www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 13: 69-77 (2013) DOI: 10.4194/1303-2712-v13_1_09

Influence of Dietary Inclusion of Full-Fat Soybean Meal and Amino Acids Supplementation on Growth and Digestive Enzymes Activity of Nile Tilapia, *Oreochromis niloticus*

Abdel-Wahab Abdel-moez Abdel-Warith[1,*], Elsayed Mohammad Younis[1], Al-Asgah, Nasser Abdualla[1]

[1] King Saud University, College of Science, Department of Zoology, Riyadh, Saudi Arabia.

* Corresponding Author: Tel.:+ 966.1 4675758; Fax: +966.1 4678514;
E-mail: aaabdelwarith@yahoo.com

Received 26 September 2012
Accepted 21 December 2012

Abstract

The influence of replacing diet fish meal LT94 (FM) with different levels of full fat soybean meal (FFSB) on growth performance and enzymes activity in stomach, hepato-pancreas and intestine were investigated for Nile tilapia *Oreochromis niloticus*. Four diets (D1, D2, D3 and D4) were formulated 0, 15, 20, and 20 g/100 protein of diets + DL-methionine by replacing FFSB with fish meal respectively. Proximate composition of the diets was determined. The growth of *O. niloticus* was significantly decreased ($P<0.05$) with substitution of fish meal by FFSB (D2, D3 and D4) compared to the control group (D1). The lowest weight gain (46.02±1.30 g), specific growth rate (SGR: 2.29%±0.03), protein efficiency ratio (PER: 2.25±0.04) and apparent net protein utilization (ANPU: 36.37%±0.29) were achieved at D4. Regression analysis did not show a significant differences ($P>0.05$) for proteolytic and trypsin activities in the intestine and stomach among the data of all fish groups. However, hepato-pancreas proteolytic and trypsin activities showed significant decrease ($P<0.05$) with increase of FFSB magnitude in the diets. Activity of amylase and lipase in hepato-pancreas, stomach and intestine displayed significant differences ($P<0.05$) between control group and fish of the tested diets groups. The carcass composition of fish did not exhibit a significant difference.

Keywords: *Oreochromis niloticus*, full-fat soybean, amino acids, enzymes activity.

Introduction

Substantial research effort in the recent years has been conducted towards the replacement of fishmeal by sustainable alternative sources, such as plant ingredients. The suitability of this replacement in terms of growth performance is highly variable among fish species and experimental conditions. Available knowledge shows that a sensible blend of different plant protein sources is needed to balance the indispensable amino acid profile, and hence minimizing the required amino acid supplementation (Kaushik *et al*., 2004; Schulz *et al*., 2007; Silva *et al*., 2009; Dias *et al*., 2009). Tilapia is one of the most important commercial aquaculture species in developing countries (FAO 2004). However, good quality feed to meet the nutritional requirements of tilapia for optimal aquaculture remains a major constraint (Fitzsimmons 2000). Economical tilapia feeds contain approximately 5–10 kg/100 kg of fish meal, but high cost, limited supplies and quality fluctuations of fish meal in the future will demand substitution ingredients as protein sources Naylor *et al.* (2000). Tilapia nutrition with emphasis on partial or total replacement of fish meal by low cost plant protein sources in tilapia diets (El-Sayed, 1999; Abdel-Warith, 2008). In general, high substitutions of plant protein sources as a complete replacement for fishmeal protein have resulted in poor growth and feed efficiency in fish (Venou *et al*., 2003). Several studies have shown that replacement of high-quality fish meal with plant protein sources causes a reduction in growth (Francis *et al*., 2001; Lin and Luo, 2011; Antolovic *et al*., 2012). Soybean meals (SBM) is low in methionine, and contain several antinutritional factors such as protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins and allergens (Francis *et al*., 2001; Hussain *et al*., 2011). However, many studies have shown considerable success in partially or totally replacing FM with SBM in diets for tilapia (El-Sayed, 1999; El-Saidy and Gaber, 2002). High trypsin inhibitor activity in inadequately heated soybean meal decreases protein and energy digestibility in Nile tilapia (Davies *et al*., 2011), growth performance in Atlantic salmon *Salmo salar* (Sorensen *et al*., 2011).

