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REPLACEMENT OF FISH MEAL BY COTTONSEED MEAL SUPPLEMENTED WITH VITAMIN E IN DIETS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

BY

Abdella, M. M.*; Hamed, M. R.; El-Sayad, G. A.*; Soltan, M. A.* and Saudy, A. M.****

* Faculty of Agriculture, Moshtohor, Benha University

** National Organization for Drug Control and Research

ABSTRACT

The present experiment was conducted to evaluate the effect of α-tocopherol as an antioxidant for avoiding the side effects of incorporation of cottonseed meal (CSM) in the diets of Nile tilapia as a replacer of fish meal (FM). Two diets were prepared, the first one contained fish meal and considered as control, in the second diet FM protein was completely replaced by CSM. The second diet was divided into ten parts, the first one served as control and the other 9 diets were enriched by increasing levels of vitamin E (1, 5, 10, 15, 20, 25, 30, 35 and 40 times of gossypol in CSM). Each aquarium was stocked with 25 fry. The experimental fish fed the diets for 90 days. Results can be summarized as follows:

- The highest average body weight (9.91 g) was recorded by the control group fed the basal diet (contained FM) and the lowest BW (5.66 g) was obtained for fish fed diet D2 in which FM was completely replaced by CSM without vitamin E supplementation. Incorporation of vitamin E in the experimental diets significantly improved the BW. Similar trend was observed for the other growth performance parameters, body length (BL), weight gain (WG) and specific growth rate (SGR).
- The best FCR was recorded for fish group fed diets D1 and D3 which were significantly different from FCR recorded for the other treatments. The worst FCR was recorded in fish group fed diet D2 in which FM was completely replaced by CSM without addition of vitamin E. Incorporation of vitamin E in the experimental diets improved FCR specially fish group fed diet D3.
- Protein content of the whole fish body ranged between 41.33 and 50.97% and the differences were significant. Fat content of fish fed diet D2 was significantly higher than those obtained for fish fed the other groups. Compared to the two control diets D1 and D2 all vitamin levels in the experimental diets increased ash content of the fish whole body and the graded levels of vitamin E in the diets significantly altered the ash content of tilapia fish.
- The highest values for hematocrite (Ht) and haemoglobin (Hb) were obtained for fish fed the control diet (D1). Incorporation of vitamin E in the experimental diets at increasing levels significantly affected Hb and Ht. Compared to control group (Diet1), AST and ALT levels were significantly increased for fish groups fed the other supplemented experimental diets.

INTRODUCTION

The high cost of fish meal in tilapia diets warrants the potential use of cottonseed meal (CSM) as an alternative source of high quality protein. Cottonseed, *Gossypium hirsute* Linnaeus, is the third leading legume seed by weight (after soybean and rapeseed) used worldwide. Owing to its high protein value for human consumption (Alford *et al.*, 1996) and animals, as well as low market price in comparison with other legumes and fish meal, cottonseed meal (CSM) consequently has an immense potential for incurporation in high-protein aquafeeds. Lee *et al.*, (2006) summarized a series of studies in rainbow trout where CSM replaced fish meal entirely over the 3-year period without significantly impacting growth rate of female and male rainbow trout. In the study of Robinson and Li (1994), channel catfish, *Ictalurus punctatus*, were fed to satiation a diet containing 51% CSM with supplemented lysine (0.65%). Results indicated that growth rate; dressing percentage and chemical composition of the fillets did not differ significantly from fish fed diets containing SBM (42%).

In tilapia *Oreochromis* sp. Rinchard *et al.*, (2002) and Garcia-Abiado, *et al.*, (2005) found that, detoxification of gossypol from CSM prevent inhibition of pepsin and trypsin, activiting as well as accelerating removal of gossypol via bile and post-absorptive transport. Impaired absorption of gossypol may allow complete utilization of CSM protein, and realization of growth-promoting effects, for instance, via flavonoid contributions (antioxidant action) or steroidogenesis, as stimulation of testosterone concentrations, have been observed in rainbow trout (Dabrowski *et al.*, 2000) and tilapia (Rinchard *et al.* 2002).

Free gossypol, when present in large quantity in the diet, has been shown to be toxic to monogastric animals including fish. Growth depression occurred in channel catfish fed diets containing more than 900 mg free gossypol/kg diet (Dorsa *et al.*, 1982), whereas a diet containing as low as 290 mg free gossypol/kg diet reduced the growth of rainbow trout (Herman, 1970). Iron as ferrous sulfate, has been successfully used to counteract the toxicity of free gossypol in diets of monogastric, terrestrial animals. However high level of supplemental iron used to counteract the toxicity of gossypol may be harmful to fish because it has been suggested that a delicate balance exists between the need of iron for host defense mechanisms and the need of iron to sustain microbial growth.

Free gossypol is known to bind lysine rendering it less bioavailable (Wilson *et al.*, 1981). Gossypol or other compounds present in CSM may have a beneficial effect by improving the immune response and the resistance of juvenile channel catfish against *E .ictaluri* infection as evidence by increased macrophage chemotaxis, improved survival and continued consumption of diets containing CSM.

Garcia-Abiado, *et al.*, (2004) reported that, fish fed 25-50% CSM protein replacement showed similar body weight and total lengths as the controls at the completion of the 16-week trial. Fish fed 75 and 100% CSM protein in replacement to FM showed a significant decline in body weight and total length. They added that fish groups fed the diets in which 25, 50, 75 or 100% FM was replaced by CSM had significant lower Ht and Hb compared with levels in fish fed FM control diet.

MATERIALS AND METHODS

Nile tilapia fingerlings were obtained from The World Fish Center at Abbassa, Sharkya Governorate, Egypt and acclimated to laboratory conditions in 1700-L fibreglass tanks. The feeding trial was performed at the Fish Nutrition Lab (Fac. Agric., Benha University, Egypt).

