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GROWTH AND PHYSIOLOGICAL, HISTOLOGICAL AND ECONOMICAL RESPONSES OF NILE TILAPIA, *Oreochromis niloticus* FRY TO DIFFERENT DIETARY LEVELS OF FABA BEAN MIDDLINGS

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ABSTRACT

Five isonitrogenous (30% protein) diets containing 0, 10, 20, 30 and 40% of faba bean middling (FBM) instead of soybean meal were investigated for 98 days. Ten glass aquaria (70-L capacity), were used to stock 10 fish / aquarium of Nile tilapia fry initially average weight of 0.37 g/fish. Fish fed the diet containing 10% FBM grew faster than those fed other diets. However, the diets had FBM levels of 20, 30 and 40 % gave similar body weight, gain and specific growth rate being relatively lower value than the control diet. No significant differences (P>0.05) were observed in protein efficiency ratio and protein productive value, body contents of protein, ether extract, ash, and plasma glucose and total lipids. However, fish fed diets containing FBM levels gave lower concentration of aspartate aminotransferase and alanine aminotransferase than fish fed the control diet. The level of 10% FBM provided healthy fish with normal liver structure and optimum gonadal development. However, liver and gonads structure of fish fed more than 10% FBM resulted in hepatic cells destruction, sperm resorption in males, and oocyte atresia in females. The 10% level of FBM was the best economical inclusion level based on incidence cost and profit index

Keywords: Nile tilapia, Faba bean middlings, Unconventional feedstuff, Growth, Feed utilization , Physiology, Histology, and Economy.

INTRODCTION

Fish production is considered as one of the most important sources of animal protein to cover the continues and increasing demand of human consumption. However, feeding cost of fish production is still the major expensive item in the fish farming industry (Magouz *et al.*, 2002). In Egypt, aquaculture production contributed by 36% of the total fish consumed during 2000 (GAFRD, 2001). Fish feed cost has been increased dramatically specially with increase prices of fishmeal, soybean meal and yellow corn which are forming the most fish feed cost. They are imported ingredients, which are not only expensive but also are difficult to be steady due to current competition among fish, poultry and animals. Therefore there is an urgent need for fish feed industry to reduce its total dependence upon the imported ingredients including fishmeal (Wassef *et al.*, 2003) and soybean meal (Hughes, 1991). Attempt to replace fishmeal with alternative cheaper protein sources have met with little success (Abdel-Hakim *et al.*, 2003). Soya protein is the principal non-animal protein source in aquafeed (Viola *et al.*, 1982). However, the economics of using the soya products in fish feeds may become less favorable (Hughes and Choct, 1999). In addition, Maldonado-Perez *et al.* (1999) reported that over the past few years world grain prices have fluctuated dramatically but on the overall have increased and continue to do so. To avoid the possible negative effects of such changes, those formulating aquafeeds need to identify other new or underutilized protein sources that could replace soya in fish rations. Therefore, there is an urgent need to investigate alternative and cheap ingredients suitable for fish feeding. El-Dakar (1999) used dried acacia leaves to replace 42% instead of soybean in rabbitfish, *Siganous rivulatus*, diets without any adverse effects on growth and feed utilization. There have been a number of excellent reviews of the more commonly available legume grains (Wiseman and Cole 1988 and Abd Elmonem *et al.*, 2004). In Egypt, faba bean, *Vicia faba*, is one of the most important crop for human consumption due to its higher nutritional value, capacity of nitrogen, fixation and ability to grow in many types of soils (Forran *et al.*, 1995). There is a large amount of its by–products, e.g. middlings have been wasted from processing of faba bean industry for human. These middlings are inexpensive and still not utilized efficiently. So, it is useful to investigate this by-product as a non-conventional feedstuff to reduce feed cost in Nile tilapia diets. Therefore, the objective of the current study was identifying the maximum dietary inclusion level that would not impair fish performance through investigating the effects of feeding graded levels of faba bean middling (FBM) on growth, feed conversion, protein and energy utilization, body composition, biochemical blood parameters, histological changes of liver and gonads and cost-benefit analysis.

MATERIALS AND METHODS

This work was carried out at the Wet Lab., Department of Animal Production, Faculty of Agriculture, Alexandria University, El-Shatby, Alexandria, Egypt. Ten glass aquaria were used in this study, each with dimensions of 70 x 30 x 40 cm and total net volume of 70 L. All aquaria were filled with dechlorinated tap water stored 24 hours before use. Fry of Nile tilapia, *O. niloticus* were obtained from the Maryot Fish Farming Co, Alexandria governorate, Egypt. Fish were transported in fiberglass tanks (300 L). After arrival, all fish were kept for one week to alleviate stresses and to be adapted to the new conditions. Ten fish in the same initial weight (0.37 g/fish) were selected and randomly allotted into each experimental aquarium. Fish were fed the control diet for two weeks, during this period healthy fish of the same weight replaced dead one.

Five experimental diets were formulated to contain 0, 10, 20, 30 and 40% of FBM. Each diet was fed to duplicate aquaria. Ingredients composition of the experimental diets is presented in Table (1). Fishmeal was home made by collecting small fish and non-soled fish named locally "Wazafa" ,dried at 60 C, ground and sieved prior to keep at –20 C. Soybean meal (48%) was used as a plant protein source. Yellow corn, wheat bran, wheat milling by-product and sunflower oil were served as energy sources. The experimental diets were prepared by mixing dry ingredients with water and were pelleted using a meat mincer with a 1-mm diameter. The pellets were air dried and stored at -20 C until use. The pellets were slightly broken into particles during the first two weeks. Fish were fed at 8, 6 and 4 % of the body weight daily for the 1-4, 5-8 and 9-14 week, respectively. The feed amount was given three times a day (at 9000, 1200 and 1500 h) in equal proportions. Feeding was performed for six consecutive days weekly, for 98 days. Fish were weighed biweekly and feed amounts were adjusted on the basis of the new weight. Fish were reared at 29±1 °C and 8.5±0.2 pH. Third water volume of each aquarium was exchanged daily except the weighing day, in which about two thirds of the water volume were changed.

Table 1 :Ingredients composition of the experimental diets.

Ingredients	Diets No.				
	1	2	3	4	5
Fish meal	18	18	18	18	18
Soybean meal	35	31.4	27.6	23.6	19.8
Yellow corn	5.0	5.0	5.0	5.0	5.0
Faba bean middling	0	10	20	30	40
Wheat milling by-product	24	18.6	12.4	6.4	0.2
Wheat bran	6.0	6.0	6.0	6 0	6.0
Starch	5.0	4.0	4.0	4 0	4.0
Sunflower oil	5.0	5 0	5 0	5.0	5.0
Vitamin mix.[1]	0.875	0.875	0 875	0.875	0 875
Ascorbic acid	0 125	0.125	0.125	0.125	0.125
Mineral mix.[2]	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100

1.Vitamin mixture (g/100 g) was 960000 IU, 160000 IU, 0.8 g, 80 mg, 0.32 g, 0.12 g, 0.8 g,, 0.8 mg 1.6 g, 80 mg, 4 mg, 40 g. of vitamin A, D3, E, K, B1, B2, B6, Pantothenic acid, B12, Niacin, Folic acid Biotin, Choline chloride, respectively.