The objective of this study was to evaluate the replacement of fishmeal with full fat soybean meal in

balanced diet formulations for tilapia *O. niloticus*. The criteria for assessment included the obvious key nutritional parameters such as growth, feed utilization and carcass composition as well as a comprehensive study to examine the effects of soybean with and without amino acids supplementation. Therefore, to determine the effect of full fat soybean meal inclusion diets on the digestive enzyme proteolytic, trypsin, amylase and lipase activities in intestine, hepato-pancreas and stomach of this fish.

Materials and Methods

Experimental System

Fingerlings of *Oreochromis niloticus* with an average body weight and average body length (n=160, 10.92±2.01 g and 8.7±0.38 cm). The fish were distributed into four equal duplicate groups in eight glass tanks being 75 litters of water. Water temperature was maintained at 28±1°C by a thermostatically controlled heater, pH values (7.1-8.0), ammonia (NH_3) (0.07-0.20 mg/L), nitrite (NO_2) (0.15-0.35 mg/L), nitrate (NO_3) (4.35-5.77 mg/L) and dissolved oxygen (5.3-6.7 mg/L) all parameters were monitored twice a week and remained at acceptable levels.

Diet Formulation

Four approximately diets were formulated for different levels of full fat soybean (FFSB) replacing by fishmeal. Table 1 displays the formulation and proximate composition of control and test diets, and Table 2 shows the essential amino acids as a % of protein for each diet. Four diets (D1, D2, D3 and D4) were formulated 0, 15, 20, and 20 g/100 protein of diets +1% DL-methionine by replacing FFSB with

Table 1. Composition and proximate analysis of the control and test diets (g 100 g^{-1} dry weight)

Ingredients	D1	D2 (1:1)*	D3(1:2)	D4(1:2)
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 $MJkg^{-1}$	20.78	20.97	20.77	20.37

*(1:1) rational of replacement fish meal protein : soybeam meal protein in the diets.
[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.

Table 2. Essential amino acids composition (expressed as % of protein) of the control and test diets fed to *Oreochromis niloticus* and their requirements

	D1	D2	D3	D4	Tilapia Requirements*
Arginine	5.99	5.39	5.33	5.76	4.20
Histidine	2.52	2.23	2.25	2.65	1.72
Isoleucine	4.01	3.79	3.55	3.65	3.11
Leucine	6.99	6.70	6.19	6.56	3.39
Lysine	6.03	4.83	4.62	5.30	5.12
Methionine	2.22	1.80	1.33	3.29	2.69
Methionine + Cysteine	2.51	2.15	1.78	3.75	
Phenylalanine	3.90	4.29	4.00	4.19	3.75
Phenylalanine + Tyrosine	6.33	7.30	6.74	7.03	
Threonine	4.36	3.79	3.38	3.84	3.75

* source: Santiago and Lovell (1988).

fish meal respectively with rational replacement of fish meal protein with full-fat soybean protein 1:1, 1:2 and 1:2 with amino acids supplementation for D2, D3 and D4, respectively.

Experimental Procedure

Fish were weighed fortnightly and fed a ration of 2.25% of body weight by hand twice a day six days a week. The experiment was conducted over a 12-week period and the feed intake adjusted according to the biomass. At the end of the feeding trial, five fish from each group were sacrificed gut, hepato-pancreas and stomach were removed then frozen at -80°C for enzymatic analysis. Also a similar group was killed for carcass composition.

Proximate Composition

Proximate chemical compositions of diets and fish tissue for moisture, protein, lipid, ash and gross energy were determined according to (AOAC, 1995).

Determination of Amino Acids

Amino acid analysis was determined using a Dionex electrochemical detector following chromatographic separation. The amino acid profiles are presented in (Table 2).

Determination of Enzymes

Trypsin activity was assayed in test tubes using benzoyl-Arg-*p*-nitroanilide (BAPNA) as substrate according to Erlanger *et al*. (1961). Total proteolytic activity was measured using the casein hydrolysis method of Kunitz (1947) as modified by Walter (1984). Lipase activity was assayed with the aid of a Sigma diagnostic test-kit. Amylase activity was determined by the starch hydrolysis method according to Tietz (1970).

Statistical Analysis

The statistical analysis of the data was done using one way analysis of variance (ANOVA) technique. The means were separated by Fisher's LSD test and compared using Duncan's Multiple Range Test (DMRT) as described by Snedecor and Cochran, (1989). Significant differences were defined at $P < 0.05$.