Experimental design:

The present study aimed to evaluate the effect of α-tocopherol as an antioxidant for avoiding the side effects of incorporation of CSM in the diets of Nile tilapia as a replacer of fish meal (FM). Two experimental diets were prepared, the first one contained FM and considered as control, while in the second diet FM was completely replaced by CSM. The second diet was divided into ten parts, the first one was considered as control one and the other 9 parts were enriched by increment levels of vitamin E (1, 5, 10, 15, 20, 25, 30, 35 and 40 times of gossypol in (CSM). Each experimental diet was fed in two aquaria (2 replicates for each diet) therefore 22 rectangular aquaria 100 × 40 × 50 cm (180 liter for each) were used in the present study and each aquarium was stocked with 25 fish (2.00 – 2.10 g).

The practical work of the present study was carried out during the period from 9 December 2006 to 9 March 2007 the fish were grouped into eleven (11) groups and each group was arranged in two aquaria and fed the experimental diets replicates.

Diets preparation

The experimental diets were prepared by thoroughly mixing the different ingredients which composed of fish meal, soybean meal, cotton seed meal, yellow corn, vegetable oil and wheat bran. In preparing the diets, dry ingredients were first ground to a small particle size. Ingredients were mixed and then water was added to obtain a 30% moisture level. Diets were passed through a mincer machine with diameter of 2 mm and the resulting pellets were sun dried for 3 days. All diets were formulated to be isonitrogenous (30% protein) and isocaloric [2700 kcal metabolizable energy (ME)/kg diet]. The composition and proximate analysis of the experimental diets are presented in Table (1).

Tilapia fry were fed the pelleted diets (2 mm in diameter) at a daily rate of 10% (during the 1st month), then gradually decreased to 7% (2nd month) and 4% (3rd month) of total biomass. Nile tilapia fed the experimental diets 6 day/week (twice daily at 9.00 am and 3.00 pm) and the amount of feed was bi-weekly re-adjusted according to the changes in body weight throughout the experimental period (90 days). About 25% of water volume in each aquarium was daily replaced by de-chlorinated aerated fresh water after cleaning and removing the accumulated excreta. Water temperature, pH and dissolved oxygen were measured daily at 2.00 pm while total ammonia was weekly measured. Water quality parameters measured were found to be within acceptable limits for fish growth and health (Boyd, 1979).

Table (1): Composition and proximate analysis of the experimental diets

Ingredients	Experimental diets										
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
Fish meal	20	0	0	0	0	0	0	0	0	0	0
Soy bean meal	30	30	30	30	30	30	30	30	30	30	30
Yellow corn	35	35	35	35	35	35	35	35	35	35	35
Cottonseed	0	26	26	26	26	26	26	26	26	26	26
Corn oil	2	2	2	2	2	2	2	2	2	2	2
Wheat bran	10	4	4	4	4	4	4	4	4	4	4
Min&vit.*	3	3	3	3	3	3	3	3	3	3	3
Vitamin E (IU) kg^{-1}	-	-	36.4	182	364	546	728	910	1092	1274	1456
Total	100	100	100	100	100	100	100	100	100	100	100
Dry matter (DM)	95.00	96.01	95.00	95.90	95.88	96.10	96.02	95.44	95.78	95.88	96.20
Crude protein (CP)	30.36	29.88	29.88	29.88	29.88	29.88	29.88	29.88	29.88	29.88	29.88
Ether extract (EE)	4.44	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Ash	8.32	9.12	9.12	9.12	9.12	9.12	9.12	9.12	9.12	9.12	9.12
Crude fiber	5.35	6.22	6.22	6.22	6.22	6.22	6.22	6.22	6.22	6.22	6.22
NFE[2]	51.53	49.78	49.78	49.78	49.78	49.78	49.78	49.78	49.78	49.78	49.78
ME (Kcal/kg diet)[3]	2748	2692	2692	2692	2692	2692	2692	2692	2692	2692	2692
P/E ratio[4]	110.48	111	111	111	111	111	111	111	111	111	111

*Vitamin & mineral mixture/kg: Vitamin D_3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin,20 mg, Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

[2] Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash)

[3] Metabolizable energy was calculated from ingredients based on NRC (1993) values for tilapia.

[4] Protein to energy ratio in mg protein/Kcal ME.

Growth and feed utilization parameters:

Growth performance and feed utilization parameters were determined according to Cho and Kaushik (1985) as follows:

Condition factor (K) = $(W/L^3) \times 100$

Where,

W = body weight in grams and L = body length in cm.

Specific growth rate (SGR) = [(LnW2-LnW1)/t] ×100, Where: - Ln = the natural log, W1= initial fish weight; W2 = the final fish weight in "grams" and t = period in days.

Feed conversion ratio (FCR) = feed intake (g)/weight gain (g),

Protein efficiency ratio (PER) = weight gain (g)/protein intake (g),

Blood samples and liver function:

Blood samples were obtained from fish at the end of experimental period. Five fish per tank were randomly chosen and anaesthetized by ethylene glycol mono-phenol ether. Blood samples were collected from the caudal vein using heparinized 27-gauge needles and tuberculin syringes. Hematocrite (Ht) was determined using the micro-Ht method described by Brown (1988). Hemoglobin (Hb) was determined using the total Hb kit (Sigma Diagnostics, Sigma, St Louis, MO. USA) which is standardized procedure using the cyanomethemoglobin method. Liver was removed, homogenized and assigned for determination of Aspartate transaminase (AST) and Alanine transaminase (ALT) according to (Reitman and Frankel, 1957).

Determination of gossypol in CSM:

Gossypol content of CSM was determined by high performance liquid chromatography (HPLC) as described by Hron *et al.* (1990).

