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Original Article

Evaluating different levels of dietary germinated corn embryo powder on reproductive performance, physiological measurements of Nile tilapia brood-fish *Oreochromis niloticus* and growth performance of their larvae

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ABSTRACT

This work was performed to investigate the effect of different levels of germinated corn as a dietary ingredient on spawning efficiency and larval performance of Nile tilapia *Oreochromis niloticus.* The experimental treatments were conducted in a private hatchery. Ten hapas, each with dimensions ($28m^3$) were used to accomplish this work. Brood-stock was randomly stocked in each hapa with a sex ratio (1 male: 3 females, i.e.15 males: 45 females). The initial average weight of males and females were 194±10g and 185±15 g, respectively. Five experimental diets were formulated and applied on the two experiments as follows: control: brood-stock and fry fed a diet without germinated corn, T1, T2, T3, and T4: fish were fed diets containing different levels of germinated corn meal (1, 2, 3, and 4%, respectively). Statistical analysis showed that fish-fed diets containing 2% Sprouted corn were significantly better in growth performance, feeding utilization indices (HSI, VSI, GSI and condition Factors) and hatching indices (number of fry produced and survival of larvae) in comparison with other treatments. Besides blood hematological and biochemical parameters, fish that were fed with dietary germinated corn had the best results compared to control treatment. Thus, it is recommended that the inclusion of 2% germinated corn meal (T2) as feed additives for tilapia brood-stock is more useful for improving their growth performance, feed utilization, and reproductive performance.

Key words: Germinated corn, tilapia, spawning, blood parameters, biometric indices

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1. INTRODUCTION

Tilapia culture has been considered as strategic goal for the aquaculture development of several countries, such as Egypt, the 3th largest world inland aquaculture producer of tilapia and the largest producer of aquatic products in Africa (FAO, 2020). Where Egypt aquaculture production in (2018) was estimated by 1561457 tons and tilapia farms represented about 60% of total production of Egyptian seafood (GAFRD, 2018). Recent studies tend to improve strains, genetic advancements, management techniques and finding alternatives to prevent the rise in disease outbreaks, particularly streptococcus infection. Many of researches used a large number of natural sexual-promoters to raise the efficiency of spawning or hatching and produce high-quality seeds.

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Whereas, herbal preparations made by Michael Babu (1999) were contributed to stimulate semen quality in male fish and gonadal maturation and high egg viability in female fish**.** Abou-Zied *et al***.** (2013) found that dietary 1% palm pollen grains had positive effects on spawning and hatching performance of red tilapia brood-stock that reared indoor. Dhas (2015) demonstrated that the combination of herbal maturation extract could be utilized for the formulation of maturation diets for *Etroplus suratensis*. Oke *et al.* (2019) found that male *C. gariepinus* brood-stock that received 1.5% of *Desmodium adscendens* leaf powder of basal feed had significantly greater sperm counts, percentage motility, and milt volumes. Moreover, male Nile tilapia dietary supplementation with different inclusions of *Tribulus terrestris* extract specially 500 and 750 mg/kg diet improves growth performance, health condition, semen quality and reproductive efficiency without any harmful effect on water quality (Hassona *et al*., 2020).

The present study is considered the first study that examined the effects of including the germinated corn grain embryos (GMGE) in brood-stock and larval feed on spawning, hatching, blood parameters and larval performance. Some studies recorded that GMGE is richness in antioxidant, osmoprotectant compounds, phyto-horomones and vitamins and minerals (Rady *et al*., 2020).

The present work was designed to evaluate the effect of different dietary germinated corn grain embryos (GMGE) levels on reproductive performance of Nile tilapia brood-stock and growth of their larval.