Results

Growth Performance

Growth performance and feed utilization data for Nile tilapia fed the four diets were designated as D1, D2, D3 and D4 Table 3. There was a significant difference ($P<0.05$) between the final average body weights between fish fed the control diet D1 and the other groups. Fish fed the fishmeal (LT94) based control diet demonstrated the highest mean final body weight (72.21 g) resulting in a 7- fold increase in weight from the initial weight. The specific growth rate (SGR%) values further supported this trend, with SGR reduced from 2.62 for the control diet fed fish to 2.33, 2.31 and 2.29 for the fish fed the other three diets which have been presented in Table 3. No mortality was observed during the experimental period and the overall health of the fish appeared normal**.**

Feed Consumption and Feed Utilization

The control diet was well accepted by the tilapia, while diets containing the partial replacement of FFSB were less palatable so that fish were fed only 2% of body weight for first six weeks increased to 2.5% for the last six weeks. Mean daily feed intake ranged between 0.93 and 0.78gfish^{-1} day^{-1}with significant difference ($P<0.05$) between control and

Table 3. Weight increase, feed consumption, nutritive utilization of *Oreochromis niloticus* fed experimental diets (mean ±SD *n=3*)

	D1	D2	D3	D4
Number of fish	40	40	40	40
Mean initial weight (g)	10.93±2.07	10.9±2.03	10.91±2.09	10.93±1.86
Mean final weight (g)	72.21±4.16^{b}	58.25±1.55^{a}	57.48±1.74^{a}	56.95±1.29^{a}
Mean weight gain (g)	61.28±4.18^{b}	47.35±1.60^{a}	46.57±1.71^{a}	46.02±1.30^{a}
Mean daily feed Intake (gfish^{-1} d^{-1})	0.93±0.04^{b}	0.80±0.02^{a}	0.80±0.01^{a}	0.78±0.01^{a}
SGR (%)[1]	2.62±0.08^{b}	2.33±0.04^{a}	2.31±0.04^{a}	2.29±0.03^{a}
FCR[2]	1.09±0.02^{a}	1.22±0.02^{b}	1.24±0.03^{b}	1.22±0.01^{b}
PER[3]	2.50±0.05^{b}	2.30±0.02^{a}	2.28±0.03^{a}	2.25±0.04^{a}
ANPU (%)[4]	43.92±0.75^{b}	38.28±0.39^{a}	36.97±0.69^{a}	36.37±0.29^{a}

Values in the same row with the same superscript are not significantly different ($P>0.05$).

[1] SGR: [Ln final bw (g) - Ln initial bw (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 intake (g) × 100

other groups. There was a noticeable effect of the dietary inclusion of alternative protein sources on feed intake (Table 3). Feed intake for tilapia fed on control diet containing the highest amount of fishmeal was significantly better than those observed for fish fed diets including FFSB even with amino acid supplementation. FCR values also differed significantly ($P<0.05$) between the control group and fish fed on diets containing FFSB. Protein efficiency ratio (PER) was noticeably different between treatments and supported the same trend. The fish fed the control diet displayed superior PER (2.50) while fish receiving the different levels of FFSB exhibited PER of 2.30, 2.28 and 2.25. Apparent net protein utilization (ANPU%) values also showed reduction when fishmeal was replaced by the FFSB source. These values ranged from 43.92 to 36.37 (Table 3).

Essential amino acids profile of the experimental diets shows a declining value for most amino acids with each FFSB increment in the diets. However, the diet supplemented with 1% DL-methionine showed the closest values to the control diet (Table 2). This was especially apparent for the total sulphur amino acids (Met+Cys) which showed 2.51 for the control diet while tilapia have a requirement for methionine of 2.69%. The other diets containing different levels of FFSB showed methionine deficiency while, diet containing 1% DL-methionine showed the highest methionine value which was 3.75, sufficient for tilapia requirement of methionine. Lysine also support this trend which has improved the lysine deficiency found in diets including 15 and 20 g/100g protein of FFSB in diet without methionine supplementation which resulted in 4.83 and 4.62% of protein respectively. Whereas, the requirements was 5.12, and these results have been listed in Table 2.

Fish Body Composition

Initial and final carcass composition of the fish fed the experimental diets is presented in Table 4. The final carcass composition showed little significant variation of their proximate composition as a result of the diet formulations. Fish fed the fishmeal based control diet and different levels of FFSB diets did not yield any variations in the moisture, protein content ($P>0.05$) but was a significant reduction in percentage lipid from 9.70 to 8.88% on the higher FFSB diets, whilst ash content showed slight differences among groups (Table 4).

