Effects of Fish Meal Replacement with Soybean Meal and Use of Exogenous Enzymes in Diets of Nile Tilapia (*Oreochromisniloticus*) on Growth, Feed Utilization, Histopathological Changes and Blood parameters

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Abstract: The present study was conducted to determine the effects of commercially prepared exogenous multi-enzyme preparations on growth performance, histopathological changes, and some blood parameters in Nile tilapia fed soybean meal plant-based diets. 180 fish were divided into 3 triplicate groups. The diets (T1 control diet with fish meal, and T2) were formulated to supply 32% crude protein and 3000 kcal digestible energy/kg diet. T3 was formulated to supply 32% crude protein and 2760 kcal digestible energy/kg diet. The used commercial enzyme complexes Pan Zyme and Phytase-plus broiler 500 were added to diets T2 and T3 without addition of fish meal. At the end of the 83-day experiment, four fish from each aquarium were individually weighed, sacrificed, blood samples were collected. Liver, kidney spleen and intestine samples were taken for histological examination. The best overall growth response was significantly obtained in tilapia fed the control diet. There were no significant differences between different groups in case of liver enzymes measurements, total protein, albumin, cholesterol, uric acid, creatinine and thyroxine. On the other hand, glucose revealed significant decrease in both groups T2 and T3. Triiodothyronine showed significant decrease in group T2 then group T3. The histopthological picture of fish at T2 and T3 showed mild to moderate enteritis. The negative effect of soybean meal on growth could be mainly explained by a general decline in feed intake combined with reduced nutrient availability that may be caused by SBM-induced enteritis. Enzymes addition could not prevent the growth retardation caused by total fish meal replacement.

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1.Introduction

Fish meal has traditionally been used as the main feed ingredient in preparation of aqua feeds, due to its high protein content and balanced amino acids profile. Because of its recent shortage in global production, coupled with increased demand and competition for its use in livestock and poultry feeds, its prices have become unaffordable (Tacon, 1993). Researchers are encouraged to use plant protein sources to replace fish meal without reducing the performance. A drawback in the use of soybean protein in fish diets is the presence of phytate (a major phosphorus component in soybean meal) which cannot be inactivated by heat treatment (Francis *et al.,* 2001). The bioavailability of mineral elements and dietary protein can be reduced by phytate. So phytate-P is not available to monogastric animals including fish without enzymatic reactions (Wang *et al.,* 2009). Increasing the availability of P from SBM is desirable to reduce the amount of supplemental P in diet formulation and limit the P loading into the environment. The incorporation of phytase into fish feeds could effectively increase the availability of P to various fish species such as common carp (Schafer *et al.,* 1995 and Nwanna *et al.,* 2007), channel catfish (Eya and Lovell, 1997; Li and Robinson, 1997; Yan *et al.,* 2002) and Korean rockfish (Yoo *et al.,* 2005). SBM also contains non-starch polysaccharides that are not efficiently digested by most fish species. The use of exogenous enzymes, such as xylanase,α-galactosidase, β-glucanase and endo-β-mannanase, to make this oligosaccharide fraction more digestible has shown positive results in some terrestrial species such as poultry (Ward and Fodge, 1996; Lobo,1999). The use of protease to improve protein digestibility has been extensively studied in poultry (Ghazi *et al.,* 2003), pigs (O-Doherty and Forde, 1999) and also in hybrid tilapia (Lin *et al.,* 2007).

No direct evidence that soybean represent a possible danger for health or for hepatocytes Manuela *et al.* (2002).Teixeira *et al.* (1998) have suggested that isolated soy protein significantly lowered total and LDL (low density lipoprotein) cholesterol and in some cases maintained HDL (high density lipoprotein) cholesterol concentrations in mildly hypercholesterolemic men. Wong *et al.* (1995) reported a significant decrease in LDL cholesterol and a significant increase in HDL cholesterol concentrations in young females supplemented with soy protein. However, not all observations have been

positive (the review by Anderson *et al.,* 1995, Gooderham *et al.,* 1996, and Jacques *et al.,* 1992).

Forsythe (1995) suggested that the elevation in blood thyroxine concentrations preceding the decline in blood cholesterol concentrations is consistent with a potential mechanism for cholesterol lowering effect of soy protein. However, no relationship betweenthe effect of soy protein and blood cholesterol concentrations via thyroid status has been observed in rats and hamsters (Balmir *et al.,* 1996, Potter *et al.,* 1996).

The present study was conducted to determine the effectsof commercially prepared exogenous multi-enzyme preparations, Pan zyme and Phytase-plus on growth performance, carcass composition, some blood parameters and histopathological changes in Nile tilapia fed plant-based diets. The questions were if these multi enzyme preparations will be able to improve nutrient utilization, and whether this improvement is enough to reduce part of the energy in the fish diet.