2.Mineral mixture (g/100g) was 12.75, 72.85, 0.55, 0.25, 0.02, 5, 2.5, 0.08, 0.05, 0.01 and 6 mg of $MgSO_4.7H_2O$, $CaHPO_4$. $2H_2O$, $ZnSO_4$. $7H_2O$, $MnSO_4$. $4H_2O$, $Cal_2.O_6$. $6H_2O$, KCl, $FeSO_4$. $7H_2O$, $CuSO_4$. $5H_2O$, $CoSO_4$. $7H_2O$, CrC_3. $6H_2O$ and NaCl, respectively.

Diets and fish samples were analyzed according to AOAC(1990) for dry matter, crude protein, ether extract, crude fiber and ash. The gross energy contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.2 kcal/g of protein, lipid and carbohydrate, respectively (NRC, 1993). Digestible energy content was calculated from standard physiological fuel values as 4 and 9 kcal/g of protein and carbohydrate and lipid, respectively (Garling and Wilson, 1976).

Blood was collected (using heparinized syringes) from the caudal vein of all the fish at the termination of the experiment. Blood was centrifuged at 3000 rpm for 15 minutes to allow separation of plasma to determine plasma total protein (Armstrong and Carr, 1969), total lipid (Frings *et al.*, 1972) and glucose (Trinder, 1969). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically by using commercial kits according to the method of Reitman and Frankel (1957).

At the end of the experiment, samples of liver, testes, and ovaries from fish are taken and fixed in 10% formal saline solution for 7 days, dehydrated in ascending series of ethyl alcohol for 30 minutes each, cleared in benzene, then embedded in two successive pure paraffin wax of melting point 56 °C, blocked, sectioned at 5 µm, stained with Haematoxylin and Eosin ,mounted in canada balsam and microscopically examined for histological changes in the liver and gonads structure.

To evaluate cost-benefit analysis of FSM inclusion in the diets of Nile tilapia fry, incidence cost (IC) and profit index (PI) were calculated according to New (1985).

Analysis of variance (ANOVA) was carried out according to Snedecor and Cochran (1982) using a completely randomized design (CRD). Differences were subjected to Duncan's Multiple Range-Test (Duncan, 1955) at a significance level of 0.05. Program of MSTAT-C was used to compute the statistical analysis (MSTAT-C, 1988).

RESULTS

Nutritive value of faba bean middling:

Faba bean middlings are rich in nitrogen free extract and have moderate percentage of crude protein and low content of crude fiber and ash (Table 2). The amino acid profile of faba been middlings compared with soybean meal is given in Table (3). It clears that, faba bean middlings are generally contain small amounts of therionine, serine, methionine, isoleucine, valine, and lysine. In general, soybean meal is rich in all amino acid profile than FBM, except the histidine and systine.

Table 2 :Chemical analysis of the experimental diets.

Item	FBM[1]	Diet No.				
		1	2	3	4	5
Dry matter %	96.67	96.4	95.66	94.9	93.94	94
% on the DM basis:-						
Crude protein	19.40	31.59	31.16	30.54	29.85	29.23
Ether extract	2.60	10.08	10.12	10.14	10.15	10.17
Crude fiber	5	2.90	3.21	3.51	3.81	4.11
NFE	68	44.57	44.82	45.37	46	46 54
Ash	5	10.86	10.69	10.44	10.19	9.95
Calculated energy (kcal/100 g diet)						
Gross energy		462	461	460	459	458
Digestible energy		395	395	395	395	395
P/E ratio mg CP/kcal DE	--	79.97	78.89	77.32	75.57	74

1-Faba bean middlings

Growth performance and feed utilization:

Fish fed the diet containing 10% FBM grew faster than those fed other tested diets. However, diets had FBM levels of 20, 30 and 40 % gave similar final body weigh, gain and specific growth rate being relatively lower than in the control diet (Table 4). No mortality was recorded within all fish groups during the experimental period. There was no significant (P>0.05) effect on feed and energy intake. However, fish fed 20,30 and 40% FBM consumed significantly less protein than those fed the control diet. Feed conversion ratio of fish fed different levels of FBM ranged between 1.27 and 1.52. The best FCR was found for fish fed the diet containing 10% FBM. While FCR of the control group recorded the highest value than the other treatments, however statistical analysis showed that the differences in FCR among fish groups were not significant (P>0.05).Results showed no significant differences (P>0.05) in protein efficiency ratio and protein productive value. But fish fed 20% FBM had the lowest energy retention than the other FBM diets. The control diet seemed the significantly lowest energy retention at all.

Table 3 :Amino acid profile of faba bean middlings and soybean meal.

Amino acid	Faba bean	Soybean meal
Aspartic	1.26	4.44
Theronine	0.74	2.01
Serine	0.76	2.16
Glutamic	1.72	7.02
Proline	0.75	2.28
Glycine	0.78	1.18
Alanine	0.85	2.31
Systine	0.42	0.28
Valine	0.88	2.42
Methionine	0.49	0.78
Isoleucine	0.72	2.30
Leucine	0.93	2.88
Tyrosine	0.75	1.72
Phenil alanine	0.77	2.38
Histidine	3.15	1.64
Lysine	0.80	2.56
Argenine	1.20	3.48

Table 4 :Growth, survival rate, feed and nutrients utilization of Nile tilapia fry fed diets containing different levels of broad bean middlings.

Item		Diet No.					MSE[1]
		1	2	3	4	5	
Growth performance							
Initial weight	g/fish	0.37	0.37	0.37	0.37	0.36	0.00^{ns}
Final weight	g/fish	15.45^{b}	17.7^{a}	14.56^{b}	14.24^{b}	14.19^{b}	0.48
Gain[2]	g/fish	15.08^{b}	17.34^{a}	14.2^{b}	13.87^{b}	13.85^{b}	0.48
SGR[3]	% /day	3.81^{b}	3.96^{a}	3.76^{b}	3.74^{b}	3.75^{b}	0.03
Survival rate	%	100	100	100	100	100	0.00^{ns}
Feed and nutrient Intake							
Feed intake	g/fish	22.83	22.02	20.3	19.28	19.9	1.08^{ns}
Protein intake	g/fish	6.95^{a}	6.57^{ab}	5.89^{bc}	5.41^{c}	5.47^{c}	0.22
Energy intake	Kcal	102	98	89	83	86	2.63^{ns}
Feed and nutrient utilization							
FCR[4]		1.52	1.27	1.43	1.40	1.35	0.04^{ns}
PER[5]		2.17	2.65	2.41	2.58	2.53	0.07^{ns}
PPV[6] %		34.98	43.56	42.31	46.58	46.32	1.70^{ns}
ER[7] %		23.88^{c}	34.01^{a}	30.01^{b}	34.17^{a}	32.76^{b}	1.38

'Values in the same row having a common superscript letter are not significantly different (P>0.05).

