficiently utilized by *C. gariepinus* this might be related to the presence of many anti-nutritional factors in plant ingredients that limit the utilization of dietary amino acids.

In addition, the palatability of FFSBM for catfish appeared to be less than has been found for tilapia (Abdel-Warith et al., 2013), as observed in daily feed intake records (Table 3), and certainly less than for fishmeal based diets for *C. gariepinus*. The results in this study were in general in accordance with data obtained for yellowtail *(Seriola quinquertidiat)* by Shimeno et al. (1993) when they also found reduced palatability in fish fed protein sources from plant replaced by fish meal. Another reason for decline the utilization of feed in the FFSBM diets is inferior in the digestibility of plant ingredients protein (Davies et al., 2011) which might be related to the high contents of anti-nutrition factors (ANFs), which inhibit the digestive enzymes.

Data in this study are in agreement with the results obtained by Luo et al. (2006) who demonstrated that diets containing more than 50% of solvent extracted cotton seed meal fed to *Oncorhynchus mykiss* had poorer growth performance since the diet contained less lysine than this fish needs. In the present study amino acid supplementation in D4 replacing 20g/100g of total protein in the diets did not improved SGR Equation 1, FCR Equation 2, PER Equation 3, and ANPU Equation 4, for *C. gariepinus* when compared with the other diets. This might be because *C. gariepinus* cannot utilize the plant ingredients because catfish is a carnivorous species; this leads us to suggest that *C. gariepinus* can utilize diets containing up to 15g/100g of total protein (about 41% of FFSBM) to observed that performance is less negatively affected at the lower level of supplementation than higher levels Shiau et al. (1990) reported that WG, FCR, PER and the digestibility of protein in hybrid tilapia (*O. niloticus* × *O. aureus*) can be improved by the substitution of up to 30% of the fishmeal in diets with defatted soybean and full-fat soybean. This study observed that the decrease of performance may be related to the high level of replacement plant materials protein leading to an imponderable of nutrients, particularly composition of protein. This may be due to an insufficient amino acid profile when FFSBM is supplemented to D2, D3 and D4. Full fat soybean has a certain amounts of both lysine and methionine, which affects the dietary contents of these amino acids, except for D4 (Table 2). ANPU values in the current study were not greatly affected except with methionine supplementation.

Protease Inhibitors especially in legumes are known to reduce the performance of growth in freshwater prawn *Macrobrachium rosenbergii* (Sriket et al., 2011). In the present study, only lower amount of proteolytic activity at acidic pH levels was found in the hepato-pancreas and intestine whilst, activity detected to be high in stomach (Figure 1) this related possibly to the fact that there are some intra-cellular enzymes which perform optimally at acidic pH. Also, the results in the current research were in accordance with those of El-Beltagy et al. (2004) who illustrated that the highest activity of partially purified acidic protease had recorded at pH 2.5 and then declined with rising of the pH. In contrast, at alkaline pHs, a higher amount of proteolytic activity was observed in both the intestine and hepato-pancreas. This agrees with data the obtained by Melo et al. (2012) who reported that the digestive tract of juvenile silver catfish showed higher activities of enzyme in the anterior section of the intestine at higher alkaline proteases. Similar results are reported in other species such as *Gptosternum maculatum* (Xiong et al., 2011).

Hidalgo et al. (1999) demonstrated that eels (*Anguilla anguilla*) had high proteolytic activity associated with a low gastrointestinal tract pH together with significant activities for proteolytic enzymes at higher gastrointestinal pH's. Das and Tripathi (1991) reported that optimum activity of protease was found when pH ranged between 7.6 and 8.4 in grass carp *Ctenpharyngodon idella* fingerling and adult.