2. MATERIALS AND METHODS

The current study included two spawning trials to investigate the effect of different levels of germinated corn as a dietary ingredient on spawning efficiency and larval performance of Nile tilapia *Oreocromis nilutics.*

2.1. Brood-stock and site of work

This trial was conducted in a private hatchery in El-Kaldia village on the west side of Lake Qarun, Fayoum governorate using its stock of Nile tilapia brood-stock and facilities (haps, water pumps…etc.). Ten hapas with dimensions (4×7×1m: W× L× H), was used to accomplished this work. A Brood-stock (60 fish) was randomly stocked in each hapa with a sex ratio (1 male: 3 females, i.e.15 males: 45 females in each hapa). The initial average weight of male and female was 194±10g and 185±15 g, respectively.

2.2. Fry collection and rearing conditions

The fry were collected, weighted using (0.0001g Analytical Balance, BA-T Series) and counted for each hapa. Fry of the 1st hatching were transferred to nursing hapa (2m^3) and stocked at a rate of 700/hapa. The initial weight of fry was 0.014±0.0003g.The fry were fed with the experimental diets of brood-stock in powder form for a period of 45 days.

2.3. Second Experiment: Study protocol and diets

One protocol was used in this study to examine five treatments designed as follows: control: brood-stock and fry fed a diet without germinated corn, T1, T2, T3, and T4: fishes were fed diets containing different levels of germinated corn meal (1, 2, 3, and 4%, respectively. Each treatment had two replicates.

2.4. Diets formulation and preparation of corn grain embryos meal

The corn grains (local Egyptian genotype) were placed in water for 4h , then, they were transferred to wet cotton and covered with a clean piece of cloth and kept moistened until germination. After then, the embryos were isolated from the germinated grains. The isolated embryos were sunny dried, and then milled using blender. The grounded embryos were mixed with the other ingredients according to study protocol.

2.4.1. Diet preparation for brood-stock and fry

Diets were formulated to contain 35% crude protein. All ingredients were milled and well mixed with grain embryos meal and some water, then pelleted using a meat mincer with a 3-mm diameter. The pellets were dried in oven at temperature of 40° C for 12 hours and stored in plastic bags at 1°C until used. On the other hand, the same tested diets with brood-stock were tested with fry, but in powdered form. Fishes were fed manually twice daily, at 9 am and 5 pm for 6 days weekly, with feeding rates of 0.7% and 10% of biomass for brood-stock and fry, respectively. Feeding rate was adjusted every two weeks through sampling of biomass for all treatments. The formulated diets and chemical composition are presented in Table 1.

2.5. Water quality parameters

Temperature, pH, salinity, and dissolved oxygen mg/l were recorded every day at 1 pm using automatic pH meter (model: lab 845/Bl29pH), digital refractometer (DR6000) and (Oxy-meter HI98198-HANNA interment, UAS). Total ammonia nitrogen, mg/l and nitrite, mg/l were determined by chemical methods (APHA, 1995).

Table (1): Ingredients and a proximate chemical composition of experimental diets.

Ingredients	Control	T1	T2	T3	T4
Yellow corn	26.0	25.5	25.0	24.5	24.0
corn grain embryo	0	1.0	2.0	3.0	4.0
Fish meal	35.0	35.0	35.0	35.0	35.0
Soybean meal	21.0	21.0	21.0	21.0	21.0
Wheat bran	7.0	6.5	6.0	5.5	4.0
Garlic meal	0.5	0.5	0.5	0.5	0.5
Starch	3.5	3.5	3.5	3.5	3.5
Molasses	1.0	1.0	1.0	1.0	1.0
Fish oil	2.0	2.0	2.0	2.0	2.0
Linseed oil	1.5	1.5	1.5	1.5	1.5
Vit.Min.Mix.[1]	2.0	2.0	2.0	2.0	2.0
Vit C	0.5	0.5	0.5	0.5	0.5
Proximate composition of experimental diets (%DM basis)					
Dry matter (DM)	92.1	91.8	92.2	91.9	92.6
Crude protein (CP)	35.31	35.39	35.47	35.55	35.66
Ether extract (EE)	12.24	12.56	12.62	12.71	12.75
NFE[2]	31.89	32.03	31.99	32.10	31.90
Crude fiber (CF)	4.0	3.9	3.6	3.5	3.45
Ash	16.56	16.12	16.32	16.14	16.24
GE(Kcal/g)[3]	4.45	4.49	4.51	4.52	4.52

1-Vitamin-mineral premix supplied as the following (vitaminIUkg^{-1} diet and mineral mg/Kg^{-1} mixture);
2- **Calculated by differences, Nitrogen free extract (NFE) = [100-(CP+EE+CF+Ash)].**
3-Estimated according to (NRC, 2011).

