Utilization of Cottonseed Meal Supplemented with Iron for Detoxification of Gossypol in Nile Tilapia, Broodstock and their Impact on the Hatchability of their Progenies

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Abstract

Nile tilapia broodstock fish of an initial average weight 72.3 g were stocked in 15 glass aquariums (500 L each) at 12 fish per aquarium were fed on cottonseed meal (40% CSM) diets as a total replacement of fishmeal. The diets were supplemented with different levels of iron (67, 67, 290, 580 and 870 mg/kg diet⁻¹) and supplemented with methionine and lysine to be similar to control diet. The fish fed twice daily at a rate of 2% of the total fish biomass daily until the end of the experiment. The results showed that final fish weight, specific growth rate and number of larvae produced increased with increasing iron level and reached maximum when fish fed diet contained 40% CSM supplemented with 580 mg Fe kg diet⁻¹ without causing, significant reduction in growth performance. In addition, the best results of larvae obtained from brood stock fed on diet contained 40% CSM supplemented with 580 mg Fe kg diet⁻¹. Red blood cell count, hematocrit and hemoglobin were increased with increasing levels of iron and significantly affected by dietary iron. Apparent digestibility coefficients of protein, fat dry matter and energy were relatively high for most diets supplemented with iron and increased by increasing iron supplementation. There were no significant ($P>0.05$) differences among fish fed diets 1 (100% FM) 4 and 5 which contained 100% CSM with additional 580 and 870 mg Fe kg diet supplemental iron. This study recommended that broodstock fed on diet contained 40% CSM supplemented with 580 mg Fe kg diet⁻¹ was comparable to fishmeal basal diet and have higher economic evaluation.

Keywords: Cottonseed; Iron; Brood stock; Nile tilapia

Introduction

Cottonseed meal (CSM) was ranks second to soybean meal in Egypt and less expensive than fishmeal and soybean meal per unit protein basis. Numerous studies have been conducted to determine the level of CSM that can be incorporated in Nile tilapia brood stock diets without affecting their growth performance [1-3]. Results have shown that the amount of CSM that can be included in Nile tilapia diets depends mainly on the levels of free gossypol and available lysine. El-Saidy [1] reported that repressed solvent extracted CSM could replace up to 50% of fishmeal in juvenile Nile tilapia diets without requiring lysine supplementation. Results Cheng et al. [4] suggest that 100% of SBM can be replaced by CSM with lysine supplement in diets contained 20% fish meal for Chinese Mitten Crab, *Eriocheir sinensis* without affecting growth performance and with decreasing level of ammonia released into the water. Free gossypol, when present in large quantity in the diet, it has shown to be toxic to monogastric animal including fish. Growth depression occurred in channel catfish fed diets counting more than 900 mg free gossypol per kg diet⁻¹ [5]. Whereas a diet containing as low as 290 mg free gossypol per kg diet⁻¹ reduced growth of rainbow trout [6]. In addition, gossypol is anti-carcinogenic activities [7,8]. Iron, as ferrous sulphate, was been successfully used to counteract the toxicity of free gossypol in diets of monogastric, terrestrial animals [9,10]. High levels of supplemental iron used to counteract the toxicity of gossypol may be harmful to fish because it was been suggested that a delicate balance exists between need of iron to sustain microbial growth. Sealey et al. [11] reported that high levels of dietary iron might lead to increased susceptibility of channel catfish to *Edwardsiella ictaluri* infection. Therefore, this study was be undertaken to evaluate the effects of total replacement of fishmeal protein by CSM protein supplemented with various levels of iron in practical diets on growth performance of Nile tilapia (*Oreochromis niloticus* L.) broodstock.