1. *Standard error of the means derived from the analysis of variance*
2. Gain = *(final weight - initial weight).*
3. Specific growth rate = *100(Ln final weight - Ln initial weight)/period in day).*
4. Feed conversion ratio = *DM intake/weight gain.*
5. Protein efficiency ratio = *weight gain/protein intake.*
6. Protein productive value % = *100(protein gain / protein intake).*
7. Energy retention % = *100 x (gross energy gain / gross energy intake).*

Body composition:

Chemical composition of whole body fish fed different levels of FBM in diets of Nile tilapia is presented in Table (5). There were no significant (P>0.05) differences in protein, ether extract and ash contents. However, energy content was significantly (P<0.05) higher in fish fed 30 and 40% FBM than those fed the lower levels.

Table 5 :Body composition (% fresh weight) of Nile tilapia fed different levels of broad bean middlings .

Composition	Initial fish	Broad bean middling levels (%)					SEM*
		0	10	20	30	40	
Moisture	79.72	72.97	69.05	69.26	67.37	66.96	0.73
Crude protein	11.57	16.06	16.38	17.4	17.85	18.15	0.31
Ether extract	2.23	7.26	10.21	9.15	10.63	10.45	0.48
Ash	3.83	4.48	4.06	4.18	4.16	4.06	0.07
Energy kcal/100 g	98.0	159c	189b	185b	202a	202a	5.48

* Standard error of the means derived from the analysis of variance.
Values in the same row having a common superscript letter are not significantly different (P>0.05).

Biochemical blood analysis:

Plasma glucose, total proteins, and total lipids were not affected by increasing FBM levels in tilapia diets (Table 6).Yet, fish fed diets containing FBM gave lower activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)(proportional to the FBM levels) than fish fed the control diet. However, differences in AST and ALT among fish fed FBM diets were not significant (P>0.05) at any level.

Table 6 :Blood biochemical parameters of Nile tilapia fed different levels of bean middling.

Item		Experimental diets					SEM*
		1	2	3	4	5	
Glucose ,	mg/dl	57.57	57.2	57.2	56.4	55.8	0.49
Total protein ,	g/dl	5.19	4.92	4.01	5.1	4.37	0.17
Total lipid ,	g/dl	4.55	4.85	5.53	4.32	4.38	2.29
AST[1] ,	u/dL	116	80	83	64	62	6 76
ALT[2] ,	u/dL	45	34	39	32	31	1.87

*Standard error of the means derived from the analysis of variance.
1. Aspartate amiontransferase
2. Alanine aminotransferase

Histological studies:

Liver of fish fed the diet containing 0% of FBM shows well-formed polygonal shaped hepatocytes with homogeneous cytoplasm, distinctive-central nucleus and prominent nucleolus (Fig.1). Changes in fish liver when replacement of soybean with faba bean occurred vary according to FBM level. At level of 10%, the hepatocytes have a more definite shape, large rounded nucleus, and great number of hepatocytes are binucleated (Fig.2). As a level of FBM increased to 20-30 % liver structure became distorted, this distortion characterized by hydropic degeneration of cytoplasm, loss of hepatic muralia orientation, nuclear shrinkage and occasionally disappearance (Fig.3). At level of 40% FBM, the hepatocytes appear as separate darkly staining patches of variable size and shape. No internal inclusion can be recognized (Fig. 4).The testis of Nile tilapia fed 0% FBM was ripe. The lobules were filled with spermatozoa. Some developmental stages (spermatids and secondary spermatocytes) are present at the margin of the lobules (Fig.5). In testis of fish fed 10% FBM, man can see spermatozoa filling all the lobules and patches of developmental stages (spermatids, primary and secondary spermatocytes) at the periphery (Fig.6). At level of 20% FBM, number of spermatozoa in lobules decreased and spermatozoa resorption can be detected (Fig.7). At level of 30 and 40 % FBM, the number of spermatozoa greatly reduced where the sperm occupy only the middle of the lobule surrounded by vacuoles of varying size indicating sperm resorption (Fig. 8).

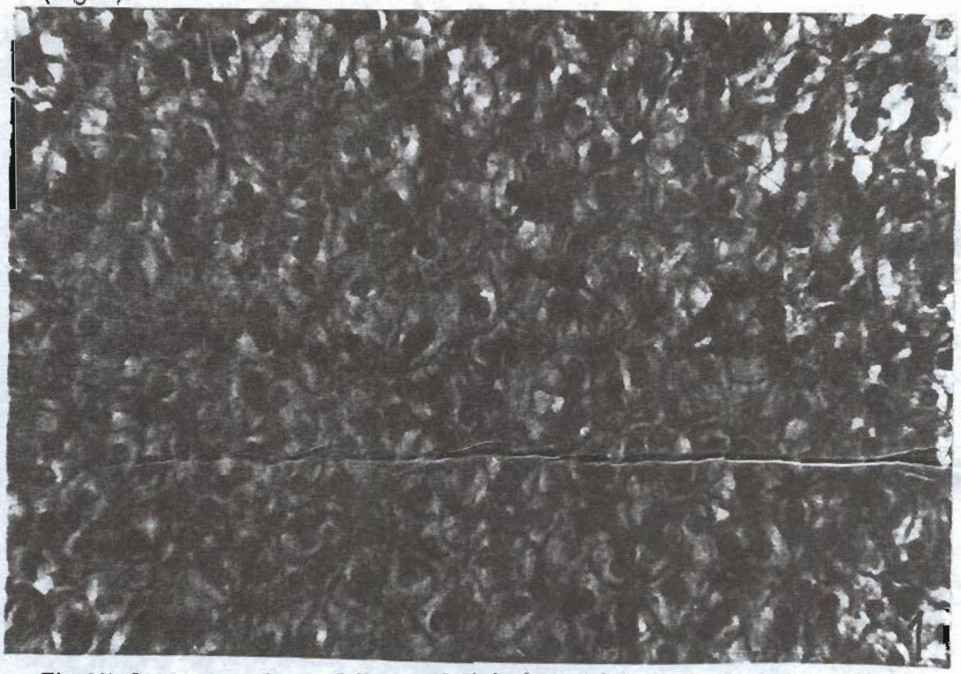

Fig.(1):Cross section of liver of fish from the control group, showing normal hepatocytes (H& E , ×250).