Chemical analysis:

At termination of the experiment, three fish were randomly sampled from each tank and subjected to the chemical analysis of whole fish body. Chemical analysis of fish, diets and feces were determined according to the methods of AOAC (1990).

Statistical analysis:

The statistical analysis of data was carried out by applying the computer program, SAS (1996) by adopting the model:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where:

Y_{ij} = the observation on the j^{th} fish eaten the i^{th} diet; μ = overall mean, α_i = the effect of i^{th} diet and e_{ij} = random error.

RESULTS AND DISCUSSION

Growth parameters:

The initial body weight (BW) of Nile tilapia fish, *O. niloticus* ranged from 2.0 to 2.1 g and the differences among these means were insignificant (Table 2). At the experiment all termination (after 90 days) the average BW ranged from 5.66 to 9.91 g and the difference in BW among the different treatments were significant ($P<0.001$) as shown in Table (2). The same trend was observed for the other growth performance parameters body length (BL), weigh gain (WG) and specific growth rate (SGR),Tables (2) and (3).

The highest average BW (9.91 g) was recorded for the control group fed the basal diet (contained fish meal) and the lowest one BW (5.66 g) was obtained for fish fed diet D2 in which fish meal was completely replaced by CSM without vitamin E supplementation. Results of the same table indicated that, incorporation of vitamin E in the experimental diet significantly improved the final BW (compared to fish group fed the diet D2) because the antioxidant effect of vitamin E in the these experimental diets and the best ratio between gossypol and vitamin E was found to be 1:1 (D3). Anderson and Sunderland (2002) reported that, α-tocopherol acetate, is the form of vitamin E commonly added to fish feed, it becomes active as antioxidant only after the hydrolysis of the acetate group in the fish body, and can not be sacrificed in the feed as an antioxidant.

As described in Table (2) the initial condition factor (K) for fish received the experimental diets ranged between 2.87 and

4.50 with significant differences. At the experimental termination (after 90 days from the experimental start) the highest average condition factor (2.71) was recorded for fish group fed diet D2 and the differences in K values of Nile tilapia significantly (P>0.05) affected by the different experimental treatments.

Table (2): Least square means and standard error for the effect of replacing fish meal by cotton seed meal and different levels of Vit. E in Nile tilapia diets on body weight, body length and condition factor.

Diets		Body weight		Body length		Condition factor	
	No.	Initial	Final	Initial	Final	Initial	Final
Diet 1 (Control)	50	2.04	9.91 a	4.10	7.59 a	2.87 c	2.18de
Diet 2 (CSM)	50	2.00	5.66 d	4.00	5.55 d	3.18 c	2.7a
Diet 3(G:Vit.E 1:1)	50	2.00	8.21 b	4.01	7.11 b	2.92 c	2.41abcd
Diet 4 (G:Vit.E 1:5)	50	2.00	7.78 b	4.10	7.03 b	3.00 c	2.23cde
Diet 5 (G:Vit.E 1:10)	50	2.03	7.12 cb	4.10	7.11 b	3.04 c	2.31bcd
Diet 6 (G:Vit.E 1:15)	50	2.00	7.00 cb	4.00	6.68 cb	2.96 c	2.43abcd
Diet 7 (G:Vit.E 1:20)	50	2.10	7.67 b	4.04	7.11 b	4.50 a	1.97e
Diet 8 (G:Vit.E 1:25)	50	2.00	6.22 cd	4.00	6.61 cb	3.30 c	2.15de
Diet 9 (G:Vit.E 1:30)	50	2.00	6.96 cb	4.00	6.61 cb	4.16 ab	2.40abcd
Diet 10 (G:Vit.E 1:35)	50	2.02	7.11 cb	4.11	6.53 cb	2.98 c	2.59ab
Diet 11((G:Vit.E 1:40)	50	2.00	7.21cb	3..88	6.65 cb	3.20 c	2.53 abc
Standard error		± 0.12	0.41	±0.11	±0.17	±0.30	±0.11

Averages followed by different letters in each column are significantly different (P<0.05)

Early studies have indicated that the amount of CSM that can be used in Nile tilapia feed depends mainly on the level of free gossypol and available lysine content of the meal. Due to unfavorable physiological effects of gossypol and to a reduction in the biological availability of lysine because of the binding properties of gossypol (Dorsa, *et al.*, 1982). Ofojekwn and Ejike (1984) and Rosbinson, *et al.* (1984) found that *O. aureus* fed CSM-based diets yield poor performance. The authors attributed the poor performance to the gossypol in CSM. On contrary repressed solvent-extracted CSM was successfully used as a single dietary protein source for *O. mossambicus* (Jackson et al., 1982) and Nile tilapia (El-Sayed 1990). and Nile tilapia (El-Sayed 1990). El-Saidy and Gaber (2003) indicated that, regardless of supplemental levels of iron, fish fed diets that contained 67% CSM (972 mg free gossypol) supplemented with lysine to level equal to that of the fish meal (FM) diet and supplemented with 972 mg Fe/kg diet exhibited better BW and SGR than those fed diet with 67%CSM without additional iron. This may be due to the addition of iron sulphate at a weight ratio of 1:1 of iron to free gossypol was effective in reducing the toxicity of free gossypol and improving their performances.

Feed utilization parameters*:*

The average feed intake (FI), FCR and PER are outlined in Table (3). As described in this table, the highest FI values were recorded for fish feed the control diet (contained fish meal) which are significantly different from those of fish fed the other diets. The high-crude fiber and poor palatability of CSM may reduce FI.

The best FCR values were recorded for fish groups fed diets D1 and D3 which were significantly different from FCR values were recorded for fish of other treatments. The poorest PER was recorded for fish group fed diet D2 in which FM was completely replaced by CSM without addition of vitamin E. Incorporation of vitamin E in the experimental diets improved PER specially fish group fed diet D3.

