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

Magdy M. Gaber\(^1\), Magdy M. Elhalfawy\(^2\) and Amal M. Ramadan\(^2\)

\(^1\)National Institute of Oceanography and Fisheries, Cairo, Egypt
\(^2\)National Institute of Oceanography and Fisheries, Suez, Egypt

**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 broad 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\(^{-1}\). 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) 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, 0.5, 1.0, and 2.0 mg iron for each mg of free gossypol 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, 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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\*Corresponding author: Magdy M. Gaber, National Institute of Oceanography and Fisheries, P. O. box 40, Shoubra, Cairo, Egypt, E-mail: gabermagdy@yahoo.com

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Each container replenished. The larvae from hatching to complete yolk sac from each treatment were counted and distributed into three glass aquarium (100 L) with total 15 glass aquarium 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 aquarium was replaced daily by aerated fresh water after cleaning and removing the accumulated excreta. All aquarium were aerated. A photoperiod of 12 h light, 12 h dark (06:00-20:00 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 each 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=100x [(%dietary CrO3/%fecal CrO3−%fecal nutrient/%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 using a hemocytometer. Hemoglobin was determined using the total Hb kit (Sigma Diagnostics, Sigma, St Louis, MO, USA) which is standardized procedure using the cyanmethemoglobin 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 2 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 chronic oxide in diets and 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 mating 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

### 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 | 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 | 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 | 0.7 |
| L-Lysine | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

### Proximate composition (%)

| Diet | Moisture | Crude protein | Crude fat | Crude fiber | Ash | Vitamin/Mineral Premix | Molasses (as Bender) | L-Methionine | L-Lysine |
|------|----------|---------------|----------|-------------|-----|------------------------|---------------------|---------------|----------|
| 1:1  | 6.21     | 25.0          | 11.94    | 7.3         | 10.3| 39.25                  | 60.00               | 870           | 580       |
| 0.5:1| 6.13     | 25.0          | 11.99    | 7.3         | 10.3| 39.25                  | 60.00               | 870           | 580       |
| 1:2  | 5.82     | 25.0          | 11.99    | 7.3         | 10.3| 39.25                  | 60.00               | 870           | 580       |

### Proximate composition (%)

| Diet | Moisture | Crude protein | Crude fat | Crude fiber | Ash | Vitamin/Mineral Premix | Molasses (as Bender) | L-Methionine | L-Lysine |
|------|----------|---------------|----------|-------------|-----|------------------------|---------------------|---------------|----------|
| 1:1  | 6.21     | 25.0          | 11.94    | 7.3         | 10.3| 39.25                  | 60.00               | 870           | 580       |
| 0.5:1| 6.13     | 25.0          | 11.99    | 7.3         | 10.3| 39.25                  | 60.00               | 870           | 580       |
| 1:2  | 5.82     | 25.0          | 11.99    | 7.3         | 10.3| 39.25                  | 60.00               | 870           | 580       |
feeces 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 (± SD): water temperature, 27.6 ± 0.9°C; dissolved oxygen, 6.5 ± 0.5 mg L⁻¹; total ammonia, 0.18 ± 0.14 mg L⁻¹; nitrite, 0.07 ± 0.05 mg L⁻¹; total alkalinity, 183 ± 45 mg L⁻¹; chlorides, 573 ± 150 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 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⁻¹ there were no liner increase in FBW and SGR. Mean body weights of fish are shown in table 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⁻¹)

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

Values are presented as means ± standard deviation. Values in the same row with superscripts are not significantly different (P ≥ 0.05).

1FBW=final body weight.
2DGR, daily growth rate=final body weight gain/30 x100
3FI=feed intake.
4PER, protein efficiency ratio=final body weight gain/protein intake X100.

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

Values are mean ± standard deviation. Values in the same row with superscripts are not significantly different (P ≥ 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 broad stock fed on diets 4 and 5 supplemented with 580 and 875 mg Fe kg⁻¹ have growth comparable to larvae obtained from broodstock fed on control diet significantly (P<0.05) higher than the larvae obtained from broad 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 matter 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⁻¹) for protein fat and energy ADC. Blood parameters of Nile tilapia broodstock fed experimental diets are illustrated in table 6. Hematocrit % (Ht), Hb and RBC were increased with increasing level of iron and significantly affected by dietary iron and not differ significantly (P>0.05) 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 (± SD): moisture, 76.4 ± 0.3; crude protein, 15.0 ± 0.2; crude fat, 4.7 ± 0.3; energy content 508.8 ± 14.1 kJ
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 exhibited superior results and comparable to the control diet. Our results are agreement with those of Rojes and Scott, Wedegaertner, Jones, Martin, El-Saïdy 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 effects 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 larval 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 Rimhard 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-Saïdy 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 with our results where digestibility of crude protein in CSM-based diets ranged from 76.3% 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 RBC and Hb as dietary CSM was 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 RBC and Hb as dietary CSM was 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 RBC and Hb as dietary CSM was 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. 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