Gastro-Intestinal Enzyme Activity

Table 5 shows total proteolytic, trypsin, amylase and lipase activities in the intestine, hepato-pancreas and stomach. Total proteolytic (sum of pHs 1.5, 3, 4, 7, 8.5, 9, and 10) activity of the intestine was higher than the activity in the hepato-pancreas and stomach and ranged between 7.92 to 10.45 μg tyrosine $minute^{-1}$ mg^{-1} protein. However, average proteolytic activity among fish fed the four experimental diets did not show any significant differences ($P>0.05$) for the intestine and stomach (Table 5). hepato-pancreas proteolytic activity was lower than stomach activity, and the mean of proteolytic activity showed a significant difference ($P<0.05$) among fish fed the control diet and test diets. Fish fed on control diet and D 2 showed similar results 0.57 and 0.50 μg $tyrosine^{-1}$ $minute^{-1}$ mg^{-1} protein respectively, however, fish fed D3 and D4 showed a significant difference ($P<0.05$) between these groups and control diets 0.26 and 0.18 respectively. Figure 1 shows the enzymatic activity determined at different pHs for tilapia fed control and test diets. For the intestine, the higher proteolytic activity was at neutral and alkaline pHs, whereas only very low activity was shown at acidic pH. However, hepato-pancreas proteolytic activity was appreciably higher at alkaline pHs only for control and D2, whereas D3 and D4 showed lower values for all pHs. In contrast, higher proteolytic activity was observed at acidic pH whereas, the lower values were observed at alkaline pH (Figure 1) in the stomach. Moreover, trypsin activity was also observed to be higher in the intestine than the hepato-pancreas, but did not show any significant differences ($P>0.05$) among groups. However, in the hepato-pancreas , trypsin activity was significantly higher ($P<0.05$) in fish fed control diet 10.44 μg tyrosine $minute^{-1}$ mg^{-1} protein than in fish fed on D3 and D4 (2.95) and 1.43) respectively, whereas fish fed on D2 and control diet did not show significant differences. Therefore, amylase activity showed higher values in hepato-pancreas than in intestine and stomach (Table 5).

The highest amylase activity was observed in hepato-pancreas of fish fed the control diet (4.99) followed by that of fish fed on D 2 (3.98). Fish fed D 3 and 4 had lower amounts of amylase (2.76 and 3.18 respectively). Only a small amount of amylase activity was detected in the intestine and stomach (Table 5). For intestinal lipase activity the lowest

Table 4. Body composition of *O. niloticus* fed graded levels of FFSB (mean ± SD *n=3*)

	Initial fish	D1	D2	D3	D4
Number of fish	20	10	10	10	10
Moisture	72.35	69.31±2.89^{a}	70.82±1.20^{a}	71.28±0.91^{a}	71.73±2.35^{a}
Protein	12.11	16.72±1.68^{a}	15.79±0.40^{a}	15.45±0.36^{a}	15.39±1.10^{a}
Lipid	7.96	9.70±0.61^{b}	9.64±0.89^{ab}	8.76±0.96^{a}	8.88±0.69^{a}
Ash	2.85	3.55±0.40^{a}	3.47±0.32^{a}	3.71±0.15^{a}	3.52±0.28^{a}

Values in the same row with the same superscript are not significantly different ($P>0.05$).

Table 5. Total proteolytic (Pro), trypsin (Tr), amylase (Am) and lipase (Li) activities in intestine, hepato-pancreas and stomach of *O. niloticus* fed control and test diets determined at 37°C (mean ±SD *n=3*)