Table (3): Least square means and standard error for the effect of replacing fish meal by cottonseed meal and different levels of Vit.E in Nile tilapia diets on growth and feed utilization parameters (feed intake feed conversion ratio and protein efficiency ratio)

Diets	No.+	Weight gain (g/fish)	Specific growth rate	Feed intake (g/fish	Feed conversion ratio	Protein efficiency ratio
Diet 1 (Control)	2	7.87 a	1.78 a	18.78 a	2.38 g	1.41 a
Diet 2 (CSM)	2	3.64 d	1.18 d	13.92 cb	3.82 a	0.87 e
Diet 3(G: Vit.E) (1:1)	2	6.40 ba	1.61 ba	15.14 b	2.36 g	1.42 a
Diet 4 (G Vit.E) (1:5)	2	5.79 ba	1.52 bc	15.88 b	2.74 fed	1.22 abc
Diet 5 (G: Vit.E) (1:10)	2	5.17 c	1.41 bcd	15.44 b	2.98 cbd	1.12 bdec
Diet 6 (G: Vit.E) (1:15)	2	5.37 bcd	1.51 bcd	13.77 cb	2.56 fg	1.31 ba
Diet 7 (G: Vit.E) (1:20)	2	4.99 bcd	1.21 bcd	15.49 b	3.10 cb	1.10 bdec
Diet 8 (G: Vit.E) (1:25)	2	4.21 cd	1.31 cd	15.33 b	3.64 a	0.92 de
Diet 9 (G: Vit.E) (1:30)	2	4.93 bcd	1.41 bcd	14.72 b	2.88 ced	1.16 abcd
Diet 10 (G: Vit.E 1:35)	2	5.26 bcd	1.40 bcd	15.22 b	2.89 ced	1.15 bcde
Diet 11 (G: Vit.E 1:40)	2	5.25 bcd	1.47 bcd	13.99 cb	2.66 fe	1.25 abc
Standard error	2	±0.56	± 0.07	±0.62	0.09	±0.08

Averages followed by different letters in each column are significantly different ($P<0.05$)
Were (NO) the number of fish from each aquarium.

Barros *et al.* (2002) carried out a 3×3 factorial experiment using three basal diets containing 0, 27.5 or 55.0% solvent-extracted CSM as replacement of 0, 50 or 100% of solvent-extracted soybean meal with three levels of iron (40, 336, 671 mg k^{-1}) the basal diets were fed to juvenile channel catfish for 10 weeks. Values of FI, FCR and PER were similar for diets containing 0 and 27.5% CSM but were significantly lower for diets containing 55.0% CSM. Dietary levels of supplemental iron had no effect on FI, FCR and PER but the interaction between CSM and iron was significant. For diets without CSM, there was a linear increase in FI, FCR and PER with increasing dietary level of iron. For diets without CSM, there was a linear increase in FI with increasing dietary level of iron. For diets containing 55.5% CSM, the effect of increasing dietary iron on FI, FCR and PER was quadratic with fish fed diet supplemented with 336 mg iron/kg having the lowest FI. At a dietary level of iron of 336 mg/kg diet, FI of the 0 and 27.5% CSM diets was similar but the value was significantly lower for the 55.0% CSM diet. At 671 mg/kg of dietary iron, FI linearly decreased with increasing dietary level of CSM.

On the contrary, Barros, *et al.* (2002) reported that channel catfish fed a diet containing 50%CSM and supplemented with 671mg Fe kg diet had no effect on growth of fish or the haematological values compared to treatments without dietary iron added. They attributed that to diets high in SBM may contain compounds or factors, which reduce iron absorption or availability. It is well known that the rate of digestion and nutrients assimilation in fish may be influenced by various physiological and a biotic factors, including fish, size, ration level and temperature (NRC 1993). EL Saidy and Gaber (2002) showed that total replacement of FM with CSM (0.145% free gossypol) reduced the nutritional value of diets. For cottonseed meal containing diets, supplemented with iron, as ferrous sulphate at 1:1 ratio of iron to free gossypol had no effects on the nutritional value of the diets. In addition, iron presents in practical diets at a level of 972mg Fe/kg diet appears to be sufficient to maintain normal function of growth performance, feed utilization, biological and haematological parameters of Nile tilapia.

Blood parameters:

The highest values for Ht and Hb were obtained for fish fed the control diet (D1). Incorporation of vitamin E in the experimental diets at increasing levels significantly decreased each of hematocrite and haemoglobin values. El-Saidy and Gaber (2004) indicated that, the response of fish based diets on Ht and Hb to dietary CSM was influenced by supplemental levels of dietary iron. For diets containing no CSM (fish meal based diets), there was an increase in these parameters. When FM was completely replaced by CSM (67%) supplemented with 972. 1458 and 1944 mg Fe/kg diet exhibited superior results of Ht, Hb and RBC to fish meal diet. Barros *et al.* (2002) found that, Ht and Hb were not significantly affected by dietary level of CSM. Ht and Hb, however, were not affected by dietary iron level. Interaction between dietary levels of CSM and iron was significant for Hb but had no effect on Ht. However Hb values decreased linearly with increasing dietary level of iron. Another major finding of this study was the similarity in pathological effects of gossypol in tilapia (low Hb and Ht levels) with the effects of vitamin E and/or vitamin C deficiency in juvenile rainbow trout (Moccia, *et al.,* 1984 & Frischnecht *et al.,* 1994). It is possible that gossypol also interferes with cell membrane integrity hence inducing erythrocyte fragility (Fu *et al.,* 1988).