Materials and Methods

Experimental diets

The experimental diets were formulated to contain 25% crude protein and 18828 kJ of gross energy kg diet⁻¹ based on feedstuff values reported by NRC (1993) [12]. The control diet (1) with 100% fishmeal protein and four diets (2-5) with 100% CSM protein (0.0145% free gossypol). Diets supplemented with iron from ferrous sulphate at 0.0, 223, 513 and 803 mg Fe kg diet⁻¹ for diets 2-5 respectively were prepared. Since the mineral premix contained 67 mg iron as ferrous sulphate per kg diet, the total level of supplemental iron were 67, 67, 223, 513 and 803 mg Fe kg diet⁻¹. The experimental diets contained 0.0, 0.5, 1.0, and 1.5 mg iron for each mg of free gossypol for diets 2-5 respectively. The ingredients and chemical composition of the diets are shown in table 1.

Experimental fish

Nile tilapia (*Oreochromis niloticus*, L.) broodstock which obtained from (Saft khaled) Behira governorate of one year old with initial average weight 72.3 ± 2.4 g were maintained on commercial diet until they selected, weighted and randomly distributed into 15 experimental glass tanks (500 L). Each tank was stocked in ratio of nine females

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Ingredients (%)	Diets				
	1	2	3	4	5
Fish meal (60% C.P.)	30.0	--	--	--	--
Cottonseed meal (41 % C.P.)	--	40.0	40.0	40.0	40.0
Yellow corn meal	20.0	27.3	27.3	27.3	27.3
Wheat bran (14% C.P.)	30.0	30.0	30.0	30.0	30.0
Soybean oil	5.0	5.0	5.0	5.0	5.0
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0
Mineral and vitamin premix ¹	2.0	2.0	2.0	2.0	2.0
Molasses (as bender)	2.0	2.0	2.0	2.0	2.0
L-Methionine	--	0.7	0.7	0.7	0.7
L-Lysine	--	1.0	1.0	1.0	1.0
Supplemented iron mg kg diet ⁻¹	--	0.0	225	513	803
Proximate composition (%) ²					
Moisture	6.21	6.13	6.14	6.46	6.48
Crude protein	25.0	25.0	25.0	25.0	25.0
Crude fat	11.94	11.9	11.98	11.82	11.8
Crude fiber	7.3	7.2	7.1	7.2	7.2
Ash	10.3	10.1	10.0	9.9	9.8
NFE ³	39.25	39.67	39.78	39.62	39.72
Gross Energy (kj/g ⁻¹ diet)	18.82	18.82	18.82	18.82	4.5
Fe supplemented (mg kg diet ⁻¹) ⁴	67.0	67.0	290	580	870
Free gossypol (mg kg diet ⁻¹) ⁵	00	580	580	580	580
Fe : gossypol ratio	00	0.0:1	0.5 :1	1:1	1.5:1

¹Premix supplied the following vitamins and minerals (according to Xie et al.)

²Values represent the mean of three sample replicates.

³Nitrogen free extract (NFE) = {100 - (moisture+crude protein+crude fat+ash +crude fiber)}

⁴Fe-values include iron supplied by mineral premix.

⁵Calculated from the free gossypol content of cottonseed meal.

Table 1: Ingredients and proximate compositions of diets containing cottonseed meal (CSM) supplemented with various levels of iron for Nile tilapia broodstock.

Ingredients (%)	Diet
Fish meal (60 % C.P.)	37.5
Soybean meal (44 % C.P.)	17.0
Brewers dried yeast (44 % C.P.)	23.4
Wheat bran	-
Starch	13.0
L-methionine	1.0
L- lysine	0.5
Vitamin/Mineral. Premix	2.0
Molasses	2.0
Vitamin C.	0.1
Fish oil	3.0
Cr2O3	0.5
Proximate analysis (%)	
Moisture	13.3
Crude protein	41.5
Crude fat	12.3
Ash	6.6
Crude fiber	5.3
NFE ¹	21.0
Gross energy(kj g ⁻¹)	19.25
Cost (Egyptian pound) ²	2.5

¹NFE (Nitrogen free extract) =100-(%moisture +%C. protein +%C. fat +%C. fiber +ash)

²Price in Egyptian pound: 1.00=US \$0.14 based on 2005 exchange prices.