	No. of fish	Pro. (mean)	Pro. Sum of pHs* (µg tyrosine/min/mg protein)	Tr. (µg tyrosine/min/mg protein)	Am. (µg maltose/ml/min)	Li. Sigma/Tietz/ unit/L)/min/ml
Intestine						
D1	5	1.13±0.71^{a}	7.92	17.94±3.37^{a}	0.80±0.21^{ab}	1.74±0.62^{b}
D2	5	1.41±0.78^{a}	9.90	17.90±1.96^{a}	0.79±0.28^{ab}	1.78±0.72^{b}
D3	5	1.39±0.88^{a}	9.74	17.82±4.45^{a}	0.65±0.04^{a}	1.67±0.36^{b}
D4	5	1.49±1.04^{a}	10.45	17.80±2.58^{a}	0.83±0.12^{b}	0.95±0.30^{a}
hepato-pancreas						
D1	5	0.57±0.30^{c}	3.98	10.44±6.20^{b}	4.99±0.79^{b}	1.18±0.30^{c}
D2	5	0.50±0.29^{bc}	3.47	9.05±8.31^{b}	3.98±1.62^{ab}	0.66±0.33^{b}
D3	5	0.26±0.04^{ab}	1.83	2.95±1.72^{a}	2.76±1.35^{a}	0.44±0.25^{ab}
D4	5	0.18±0.02^{a}	1.25	1.43±0.37^{a}	3.18±1.32^{a}	0.26±0.19^{a}
Stomach						
D1	5	0.68±0.36^{a}	4.76	ND	0.59±0.06^{ab}	ND
D2	5	0.70±0.42^{a}	4.92	ND	0.64±0.08^{b}	ND
D3	5	0.77±0.39^{a}	5.40	ND	0.52±0.12^{a}	ND
D4	5	0.63±0.58^{a}	4.38	ND	0.67±0.15^{b}	ND

Values in the same column with the same superscript are not significant (P> 0.05).
ND: not detected
*: Total proteolytic activity was obtained as the sum of those determined at pH 1.5, 3, 4, 7, 8.5, 9, and 10.
Total proteolytic activity was obtained as the sum of those determined at pH 1.5, 3, 4, 7, 8.5, 9, and 10.
Proteolytic activity was expressed as the amount of tyrosine (µg) digested by 100µl of enzyme solution /minute/mg protein at acid, natural and alkaline pHs at 37°C.
Trypsin activity was expressed as the amount of tyrosine (µg) liberated by 0.5ml of enzyme extract per minutes /mg protein at 37°C.
Amylase activity was expressed as the amount of maltose liberated by 50µl of enzyme extract /minute/ml at 37°C.
Lipase activity was expressed as the amount of fatty acids neutralized by 0.05 NaOH liberated by 1ml enzyme solution /minute at 37°C.

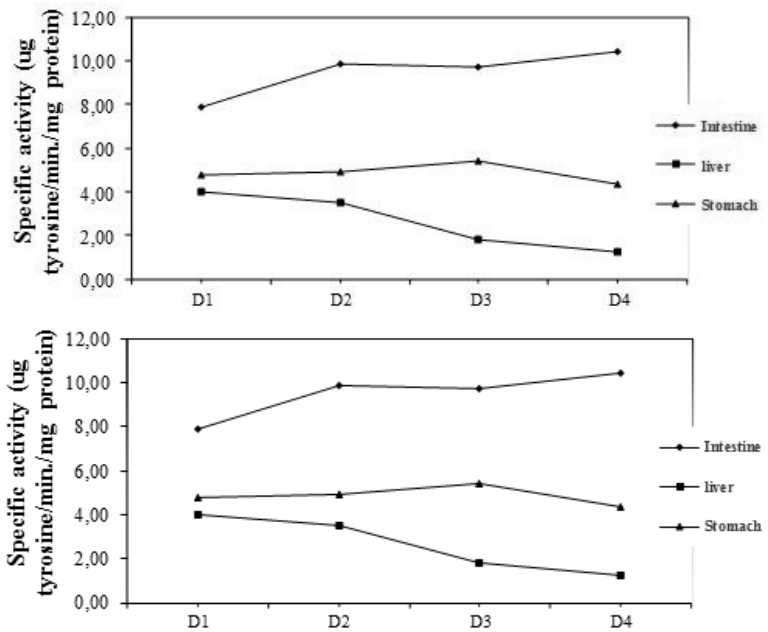

Figure 1. Total proteolytic activity in intestine, hepato-pancreas and stomach in tilapia fed different levels of FFSB (Top) is total proteolytic activity (PA) for control and test diets, (Bottom) is average PA affected by different pHs (mean values ±SD *n=3*).

level was observed in fish fed D 4 (0.95) followed by D3 (1.67) whereas fish fed control diet and D2 showed slightly higher values (1.74 and 1.78 respectively) (Table 5). hepato-pancreas lipase activity was significantly (P<0.05) higher in fish fed the control diet 1.18 whereas fish fed on diets 2, 3, and 4 exhibited low values 0.66, 0.44, and 0.26 respectively.