Tilapia fish *Oreachromis spp,* was reared semi-intensively and intensively by (Vialo and Zohar 1984; Middendorp and Huisman 1995; Middendorp 1995 a, b) they reported that, a polyphenolic substance with known toxic effects in fish that include growth depression, reduced haematocrit and haemoglobin, as well as total plasma protein, and liver and kidney damage (Herman 1970). Free gossypol is a membrane active agent with cytocidal properties and has the ability to inhibit membrane bound enzymes, causing hemolytic anemia at high concentrations in mammals. The cytotoxic effects of gossypol include increase in red blood cell fragility and accurrence of hypokalaemia in animal models (Colin-Negrete, *et al.,* 1996; Randel, *et al.,* 1996; Nikokyris, *et al.,* 1999).

Rinchard *et al.* (2000) showed that haemoglobin and haematocrit decreased significantly when adult rainbow trout, *Oncorbynchus mykiss*, were fed a diet containing 100% CSM protein as fish meal protein replacement. This suggests that the properties of fish blood are sensitive to physiological and pathological changes (Hibiya 1982) that are associated with gossypol toxicity.

Garcia-Abiado, *et al.* (2004) showed that increasing concentration of total gossypol in the diets (0.11-0.44%) resulted in a proportional increase of total gossypol in fish liver. And they provides supporting evidence on the deleterious effects of gossypol on Ht and Hb levels in tilapia blood, frequency of immature/abnormal erythrocytes, and accurrence of spleen abnormalities.

It has been shown that free gossypol traps iron causing anaemia and erythrocyte fragility (Nikokyris *et al.,* 1999). In swine, gossypol feeding resulted in iron deficiency anemia, because of binding of iron by the gossypol, which resulted in low Hb and Ht and the high iron-binding capacity of blood serum (Nikokyris *et al,* 1999). Erythrocyte fragility of Holstein dairy heifers fed diets containing 0 or 15% whole cottonseed were similar, but lower than that for heifers fed the 30% whole cottonseed diets, suggesting that effects of gossypol are concentration dependent (Colin *et al.,* 1996).

Liver functions:

Changes in liver enzymes (AST and ALT) are outlined in Table (4). As described in this table, compared to control group (Diet1) AST and ALT levels were significantly increased for fish groups fed the other experimental diets and the differences were significant.

Proximate analysis of whole fish of Nile tilapia:

The highest moisture content of fish whole body (Table, 4) was recorded for fish groups fed diets 7 and 10. And the lowest moisture content was recorded in fish groups fed the diets 3 and 5. The increasing levels of vitamin E in the experimental diets did not significantly affected moisture content of the fish whole bodies. Protein content of the whole fish ranged between 41.33 and 50.97% and the differences in CP were significant (P<0.05). Incorporation of vitamin E in the experimental diets (diet2, diet3 and diet4) significantly increased protein content of fish bodies as compared to fish fed the control diet (diet 1) while the highest incorporation levels of vitamin E in the experimental diets (D6, D7, D10 and D11) significantly (P<0.05) reduced protein content of fish whole body compared to fish of the control group.

Table (4): Least square means and standard error for the effect of replacing fish meal by cotton seed meal and different levels of Vit.E in Nile tilapia diets on blood parameters.

Diets	No.+	Ht	Hb	AST	ALT
Diet 1 (Control)	4	26.73 a	11.16 a	110.0 e	81.66 c
Diet 2 (CSM)	4	17.30 e	4.96 h	147.66 c	152.0 a
Diet 3(G: Vit. E 1:1)	4	20.63 c	7.33 ed	155.0abc	154.0 a
Diet 4 (G: Vit E 1:5)	4	14.22 f	5.00 h	150.66bc	155.6 a
Diet 5 (G: Vit.E 1:10)	4	20.65 c	5.15 g	155.0abc	155.3 a
Diet 6 (G: Vit.E 1:15)	4	26.45 a	9.35 bc	150.16abc	153.5 a
Diet 7 (G: Vit.E 1:20)	4	19.40 d	7.10 e	149.66 bc	146.5 a
Diet 8 (G: Vit.E 1:25)	4	23.37 b	9.03 c	158.0 ab	148.0 a
Diet 9 (G: Vit.E 1:30)	4	20.20 c	9.60 d	137.83 d	137.303b
Diet 10 (G: Vit.E 1:35)	4	19.43 d	7.56 d	149.33 c	152.33 a
Diet 11 (G: Vit. E 1:40)	4	20.54 c	7.36 ed	161.0 a	152.33 a
Standard error		±0.22	± 0.14	±2.59	±3.15

Averages followed by different letters in each column are significantly different (P<0.05)
Were (NO) the number of fish from each aquarium.

The highest fat content of fish whole body was recorded in fish group fed diet D2 (in which fish meal was completely replaced by CSM without incorporation of vitamin E in the diet). The fat content of fish fed diet D2 was almost significantly higher than that of fish fed in other diets. Compared to the two control diets D1 and D2 not all vitamin levels in the experimental diets increased ash content of the fish whole body and the graded levels of vitamin E in the diets significantly altered the ash content of tilapia fish.

CONCLUSION

Based on the obtained results, the complete replacement of the high price fish meal by cottonseed meal could be safely achieved through avoidance of gossypol toxicity, the matter that was obtained by adding Vit. E in an equal amount of gossypol, utilizing an international unit of Vit. E in correspondence to a one mg of gossypol in the diets it is also worth noting that using the cotton seed meal diet have quite similar K factor and PER values as for fish fed the fish meal diet.

Percentages of changes in growth parameters values followed feeding with CSM with 1 to 1 gossypol and Vit. E as compared to those of feeding with FM. A Comparison of the growth parameter values in case of fish fed on CSM (1:1) gossypol and Vit.E diet with fish fed on FM diet showed a high degree of similarity in the growth outcome of the two types of feeding. The values of growth parameters followed feeding CSM were in the range of 80.62-110.50% of those of values of feeding FM. Moreover, the k factor and values PER were almost the same,. These findings indicate the economic importance of using CSM in fish feeding in the way formulated in the present work, as shown in Table 6. Thus, the two types of diets have comparable effects on growth.

