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EFFECT OF ADDING ROCKET SEEDS (ERUCA SATIVA) POWDER TO DIETS OF NILE TILAPIA BROODSTOCK IN CONCRETE PONDS ON PRODUCTIVE, REPRODUCTIVE PERFORMANCE AND SOME PHYSIOLOGICAL ASPECTS

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Abstract

The present study were conducted to evaluate the effect of dietary graded levels0, 1, 2, 3 and 4% Rocket seed (*Eruca sativa*) on the reproductive performance, feed utilization, and some physiological parameters of Nile tilapia, *Oreochromis niloticus* broodstock for 75 days. A total of 480 broodstock *O.niloticus* with an average initial body weight of 219.7±0.153 g were randomly distributed into five treatments T_0, T_1, T_2, T_3 and T_4. Results revealed that T_4 was the best treatments for number of fry produced than other treatment and different significantly than control treatment (T_0). Regarding broodstock growth performance the results showed that T_3 and T_4 were significantly improved ($P \leq 0.05$) than other treatments. For blood hematological and biochemical parameters among all treatments dietary Rocket seed gave the best results compared to control treatment. Thus, it is recommended that the inclusion of 4 % seed (T_4) as feed additives for broodstock are more useful for improving their growth performance, feed utilization, reproductive performance and physiological responses.

INTRODUCTION

Globally, aquaculture is a vital industry that provides essential cheap animal protein to a rapidly growing population. Global production of Nile tilapia (*Oreochromis niloticus*) has dramatically increased year by year to provide staple protein source for world demand of fish products (FAO, 2014).

Tilapia farming is a growing industry, given that tilapia is the second largest group of freshwater fish cultivated worldwide. Production of tilapias has a wide distribution, notably in Asia, Africa, and Americas. The global production of tilapia reached 4,507,002 mt in 2012, representing 10.2%

of total farmed fish production (FAO, 2014). Egypt is the world's third largest producer of farmed tilapia.

Egypt has the largest aquaculture industry in Africa. Egyptian aquaculture currently provides almost 77percent of the country's fish needs, with almost all the output coming from small and medium sized privately-owned farms (GAFRD, 2015).

Some studies have been done in which herbs, as dietary additives, were fed to fish. The converge of these studies contain their use as food attractants (Harada, 1991) and their performance on growth rate (Lee *et al.*, 2001), survival (Kim *et al.*, 2003) and immune system verve (Immanuel *et al.*, 2004).

Eruca sativa seed is rubefacient and stimulant. All phytochemicals found in seeds are responsible for different bioactivities including antimicrobial activity against various pathogenic microorganisms (Gulfraz *et al.*, 2011). The presence of phenolic compound in the seed indicates its antimicrobial properties against pathogenic bacteria (Khoobchandania *et al.*, 2010) while Tannins are reported to exhibit antiviral, antibacterial and antitumor activity and also used as diuretic. Similarly Cardiac glycosides are helpful to overcome various human diseases, and Saponin has the property of precipitating and coagulating red blood cells (Gulfraz *et al.*, 2011). Recent studies suggest that rocket has already shown anticancer activities (Hussein, 2013).

Marwat *et al.* (2016) reported that the Rocket medicinally used for increasing fertility and sperm production, eye infection (antibacterial). It is also helpful in digestive process and kidney activities. *E. sativa* is mainly responsible for different biological and pharmacological activities such as: antimicrobial, antibacterial, anti-fungal, antitumour, analgesic, antioxidant, antidiabetic, antidiarrhoeal, anti-inflammatory, anti-giardial activity etc.

Al-Qudah (2017) reported that ethanolic extract of rocket leaves could increase fertility potential and testosterone concentration in male rats. This plant

seems to reduce serum cholesterol levels with some alterations in the structure and ultrastructure of the testes when this dose (500 mg /kg).

In tilapia diets Khalil*et al.*, 2015 cited that the inclusion of 3% Rocket leaves or 2% Rocket seeds as feed additives of all male monosex *O. niloticus* are more useful for improving their growth performance, feed utilization, chemical composition of the whole fish body and physiological responses.