The microscopic features of ovary of fish fed 0% FBM have ripe oocyte. Oocytes has an intact follicular wall formed from an outer follicular epithelial cells (follicular theca), then inner granulosa cells (Figs.9&10). Ovary of fish fed 10% FBM has the same structure as in the control group (Figs.11&12). Up to level of 40% FBM, number of atretic oocytes increased. Atresia of oocytes characterized by hyperplasia of follicular granulosa cells (Fig.13). Hyperplasia begins by disappearance of cells septum (Fig.14), than the granulosa cells increased in number and invade the ooplasm (Fig.15). The hyperplasia of granulosa cells increased at level of 40% FBM and the oocyte was occupied by debris of ooplasm and cells derived from granulosa layer (Figs.16 &17).

Fig.(2): Cross section of liver of fish fed FBM at level of 10% , showing binucleated hepatocytes (arrows) (H &E , ×250).

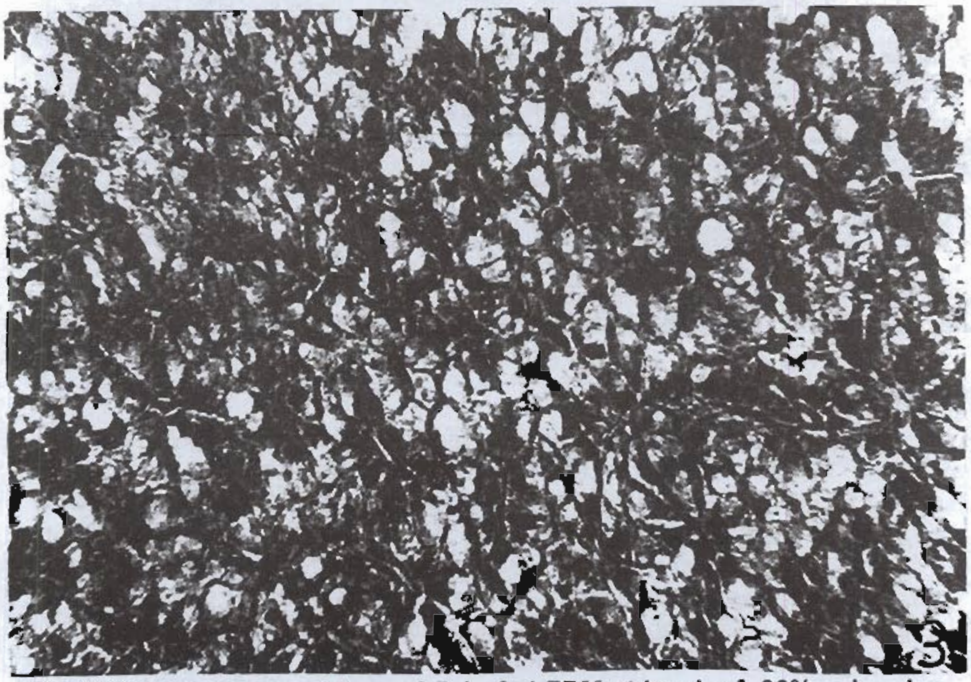

Fig.(3):Cross section of liver of fish fed FBM at level of 30% , showing destruction of hepatocytes, and nuclear shrinkage (H& E,×250).

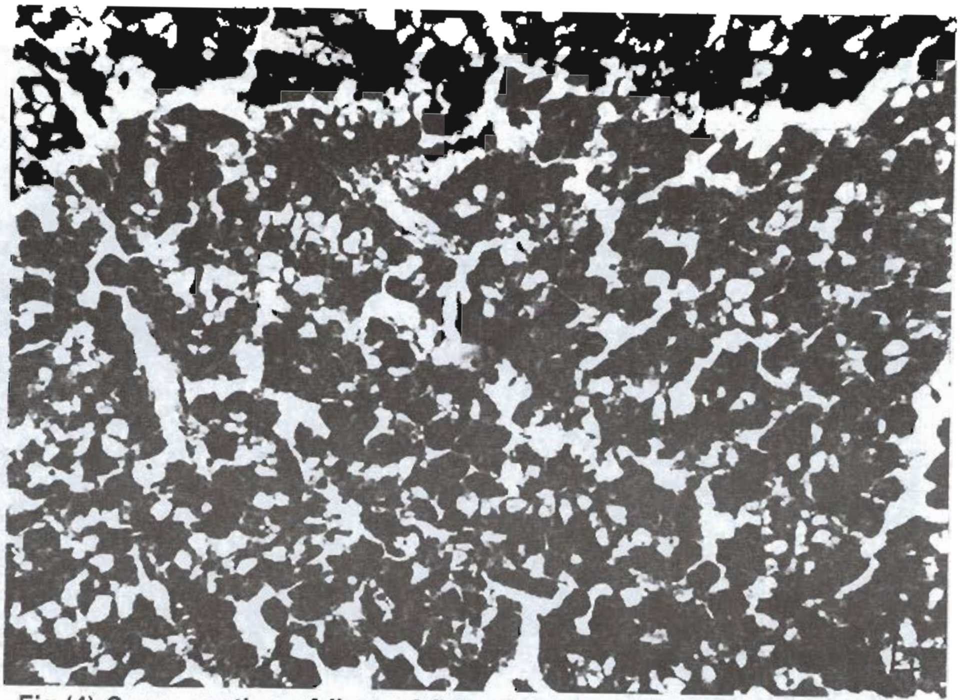

Fig.(4):Cross section of liver of fish fed FBM at level of 40%, showing darkly staining hepatocytes with no internal inclusions (H&E, ×250).

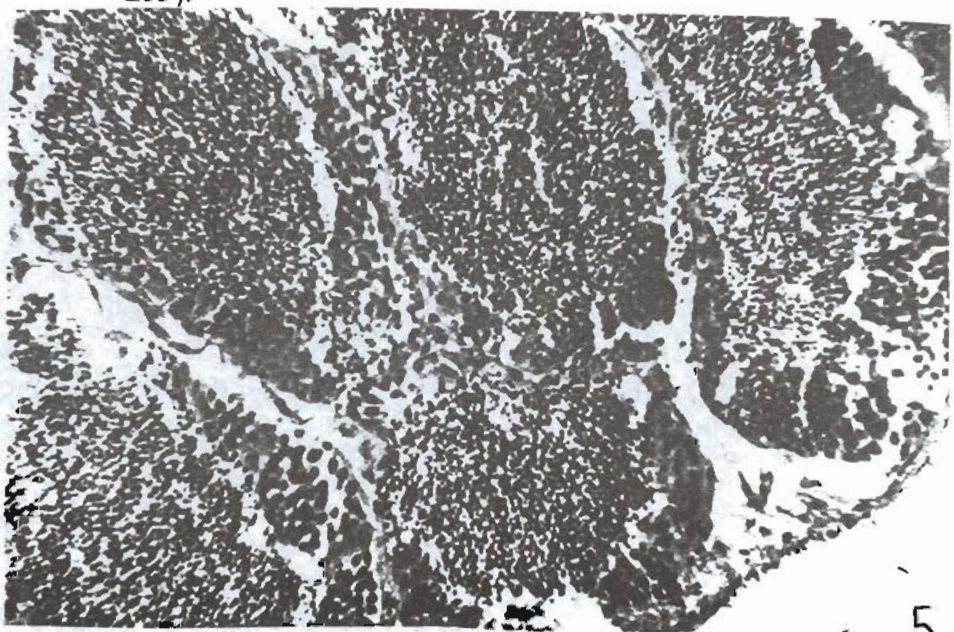

Fig.(5): Cross section of testis of fish from the control group , showing ripe testis full with spermatozoa (A), spermatids (B), and secondary spermatocytes (C) (H &E , ×400).