2.Material and Methods

Experimental procedure

Experimental fish were obtained from a fish farm in El-Tal El- Sagher, Ismailia, Egypt. All Nile tilapia (*Oreochromisniloticus*) were reared in 500 L tank filled with aerated fresh water for 2 weeks to acclimate to the experimental conditions. Fish were fed twice daily on the control pelleted diet. At the start of the experiment, the fish were fasted for 24 hrs and weighed individually. Ten fish were sampled randomly for determination of whole body proximate composition. Twenty (9.29 g average initial body weight) fish per glass aquarium (80 x 40 x 45 cm) were used with three aquaria per treatment.

Fish were hand fed twice daily (at 9 am and 3 pm) 7 days a week, at a rate equal 3% of their body weight. Fish were bulk weighed every 2 weeks and the diet adjusted accordingly. Each aquarium was supplied with low-pressure automatic aerator to provide continuous aeration via air stone. During the experimental period, the water temperature was maintained at 27±1ºC, pH 7.21 ± 0.12 and the photoperiod used was a 14 h light/10 h dark cycle. Each aquarium was cleaned every other day at morning prior to feeding by siphoning the wastes which had accumulated on the bottom. About two-thirds of the water was replaced by aged aerated water from the storage tanks every other day. Dead fish were recorded and removed daily. At the end of the 83-day experiment, four fish from each aquarium were individually weighed, sacrificed. Blood samples were collected in a clean centrifuge tube for serum separation. The viscera were removed. Then the liver and the carcass were weighed individually. These data were used to calculate dressing percentage and hepatosomatic index (HSI). Liver, kidney, spleen and intestine samples were taken for histological examination. Further on day 83, four fish were sampled randomly from each replicate group, sacrificed and frozen for determination of whole body proximate composition.

Diets preparation

The diets (T1 control diet, and T2) were formulated to supply 32% crude protein and 3000 kcal digestible energy/kg diet (NRC, 1993), T3 was formulated to supply 32% crude protein and 2760 kcal digestible energy/kg diet (less energy and no oil) (Table 1). The used commercial enzyme complexes Pan Zyme(multienzymes) and Phytase-plus broiler 500 were added to diets T2 and T3 (SBM plant based diets). The diet ingredients were finely ground and thoroughly mixed. Then sufficient amount of cool water (about 400 ml/kg diet) were added and mixed to obtain stiff dough. The dough was passed through die (2 mm) of a meat mincer. The pelleted diets were air dried by electric fan at room temperature for 24 hrs, then packed in plastic bags and refrigerated at 4 ºC until use (El-Ashram and El-Boshy, 2008).

Studied parameters

Fish performance was assessed by the following:

1- Initial and final mean body weight (IBW, FBW, g).

2- Body weight gain (WG) = FBW- IBW (g/fish)

3- Specific growth rate (SGR) = 100x [logFBW (g) - logIBW (g)]/ time (days)

4- Feed consumed (g/ fish) =total feed consumed over 83 days (g)/ number of fish.

5- Feed efficiency ratio (FER) (g/g) = WG (g)/feed consumed (g).

6- Protein efficiency ratio (PER) (g/g) = WG (g)/ protein consumed (g).

7- Apparent energy utilization (AEU) (%) = energy gain (MJ/fish) x 100/energy intake (MJ/fish).

8- Survival rate percentage = 100x (total number of fish at the end of the experiment/ total number of fish at the start of the experiment).

9- Dressing percentage = 100x (dressed carcass weight (g)/live weight (g).

10- Hepatosomatic index (HIS) (%) = 100x (liver weight (g)/body weight (g)).

Serum biochemical parameters

Serum was separated for biochemical studies: serum total proteins (TP), serum albumin (ALB), alanine aminotransferase (ALT), aspartate amino transferase (AST) activities according to (Tietz 1986). The above mentioned serum biochemical parameters were assayed using reagent kits supplied by StanBio-Laboratories incorporation, USA. Serum glucose was assessed according to Trinder (1969) using kits supplied by Bichon Analyticon, Germany. Serum urea

and creatinine were determined using kits of Bio Merieux (France) according to Caraway (1963). Serum cholesterol measured by the enzymatic method described by Richmond (1973). Serum hormone concentrations were assayed radioimmunologically using kits, thyroid hormones from CIS Bio International (France).

Table (1): Ingredients and composition of experimental diets

Ingredients %	T1	T2	T3
Fish meal (60.05)[1]	20.00	-	-
Soya bean meal (45%)[1]	23.86	43.00	43.00
Corn gluten (62)[1]	8.00	14.97	14.13
Ground yellow corn (8.5)[1]	16.84	17.06	14.18
Wheat flour (11.43)[1]	25.30	16.77	23.50
Vegetable oil	3.00	3.00	-
DL-Methionine	-	0.20	0.17
Dicalcium phosphate	-	0.57	0.63
Ground limestone	-	1.23	1.19
Minerals and vitamins premix[2]	3.00	3.00	3.00
Vitamin C (mg/kg diet)	50	50	50
Pan Zyme[3]	-	0.1	0.1
Phytase-plus broiler 500[4]	-	0.1	0.1
Calculated composition:			
Crude protein %	32	32	32
DE (kcal/kg)	3000	3000	2760
P/E ratio (mg protein/kilocalories DE)	106.7	106.7	115.9

[1]Determined according to AOAC, 1995.