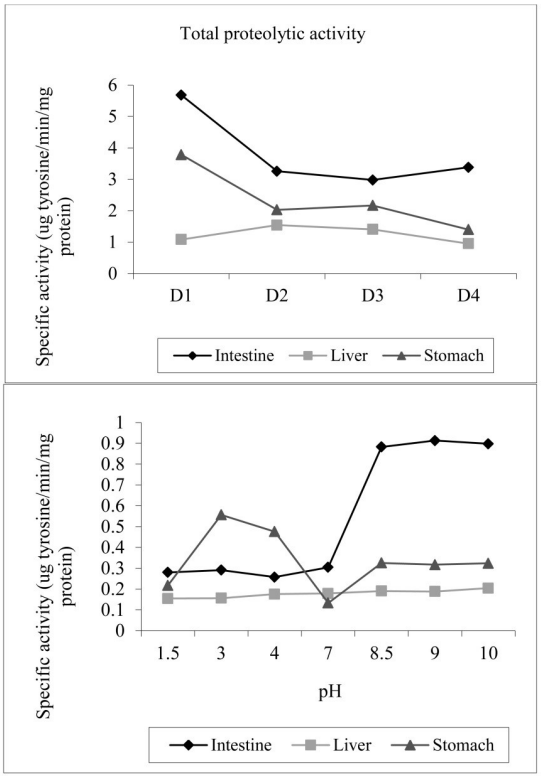

Figure 1. Total proteolytic activity (PA) in intestine, liver and stomach in *Clarias gariepinus* fed different levels of FFSBM (Top) is total proteolytic activity (PA) for control and test diets, (Bottom) is average PA affected by different pHs (mean ±SD).

In this experiment, however, the optimum proteolytic activity was recorded in different organs. I.e. in the catfish intestine optimum pH ranged between 8.5 to 10 Also, the catfish stomach showed optimum proteolytic activity at pH 3.0 and 4.0 however, Abdel-Warith et al. (2013) reported that the data obtained of tilapia showed optimum proteolytic activity in tilapia intestine was at pH 7.0-8.5, whereas, in the stomach was at pH 1.5-3.0, this gives an indicator that in thick-walled muscular stomachs, such as *C. gariepinus*, the pH is quite high at around 4.

Lovatto et al. (2017) argued that higher trypsin activity in silver catfish (*Rhamdia quelen*) fed diets containing pumpkin seed meal represents the body's attempt to increase the digestibility of protein, that adverse in increased activities of the proteolytic. Alarcon et al. (2001) who found that the connection between the trypsin activities in the intestinal and the digestibility of protein in *L. argentiventris* and *L. novemfasciatus*, inhibitor activity of the enzyme appears to be offset by raising the secretion of enzymes proteolytic and increased protein absorption in distal parts of the intestine. In the contrary, the activities of trypsin were higher in *Salmo salar* fed pea protein concentrate (Penn et al., 2011). Song et al. (2014) also found that in *Platichthys stellatus* fed diets containing 15–70% of soybean protein hydrolysate replaced by fish meal showed higher trypsin activity.

Amylase activity in different organs (stomach, liver and intestine) also varied for *C. gariepinus* in the present study. The highest values were measured in the liver compared with the stomach and intestine. Fish fed high inclusion levels of FFSBM effected on the activities of amylase in the liver, with fish fed D4 (containing a high level of FFSBM with amino acid supplementation) showed higher values than those on FFSBM diets without AA supplementation. This indicated that the supplementation of amino acid improved enzyme activities. The lower value of amylase in the stomach, meanwhile, indicates that small amount of starch is digested before the food reaches the foregut. Al-Owafeir (1999) found that activity of α-amylase was especially existing in Nile tilapia; this might be indicate that the tilapia is more ability to using and digesting carbohydrates than African catfish. Relatively few investigations have been undertaken of lipase activity in African catfish.

Lipase activity in this study was shown to be slightly higher in the intestine than in the liver and stomach. Tengjaroenkul et al. (2000) reported that activity of lipolytic enzymes absolutely exists in Nile tilapia *O. niloticus*, and occurs fundamentally in the cranial half of the intestinal in the digestive tract. While Melo et al. (2012) reported that lipase was stimulated by the lipid content in the diets.

Based on the results of this study we conclude that African catfish responded to diets of varying levels of FFSBM incorporation and grew favorably up to an inclusion level of about 41%, of the original fishmeal component with less negatively affected at the lower level of supplementation than it is at the higher levels affected growth adversely, however, even with AAs supplementation, and also resulted in changes in the digestive enzyme activities. Anti-nutritional factors (ANF's) associated with FFSBM possibly caused a further depression in growth rate, feed utilization efficiency and also negative changes to several key enzyme activity levels associated with the gastrointestinal tract. Futures studies should consider how to improve the utilization of plant ingredients by adding some materials to the diets such as phosphorus, a different ratio of amino acids, minerals and other new additives such as prebiotics and probiotics to enhance digestive enzymes and immune responses.

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