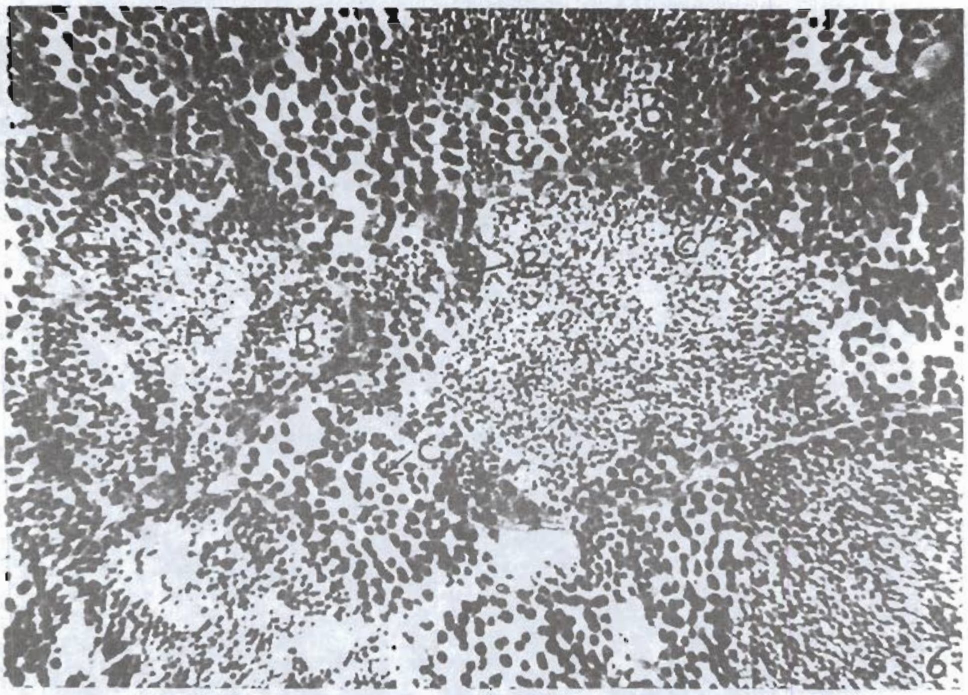

Fig.(6): Cross section of testis of fish fed FBM at level of 10 % , showing ripe testis with great number of spermatozoa (A), spermatids (B), and primary (D), and Secondary spermatocytes (C) (H& E , ×400).

Fig.(7):Cross section of testis of fish fed FBM at level of 20% , showing spermatozoa resorption (H & E , ×400).

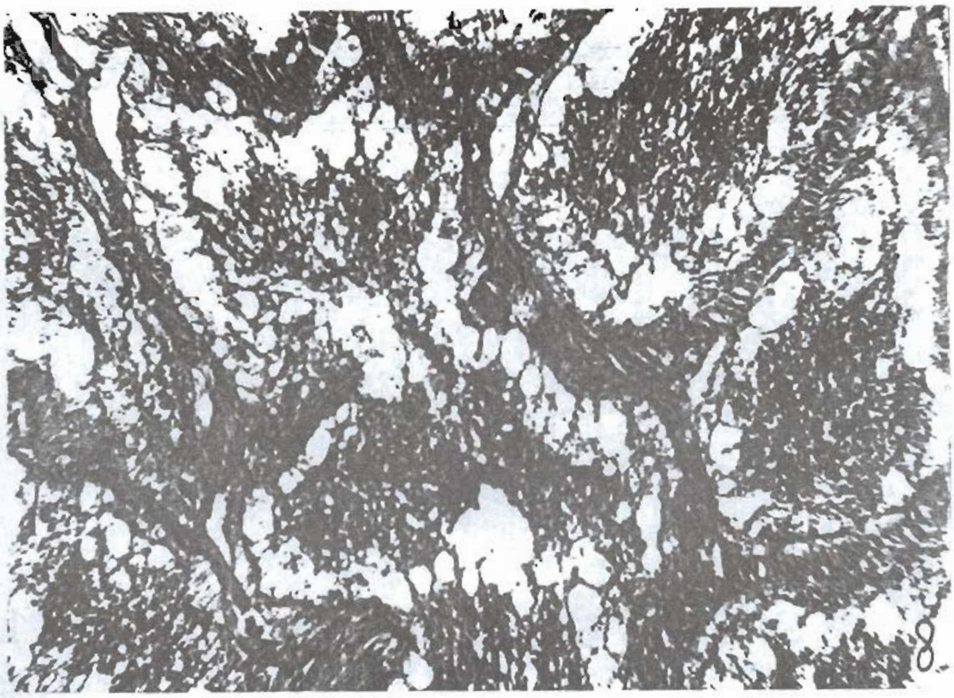

Fig.(8): Cross section of testis of fish fed FBM at level of 40%, showing reduced number of spermatozoa at middle of lobule (H& E , ×400).