2.6. Measurements

- Body weight gain WG (g) = (FW-IW)
- FW=Final weight (g) IW=Initial weight (g)
- Specific growth rate (SGR) (%/day) = [ln FW – ln IW)/days] ×100,
  where ln = Natural log
- Survival rate (SR %) = (final fish numbers/initial numbers of fish)×100
- Condition factor (CF) = [FW/ (final length) ^{3}] x 100
- Feed conversion ratio (FCR) =feed intake (g)/weight again (g).

- Gonad somatic indexes GSI %= (gonads weight / body weight) × 100.
- Hepatosomatic index (HSI)=
- (liver weight / FW) × 100
- Viscerosomatic index (VSI) = [viscera weight (g)/ FW]x 100

2.7. Chemical analysis

2.7.1. Feeds

Moisture, crude protein (CP), ether extracts (EE), crude fiber (CF), and total ash content were determined according to (AOAC, 2010). Nitrogen free extract (NFE) was calculated by difference. Gross energy (GE) was estimated for formulated diets the factors 5.64, 9.44 and 4.11 Kcal/g for CP, EE and carbohydrates, respectively (NRC, 2011).

2.7.2. Germinated corn embryo meal

Total soluble sugars were measured by the anthrone reagents method (Irigoyen,1992).The metals such as calcium, magnesium, potassium, manganese, phosphorus, zinc, copper, iron, and iodine were measured by using Inductively Coupled Plasma Emission Spectrometer (ICP) (ICAP- 6300 Duo). Protein and nitrogen according to (AOAC, 2006), proline (Bates *et al.*, 1973), total free amino acids (Irigoyen *et al.*, 1992), B-group vitamins (Chen *et al.*, 2007), zeatine (Novák *et al.*, 2008), cytokinins (Zemanova, 2019) and ascorbic acid content were estimated using methods of (Mukherjee and Choudhuri, 1983) Table 2.

Table (2): Chemical constituents of corn grain embryo on dry weight basis.

The component	Value
Soluble sugars (mg/g)	101.4
Proline (mg/g)	15.9
Total free amino acids (mg/g)	79.2
Protein (mg/g)	205
Nitrogen (mg/g)	42.3
Phosphorus (mg/g)	10.4
Potassium (mg/g)	45.2
Magnesium (mg/g)	10.2
Calcium (mg/g)	10.7
Iron (mg/g)	4.1
Manganese (mg/g)	2.2
Zinc (mg/g)	1.9
Iodine (mg/g)	2.1
Copper (mg/g)	1.1
Total B-group vitamins mmol/g	2.2
Vitamin C mmol/g	52
Vitamin E mmol/g	2.8
Total cytokinins mg/g	5.2
Zeatin mg/g	3.2

2.8. Blood analysis:-

At the end of the trials, four brood stock (2 male and 2 female) of each treatments were placed in plastic tank and were anesthetized using clove oil at a dose of 0.05ml/l (El-Dakar, 2021). Blood was taken from the caudal vein using 3-ml syringes and was placed in tubes containing EDTA as anticoagulant. Red blood cells (RBC), white blood cells (WBC), hematocrit (Hct), hemoglobin (Hb) and lymphocytes (LYM) were determined using the fully automatic hematological analyzer according to (Dacie and Lewis, 2001).

2.8.1. Hormonal analysis:-

Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH), as well as the sex steroids hormones,

progesterone (P4) and testosterone were quantitatively analyzed by i-CHROMA reader system, estradiol was determined by enzyme immunoassay using stander estradiol (0, 20, 100, 300, 800 and 3200 pg/ml) (Biocheck, Inc. Foster City, CA 94404U.S.A).