Table (5): Least square means and standard error for the effect of replacing fish meal by cotton seed meal and different levels of Vit.E in Nile tilapia diets on proximate analysis of whole fish.

Diets	No.+	Moisture	CP	EE	Ash
Diet 1 (Control)	8	72.38bdc	45.77 b	21.44 b	10.79 b
Diet 2 (CSM)	8	73.50 ba	50.97a	23.62 a	10.87 ba
Diet 3(G: Vit. E) (1:1)	8	71.68 ed	50.72a	21.76 b	11.24 ba
Diet 4 (G: Vit. E) (1:5)	8	72.46 bdc	50.23a	22.75 ba	11.04 ba
Diet 5 (G: Vit. E) (1:10)	8	71.83 edc	46.72b	21.74 b	11.54 ba
Diet 6 (G: Vit. E) (1:15)	8	72.40 bdc	41.99cd	21.95 b	12.01 a
Diet 7 (G: Vit. E) (1:20)	8	74.23 a	44.47cb	21.28 b	11.25 ba
Diet 8 (G: Vit. E) (1:25)	8	73.45 ba	45.10b	22.63 ba	11.10 ba
Diet 9 (G: Vit. E) (1:30)	8	73.12 bc	47.46b	21.47 b	12.05 a
Diet 10 (G: Vit. E) (1:35)	8	74.42 a	41.33d	21.38 b	11.57 ba
Diet 11 (G: Vit. E) (1:40)	8	73.50 ba	41.33d	22.26ba	11.29 ba
Standard error		±0.44	±0.97	±0.531	±0.37

Averages followed by different letters in each column are significantly different ($P<0.05$)
Were (NO) the number of fish from each aquarium.

Table (6): Percentage of change of growth parameters after feeding CSM (G:Vit.E) (1:1) as compared to feeding with FM.

Growth Parameters	Types of diet		Relative growth parameter %
	FM	GSM	
Weight gain	7.87	6.40	81.32
Specific growth rate	1.78	1.61	90.50
Feed intake (g/fish)	18.78	15.14	80.62
Feed conversion ratio	2.38	2.36	99.20
Protein efficiency ratio	1.41	1.42	100.70
Body weight	9.91	8.21	82.80
Body length	7.59	7.11	93.67
Condition factor	2.18	2.41	110.50

REFERENCES

Alford, B.B.; Liepa, G.U. and Vanbeber, A. D. (1996): Cottonseed protein: what does the future hold? Plant Foods Human Nutrition, 49:1-11

Anderson, J. S. and Sunderland, R., (2002). Effect of extruder moisture and dryer processing temperature on vitamin C and E and asta-xanthin stability. Aquaculture 207, 137 – 149.

AOAC Association of Official Analytical Chemists, (1990): Official Methods of Analysis. 15th ed. AOAC, Arlington, VA, USA.

Barros, M.M.; Lim, C. and Klesius, P.H. (2002): Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri* challenge. Aquaculture, 207: 263-798.

Boyd, D. (1979): Water Quality in Warm-water Fish Ponds. Auburn University Agricultural Experiment Station, Auburn, AL.

Brown, B.A. (1988): Routine hematology procedures. In: Hematology: Principle and Procedures (ed, by B. A. Brown). Pp. 7-122. Leo and Febiger, Philadelphia, PA. USA.

Cho, C. Y. and Kaushik, S. J. (1985): Effect of protein intake on metabolizable and net energy values of fish diets. In: Nutrition and Feeding in Fish (ed. by C.B. Cowey, A.M. Mackie & J.G. Bell), pp. 95– 117. Academic Press, London, UK

Colin-Negrete, J.; Kiesling, H.E.; Ross, T.T. and Smith, J.F.(1996): Effect of whole cottonseed on serum constituents, fragility of erythrocyte cells, and reproduction of growing Holstein heifers. *Journal of Dietary Science*79:2016-2023.

Dabrowski, K.; Rinchard, J.; Lee, K.-J.; Blom, J.H.; Ciereszko, A. and Ottobre, J. (2000): Effects of diets containing gossypol on reproductive capacity of rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod,. 62:227–234.

Dorsa, W.J.; Robinette, H.R.; Robinson, E.H. and Poe, W.E. (1982): Effects of dietary cottonseed meal and gossypol on growth of young channel catfish. Trans. Am. Fish. Soc,. 111: 651– 655.

El-Saidy D.M. and Gaber M.M. (2002): Complete replacement of fish meal by soybean meal with the dietary L-lysine supplementation in Nile tilapia fingerlings. Journal of the World Aquaculture Society 33:297-306.

El-Saidy, D.M. and Gaber, M.M. (2003): Replacement of fish meal with a mixture of different plant protein sources in Juvenile Nile tilapia diets. Aquaculture Research 34. 1119-1127.

El-Saidy D.M. and Gaber M.M. (2004): Use of cottonseed meal supplemented with iron for detoxification of gossypol as a total replacement of fish meal in Nile tilapia *Oreochromis niloticus* (L.) diets. Aquaculture Research,. 35: 859-865.

El-Sayed, A. M. (1990): Long-term evaluation of cottonseed meal as a protein source for Nile tilapia *Oreochromis niloticus,* (Linn.). Aquaculture, 87:315–320.

Frischknechet R.; Wahli, T. and Meier, W. (1994): Comparison of pathological changes due to deficiency of vitamin C, vitamin E and combination of vitamin C and E in rainbow trout, (*Oncorhynchus mykiss*). (Walbaum). J. Fish Diseases,. 17:31-45.