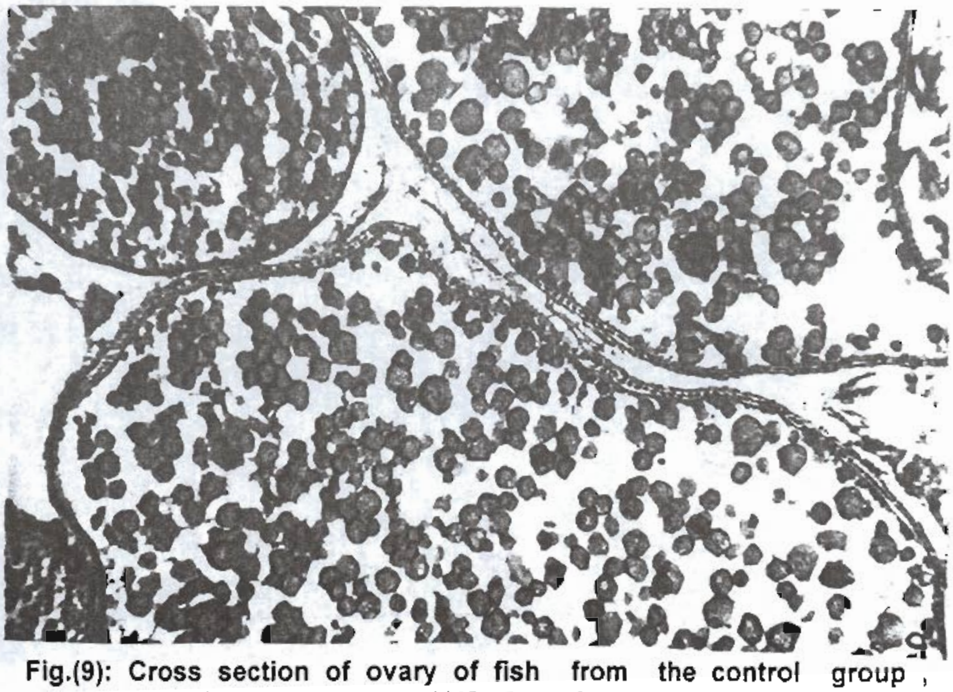

Fig.(9): Cross section of ovary of fish from the control group , showing ripe oocytes (H& E , ×100).

Fig.(10): High magnification of the previous section, showing follicular wall, follicular epithelia (FE) cells, and granulosa cells (GR) (H &E , ×250).

Fig.(11): Cross section of ovary of fish fed FBM at level of 10 %, showing mature oocytes (H &E , ×100).

Fig.(12): High magnification of the previous section, showing follicular wall , follicular epithelial cells,(FE), and granulosa cells (GR) (H& E , ×250).

Fig.(13): Cross section of ovary of fish fed FBM at level of 20% showing hyperplasia of granulosa cells (see arrows) (H & E , ×100).

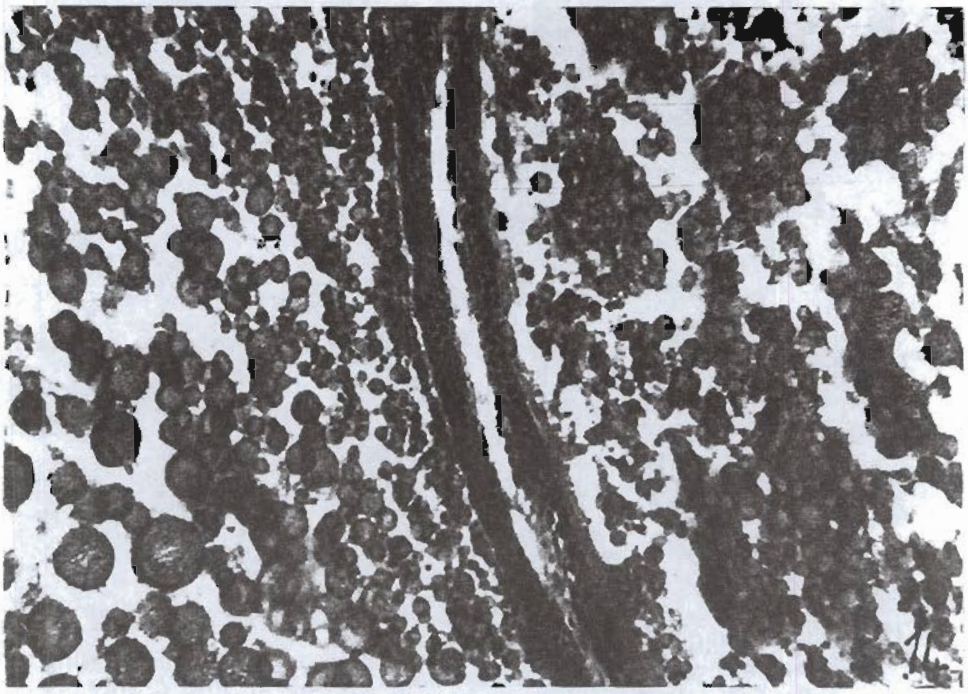

Fig.(14): High magnification of the previous section (broken arrow), showing disappearance of cells septum (H & E , ×250).

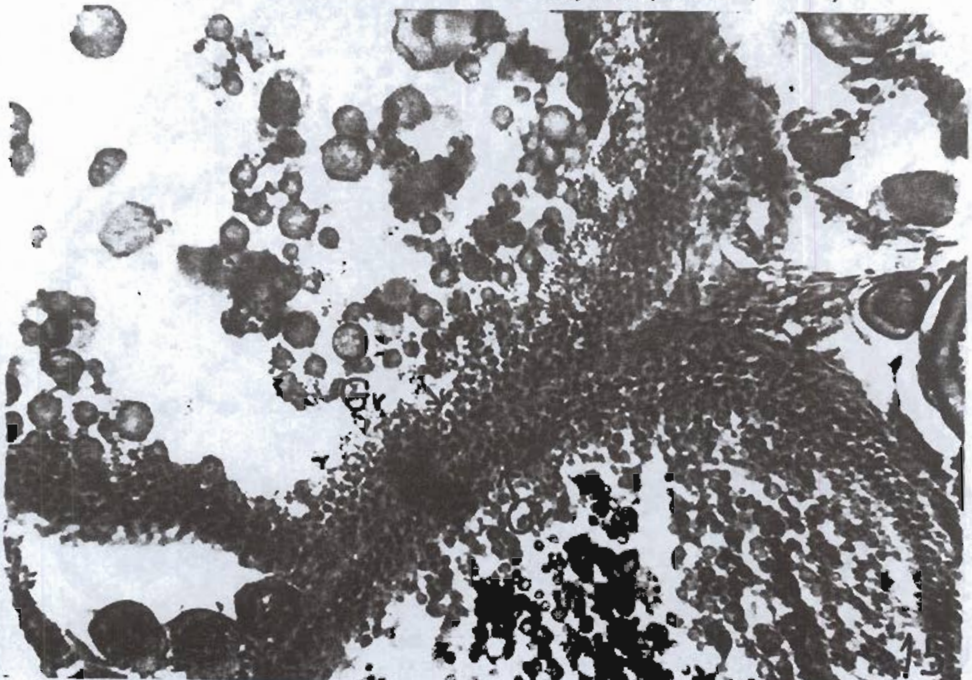

Fig.(15):Cross section of ovary of fish fed FBM at level of 30%, showing hyperplasia of granulosa cells (GR) (see arrows) (H &E, ×250).