2.9. Statistical analysis:

All data were analyzed by one way analysis of variance (ANOVA) and Significant level ($P \leq 0.05$). Using Statistical Package for Social Sciences (SPSS) (Version 20, Inc., Chicago, USA).

3. RESULTS AND DISCUSSION

3.1. Water quality parameters:-

As shown in (Table, 3), the mean values of different parameters of water quality in ponds during the spawning period and fry raring were optimal for rearing tilapia as recorded by (El-Sayed, 2006).

Table (3): Means of water quality of ponds during the experimental trial

Items	Temperature °C	Salinity ppt.	pH	DO, mg/l	TAN, mg/l	NO_2, mg/l
Control	28.24	2.32	7.52	7.6	0.023	0.06
T1	28.15	2.34	7.50	7.8	0.024	0.04
T2	28.22	2.31	7.51	7.4	0.021	0.06
T3	28.24	2.33	7.48	7.0	0.023	0.04
T4	28.25	2.35	7.46	7.3	0.025	0.07
PSE*	± 0.15	± 0.04	± 0.35	± 0.18	± 0.002	± 0.03

*Pooled standard error.

3.2. Brood-stock hatching of females

The hatching performance of females Nile tilapia was tested in four batches through two months as detected in (Table 4). The high total weight and fry number were obtained with females fed (T2) with a significance ($P<0.05$) differences compared to other treatments. This enhancement in the present trial may be due to the sufficient percent of germinated corn meal from zeatin, vitamin C and E.

Nutrition can be evaluated by assessing the quality of eggs and larvae, reproductive parameters, and blood variables of Nile tilapia (Pamungkas *et al.*, 2014 and Sarmento *et al.*, 2018). The high reproductive performance in tilapia was recorded by using carica papaya (Abdelhak *et al.*, 2013 and Solomon *et al.*, 2017); palm pollen grain (Abou Zied *et al.*, 2013) and herbal extract (Gabriel, 2019). However, there is no studies recorded the effects of germinated corn meal in animals or fish performance, but there is a report for zeatin as plant hormone that induced enrichment of *green algal*. omega-3 fatty acids, the main important fatty acids in brood-stock diets as recorded by (Han *et al.*, 2018 and Sivaramakrishnan and Incharoensakdi, 2020 Moreover, the benefit effects of zeatin adenosinergic pathways were recorded in rat (Oz *et al.*, 2020). 3.3. Brood-stock indices of males:-

The high significance differences in male GSI, HIS and VSI ($P<0.05$) between males in were obtained with T2 compared with the other treatment groups as presented in (Table, 5). Data also showed insignificant differences in conduction factor but significant in feed conversion ratio between fish fed different diets.

3.4. Brood-stock indices of females

The reproductive performance of females represented an increase in weight gain and GSI in fish fed 2% of germinated corn embryo diet as shown in (Table 6). Similar increases were reported in brood-stock tilapia (Yones *et al.*, 2019). Insignificance differences in HIS, VSI, conduction factor and feed conversion ratio were recorded in all females fed different diets.