Nowfel and Al-Okaily (2017) reported that the provide an evidence for a prophylactic and stimulatory effects of *E. sativa* on pituitary-gonadal axis. Thus the potential of E. sativa leaves extract improved male reproduction. In laying breeder male Al - Daraji and Razuki (2012) stated that supplementing diet with rocket salad seeds especially at the level of 3 Kg/ton of diet resulted in improvement as concerns certain seminal plasma constituents of roosters. Therefore, rocket salad seeds could be used as an important tool for improving reproductive performance of roosters. In male Awassi lambs Al-Fityin and ALSaig (2009) mentioned that testes circumferences recorded significantly ($P \leq 0.05$) higher by using *Eruca sativa* seeds in diets.

Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Zhou *et al.*, 2009).

MATERIALS AND METHODS

The present study was carried out in concrete ponds in the Central Laboratory for Aquaculture Research at Abassa, Sharkia Governorate, Egypt, to evaluated the effect of Rocket (*Eruca sativa*) seeds powder on productive and reproductive performance and some physiological blood characteristics of Nile tilapia broodstock in concrete ponds.

Nile tilapia (*Oreochromis niloticus*L.) broodstock with an average initial body weight 219 g /fish were obtained from the same hatchery then distributed

randomly into ten concrete ponds each measuring 2.5 x 8 m, water level was maintained at 0.8 meter level throughout the whole experimental period (75 days).

Five treatments, each treatment was performed in duplicate were applied in the experimental concrete ponds. These were (T_0) without Rocket addition (control), (T_1) Rocket addition at 1%, (T_2) Rocket addition at 2%, (T_3) Rocket addition at 3% and (T_4) Rocket addition at 4%, . All ponds were stocked with 48 broodstock/ pond (16 ♂ + 32 ♀) diets was offered twice daily at a rate of 1% of total body weight. The chemical analyses of diets and Rocket was analyzed according to the methods of AOAC (1990) are illustrated in Tables (1 and 2).

Table 1. Formulation and proximate composition of the tested diets used in the Trial.

Ingredient	Rocket seed level %				
	T_0	T_1	T_2	T_3	T_4
Fish meal	30	30	30	30	30
Soybean meal	15	14.5	14	13.5	13
Rice bran	21	20.5	20	19.5	19
Wheat bran	16	16	16	16	16
Yellow corn	14	14	14	14	14
Molasses	2.75	2.75	2.75	2.75	2.75
Rocket seed	0	1	2	3	4
Dicalcium phosphate	1	1	1	1	1
Vit& Min. premix	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Proximate composition (dry weight), %					
Dry matter (DM)	89.57	89.4	90.12	88.73	89.71
Crude protein, CP	34.42	34.45	34.48	34.5	34.55
Crude fat, EE	6.2	6.53	6.62	6.5	6.73
Crude fiber, CF	6.13	6.2	6.53	6.14	6.03
NFE1	45.55	45.12	44.45	45.12	45.27
Ash	7.7	7.7	7.92	7.74	7.42
GE. Mcal/kg2	4.651	4.669	4.665	4.666	4.692
GE. Mcal/kg3	4.399	4.414	4.396	4.414	4.445

1, Calculated by difference.2, Including CF energy.3, Excluding CF energy.

Table 2. Proximate analysis of Rocket (*Eruca sativa*) seeds.

Ingredient	%
Dry matter (DM)	93.40
Crude protein, CP	32
Crude fat, EE	20
Crude fiber, CF	16.88
NFE	26.82
Ash	4.3

Water temperature, dissolved oxygen and pH were measured weekly at 6 a.m. and 12 p.m. using thermometer, dissolved oxygen meter (YSI model 57) and pH meter (model Corning 345), respectively. Samples were collected from different sites of the experimental ponds randomly. Live body weight measured monthly and number of fry were recorded upon termination of the experiment; all fish in each pond were collected, weighted and counted. Growth performance parameters were determined and blood samples was taken to analyzed.

Blood sample collection:

At the end of the experiment fish were individually captured from pond and anaesthetized with diluted Ms222. Blood was collected via the caudal vein into plastic syringe .The blood sample were divided into two parts, first part was immediately transferred to an Ependorf tube coated with lithium heparin as anticoagulant and it used as it is for measuring out Hb, RBCs and hematocrit immediately .The second part was put in clean and dry tube to centrifuge at -20^{o}C.