Fu, Y.F.; Zhang, S.L.; Lu, Z.M. and Wang, W.(1988): Effect of gossypol on the activity of kidney (Na^+ + K^+)-ATPase and the function of erythrocyte membrane. *Contraception,* 37:179-184.

Garcia-Abiado, M.A.; Mbahinzireki, G.; Rinchard, J.; Lee, K. J. and Dabrowski, K. (2004): Effect of diets containing gossypol on blood parameters and spleen structure in tilapia, *Oreochromis sp.,* reared in a recirculating system. *Journal of fish Diseases* .27: 359-368.

Garcia-Abiado, M.A.; Mbahinzireki, G.; Rinchard, J.; Lee, K.J. and Dabrowski, K. (2005): Effect of diets containing gossypol on blood parameters and spleen structure in tilapia, *Oreochromis* sp., reared in recirculating system. Journal of Fish Diseases, 27:359-368.

Herman, R.L. (1970): Effects of gossypol on rainbow trout (*Salmo gairdneri*). Journal of Fish Biology, 2:293-297.

Hibiya T. (1982): An Atlas of Fish Histology-Normal and Pathological Feattures. Kodanasha Ltd, Tpkyo.

Hron, R. J; Kuk M S and Abraham, G. (1990): Determination of free and total gossypol by high performance liquid chromatography. J. AOCS, 67: 182-187.

Jackson, A.J.; Capper, B.S. and Matty, A.J. (1982): Evaluation of some plant proteins in complete diets for the tilapia. *mossambicus*. Aquaculture,. 27: 97–109.

Jones, L. A., (1987). Recent advances in using cottonseed products . Proceedings of the Florida Nutrition conference, 12 – 13 March 1987, Daytona Beach, FL, PP . 119 – 138 .

Lee, K.J.; Rinchard, J.; Dabrowski, K.; Babiak, I.; Ottobre, J.S. and Christensen, J. E. (2006): Long-term effects of dietary cottonseed meal on growth and reproductive performance of rainbow trout: three-year study. Animal Feed Science and Technology, 126:93-106.

Martin S. D. (1990): Gossypool effects in animal feeding can be controlled. Feedstuffs, 62:14- 17.

Mbahinzireki, G. B. Dabrowski K.J.; EL-Saidy D. and Wisner E.R. (2001) Growth, feed utilization, and body composition of tilapia (*Oreochromis sp.*) fed cottonseed meals-based diets in a recalculating system. *Aquaculture Nutrition* 7: 189-200.

Mbahinzireki, G.B.; Dabrowski, K.; Lee, K.-J.; El-Saidy, D. and Wisner, E. R. (2000): Growth, feed utilization and body composition of tilapia (*Oreochromis* sp.) fed cottonseed meal-based diets in a recirculating system. Aquacult. Nutr. 7:189–200.

Middendop, A.J. (1995a) Pond farming of Nile tilapia *(Oreochromis niloticus)*(L.) in northern Cameroon. Feeding compination of cottonseed cake and brewery waste in fingerling culture, hand-sexed male monosex culture, and mixed culture with police-fish, *Clarias gariepinas* (Burchel). Aquaculture Recearch 26: 715-722.

Middendop A. J. (1995b): Pond farming of Nile tilapia,*(Oreochromis niloticus)*(L.) in northern Cameroon. Mixed culture of large tilapia (>200 g) with cattle manure and cottonseed cake as pond inputs, African catfish *Clarias gariepinus* (Burchell), as police-fish. Aquaculture Research 26: 723-730.

Middendorp, A. J. and Huisman, E. A. (1995): Pond farming of Nile tilapia, *Oreochromis niloticus* in northern Cameroon. Comparing two different strategies for feeding cottonseed cake in tilapia male monosex culture. *Aquaculture Research* 26, 731-738.

Moccia, R. D.; Hung, S.S.; Slinger, S.J. and Ferguson, H.W.(1984): Effect of oxidized fish oil, vitamin E and ethoxyquin on the histopathology and hematology of rainbow trout, *Salmo gairdnerii* Richardson. Journal of Fish Disease, 7: 269-282.

Nikokyris, P.N.; Kandylis, K. and Deligiannis, K. (1999): Effect of varying levels of dietary free gossypol in whole cottonseed on physiological response of growing-fattening lambs. Journal of the Science of Food and Agriculture, 79: 1969-1981.

NRC (National Research Council) (NRC) (1983): nutrient requirement of warm water fish and shellfish. National Academy of Sciences, Washington D.C.

(National Research Council), (NRC) (1993): nutrient requirements of fish. National Academy Press Washington D.C.

Ofojekwu P . C . and Ejike, C. (1984): Growth response and feed utilization in tropical cichild *(oreochromis niloticus)* (LIN) fed on cottonseed–based artificial diets. Aquaculture.42: 27-36.

Randel, R.D; Willard, S.T; Wyse, S.J. and French, L.N. (1996): Effects of diets containing free gossypol on follicular development embryo recovery and corpus luteum function in Brangus heifers treated with bFSH. Theriogenology, 45: 911-922.

Reitman, S. and Frankel, F. (1957): A colorimetric method for determination of oxaloacetic transaminase and serum glutamic puruvic transaminase. A.J. Clin. Path, 28: 56- 60.

Rinchard, J.; Mbahinzireki, G.; Dabrowski, K.; Lee, K. J.; Garcia-Abiado, M.A. and Ottobre J.S. (2002) Effect of dietary substitution of animal protein with plant protein on reproductive parameters of tropical fish tilapia. *Oreochromis* sp. Aquaculture International 10:11-28.

Rinchard, J.; Mbahinzireki, G.; Dabrowski, K.; Lee, K.-J.; Garcia-Abiado, M. A. and Ottobre, J. (2002): Effects of dietary cottonseed meal protein level on growth, gonad development and plasma sex steroid hormones of tropical fish tilapia *Oreochromis* sp. Aquacult. International,. 10:11–28.