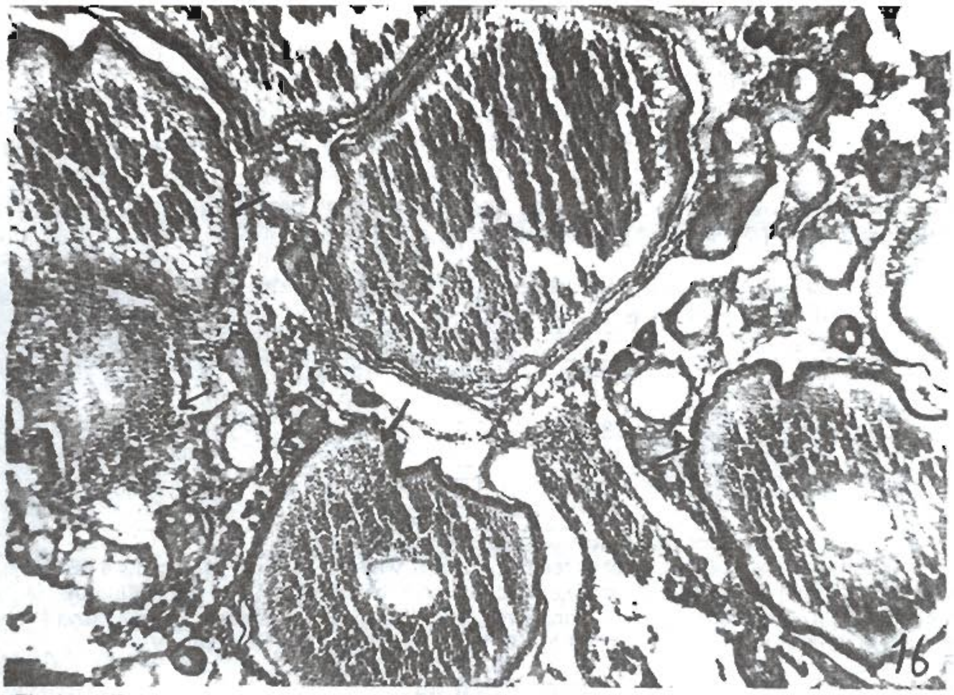

Fig.(16): Cross section of ovary of fish fed FBM at level of 40 % showing varying degrees of granulosa cells hyperplasia. (see arrows) (H & E , ×100).

Fig.(17): High magnification of the previous section, showing hyperplasia of granulosa cells invading oocyte and degenerated ooplasm (see arrows) (H & E , × 250).

Cost-benefit analysis:

Using of FBM in Nile tilapia diets resulted in a decrease of feed cost to be 97, 94, 91 and 88% of the control diet for diets containing 10, 20, 30 and 40% FBM, respectively. The incidence cost take the same trend, it decreased with fish fed the FBM diets than the control group. Consequently, profit index of fish fed diets containing FBM was higher than the control diet. The diet gave lowest incidence cost and the highest profit index was that contained 10% FBM(Table 7).

Table 7 :Cost-benefit analysis of feeding Nile tilapia on different levels of bean middling.

Items / Diet	1	2	3	4	5	MSE
Cost per kg feed, LE	2.51	2.43	2.36	2.28	2.21	-
Change %	100	97	94	91	88	-
Incidence cost[1]	3.81	3.09	3.38	3.18	3.18	0.11
Change %	100	81	89	83	83	-
Profit index[2]	1.58	2.12	1.78	1.91	1.89	0.08
Change %	100	123	113	121	120	

1- Incidence cost = feed cost to produce 1 kg fish

2- Profit index = value of fish /cost of feed consumed, where 1 kg fresh fish equals 6 LE

Cost of 1 Kilogram fishmeal, soybean meal, yellow corn, bean middling, wheat milling by-product, wheat bran, corn starch, sunflower oil, vitamin premix, ascorbic acid and minerals premix were 5.0, 2.1, 1.1, 1.0, 1.5, 0.8, 1.9, 3, 8, 10 and 8 LE, respectively.

DISCUSSION

The amino acid profile of FBM is in good agreement with the data given by Forran *et al.* (1995) and Maldonado-Perez *et al.* (1999). They found that the most nutritionally amino acids in faba bean protein appear to be sulfur amino acids. All diets containing faba been well accepted by the fish, as deduced from feed intake and survival rate data which were nearly similar to those obtained with the control diet, from both nutritional and palatability points of view. These results partially agreed with those of D'Mello *et al.* (1990) who reported that jack bean is a relatively good source of protein and apparent metabolizable energy. Survival rate was found to be 100% for all diets. Similarly, there was no mortality among hens fed diet containing faba bean at different levels (Ergun *et al.*, 1990; Perez-Lanzac and Castanon, 1990 and Forran *et al.*, 1995).

There was a tendency for slightly lower feed intake at higher FBM levels (diets 3, 4 and 5) than 10% FBM (diet 2). It could be due to a difficult adaptation of fish to the organoleptic properties of such diets. The presence of a potent heat-labile lectin (two restricted toxic amino acids, i.e. canavanine and canaline) has restricted the exploitation of this legume as a component of animal diets (D'Mello, 1989). Yet, at levels of 20-40% FBM, feed intake was similar to value of the control diet, it is suggested that fish can consume feed to fulfill their energy requirements (Tacon and Cowey, 1985). In this case, distinguishing nutrient deficiency of the tested ingredients is covered by the compensatory feed intake (Farhangi and Carter, 2001).

Morales *et al.* (1994) reported a higher feed intake of a diet containing 40% lupine by rainbow trout compared with other plant proteins.

However, they related the higher feed intake to lower availability of energy rather than higher palatability of lupine. In the present study, levels of 20-40% FBM were used without negative effects on growth performance, feed conversion and nutrient utilization might be attributed to the increased amount of amino acid profile in the diets. These results are in agreement with those obtained by Eusebio (1991) who reported that the leguminous seeds show promise with respect to their protein content and essential amino acid index. Similarly, an inclusion level of 40% dehulled lupine was demonstrated to have no significant effect on growth rate of rainbow trout (Hughes, 1991). The same pattern was demonstrated with sea bream up to 30% of lupine. However, biological assays on rats indicated that protein digestibility of legumes is very poor (Eusebio and Eusebio, 1984). This could be due to the presence of protease inhibitors and other toxic substances in the beans (Mendoza *et al.*, 1980). The increase of growth performance, feed conversion and nutrient utilization observed in the present study may be attributed to the associative effects of the digestibility of legume proteins and digestibility of other protein sources in the diet such as fish meal and soybean meal. Successful incorporation of lupine up to a level of 50% in trout diet was accepted when diets were offered to fish *ad lib* and supplemented with other protein sources (Hughes, 1991; Burel *et al.*, 1998), amino acids (de la Higuera *et al.*, 1988) or highly unsaturated fatty acids (Robaina *et al.*, 1995).