Total protein concentration in fish serum was determined by the (Lowry *et al.*, 1951), using BSA as the standard. Hematocrit was measured as packed cells volume by using a Haemofugemicr centrifuge (Heraeus, Christ, Osterrode, Germany). Hemoglobin (Hb) concentration was determined immediately

according (Handy *et al.*, 1999). PCV% was measured according to Stoskopf (1993)

Statistical analysis:

Statistical analysis for the experimental results was carried out using SPSS (2007).

RESULTS

Water quality parameters:

Results of water quality parameters of the experimental ponds during the experimental period (75 days) as averages of the monthly samples are summarized in Table (3). In general, water temperature ranged from 27 through 29 °C Gui*et al.* (1989) found that an average temperature of 28 °C was optimal for growth of Nile tilapia. Dissolved oxygen ranged between 5.21 and 5.85 mg /L. Denzer (1968); AIT (1986) and Hasssan *et al.* (1997) reported that 2.3 mg DO /l is above the normal tolerance level of tilapia. pH ranged between 8.1 and 8.20. Ellis (1937) and Boyd (1998) reported that water with a pH range of 6.5 – 9 is the most suitable for fish production.

Table 3. Average water quality parameters during the experimental period (75 days) in concrete ponds stocked with Nile tilapia broodstock.

Parameter	Treatment				
	T_0	T_1	T_2	T_3	T_4
Temperature (^oC)	28	28	28	28	28
Dissolved oxygen (mg/l)	5.56	5.53	5.5	5.52	5.5
PH	8.2	8.1	8.1	8.1	8.1

Broodstock reproductive performance:

Reproductive performance parameters of Nile tilapia broodstock as affected by adding Rocket seed powder to diets is shown in Table 4. Significant differences were found in reproductive performance of broodfish due to the tested diets. In general addition of Rocket seed powder to diets resulted in better

reproductive performance than the control with Nile tilapia broodstock. However, the broodfish fed at a rate of 4.0% of Rocket diets had higher ($P \leq 0.05$) number of fry/female. These maybe related to the effect of polyphenoliclavonoid compounds in leaves extract on pituitary-testicular axis that alleviate the negative effects of H_2O_2 (Ismail and Al-Nahri, 2009 and Arafa *et al.*, 2014). Few observations reported that the presence of sterols, flavonoids, quercetin and saponins in *E. sativa* scaveng or remove FRs and secondary acts to an improvement of thefertility and testicular functions (Agarwal and Allamaneni 2011 and Ansari *et al.*, 2014) and, thereby augmentation of sexual desire (Ates and Erdogrul, 2003).

Recently Al-Tohamy *et al.* (2010) showed that the presence of glucosinolates and other stimulant materials in *Eruca sativa* seeds have several biological activities and potentially capable for protecting the cells against oxidative stress led to ameliorate semen characters and fertility in male rabbits (Talalay and Fahey, 2001 and Martinez-Sanchez *et al.*, 2007), accordingly, the positive actions of *E. sativa* on the hormonal profile support the folkloric beneficial effect of the plant in the management of reproductive dysfunction. These results was agreement with several studies which found that the presence of flavonoids, saponine and alkaloids in rocket extract caused a significant increase in sperm activity (Barillari *et al.*, 2005), as well as increase spermatogenesis (Homady *et al.*, 2000).

Table 4. Reproductive performance of Nile tilapia brood stock as affected by Rocket seed powder (± SE).

Parameters	Rocket seed powder %				
	T_0	**T_1**	**T_2**	**T_3**	**T_4**
Females number/pond	32	32	32	32	32
Fry No/pond	51280^{b} ±1296	56480^{ab} ±544	57920^{ab} ±352	58784^{ab} ±1120	66128^{a} ±592
Fry No/ female	1602.5^{b} ±40.5	1765^{ab} ±17.0	1810^{ab} ±11.0	1837^{ab} ±35.0	2066.5^{a} ±18.5
Female average weight	239.50	242.75	246.00	249.25	250.750

* Average in the same row having different superscripts differ significantly P0.05.