[2] Each 3 kg contain the following vitamins and minerals: Vit.A 15mIU, vit.D3 2 mIU, vit.E 1000mg, vit.k3 1000mg, vit.B1 1000mg, vit.B2 5000mg, vit.B6 1500mg, vit. B12 10mg, biotin 50mg, pantothinic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 60000mg, zinc 50000mg, iron 30000mg, copper 4000mg, iodine 300mg, selenium 100mg, cobalt 100mg, carrier(CaCO3) to 3kg. (Golden premix- Selim Pharm Elasher, Egypt).

[3]Pan Zyme (multiple enzymes) each 1 kg contains: xylanase enzyme 15.000.000 I.U, acidic proteinase 540.000 I.U, neutral proteinase 450.000 I.U, cellulase 600.000 I.U. produced by Bytara for Pharmaceuticals Technology under license of VTR Company Sadat Industrial City, Egypt.

[4]Phytase-plus broiler 500, each 1 kg contains phytase enzyme 500.000 I.U, vitamin $D_3$2.000.000 I.U, wheat bran and calcium carbonate up to 1 kg produced by Bytara for Pharmaceuticals Technology under license of VTR Company Sadat Industrial City, Egypt.

Histopathological parameters

Liver, kidney,spleenand intestine were examined then dissected and fixed in 10% neutral buffered formalin. Following fixation the specimens were carefully washed in running tap water, dehydrated in an ascending series of alcohol, cleared in xylene and then embedded in paraffin wax. Sections of 5µ thickness each were cut and stained with haematoxylin and eosin. Sections were then investigated under light microscope according to Bancroft and Gamble(2007).

Statistical analysis

Obtained data were analyzed by one-way ANOVA. Differences between means were tested at the 5% probability level using Duncan Multiple Range test. All the statistical analyses were done using SPSS program version 16 (SPSS, Base for windows.2007. Chicago, IL, USA) as described by Dytham (1999).

3.Results

Growth response, feed efficiency, protein and energy utilization by tilapia are presented in table 2, which shows that the best overall growth response was significantly obtained in tilapia fed the control diet, T1. Feed utilization indices followed a similar pattern as the growth in the treatments (Table 2). However there was a trend that growth, feed utilization indices were higher in T2 compared to T3. Moreover, PER was significantly higher in T2 compared to T3. Survival rates and HSI values did not show any differences relating to diet treatment. No significant differences occurred in body composition of tilapia fed different experimental diets, except in the crude protein content that was significantly higher in T3 and slightly higher in T2 compared with the control group T1 (Table 3).

In this study we noticed that there were no significant differences between different groups in case of liver enzymes (ALT and AST) measurements. The same occurred in assaying total protein and albumin. On the other hand, glucose revealed significant decrease in both groups T2 and T3.In our research cholesterol showed insignificant reduction among different groups. Concerning kidney functions (uric acid and creatinine) no change occurred between different groups. Triiodothyronine (T3) hormone showed significant decrease in group T2 then group T3 while thyroxine (T4), revealed no significant change between different treatments (Table 4).

Liver:

Liver of fish inT1 (control diet) showed normal polyhedral heptocytes with normal cytoplasm and centrally located nuclei (Figure 1 A). The liver of T2 revealed focal to diffuse areas of vacuolar degeneration (Figure 1 B). Whereas T 3 showed mild focal necrotic cells with pyknosis and karryorhexis of their nuclei (Figure 1 C).

Table (2): Growth performance of Nile tilapia fed different experimental diets

Parameters	T1	T2	T3
Initial weight (g.fish-1)	9.30	9.29	9.30
Final weight (g.fish-1)	41.99±1.27 a	26.61±0.92 b	23.99±0.21b
Body weight gain (g.fish-1)	32.69±1.27 a	17.33±0.92b	14.69±0.21b
Specific growth rate (SGR,%BW/day)	0.78±0.02 a	0.54±0.02 b	0.49±0.01 b
Feed consumed (g.fish-1)	47.94±0.73a	41.32±1.12b	38.40±0.60 b
Feed Efficiency ratio (FER)	0.68±0.02 a	0.42±0.03b	0.38±0.00 b
Protein Efficiency ratio (PER)	0.65±0.12 a	0.45±0.17 b	0.41±0.01c
Apparent energy utilization (AEU)%	23.32±1.63 a	15.27±1.80 b	14.38±0.40 b
Survival rate %	90.00±2.88	86.67±1.67	93.33±1.67
Dressing percentage	84.25±0.67 a	81.52±0.69 b	82.23±0.65 b
Hepatosomatic index (HSI)	2.82±0.32	2.60±0.18	3.15±0.24

a-c Means in the same row with different superscripts are significantly different ($p \leq 0.05$); values are presented as means ± SE.