Table 2: Feed formulations and proximate composition of experimental diet used in feeding larvae (percentage on wet weight bases).

to three males. The fish were starved for one day prior to the start of experiment and five experimental diets namely 1-5 were assigned each to triplicate groups. Each group contained nine females' three males. When the females were ready for spawning about 5 days prior to spawning, the exiting males were used for mating in all treatment. The wire mesh between the males and females were removed after observing their courtship behavior. The day after spawning males were separated, returned to the original tanks. The eggs were collected from the female's buccal cavity and transferred to a strainer with, fine mesh, kept in plastic bowl containing dechlorinated water and aerated continuously. Every day, dead eggs were removed and halve of dechlorinated water in

each container replenished. The larvae from hatching to complete yolk sac from each treatment were counted and distributed into three glass aquaria (100 L) with total 15 glass aquaria were stocked with 200 larvae with an average weight 10 ± 2 mg. The larvae were fed on a diet for one month (Table 2). The larvae were fed 6 days a week with a rate 10 % of body weight until the end of experiment.

The experimental setup

During the experiment, the aquaria were supplied with fresh water about one-third of water volume in each aquaria was replaced daily by aerated fresh water after cleaning and removing the accumulated excreta. All aquaria were aerated. A photoperiod of 12 h light, 12 h dark (08:00-20:00 hours) was used. The illumination was supplied by fluorescent ceiling light. Each group of fish was weight at the beginning and every 2- week through the experimental period (12 weeks). The brood stocks were fed 6 days a week with a rate 2% of body weight until the end of experiment. At the end of experiment three fish from each group (nine fish per each treatment) was killed homogenized and frozen. During last month, feces were collected from each aquarium every morning before feeding. The feces were collected on filter paper for drying and subsequent chemical analysis. The apparent digestibility coefficients (ADCs) for protein, lipid, dry matter and energy were calculated using the formula of Maynard and Loosli [13]. The $ADC = 100 \times [1 - (\% \text{dietary } Cr_2O_3 / \% \text{fecal } Cr_2O_3 - \% \text{fecal nutrient} / \% \text{dietary nutrient})]$

Hematological assay

Blood samples were obtained from broodstock at the end of experimental period. Four fish per group were randomly chosen and anaesthetized with tricainemethanesulphonate (MS-222, Argent Chemical Redmond, WA, USA) at 125 mg L⁻¹. Blood samples were collected from the caudal vein using heparinized 27- gauge needles and tuberculin syringes (20 Um L⁻¹) for determination of hematocrit (Ht), red blood cell count (RBC) and hemoglobin (Hb). Hematocrit was determined using the micro-Ht method described by Brown [14]. Total RBCs were determined by diluting whole blood and enumeration in hemocytometer. Hemoglobin was determined using the total Hb kit (Sigma Diagnostics, Sigma, St Louis, MO, USA) which is standardized procedure using the cyanomethemoglobin method.

Chemical analyses

Analysis of samples was made as follows: dry matter after desiccation in an oven (105°C for 24 h); ash (incineration at 550°C for 12 h); crude protein (microkjeldahl, N x 6.25); crude lipid (ether extract using soxhlet method) crude fiber (AOAC 1995) and gross energy (Ballistic bomb calorimeter, Gallenkamp, UK). The chromic oxide in diets and

Parameters	Diets				
	1(control)	2	3	4	5
FBW ¹	170.6 ± 2.2 ^b	151.8 ± 2.5 ^d	161.3 ± 4.2 ^c	180.7 ± 5.8 ^a	171.2 ± 3.1 ^b
FI (g fish-1) ²	151.7 ± 0.3 ^{ab}	146.2 ± 0.8 ^c	146.1 ± 0.1 ^c	154.5 ± 0.1 ^a	150.7 ± 0.1 ^b
SGR (%/day) ³	1.02 ± 0.1 ^b	0.9 ± 0.1 ^c	0.9 ± 0.1 ^c	1.1 ± 0.1 ^a	1.02 ± 0.04 ^b
FCR (FI/WG) ⁴	1.54 ± 0.1 ^a	1.92 ± 0.03 ^d	1.82 ± 0.1 ^c	1.67 ± 0.1 ^b	1.73 ± 0.1 ^{bc}
PER ⁵	2.58 ± 0.1 ^{ab}	2.16 ± 0.1 ^c	2.42 ± 0.1 ^b	2.8 ± 0.2 ^a	2.6 ± 0.1 ^a

Values are mean ± standard deviation. Values in the same row with same super-scripts are not significantly different (P ≥ 0.05).