Rinchard, J.; Mbahinzirereki, G.; Dabrowski, K.; Lee, K-J.; Garcia-Abiado, M.A. and Ottober, J., (2000): effects of partial or total substitution of fish protein with cottonseed meal in diets on reproductive parameters of tilapia *Oreochromis* sp. Aquaculture America 2000, Feb 2-5, 2000 New Orleans. P-282.

Robinson, E.R.; Rawles, S.D.; Oldenburg, P.W. and Stickney, R.R. (1984): Effects of feeding glandless or glanded cottonseed products and gossypol to Tilapia aurea. Aquaculture.28, 145–154.

Robinson, E. H. and Li, M. H. (1994): Use of plant proteins in catfish feeds: replacement of soybean meal with cottonseed meal and replacement of fish meal with soybean meal and cottonseed meal. J. World Aquac. Soc, 25: 271–276.

Robinson, E. H. and Rawies, S. D.(1983): use of defatted glandless flour and meal in channel catfish diets. In proceeding of animal Conference of Southeastern Association of fish and wildlife Agencies. 37: 358-363.

Robinson, E.H., (1991): Improvement of cottonseed meal protein with supplemental lysine in feeds for channel catfish. J. Appl. Aquacult. 1: 1-14.

SAS (1996): SAS procecdure guide, "ver sion 6 – 12 Fd ". SAS Institute Inc. .Cary, NC, USA.

Sealey, W.M.; Lim, C. and Klesius, P.H.(1997): Influence of the dietary level of iron from iron methionine and iron sulfate on immune response and resistance of channel catfish to Edwardsiella ictaluri. J. World Aquacult. Soc, 28: 142-149.

Viola, S. and Zohar G. (1984): Nutrition studies with market size hybrids of tilapia *Oreochromis* in intensive culture. *Bamidgeh,* 36: 3-15.

Wilson, R. P.; Robinson, E.H. and Poe, W.E., (1981): Apparent and true availability of amino acids from common feed ingredients for channel catfish. J. Nutr, 111: 923-929.

إحلال مسحوق السمك بكسب القطن المضاف إليه فيتامين هـ في علائق أسماك البلطي النيلي

محمد محمد عبدالله *، محمد رؤوف حامد، جمال علي الدين الصياد*، مجدي عبدالحميد سلطان*، عبدالناصر محمد سعودي**

* قسم الإنتاج الحيواني ـ كلية الزراعة بمشتهر جامعة بنها.

** الهيئة القومية للرقابة والبحوث الدوائية

أجريت هذه التجربة بهدف تقييم تأثير إضافة فيتامبن هـ إلى علائق أسماك البلطي لإزالة سمية تأثير الجوسيبول من كسب القطن كبديل لمسحوق السمك. وفي هذه التجربة تم تكوين ١١ عليقة. العليقة الأولى أستخدمت كعليقة ضابطة (تحتوى على مسحوق السمك) وفي العليقة الثانية تم إحلال مسحوق السمك إحلالاً تاماً بكسب بذرة القطن وتم تقسيم هذه العليقة إلى ١٠ علائق الأولى تركت للمقارنة والعلائق التجريبية الأخرى تم إضافة فيتامين هـ إلى العلائق بتركيزات متزايدة ١، ٥، ١٠، ١٥، ٢٠، ٢٥، ٣٠، ٣٥، ٤٠م ن مثل محتواها من الجوسيبول وتم تغذية كل عليقة لمجموعتين من الأسماك (مكررين) إستمرت التجربة ٩٠ يوم. وكان من أهم النتائج المتحصل عليها مايلي:

- كان أعلى متوسط لوزن الجسم قد تم الحصول عليه لمجموعة الكنترول (العليقة المحتوية على مسحوق السمك) بينما أعطت المعاملة الثانية أقل مقياس لوزن الجسم. وقد أدت إضافة فيتامين هـ إلى العلائق إلى تحسين وزن الجسم وقد أعطت صفات طول الجسم، الزيادة في وزن الجسم ومعدل النمو نتائج مشابهة لتلك المتحصل عليها بالنسبة لوزن الجسم عند نهاية التجربة.
- أظهرت النتائج أن أفضل معدل لتحويل الغذاء قد أظهرتها الأسماك التي تغذت على العلائق ١، ٣ كما أعطت الأسماك التي تغذت على العليقة ٢ أقل قيمه لكفاءة تحويل الغذاء وقد أدت إضافة فيتامين هـ إلى تحسين كفاءة تحويل الغذاء.
- أظهرت نتائج التحليل الكيماوي لجسم السمكة أن نسبة البروتين قد تراوحت بين ٣٣ر٤١-٩٧ر٥٠% وكانت الفروق بين المعاملات معنويه. أما بالنسبة لمحتوى جسم السمكه من الدهن فقد وجد أن الأسماك التي تغذت على العليقة ٢ قد أعطت أعلى نسبة للدهن في جسم السمكة. أما بالنسبة للرماد فقد وجد أن إضافة فيتامين هـ إلى العلائق قد أدى إلى زيادة نسبة الرماد في المادة الجافه.

بالنسبة لنسب الهيموجلوبين والهيماتوكريت في الدم فقد وجد أن أعلى قيم لهذه المقاييس تم الحصول عليها في عليقة الكنترول ١ كما أدت إضافة فيتامين هـ إلى العليقة إلى زيادة نسبة كلا من الهيموجلوبين والهيماتوكريت وقد أظهرت نتائج تحليل السيرم أن كانت إنزيمات الكبد نتائج مشابهة لتلك المتحصل عليها بالنسبة للهيموجلوبين والهيماتوكريت