Discussion

The findings of the present study, the results exhibit that plant protein sources such as full fat soybean are unable to substitute 15g/100 protein of a high quality fishmeal protein in the diets of Nile tilapia *O. niloticus*. Growth and feed utilization decreased with diet (D2, D3 and D4) which contain 15 or more of the total protein from fishmeal was substituted with the full fat soybean meal these might be the ration of replaced is high also, these diets contain amino acids lower than the minimum requirements of this fish. These results were in agreement with data obtained by Santigosa *el al*. (2008) they reported that replacement of fish meal by plant proteins caused a decrease in growth in both trout *Oncorhynchus mykiss* and sea bream *Sparus aurata*. Additionally, tilapia fed with D4 (FFSB with DL-methionine supplementation) did not show any improvement in the growth performance compared to the unsupplemented diet these might be the amount of amino acids supplemented to diet 4 is not sufficient to enhance the growth. Similar findings were also reported by Davies and Morris, (1997). We are view that supplemented crystalline amino acids might not be efficiently utilized by Nile tilapia due to the presence of several anti-nutritional factors limited dietary amino acid utilization. A similar results were obtained by Luo *et al*. (2006), they reported that *Oncorhynchus mykiss*, fed on diets containing solvent extracted cotton seed meal up to 75% (SCSM75), declined the growth performance when the dietary lysine levels were lower than 21.1 g/ kg^{-1}). Other investigations have suggested that weight gain, feed conversion ratio, protein efficiency ratio and protein digestibility in hybrid tilapia (*O. niloticus* × *O. aureus*) can be supported by diets containing full-fat soybean and defatted soybean levels up to 30% replacement of fishmeal (Shiau *et al*., 1990). Therefore, Abdel-Warith (2008) concluded that Nile tilapia is able to utilize plant protein based diets from different types of soybeans (SPC, SF, SBM and FFSB) up to 50% of total protein in the diets. In addition, Antolovic *et al*. (2012) reported that, inclusion of soybean meal up to 34% in saddled bream diets did not affect the growth rate when compared with the control diet containing 84% fish meal. In accordance to results obtained by (El-Saidy and Gaber, 2002) they demonstrated that soybean meal supplementation with 10 g/kg methionine and 0.5 g/kg lysine could replace total FM protein in Nile tilapia diets these due to the high amino acids supplementation. Imbalanced dietary amino acid could affect the digestion, absorption and metabolism of these nutrients also can occurs inadequate between digested protein and crystalline amino acids (Aragao *et al*., 2004; Dabrowski *et al*., 2007). In the present study, the poor performance might be associated with the high substitution of plant proteins is an imbalance of nutrients, especially protein composition. This may be related to a less adequate dietary amino acid profile when FFSB is added to the formula as this latter ingredient is considered deficient in both lysine and methionine. The data obtained with tilapia in this study indicated that methionine supplementation was insufficient to improve the protein quality.

Therefore, certain anti-nutritional factors (ANF's) are also known to specifically interfere with the digestive enzymes in the gastro-intestinal tract and associated organs such as the hepato-pancreas and pancreas thereby suppressing digestion and absorption. Furthermore, in relation to the present study a decrease in growth rate was observed in all fish fed FFSB diets when compared with the fishmeal reference diet.

Investigations of digestive enzyme activities constitute an essential aspect of understanding the physiology of the digestive tract and the nutritional requirements of specific stages of development (Le Moullac *et al*., 1997). Present study, in which we observed a high amount of proteolytic actually present in the intestine and stomach compared to the hepato-pancreas.

Protease inhibitors are common anti-nutrient substances in many plant derived nutritional staff of potential value, especially the legumes (Norton, 1991). Also protease inhibitors particularly in oil seeds are known to decrease the growth performance in fish (Liener, 1994; Sriket *et al*., 2011). However, Kuz`mina (1990) observed a high proteolytic potential in non-carnivorous fish. This may be understood, on the basis that plant proteins are more difficult to digest by fish compared to animal protein and fish meal. Moyano *et al*. (1999) demonstrated that *O. niloticus* displayed a more sensitivity to protease inhibitors present in defatted soybean meal, corn gluten meal and wheat bran on alkaline protease activity than sea bream *Sparus aurata* and African sole *Solea senegalensis*. Only minor activity at acidic pH was detected in the intestine and hepato-pancreas whereas a high amount was actually observed in the stomach. It was interesting that the hepato-pancreas showed the lowest amount of protease at acidic pH due possibly to the fact that there are some intra-cellular enzymes with an optimal acidic pH (Kuz`mina, 1990). Data in the present study were also in agreement with (El-Beltagy, 2004) who reported that the partially purified acidic protease had the highest activity at pH 2.5 and it then decreased with increasing of pH. These related to the acidity in the stomach also causes lysis of plant cell walls in macrophyte feeding fish such as tilapia. Hydrolysis of the cell walls by HCl, facilitated by partial crushing of the ingested material by pharyngeal teeth, allows the plant cell contents to be subjected to the actions of the proteolytic enzymes (De Silva and Anderson, 1998). In contrast, at alkaline pHs intestine and hepato-pancreas both showed a higher proteolytic activity.