Table (3): Body composition of Nile tilapia fed different experimental diets (on % wet basis) 1

Parameters	T1	T2	T3
Moisture	70.69±0.74	71.53±0.65	71.71±0.22
Crude protein	15.71±0.41 b	16.16±0.24ab	16.87±0.05 a
Ether extract	5.12±0.31	5.08±0.53	4.08±0.29
Ash	3.67±0.14	3.34±0.12	3.50±0.24
Gross Energy (MJ.Kg-1)2	5.74±0.21	5.83±0.27	5.60±0.12

1. Composition of the fish killed at the beginning of the experiment (moisture, 74.65 %; crude protein, 14.41 %; ether extract, 3.52%; ash, 4.27 % and gross energy, 4.80 MJ.Kg-1). Determined according to AOAC, 1995.
2. The gross energy content of fish was calculated from the fat and protein contents, using the equivalents of 39.54 MJ.kg-1 for fat, and 23.64 MJ.kg-1 for crude protein (Kleiber, 1975).
a-b Means in the same row with different superscripts are significantly different ($p \leq 0.05$); values are presented as means ± SE.

Table (4): Effect of different experimental diets on serum parameters of Nile tilapia fed

Parameters	T1	T2	T3
ALT	6.33±0.67	6.67±2.9	11.00±2.00
AST	12.67±1.45	10.00±3.01	12.83±1.36
Total Protein	3.13±0.43	2.83±0.12	3.13±0.43
Albumin	1.00±0.17	1.00±0.06	1.17±0.14
Glucose	72.00 ±7.51 a	49.33 ±4.84 b	54.00 ±4.16ab
Cholesterol	158.00±7.51	138.33±26.56	112.67±11.62
Uric acid	0.67±0.42	0.97±0.52	0.95±0.48
Creatinine	0.7±0.15	0.3±0.06	0.47±0.17
Triiodothyronine (T3)	234.23±26.03 a	146.1 ±18.96 b	175.93 ±10.33ab
Thyroxine (T4)	12.33±1.41	8.94±1.43	8.12±3.05

a-b Means in the same row with different superscripts are significantly different ($p \leq 0.05$); values are presented as means ± SE.

Kidney:

Kidney of T1 showed mild focal degeneration of epithelial lining renal tubular epithelium (Figure 2 A). Kidneys of T 2 mild degeneration in addition to congestion of peritubular capillaries (Figure 2 B). Kidneys of T3 showed mild degeneration and hyperplasia of melano-macrophage centers (MMC) (Figure 2 C).

Spleen:

Spleen of T1 showed normal population of both white pulp and red pulp (figure 3 A). Mild hyperplasia of MMC was observed in spleen of both T2 and T3 (Figure 3 B&C).

Intestine:

Intestine of T1 showed normal intestinal mucosa while T2 showed mild degeneration of intestinal mucosa along with focal detachment of epithelial limning. Degeneration of intestinal mucosa & mild enteritis characterized by aggregation of mononuclear cells mainly lymphocytes were observed in T3 (Figure 4).

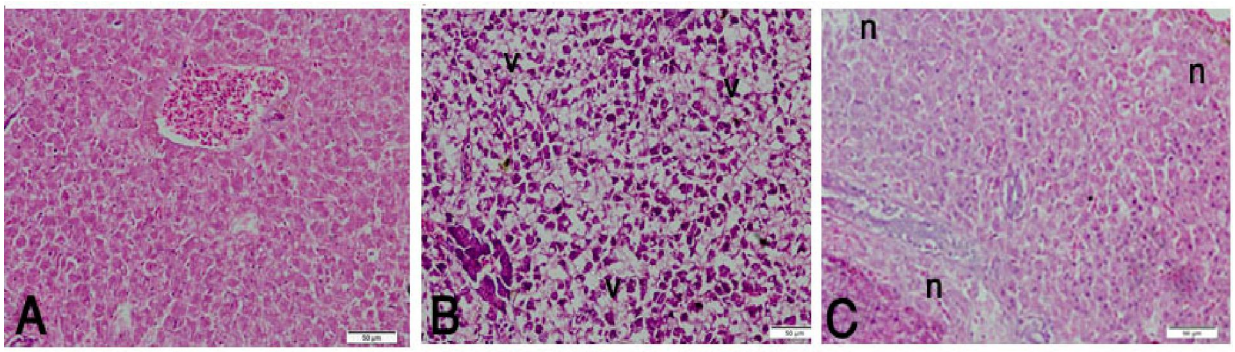

Figure (1): Liver of fish stained with H&E, A received (T1) and showing normal hepatocyte, B received T 2 and showing focal to diffuse areas of vacuolar degeneration (v). C received T3 and showing focal necrotic cells (n). x400.