¹FBW=final body weight.

²FI= feed intake.

³SGR, specific growth rate= (Ln FBW-Ln IBW)/90x100.

⁴FCR, feed conversion ratio=dry feed fed/ body weight gain.

⁵PER, protein efficiency ratio= final body weight gain/protein intake X100.

Table 3: Growth performance and nutrient utilization of Nile tilapia Broodstock fed the experimental diets.

feces was determined using the method of Zhou et al. (2004). Gossypol content of CSM was analyzed using standard method of American oil chemists (AOCS 1998) [15].

Water quality

Water temperature and dissolved oxygen were measured every other day using an YSI Model 58 oxygen meter (Yellow Springs Instrument, Yellow Springs, OH, USA). Total ammonia and nitrite were measured twice weekly using a DREL/2000 spectrophotometer (Hash Company, Loveland, CO, USA). Total alkalinity and chloride were monitored twice weekly using the titration method; pH was monitored twice weekly using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH, USA). During the 30-week feeding trial, the water-quality parameters averaged ($\pm$ SD): water temperature, $27.6 \pm 0.9^\circ\text{C}$; dissolved oxygen, $6.5 \pm 0.5 \text{ mg L}^{-1}$; total ammonia, $0.18 \pm 0.14 \text{ mg L}^{-1}$; nitrite, $0.07 \pm 0.05 \text{ mg L}^{-1}$; total alkalinity, $183 \pm 45 \text{ mg L}^{-1}$; chlorides, $573 \pm 150 \text{ mg L}^{-1}$; pH, 7.9 ± 0.2 .

Calculations and Statistical Analysis

Calculations of growth parameters followed those described in a previous work [16]. Data were analyzed using analysis of variance (ANOVA) using the SAS ANOVA procedure (Statistical Analysis System 1988). Duncan's multiple range tests was used to compare differences among individual means. Treatment effects were considered significant at $P < 0.05$. All percentage and ratio were transformed to arcsin values prior to analysis [17].

Results

The results of average final body weight (FBW) specific growth rate (SGR) food conversion (FCR) and protein efficiency ratio (PER) are presented in table 3. At the start experiment (broodstock) there were no significant difference ($P > 0.05$) in average body weight which indicates that there were homogeneity among these groups. At the end of the experiment, the average FBW and SGR showed that the groups of fish fed diet 2 contained 40% CSM without iron supplement had the lowest value of FBW and SGR, when compared with groups of fish fed control diet 1 (100% FM protein). In addition, when compared with GSM-based diets (4-5) supplemented with iron at rate of 580 and 870 mg Fe/kg respectively. Among diets containing CSM, the response of fish to increasing levels of dietary iron above 580 mg Fe/kg diet⁻¹ there were no linear increase in FBW and SGR. Mean body weights of fish are shown in figure 1. Nile tilapia growth rates began to differ in week 6 and became distinctly different between weeks 8 and 12. Groups of fish fed diet 4 (40% CSM supplemented with 580 mg Fe/kg diet⁻¹)