*Fry No collection = 5

Broodstock growth performance and feed utilization:

Table (5) presents growth performance and feed utilization of Nile tilapia broodstock as affected by Rocket seed powder. It was observed that as the level of Rocket seed increased as Nile tilapia final weight and gain increased. However, such increase was not great specially when looking to specific growth rate (% SGR).

Table 5. Growth performance and feed utilization efficiency of Nile tilapia brood stock as affected by feeding regime (± SE).

Item (per fish)	Rocket seed powder %				
	T_0	T_1	T_2	T_3	T_4
Initial mean weight, g	220±0.001	219.5±0.5	219.5±0.5	220±0.001	219.5±0.50
Final mean weight, g	259^{d}±1.0	266^{cd}±1.0	272.5^{bc}±2.5	278.5^{ab}±1.5	282^{a}±4.0
Total gain, g (1)	39^{d}±1.0	46.5^{cd}±0.5	53^{bc}±3.0	58.5^{ab}±1.5	62.5^{a}±3.5
Daily gain, g (2)	0.52^{d}±0.15	0.62^{cd}±0.07	0.71^{bc}±0.04	0.78^{ab}±0.02	0.83^{a}±0.05
SGR, % (3)	0.22^{d}±0.005	0.26^{cd}±0.002	0.29^{bc}±0.017	0.31^{ab}±0.008	0.33^{a}±0.018
Total feed used, kg/pond	7.59±0.015	7.69±0.025	7.82±0.0001	7.90±0.025	7.94±0.07
Feed required to produce 1000 fry, kg	0.15±0.003	0.14±0.001	0.14±0.008	0.13±0.002	0.12±0.002
The cost of feed/1000 fry, LE	0.74±0.017	0.68±0.008	0.68±0.004	0.67±0.01	0.60±0.01

* Average in the same row having different superscripts differ significantly P0.05.
* period = 75 days
(1) = Final weight - Initial weight
(2) = (1) /period in days.
(3) = 100 (ln Final weight - ln Initial weight) / period in days, where ln is the natural log.

Blood analysis:

Data in Table 6 indicated that the addition of Rocket seed powder in diet at level (4%) significantly (P≤ 0.05) increased Hb (Hemoglobin) concentration, RBCs (Red blood cells) count, PCV% (Packed cell volume) and WBCs (White blood cells) count among other levels and compared with control. While, fish fed control diets (without Rocket seeds) had highest significant (P≤0.05) values

of MCH (Mean corpuscular hemoglobin) compared with diets contained Rocket seed powder.

On the other side, increasing the level of addition of Rocket seed powder led to significantly (P≤0.05) decreased blood indices MCH and MCHC (Mean corpuscular hemoglobin concentration).

Table 6. Hematological parameters of *O. niloticus* broodstock fed different levels of Rocket seeds (± SE).

Parameter	Rocket seed level %				
	T_0	T_1	T_2	T_3	T_4
Hb(gdL^{-1})	5.20^{c}±0.1	5.50^{bc}±0.1	5.55^{bc+}±0.15	5.80^{ab}±0.1	6.05^{a}±0.15
RBCs(×10^{6} mm^{-3})	1.68^{c}±0.025	1.74^{bc}±0.01	1.78^{bc}±0.005	1.82^{ab}±0.03	1.91^{a}±0.05
PCV (%)	10.54^{d}±0.125	11.50^{c}±0.20	11.99^{c}±0.11	12.90^{b}±0.30	14.05^{a}±0.15
MCV(µ)	64.71^{ab}±0.40	65.75^{a}±0.45	65.80^{a}±0.50	64.00^{ab}±0.20	62.95^{b}±0.85
MCH(pg)	32.40^{a}±0.20	32.30^{a}±0.20	30.35^{b}±0.65	27.75^{c}±0.65	26.20^{c}0.30
MCHC(%)	50.07^{a}±0.004	49.13^{a}±0.64	46.12^{b}±0.63	43.36^{c}±0.88	41.62^{c}±0.08
WBCs(×10^{6} mm^{-3})	407.50^{d}±2.5	437.50^{c}±7.5	459.00^{bc}±3.0	476.00^{b}±6.0	501.00^{a}±11.
Albumin(gdL^{-1})	3.15^{c}±0.05	3.65^{c}±0.15	4.55^{b}±0.35	5.15^{ab}±0.05	5.65^{a}±0.05
Total protein(gdL^{-1})	7.40^{d}±0.10	8.00^{c}±0.10	8.75^{b}±0.15	9.65^{a}±0.15	10.15^{a}±0.17

* Average in the same row having different superscripts differ significantly P0.05.