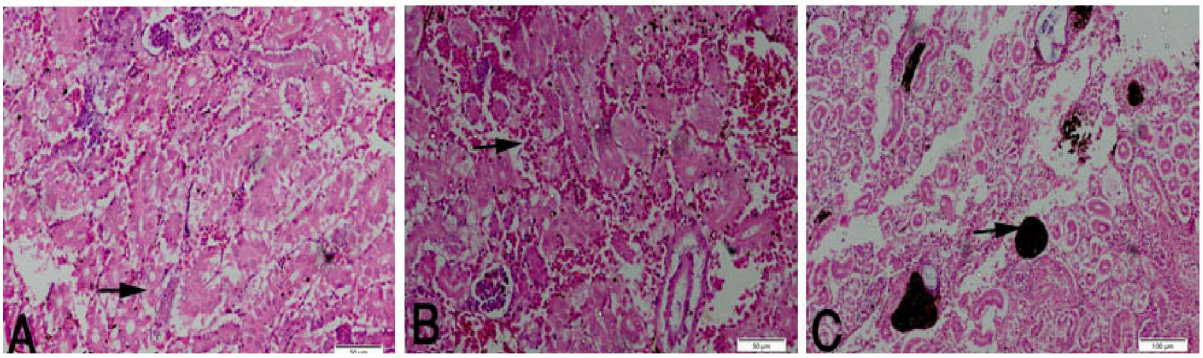

Figure (2): Kidney of fish stained with H&E, A received (T1) and showing mild degeneration of tubular epith. (arrow), B received T2 and showing areas of congestion and degeneration (arrow). C received T3 and showing degeneration and hyperplasia of MMC (arrow). X400.

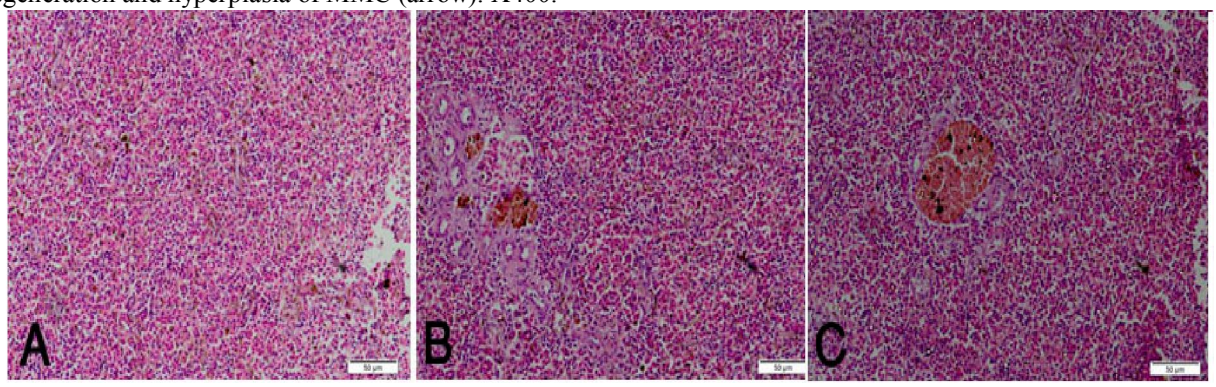

Figure (3): Spleen of fish stained with H&E, A received (T 1) and showing normal spleen, B received T 2 and showing mild hyperplasia of MMC. C received T3 and showing mild hyperplasia of MMC. X400.

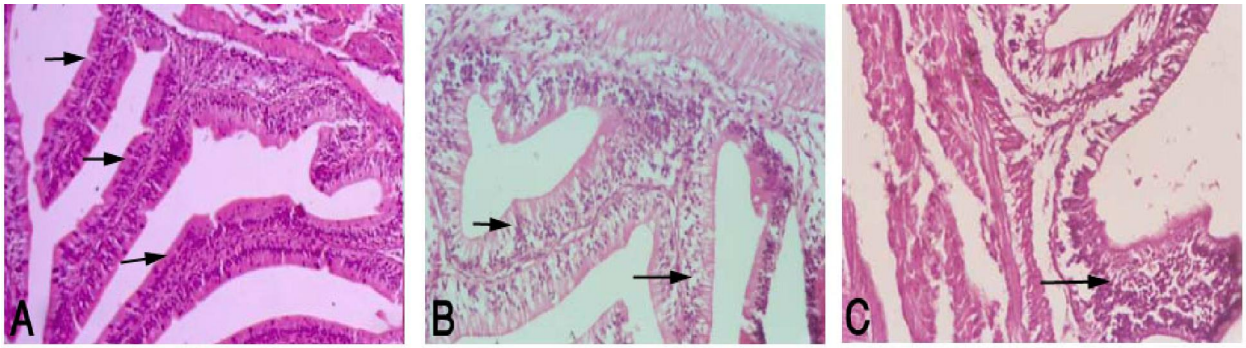

Figure (4): Intestine of fish stained with H&E, A received (T 1) and showing normal intestinal mucosa (arrows). B received diet T2 and showing mild degeneration of intestinal mucosa (arrows). C received diet T3 and showing degeneration of intestinal mucosa & mild aggregation of mononuclear cells (arrows). X 200, 400, 400.