Parameters	Treatments				
	1 (control)	2	3	4	5
FBW ¹	7.2 ± 0.7^a	4.5 ± 0.1^c	5.8 ± 0.1^b	7.1 ± 0.5^a	6.5 ± 0.6^{ab}
DGR (%day ⁻¹) ²	23.9 ± 2.1^a	14.9 ± 0.4^c	19.3 ± 0.3^b	23.6 ± 1.7^a	21.4 ± 2.4^{ab}
FI (g fish ⁻¹) ³	14.1 ± 0.2^a	11.5 ± 0.4^c	12.1 ± 0.3^b	13.9 ± 0.3^a	12.4 ± 0.4^b
FCR (FI/WG) ⁴	2.0 ± 0.2^b	2.6 ± 0.1^a	2.1 ± 0.1^b	2.0 ± 0.1^b	1.9 ± 0.1^b
PER ⁵	1.25 ± 0.1^a	1.0 ± 0.02^c	1.19 ± 0.04^b	1.27 ± 0.06^a	1.9 ± 0.1^a
Survival (%)	85.4 ± 1.3	71.4 ± 1.4	71.9 ± 1.1	84.2 ± 2.1	74.3 ± 3.4

Values are mean $\pm$ standard deviation. Values in the same row with same superscripts are not significantly different ($P \geq 0.05$).

¹FBW=final body weight.

²DGR, daily growth rate=final body weight gain/30 x100

³FI=feed intake.

⁴FCR, feed conversion ratio=dry feed fed/body weight gain.

⁵PER, protein efficiency ratio=final body weight gain/protein intake X100.

Table 4: Growth performance and nutrient utilization of Nile tilapia larvae fed the experimental diets.

Figure 1: Effect of total replacement of fishmeal with cottonseed meal supplemented with different levels of iron on growth of Nile tilapia Broodstock.

Parameters	Treatments				
	1 (control)	2	3	4	5
Dry mater	74.0 ± 0.6^a	63.8 ± 0.6^b	65.1 ± 0.8^d	72.0 ± 0.2^c	73.2 ± 0.1^b
Protein	86.1 ± 0.3^{ab}	76.5 ± 0.5^d	78.7 ± 0.4^c	86.4 ± 0.2^a	86.4 ± 0.2^a
Fat	78.0 ± 0.4^a	62.4 ± 0.6^d	63.8 ± 0.8^c	77.2 ± 0.6^a	78.4 ± 1.1^a
Energy	76.7 ± 0.6^c	60.1 ± 0.3^c	60.4 ± 0.7^c	70.3 ± 0.9^{ab}	72.1 ± 0.1^a

Values are mean $\pm$ standard deviation. Values in the same row with same superscripts are not significantly different ($P \geq 0.05$).

Table 5: Apparent digestibility of dry matter, fat, protein, and energy for Nile tilapia broodstock fed the experimental diets.

Parameters	Diets				
	1 (Control)	2	3	4	5
Ht (%)	74.0 ± 0.6^a	63.8 ± 0.6^b	65.1 ± 0.8^d	72.0 ± 0.2^c	73.2 ± 0.1^b
Hb (mg/ml)	86.1 ± 0.3^{ab}	76.5 ± 0.5^d	78.7 ± 0.4^c	86.4 ± 0.2^a	86.4 ± 0.2^a
RBC (10 ⁹ /ml)	78.0 ± 0.4^a	62.4 ± 0.6^d	63.8 ± 0.8^c	77.2 ± 0.6^a	78.4 ± 1.1^a

Values are mean $\pm$ standard deviation. Values in the same row with same superscripts are not significantly different ($P \geq 0.05$).

Table 6: Blood parameters for Nile tilapia Broodstock fed the experimental diets.

had significantly ($P < 0.05$) the best values FCR and PER, the poorest result were recorder with groups of fish fed diet 2 (100% CSM protein) without additional iron. Larvae growth performance is represented in table 4. It showed that the larvae obtained from brood stock fed on diets 4 and 5 supplemented with 580 and 875 mg Fe/kg diet⁻¹ have growth comparable to larvae obtained from broodstock fed on control diet significantly ($P < 0.05$) higher than the larvae obtained from brood stock fed on other diets. The result ADCs of protein, fat, dry matter and energy for Nile tilapia fed experimental diets are presented in table 5. Apparent digestibility coefficients of protein, fat, dry mater and energy were relatively high for most treated diets with iron and increased with increasing level of iron. There were no significant differences ($P > 0.05$) among groups of fish fed control diet (100% FM) and diet 5 (which contained 100% CSM with additional 803 mg Fe/kg diet) for protein fat and energy ADC. Blood parameters of Nile tilapia broodstock fed experimental diets are illustrated in table 6. Hematocrit % (Ht), Hb and RBCs were increased with increasing level of iron and significantly affected by dietary iron and not differ significantly ($P < 0.5$) when compared with control diet contained 100 % FM protein. Whole-body moisture, crude protein, crude fat and energy content were not significantly influenced by dietary treatments. The results of whole-body proximate analysis, expressed on a wet basis %, for Nile tilapia fed experimental diets averaged ($\pm$ SD): moisture, 76.4 ± 0.3 ; crude protein, 15.0 ± 0.2 ; crude fat, 4.7 ± 0.3 ; energy content $508.8 \pm 14.1 \text{ kJ}$