Results of this study indicated that fish given 10% FBM seemed to be better in growth performance and feed efficiency than on the other diets. These observations might be attributed to the presence of antinutritional factors present in the cotyledon of the bean. Tannins in faba bean have also been cited as the antinutritional factor responsible for poor chick performance. However, work of Wareham *et al.* (1993) would indicate that these might not be the causative factor. Hew *et al.* (1997) suggested that digestibilities of protein and amino acids were lower in field peas, faba beans and chick peas than in sweet lupines. Lysine was particularly poorly digested in faba beans (Farrell *et al.*, 1999). In this connection, the beans also contain a lipoxygenase enzyme that catalyses the oxidation of polyunsaturated fatty acids to form hydroxyperoxidase which are responsible for the beany flavor (Eusebio, 1991). The hydroxyperoxides and the secondary products can react with amino acids or proteins and certain vitamins to lower the nutritive value of the feed (Gardner, 1979).

Although FCR generally decreased at 10% FBM inclusion level, it was not significantly different up to 40% inclusion level in the diets. This is partially in agreement with other findings for other legumes (Burel *et al.*, 1998 and Farhangi and Carter, 2001). Values of PER and PPV were not significantly different among the experimental diets. Findings of Wryawan and Dingle (1999) support this hypothesis, as the metabolizable of lupin ranked the second lower after the faba bean for growing chick in spit of its higher gross energy content comparing with all other legume grains. No significant differences ($P>0.05$) were observed in protein, ether extract and ash contents of whole body among all FBM tested levels. Similar results were obtained in case of using sesame hulls at graded levels up to 30% in tilapia feeding (Abd Elmonem et al, 2004). Plasma glucose and total lipids concentrations were not affected by different levels of faba bean middlings. On the other hand, plasma total proteins concentration decreased at higher levels of FBM. These data may show gluconeogensis at higher FBM levels of faba bean middlings. This is in agreement with the findings of Brauge *et al.* (1994) which attributed the high glucose level of trout 16 and 30 hrs after being fed low digestible carbohydrates to gluconeogensis. Similar results were obtained by Farhangi and Carter (2001) with trout fed graded levels of lupine legume.

The most prominent feature of liver histological response of tilapia fed levels of 20-40% faba bean inclusions was distortion of hepatic cells and disintegration of cells inclusions. This variability no doubt reflect: (1) dietary input of disproportional levels of specific amino acids. This result is in line with the results obtained by Walton *et al.* (1982) and Watanabe (1987) for rainbow trout, (2) the presence of amino acid cystine in excess value in faba bean rendered the animal feeding which is suffering from methionine deficiency caused by an excess of dietary cystine, antagonism effect (Harper *et al.*, 1970), and (3) liver destruction at higher levels of FBM may by due to the presence of toxic amino acids in bean. The improvement in liver structure in fish fed FBM at a ratio of 10% may be due presence of other protein sources in this diet.

Gonads of fish that fed 20-40% FBM levels developed atretic oocytes characterized by ooplasm degeneration and hyperplasia of granulosa layer may be due to disproportional level of amino acids or toxic substances as L-canavanine in faba bean. Washburn *et al.* (1990) recorded ovaries abnormalities in rainbow trout due to dietary imbalances. Testis shows resorption of spermatozoa. The decreases in number of sperm in male might be ascribed to inadequate levels of amino acids specially arginine in FBM to fulfill growth and development of testis. As arginine accelerate basal secretion of sex hormone and pituitary activity in fish (Gobletti and Zerani, 1999). Alanine and arginine are the major and highest amino acids content of maturing testis in *Dicentrarchus labrax* , and *Solea impar* (Assem, 2001 and Hassan, 2003).

As a conclusion it can be said that 10% FBM provided normal healthy fish with optimum gonadal development. Although FBM in levels greater than 10% don't have negative effect on fish growth rate, but it is undesirable on long run as an unique source of plant protein, it decreases fertility of males and caused atresia in oocytes of females. A proper balance of amino acid profile should be put in mind in preparing fish diet for rearing fish for reproduction.

Up to the 40% inclusion level of faba bean middlings was shown in this study to be adequate, with no extra supplementation being needed for Nile tilapia. In this case, feeding cost and incidence cost were dramatically decreased and the profit index increased than the control group. The 10% level of FBM was the best economical inclusion level based on incidence cost and profit index. However, further studies are needed to find out the maximum utilization of FBM using other feeding regimes (e.g. *ad lib*), different feeding frequency and different types of pellet making (e.g. extrusion), considering the efficient utilization of essential nutrients through using supplemented amino acids, fatty acids and/or digestive enzymes.

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استجابة زريعة أسماك (البلطى النيلى من حيث النمو وفسيولوجياً وهستولوجياً واقتصادياً) للعلائق المحتوية على مستويات مختلفة من كسر فول الحقل.

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فى تجربة غذائية أجريت لدراسة تأثير إضافة مستويات مختلفة من مسحوق كسر الفول على النمو والاستفادة من الغذاء والعناصر الغذائية والجلوكوز والدهون الكلية والبروتينات الكلية للبلازما والتركيب الكيماوى لجسم الأسماك وتحليل التكلفة لأسماك البلطى النيلى.

استخدمت ١٠ أحواض زجاجية بأبعاد ٧٠ × ٣٠ × ٤٠ سم لتخزين ١٠ أسماك لكل حوض بمتوسط وزن ٣٧ر٠ جم لكل سمكة. تم تركيب ٥ علائق تجريبية متزنة فى البروتين (٣٠%) والطاقة لتحتوى على صفر، ١٠، ٢٠، ٣٠، ٤٠% من مسحوق كسر الفول. قدمت العلائق ثلاثة مرات يوميا فى ستة أيام لكل أسبوع لمدة ٩٨ يوما. وكانت الأسماك توزن كل أسبوعين ويعدل على أساسها كميات الغذاء.

وقد أشارت النتائج إلى أن الأسماك المغذاة على عليقة ١٠% مسحوق كسر الفول قد سجلت زيادة معنوية فى كل من وزن الجسم والزيادة الوزنية ومعدل النمو النوعى، ومعدل التحويل الغذائى وكفاءة الاستفادة من البروتين والطاقة مقارنة بالعلائق المحتوية على المستويات الأخرى من كسر الفول والمقارنة فى حين أن العلائق المحتوية على ٢٠، ٣٠، ٤٠% من كسر الفول قد سجلت انخفاضاً معنويا فى كل من كفاءة النمو والقياسات الغذائية الأخرى مقارنة بالعليقة المقارنة.

كما أظهرت النتائج أنه لا توجد اختلافات معنوية فى محتوى الجسم (الماء والبروتين والمستخلص الإثيرى والرماد والطاقة) للأسماك التى تغذت على مستويات مختلفة من كسر الفول فى حين أن الجلوكوز والبروتينات الكلية والدهون الكلية للبلازما قد تناقصت بزيادة مستوى كسر الفول فى العلائق. وأوضح تحليل التكلفة والربح أن ١٠% من كسر الفول كان أفضل مستويات الإضافة من الناحية الأقتصادية.