The present findings agree with results observed by Moyano *et al*. (1999) who reported that the high

sensitivity for protease activity in the digestive tract of tilapia were optimum at alkaline pH. On the other hand, Hidalgo *et al*. (1999) reported that the proteolytic activity in the digestive tract of eel was detected at an acidic pH (pH 1.5) however trout showed opposite results which were more active at alkaline pHs. These variations between tilapia and other species might due to the construction of digestive tract and nutritional habits. However, in the present work, the optimum protease activity was recorded in different organs which showed a different activity i.e. for intestine and hepato-pancreas, optimum pH ranged between 7.0-8.5, stomach 1.5-3.0 in tilapia. However, in the thick walled muscular stomach such as in the African catfish the pH is fairly high, around 4 (Uys and Hecht, 1987). Therefore, present results have demonstrated that the higher enzymatic activity at pH 8.5 than at 7.0 for tilapia agree with other species such as carp, trout, and sea bream (Hidalgo *et al*., 1999). The detected results at high alkaline pHs (9.0 and 10.0) might be attributed to alkaline protease having carboxypeptidase, elastase or collagenase-like activities.

The study on this species can be seen that quite sensitive to the amount of trypsin inhibitor in intestine and hepato-pancreas. Generally a considerable reduction of trypsin activities was found in the hepato-pancreas for tilapia fed high inclusion levels of FFSB. In agreement with these findings (Robaina *et al*., 1995) observed that gilthead sea bream fed diets containing soybean meal showed a reduction in trypsin activity and protein digestibility when substitution levels increased.

The amylase activities in various organs (intestine, hepato-pancreas and stomach) also varied for tilapia in this study. The higher levels were shown in the hepato-pancreas rather than intestine and stomach. However, amylase activity in hepato-pancreas was affected in fish fed high FFSB inclusion levels in diet formulation for tilapia. The low amount of amylase in the stomach indicates that very little starch is digested before the food reaches the foregut. This supports the findings of other authors (Uys and Hecht, 1987). Al-Owafeir (1999) reported that α-amylase activity was present in Nile tilapia, may indicated that Nile tilapia is more adapted to utilization and digestion of carbohydrate more than in the African catfish. In contrast, (Santigosa *et al*., 2008) reported that the replacement of fish meal by plant protein did not affect α-amylase activity in both trout and sea bream. These variations are due to (feeding habits) carnivores, omnivorous fish and diets formulation. Tengjaroenkul *et al*. (2000) suggested that lipolytic activity in *O. niloticus* is definitely present, and occurs mainly in the anterior half of the intestinal tract. The relatively restricted distribution of lipase enzyme in the Nile tilapia may be due to the fact that lipase activity is lowest in herbivorous fish (Opuszynski and Shireman, 1995), related to the low fat content in plant materials naturally consumed by tilapia. Das and Tripathi, (1991) reported that lipase activity was generally highest in the hepatopancreas of both the adult and fingerling grass carp *Ctenopharyngodon idella.* This latter study supported the present findings, which found lipase activity was present in intestine and hepato-pancreas for tilapia, therefore, the amount of lipase was decreased by the substitution of FFSB. In contrary, Lipase activity is almost non-existent in the stomach, and in the intestine the principal site for lipase is the mucosal layer.

In conclusion, the investigation has demonstrated that tilapia is unable to grow fairly effectively with high replacement of the fishmeal component of the diet with full fat soybean meal. Growth was appreciably compromized and feed utilization was much lower than the control diet group of fish. The physiological processes of digestion were noticeably impaired and specific gastro-intestinal enzymes were affected by the change in diet composition. So, future investigation should focused to use these ingredients with supplementation highly amount of amino acids, phosphorus, phytase and some enzymes to improve the performances of plant protein such as oil seeds.

Acknowledgments

This project was supported by the Research Center, College of Science, King Saud University.

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