4.Discussion

It is expected that growth of fish fed diets containing similar levels of digestible protein and digestible energy should be identical (Kim and Kaushik, 1992). Growth retardation and poor feed utilization was however observed in diets which SBM totally replaced fish meal. Reduced growth response and feed utilization have been explained by suboptimal amino acid balance, inadequate levels of phosphorus, inadequate levels of energy, low feed intake caused by palatability, presence of endogenous anti-nutrients or dietary level of fish oil (Lim and Dominy 1991) and recently explained by SBM-induced enteritis (Krogdahl *et al.,* 2003; Merrifield *et al.,* 2009). Lower growth at total fish meal replacement with SBM in this study may have been caused by one or some of these factors.

According to Liener, 1975 and Tacon, 1993, oilseed meals contain many thermolabile anti-nutrients, most importantly enzyme inhibitors and haemagglutinins. Whether these or other anti-nutrients factors weretotally inactivated was not determined in this study. Since T1 and T2 diets were isocaloric, the problem of low digestible energy value of oilseed meals may not be relevant, furthermore presence of exogenous enzymes such as xylanase should make oligosaccharide fraction in SBM more digestible. The phosphorus requirement for tilapia was met in the diets and presence of phytase enzyme should increase the availability of phytate phosphorous in SBM. As feed consumption was significantly reduced in diets lacking fish meal, (Table 2) palatability seems to pose a problem. The effect on growth may be mainly explained by a general decline in feed intake. A reduction in feed intake was regarded as the primary factor responsible for the depressed growth observed in rats (Jiang *et al.,* 2009). Gomes and Kaushik (1992) and Gomes *et al.* (1995) have previously reported this type of depression of voluntary feed intake with a 100% replacement of FM that led to a decrease in the growth performance of rainbow trout. Boonyaratpalin *et al.* (1998) reported that the lower growth obtained in fish fed the extruded or steamed full-fat SBM diets as compared to that of the control fish meal diet could not be attributed to protein digestibility, but it could be due to the lower feed intake during the first two weeks suggesting that palatability was possibly a factor. Also Mambrini *et al.* (1999) showed that the growth rate and nutrient utilization of rainbow trout, reduced when more than 50% of the dietary protein was of soy origin. Compared to the control group, Atlantic salmon fed the diet with 32% SBM ate 18% less, grew 30% slower, had 24% poorer feed efficiency ratio, and also suffered from serious SBM-induced enteritis, diarrhea, and reduced capacity to digest lipid (Refstie *et al.,* 2010). The significant reduction in feed intake may be explained by presence of other thermostable anti-nutrients in SBM, asisoflavones or phytoestrogens. Rats fed soy phytoestrogens have significantly decreased body and adipose tissue weights and feed intake compared with rats fed a phytoestrogen-free diet (Lephart *et al.,* 2004; Cederroth *et al.,* 2007 and Abd El-Razik, 2011). Reduction in feed intake may be due to the appetite repressing action of estrogen, as dietary phytoestrogens decrease feed intake and hence decrease body weight (Wade, 1975). Mai *et al.,* 2012 proved that high dietary soy isoflavones level significantly depressed weight gain, FER, whole-body crude lipid content of Japanese flounder and apparentdigestibility coefficient of nutrients. SBM is also known to contain allergenic or antigenic factors such as α-conglycinin, and β-conglycininwhich are known to trigger specific and non-specific immune response in several farm animals. β-conglycinin accounts for about 30% of the total soybean proteins (Utsumi *et al.,* 1997). It has been identified as one of the major allergenic proteins in soybean causing growth depression in animals (Hao *et al.,* 2009). However, these immunologically active globulins were not detected in soya protein concentrate

produced by leaching with aqueous alcohol, improving their inactivation. Zhang *et al.* (2013)fish study showed that the feed intake in the β-conglycinin group was significantly lower than that of the control group, which was in accordance with the results for rats (Nishi *et al.,* 2003). This result suggested that the growth reduction caused by β-conglycinin was most likely attributed to the suppression of feed intake. They proved that β-conglycinin can cause inflammation and oxidative damage (impair antioxidant system), and thus lead to damage and poor growth of digestive organs, subsequent by dysfunction of digestion and absorption, and finally reduce juvenile Jian carp growth. Saponin, another anti-nutritional factor present in SBM, is principally responsible for enteritis in Atlantic salmon (Baeverfjord and Krogdahl, 1996; Bakke-Mckellep *et al.,* 2000; Krogdahl *et al.,* 2003; Knudsen *et al.,* 2008). This was confirmed by Hedrera *et al.,* (2013) results which determined that is not the soy protein present in the diet but the soy saponin that is primarily responsible for triggering the inflammatory immune response. Bone, (2013) showed that pathological changes observed in fish fed the soybean meal equivalent replacement diets may be due to higher amounts of anti-nutritional factors in these diets or to additive or synergistic impacts of several anti-nutritional factors.In the present study the histopthological picture of fish fed diet containing SBM showed mild to moderate enteritis that may interfere with absorption of nutritive substances.