DISCUSSION

In the present study the reproductive performance of the broodstock fish was improved as Rocket seed powder addition increased in the diets. Fish of T_4 showed the best performance than fish of other groups and the lowest values obtained with control group (without Rocket). In the present study broodfish of Nile tilapia that reared in concrete ponds had higher fecundity or relative fecundity than that reported by Camps-Mendoza *et al.* (2004) and Lu and Takeuchi (2004). Such trend could be attributed to different factors.

Gunasekera *et al.* (1995) tested the effect of diet protein on oocyte maturation. They indicated that 32% protein diet seems to be adequate for

tilapia broodstock. In the present study a dietary protein levels of 34% in the diet was administered to improve broodstock fecundity.

Results in Table (6) showed that fish fed 4, 3, 2, and 1% Rocket seed powder had significantly (P≤0.05) increased of Hb, RBCs, PCV, WBCs, plasma total protein and albumin compared to the control group. While, the blood indices (MCH and MCHC) were significantly decreased. These promising results in blood hematological and biochemical parameters led to increasing immune responses and healthy status of fish. These positive findings were related to rocket inclusion of antioxidant constituents; carotenoids, vitamin C, flavonoids such as appiin and luteolin and glucosinolates the precursors of isothiocyanates and sulfaraphene (Barillari*et al.*, 2005; Hanafi*et al.*, 2010), volatile oils like myristicin and apioleβ-phellandrene (Leung and Foster, 1996). Glucosinolates were found to have several biological activities including anticarcinogenic, antifungal and antibacterial plus their antioxidant action (Kim *et al.*, 2004). The major glucosinolate in seeds of rocket which is potentially capable of protecting cells against oxidative stress. In addition, rocket contain Zn, Cu, Fe, Mg, Mn and other elements (Abdo and Zeinab, 2003) which increase immune response. On the other hand, rocket is a good source of beta-carotene (Rinzler, 1990). Therefore, rocket seeds are rich source of vitamin A which is considered the most important vitamin in the body for normal growth,

CONCLUSION

Under the study conditions, feeding fish on Rocket seed powder at level of 4.0% is preferable followed by 3% and the lowest was control.

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تاثير اضافة مسحوق بذور الجرجير لعلائق امهات البلطى النيلى فى الاحواض الاسمنتية على الاداء الانتاجى والتناسلى وبعض الجوانب الفسيولوجي

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الملخص العربى

اجريت هذه التجربه لتقييم تأثير إضافة مستويات مختلفه من مسحوق بذور الجرجير (0% ، 1% ،2 % ،3% ، 4%) على الكفاءة التناسليه وكفاءه استعمال الغذاء وبعض الجوانب الفسيولوجية لإمهات اسماك البلطى النيلى فى الاحواض الاسمنتيه لمده 75 يوم . اجمالى عدد الاسماك فى التجربه 480 سمكه وكان متوسط وزنها 219.7 جم تم توزيعها عشوائيا على خمسه معاملات.

وقد اظهرت النتائج ان المعامله % 4 هى احسن المعاملات بالنسبه لعدد الزريعه بالمقارنه بالمعاملات الاخرى وظهر ذلك فى اختلاف المعنويه بينها وبين الكنترول. وبالنسبه لمعدل النمو اظهرت المعاملات 3% و 4% هم احسن المعلاملات وايضا بعض الجوانب الفسيولوجية لمعاملات بذور الجرجير اظهرت نتائج افضل من الكنترول.

وبذلك فإن إضافه بذور الجرجير الى علائق امهات البلطى النيلى بنسبه 4% ادت الى تحسن انتاج الزريع والاستفاده من الغذاء وبعض الجوانب الفسيولوجية.