Item	Diets				
	1 (control)	2	3	4	5
No. fish stocked/m ³	24	24	24	24	24
No fish harvested/m ³	24	24	24	24	24
Harvested (kg/m ³)	4.1	3.6	3.9	4.3	4.1
Food used(kg/m ³)	3.6	3.5	3.5	3.71	3.62
Fingerling cost (LE) ¹	24	24	24	24	24
Food cost (LE) ²	18.0	5.3	5.3	5.6	5.4
No. of larvae harvested/m ³	4166	2250	2746	4122	4120
Fish cost (LE) ¹	42	29.3	29.3	29.6	29.4
Larvae cost/(LE) ¹	104	56.3	68.7	103.3	103
Total cost (LE) ¹	41	36	39	43	41
Value of harvest (8.6LE. kg ⁻¹)	145	92.3	107.7	146.3	144
Net profit (LE) ¹	103	63	78.4	116.	114.6

¹LE= Lever Egyptian, one Dollar equal 6.12 LE.

²Food cost equal 2.80 and 2.25 LE for diets.

Table 7: Economic information of Nile tilapia Broodstock.

(100 g⁻¹); crude ash 3.5 ± 0.4 . There was a significant ($P < 0.05$) change in ash content associated with diets supplemented with different levels of iron. The economic calculation from the study is presented in table 7. The feed cost and the total cost (Lever Egyptian) increased with fish fed fishmeal protein (Diet 1). From the economic information, it can be concluded that the highest net profit was achieved with fish fed diets that contained 40% CSM supplemented with 580 mg Fe kg diet.

Discussion

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 lead to a reduction in the biological availability of lysine because of the binding properties of gossypol. Ofojekwu and Ejike [18] found that *O. aurosus* fed CSM-based diets yielded poor performance. The authors attributed the poor performance to the gossypol contained in glanded and glandless CSM respectively. On the contrary, solvent extracted CSM was successfully used as single dietary protein source for *O. mossambicus* [19] and Nile tilapia [2,20]. Also El-Saidy and Gaber [20] reported that CSM supplemented with iron was successfully used as single dietary protein for Nile tilapia fry. In the present study, regardless of supplemental levels of iron, fish fed diets that contained 40% CSM (580 mg free gossypol) supplemented with lysine to a level equal that of the FM diet and supplemented with 580 mg Fe kg diet exhibited better FBW and SGR than those fed diet 2 (40% CSM without additional iron) in feeding broad stock. This may be addition of iron sulphate at weight ratio of 1:1 of iron to free gossypol, which lead to improving their performance diet 4 (Table 1). Although a diet contained 40% CSM (diet) supplemented with lysine and 223 mg iron, it did not improve fish performance Robinson and Rawles [21] showed that supplementation of lysine to diet containing 44.6% glandless cottonseed flour or 44.4% glandless CSM as total replacement of soybean meal (SBM) did not improved growth and feed conversion. However when glanded CSM with 0.022% free gossypol was used to totally replace SBM, supplementation of lysine is, need to improve the nutritional value channel catfish feed to be level comparable with that of control [22]. Also El-Saidy and Gaber [20] reported that adding 972 mg Fe Kg diet⁻¹ from ferrous sulphate CSM-based diet that contained 972 mg free gossypol for Nile tilapia reduced the negative effects of gossypol and improved growth performance. In our study even though methionine and lysine were added total replacement of FM with CSM (0.145% free gossypol) without additional iron (2) resulted in reduced FBW and SGR. The higher concentration of free gossypol in CSM in our study may account for difference between results of this study and that of Robinson [22]. The response of broad stock to larval production