Methionine was identified as the limiting amino acid (NRC, 1993) when the level of FM dropped below 60% in the diet. Variable results have been reported in the literature about the replacement of FM with SBM in the diets of tilapia with or without the supplementation of essential amino acids. Some researchers concluded that methionine supplementation improves growth performance with FM partial replacement (Jackson *et al.,* 1982 and Tacon *et al.,* 1983). Shiau *et al.* (1987) and Mambrini *et al.* (1999) demonstrated that at the optimum level of dietary protein depressed growth and feed efficiency can be restored by addition of methionine to the partially FM replacement diet mainly by enhancing the intake. On the contrary, methionine addition has no effect on growth at total FM replacement with SBM. Viola andArieli (1983) and Teshima and Kanazawa (1986) reported that supplementing tilapia diets with crystalline essential amino acids did not improve fish performance. Viola *et al.*(1988) concluded that only phosphorus supplementation of the SBM-based diet was required to achieve weight gain and feed efficiency responses similar to that of fish fed a control diet. Tilapia fed diets without FM, had similar weight gain and feed efficiency as fish fed diets containing as much as 6% fish meal(Wu *et al.,* 1995 and Tudor *et al.,* 1996). Methionine did not appear to be limiting in practical diets using typical levels of cotton seed meal, dehulled solvent-extracted soybean meal, and meat and bone meal as primary protein sources, compared with a diet containing 6% FM (Nguyen *et al.,* 2009). Al-Ogaily (2002) proved that the diet with 0% FM showed the poorest results. He concluded that when 47% of FM was substituted with SBM in practical diets for tilapia, it produced the best results. Also, the diets, either meeting the essential amino acids requirements or without FM, did not show any beneficial effect of supplementary amino acids.Lin and Luo, (2011) observed that tilapia fed the diet with 100% protein from SBM had lower relative weight gain ratio, specific growth rate and protein efficiency ratio than the other groups. Feed conversion ratio was higher than other groups with a 100% substitution level. The results of the current study are in parallel with the above findings that demonstrated that total replacement of FM with SBM led to poor growth response. Keeping in mind that the lower methionine level in SBM-based diets was compensated for by addition of methionine in T2 and T3 (Table 1), Lysine content of the SBM-based diets was similar to or higher than that of the control diet. That indicated that not only the limiting amino acids but also the poor nutrient availability might have been responsible for this. Nordrum *et al.* (2000) reported that SBM causes decreased carrier mediated transport and increased permeability of distal intestinal epithelium for nutrients. The capacity of this region to absorb nutrients was diminished. Also Krogdahl *et al.* (2003) confirmed that all enzyme activities in the distal intestinal mucosa decreased dose-dependently with increasing SBM inclusion. The activities of protease in both intestine and hepatopancreas decreased with increasing dietary SBM level as reported by Lin and Luo, (2011). The protein efficiency ratio largely depends both on the quantity and quality of dietary protein (Davis and Stickney, 1978). The level of protein in all the diets was similar, however the quality and availability of the protein varied with FM replacement, which might be responsible for these results.

The body composition of fish is primarily influenced by diet composition, feeding practices, fish size, and can be controlled through nutrition (Burtle, 1990). The results of the present study indicated that substituting FM with SBM did not affect the moisture, ash, and GE. This is in agreement with findings of Al-Ogaily (2002). Crude protein was significantly higher in T3 compared to the control T1 diet. This may be explained by the lower levels of

carcass lipids in fish fed FM free diets coupled by omitting oil from T3 (Table 3). The lower levels of carcass lipids in fish fed FM free diets may be attributed to poor growth performance of fish. Although the control T1 and T2 experimental diets were isoenergetic, FM free diet T2 had a little lower fat content. Body fat content seems to be more related to growth rate or lipid level in the diet rather that to dietary energy content (Sargent *et al.,* 1989; Hanley, 1991).

Hepatocytes one of the most important tool in estimation of liver function and this occur through estimation of liver enzymes ALT and AST. These enzymes are sensitive indicators of liver cell damage (hepatocytes). Our results match with Manuela *et al.* (2002) who recorded no changes in the level of both ALT and AST in mice fed genetically modified soybean meal. Also, Vijay *et al.* (2002) mentioned that there were no changes in liver function tests in women fed soya for 12 weeks. Also our histopatholgical examination revealed mild hepatic necrosis of the liver in all the treatments except the control. The effect was mild enough that hematological parameters indicated no significant differences in liver enzymes.Our results showed mild hyperplasia of MMC in fish fed T2 and T3.This is in accordance with Evans *et al.* (2005) who concluded that feeding channel catfish a diet containing 450 g kg^{-1} non-heat-treated raw SBM did not cause severe histopathologic changes in liver and spleen associated with soybean meal anti-nutritional factors as have been reported in salmonids.