Table (4): Hatching performance of Nile tilapia fed different experimental diets

Treatments	Control	T1	T2	T3	T4	PSE*
Hervest1						
Total weight of Fry/Hapa(g)	128.5^{c}	153.00^{b}	186.5^{a}	159.5^{b}	152.5^{b}	± 6.38
Number of Fry/Female	200^{c}	237^{b}	290^{a}	247^{b}	237^{b}	± 9.89
Number of Fry/hapa	9000^{c}	10688^{b}	12950^{a}	11138^{b}	10688^{b}	± 4.37
Hervest2						
Total weight of Fry/Hapa(g)	106.00^{c}	135.5^{ab}	151.5^{a}	122.5^{bc}	130.00^{abc}	± 5.45
Number of Fry/Female	165^{c}	210^{ab}	235^{a}	190^{bc}	202^{abc}	± 8.48
Number of Fry/hapa	7425^{c}	9450^{ab}	10575^{a}	8550^{bc}	9125^{abc}	± 3.81
Hervest3						
Total weight of Fry/Hapa(g)	108^{c}	130^{b}	148.5^{a}	120.5^{bc}	127^{b}	± 4.6
Number of Fry/Female	167^{c}	202^{b}	230^{a}	187^{bc}	197^{b}	± 7.15
Number of Fry/hapa	7537^{c}	9125^{b}	10350^{a}	8437^{bc}	8887^{b}	± 3.22
Hervest4						
Total weight of Fry/Hapa(g)	107.5^{c}	125.5^{b}	145.00^{a}	117.5^{bc}	120.5^{b}	± 4.27
Number of Fry/Female	167^{c}	202^{ab}	225^{a}	182^{bc}	187^{bc}	± 6.96
Number of Fry/hapa	7387^{c}	8725^{b}	10125^{a}	8312^{bc}	8312^{bc}	± 3.08

Within each raw, mean values with different superscripts are significantly different (P<0.05).
*Pooled standard error.

Table (5): Reproductive indices of experimental males fed different experimental diets

Treatments	Control	T1	T2	T3	T4	PSE
Initial weight , g	194.0	197.0	195.0	193.0	194.0	± 0.89
FW, g	249.5^{c}	261.5^{b}	279.00^{a}	259.00^{b}	253.00^{c}	± 3.45
Gain, g	56.00^{d}	64.00^{cb}	84.00^{a}	67.5^{b}	58.00^{cd}	± 3.39
Feed Intake, g	80	82	93	80	82	± 0.11
FCR	1.44^{a}	1.28^{b}	1.11^{c}	1.22^{bc}	1.42^{a}	± 0.12
Initial length, cm	19.16	19.50	19.50	19.33	19.10	± 0.11
Final Length, cm	22.25	21.83	21	21.33	20.5	± 0.41
GSI, %	0.79^{d}	0.95^{c}	1.42^{a}	1.13^{b}	1.44^{a}	± 0.24
HSI,%	0.99^{c}	1.22^{b}	1.53^{a}	1.35^{ab}	1.31^{b}	± 0.09
VSI, %	6.91^{a}	5.20^{b}	4.12^{d}	4.22^{c}	4.27^{c}	± 0.27
Conduction Factor, %	1.88	2.07	2.31	2.16	2.43	± 0.19

Within each raw, mean values with different superscripts are significantly different (P<0.05).
*Pooled standard error.

Table (6): Reproductive indices of females fed different experimental diets

Treatments	Control	T1	T2	T3	T4	PSE*
Initial weight, g	183	185	186	186	185	± 0.61
Final body weight, g	225.00^{b}	231^{ab}	237.00^{a}	231.5^{ab}	235.00^{a}	± 1.25
Final Weight Gain	42.5^{b}	46.6^{ab}	51.00^{a}	45.5^{ab}	49.5^{ab}	± 0.95
Feed Intake	81	80	82	81	82	± 0.11
FCR	1.90^{a}	1.72^{b}	1.58^{c}	1.79^{ab}	1.65^{c}	± 0.14
Initial length, cm	16.83	17.66	17.5	16.16	17.00	± 0.21
FL, cm	19.23	18.16	18.12	18.25	18.00b	± 0.25
GSI, %	2.22^{b}	2.73^{a}	2.71^{a}	2.45^{a}	2.10^{b}	± 0.32
HSI,%	1.41^{c}	1.81^{b}	1.96^{a}	1.84^{b}	1.87^{ab}	± 0.07
VSI, %	7.45^{a}	6.13^{b}	5.11^{d}	5.22^{c}	5.34^{ab}	± 0.25
Conduction Factor	2.52	2.45	2.51	2.58	2.44	± 0.28

Within each raw, mean values with different superscripts are significantly different (P<0.05).
*Pooled standard error.