was influenced by supplemental levels of dietary iron. For diet 1 containing no CSM (FM-based diet), there was an increase in larval production. When FM was totally replaced by CSM (40%), the diets 4 - 5 supplemented with 580-870 mg Fe Kg diet⁻¹ exhibited superior results and comparable to the control diet. Our results are agreement with those of Rojes and Scolt , Wedegaertner, Jones, Martin, El-Saidy and Gaber [9,10,20,23,24]. They found that addition of iron sulphate at weight ratio of 1:1 of iron to free gossypol in pigs, broilers and Nile tilapia was effective in reducing the toxicity of free gossypol and improving animal performance. They suggested that iron inactivates gossypol by forming a strong complex compound in the intestinal tract thus preventing it from absorbed [24]. Result of the present study indicate that of larvae of female fish of Nile tilapia significant influenced with iron supplement and the lowest value we recorded with group of fish fed diet 2 (100% CSM without iron supplementation). The same results were reported by Dabrowski et al. [8], Bloom et al. [7] and Rincharde et al. [25] in their studies on rainbow trout fish. The present, study showed that ADC value of nutrients in CMS was comparable with those in other oil seed meals. El-Saidy and Gaber [20] reported ADC of crude protein in CSM was 78.7-88.9% for Nile tilapia when supplemented with methionine and lysine. Cheng and Hardy [4] found the ADC of protein in CSM was 81.6-87.9% for rainbow trout. These values agree of our results where digestibility of crude protein in CSM-based diets ranged from 76.5% to 88.2%. The results also in agreement with Mbahinzireki et al. [3], where reported that ADCs of crude protein decreased as dietary gossypol level increased in tilapia (*Oreochromis* sp). The response of broodstock based on RBC. He and Hb to dietary CSM were influenced by supplemental levels of dietary iron. For diets containing no CSM (FM based diets), there was an increase in these parameters. When FM was totally replaced by CSM (40%) the diets 4, 5 supplemented with 580 and 870 Fe Kg diet⁻¹ exhibited superior results of Ht, Hb and RBC to fish meal diet. Our results are in agreement with those of Martin [10] in catfish. On the Contrary to our results of the hematological values with Nile tilapia in the present study, Barros, Lime and Klesius [26] reported that channel catfish fed a diet containing 50% CSM supplemented with 671 mg Fe Kg diet⁻¹ had no improvement in growth of fish or hematological values with treatments without diet iron added. They attributed that to diets high in CSM contain compounds or factors, which reduce from iron absorption or availability. Also, Results of study Nguyen et al, [27] indicated that cotton seed meal and soybean meal could totally replace FM's inclusion rate in commercial diets for juvenile tilapia. Furthermore, methionine did not appear to be limiting in practical diets using typical levels of CSM, DSESM, and MBM as primary protein sources. Production estimated, in the present experiment are based on biomass estimates which adjusted for mortality and corrected for growth rate [28]. It is the basis for estimating economic yield for both fish culture operation and for natural fish population. Because production and harvest value were dependent on protein, source. In addition, final harvest and production values were directly related to protein source and iron supplemental level at which growth rate is reduced and when it occurs, production will be reduced. The critical level in our experiment was fish fed diets that contained 40% CSM supplemented with 580 mg Fe kg diet exhibited maximum growth in broodstock of Nile tilapia. Results of these studies indicate that total replacement of FM with CSM (0.145% free gossypol) reduced the nutritional value of the diets. For CSM 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 2 mg Fe kg diet 5 appears to sufficient to maintain normal function of growth performance, feed utilization and biological and hematological parameters of Nile tilapia.

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