The most recorded beneficial effects of soybean is lowering lipid of the blood Vijay *et al.* (2002) who observed significant improvements in total cholesterol, LDL cholesterol, and cholesterol-to-HDL ratio but no change in triglyceride levels. Also, Anderson *et al.* (1995) mentioned that when human clinical trials using on average 47 g of soy protein daily showed significant reductions in total cholesterol (9%), LDL cholesterol (13%), and triglycerides (11%). Hermansen *et al.* (2001)mentioned that when 14 women and 6 men, treated for 6 weeks with soy protein (50 g/day), isoflavones (165 mg/day), and cotyledon fiber (20 g/day) they revealed an improvement in lipid parameters. In the current study serum total cholesterol was non-significantly improved by soybean consumption (insignificant decrease in cholesterol level). Nestel *et al.* (1997) reported that administration of genistein (45 mg for 5–10 wk) in menopausal women resulted in, unchanged level of plasma lipid concentrations by the consumption of soy isoflavones. Pipe *et al.* (2009) found that soybean isolate (SPI) did not affect serum total cholesterol, HDL cholesterol, triacylglycerol only serum LDL cholesterol was significantly reduced by SPI consumption. In contrast, 4 other studies did not find significant changes in serum LDL cholesterol in adults with type 2 diabetes following consumption of extracted soy isoflavones (Oh *et al.,* 2005 and Gonzalez *et al.,* 2007), SPITeixeira *et al.* (2004) and soy protein Anderson *et al.* (1995).

In the current study serum total cholesterol was not significantly affected by soybean consumption. This result is consistent with 6 previous soy intervention studies that investigated lipid-altering effects of soy protein Hermansen et al *et al.* (2001) and Li *et al.* (2005) and extracted isoflavones (Gonzalez *et al.,* 2007,Howes *et al.,* 2003 and Oh *et al.,* 2005)for periods of 6 weeks to 12 months.

Bayoumy (2013)observed that levels of total protein and albumin were significantly increased when male nourished rats fed diet containing soya bean for 30 days after malnutrition. We found no changes in levels of both total protein and albumin and this matches withYan *et al.,* (2012) who noticed no change in level of albumin in rats fed soy protein for 6 weeks**.**

Vijay *et al.* (2002) stated that the mean free thyroxin levels decreased significantly with soy and serum thyroid-stimulating hormone and free triiodothyronine levels were unchanged. On the other hand, Forsythe (1995)recorded increase in thyroid hormones when pigs were fed on soya protein diet for 14 weeks. Also, Barth *et al.* (1990) reported elevated total T4 and free T4 when soy protein was fed as compared with when casein was fed. In their study with rats, they also found that soy protein increased total T3 and free T3 compared with casein. Several investigators reported that the source of dietary protein alters plasma thyroid hormone concentrations. Akiba *et al.* (1982) reported that laying hens fed a corn-soy protein-based diet had greater plasma T4 concentrations than did laying hens fed casein as the dietary protein source. In a follow-up study Akiba and Jensen (1983) conducted a crossover experiment. Their results clearly show that plasma T4 concentrations respond to the source of dietary protein fed. Laying hens fed fish-meal protein had significantly lower plasma T4concentrations after only 7 d of feeding than did hens fed corn-soy protein. After 14 d, the groups switched diets. Hens switched from the corn-soy to the fish-meal protein had significant falls in their plasma T4 concentrations; whereas those switched to the corn-soy from the fish-meal protein had significant increases.

Our study revealed decrease in the level of T3 hormone which may be due to presence of an acidic methanolic extract of soybeans contains compounds that inhibit thyroid peroxidase- (TPO) catalyzed

reactions essential to thyroid hormone synthesis (Rao *et al.,* 1997).

On the contrary of our data Hermansen *et al.* (2001)reported no difference in glucose levels when 14 women and 6 men, treated for 6 weeks with soy protein (50 g/day), isoflavones (165 mg/day), and cotyledon fiber (20 g/day).While, Sada et al., (2013) recorded a significant reduction in the blood glucose levels in groups of rats fed with 25% and 50% of the soya beans supplement for two weeks. They explained the improved glucose levels by the function of various components of the soya beans, as soya bean fiber contains pectins, galactomannans and arabinogalactans with high viscosity. These substances delay gastric emptying and glucose absorption.

In the same line as we found Vijay *et al.* (2002) discovered thatthere was no changes in renal function as measured by serum creatinine. Also our histopathological examination showed mild degeneration in the kidney by the dietary treatments but did not affect kidney function. On the other hand, Yan *et al.,* (2012) mentioned that the level of uric acid and creatinine reduced significantly when hyperuremic rats fed diet containing 11% soy protein for 6 weeks**.**

Conclusion

This study provides evidence that the possibility of obtaining similar results with diets having the same levels of DE and DP was reduced. SBM effect on growth could be mainly explained by a general decline in feed intake combined with reduced nutrient availability that may be caused by SBM-induced enteritis. Enzymes addition could not prevent the growth retardation caused by total fish meal replacement. The interaction between feed intake and digestible utilization of SBM in fish needs more detailed study. SBM commercially produced at present can only partially replace FM. We need to improve processing methods and to develop new products that will be suitable for animal consumption and utilization, reduce the indigestible carbohydrate content, neutralize or minimize residual anti-nutritional factors, improve the palatability, flavor and improve nutrient digestibility, which is the main determinant of nutrient availability.

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