3.5. Hematological parameters of brood-stock during spawning period

All hematological parameters of brood-stock showed significance differences between treatments as presented in (Table, 7). These results were in agreement with the results of (Solomon *et al.*, 2017) in tilapia.

Table (7): Means of hematological parameters of females

Treatments	Control	T1	T2	T3	T4	PSE*
Hb (gdl^{-1})	5.55^{ab}	4.9^{b}	6.45^{a}	4.3^{b}	4.35^{b}	± 0.40
RBC ($x10^{6}$ mm^{-3})	1.435^{a}	1.245^{a}	1.345^{a}	0.975^{a}	1.09^{a}	± 0.84
Hct (%)	24.8^{a}	20.05^{b}	23.7^{ab}	17.1^{c}	19.2^{b}	± 1.43
WBC ($x10^{3}$ mm^{-3})	47.15^{a}	47.95^{a}	49.8^{a}	48.6^{a}	33^{b}	± 7.49
LYm	69.49^{ab}	35.96^{c}	46.28^{bc}	79.85^{a}	42.16^{c}	± 6.14

Within each raw, mean values with different superscripts are significantly different (P<0.05).
*Pooled standard error.

3.6. Hormonal indices of females during spawning period.

The sexual hormone levels especially testosterone and progesterone were different between treatments (Table 8). Also, the values of FSH and LH showed significant-differences between females fed different diets. Zeatin, an adenine-derivative cytokinin has well-established functionally in plants. It is also suggested to activate A2A receptors in animals; however, there is limited knowledge of its effects. In addition, zeatin might have a potential therapeutic use in depression, acting via adenosinergic pathways. The benefit effect of zeatin was evident in rat performance (Öz *et al.*, 2020).

Table (8): Means of sexual hormones in females during the spawning period.

Treatments	Control	T1	T2	T3	T4	PSE*
FSH, mIU/ml	0.55^{b}	0.52^{c}	0.60^{a}	0.58^{b}	0.51^{c}	± 0.06
LH, mIU/ml	0.157^{c}	0.181^{b}	0.194^{a}	0.185^{b}	0.188^{ab}	± 0.211
Testosterone, ng/ml	0.400^{c}	0.940^{b}	1.780^{a}	0.800^{b}	0.520^{c}	± 0.250
Progesterone, ng/ml	0.12^{b}	0.07^{c}	0.30^{a}	0.05^{c}	0.26^{a}	± 0.450

Within each raw, mean values with different superscripts are significantly different (P<0.05).
*Pooled standard error.

3.7. Growth trial of fry

As can be seen, the fry performance of final weight and SGR were significantly higher in T2 diet as shown in (Table 9). Also, the best survival rate was obtained with the fry fed diet T2, where its survival was 92%. From table (9) it is evidenced that the high fry performance and survival rate were obtained by using 2% of corn grain embryo as feed additive. This enhancement could be due to the presence of adequate values of vitamin E, C and zeatin in this diet. Comparable results were obtained in fry of Tilapia by (Yones *et al.*, 2019).

Table (9): Growth performance of larval.

Treatments	Control	T1	T2	T3	T4	PSE*
Initial W, g	0.0138	0.0117	0.0136	0.0156	0.0158	± 0.014
FW, g	4.0^{b}	5.0^{ab}	6.0^{a}	4.5^{b}	4.0^{b}	± 0.34
WG, g	3.98^{b}	4.98^{ab}	5.98^{a}	4.48^{ab}	3.98^{b}	± 0.29
SGR, %/ day	12.59^{c}	13.46^{b}	13.53^{a}	12.56^{c}	12.20^{d}	± 0.25
SR, %	79.77^{d}	78.8^{d}	92.19^{a}	88.12^{ab}	86.64^{bc}	± 7.21

Within each raw, mean values with different superscripts are significantly different (P<0.05).
*Pooled standard error.

4.CONCLUSION

It could be concluded that 2% of germinated corn grain embryos as dietary incorporated in feed of brood-stock or larval lead to improve physiological status, improving reproductive performance and larval performance.

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