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DIETARY COMBINATION OF VITAMIN E, SELENIUM, AND ZINC EFFECT ON THE REPRODUCTIVE EFFICIENCY OF NILE TILAPIA (*Oreochromis niloticus*)

El-Sayed M. Abou El-Fotoh^{1*}, G.A. Abd El-Rahman², M.E. Farag², B.A. Khalil² and M.S. Ayyat²

1. Fish Physiol. Dept., Cent. Lab. Aquac. Res., Abbass, Sharkia, Egypt

2. Anim. Prod. Dept., Fac. Agric., Zagazig Univ., Egypt

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ABSTRACT: This experiment was conducted to study the effect of combinations between 350 mg vitamin E with 120 mg zinc/kg diet, or 0.3mg selenium/kg diet as feed additives on the reproductive performance of female Nile tilapia (*Oreochromis niloticus*) diet for 144 days. Growth rate, average weight and number of eggs, egg weight, Relative fecundity, hatching percentage, survival rate of the fry and the concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the blood serum at the different treatments compared to the control group. The results showed that the increase in the final body weight with vitam. E+Zn and vitamE +Se treatments was 141.80±0.92 and 142.00±1.15 g and body length was 20.60±0.06 and 20.60±0.06 cm compared to the control groups (136.00±0.92g, 20.30±0.05cm, respectively. The results of spawning efficiency showed an increase in average weight of eggs with vitam. E +Zn and vitam. E +Se treatments valued as much as 2.613±0.085 and 2.643±0.018 g and the number of eggs produced 453.33±18.05and 475.67±4.05 egg/female/spawning compared to the control group (2.043±0.074 g and 365.67±15.81 egg/female/spawning, respectively). The relative fecundity of female's recorded the highest value (3.20±0.12 and 3.35±0.03 egg/g of body weight) with vitam. E +Zn and vitam. E +Se treatments compared to the control group (2.70±0.12 egg/ g of body weight). On the other hand, the hatching percentage of eggs in treatment fed on supplementation diet with combination (vitam. E+ Zn) was higher (87.13±0.08%) than the other treatments. Fish fed diets supplemented with vitem. E+ Zn recorded the highest value of FSH, unlike the released hormone, which increased its concentration in blood fish in all treatments. The highest rate of increase in the body weight of fish larvae fed with (vitamin E +Zinc), the highest survival rate was with the fish group fed with a dietary supplement of vitam. E+ selenium).That is to say, female Nile tilapia (*O. niloticus*) feeding on diet combined with vitamin E, selenium and zinc had the positive effect on growth and reproductive performances in spawning season.

Key words: Reproductive, Nile tilapia, dietary, vitamin E, selenium.

INTRODUCTION

The culture of tilapia is the major interest among many freshwater fishes due to several factors such as high resistance to diseases, excellent growth rate, high yield, tolerance to a wide range of environments and acceptability of natural and artificial feed, several methods have been developed to control reproduction for cost-

effective production (El-Sayed, 2006; Olufeagba and Okomoda, 2015; FAO, 2016).

Feed additives, such as micronutrients, and vitamins are commonly administered in feed to improve productive and reproductive performance and to control the outbreak of diseases in aquaculture (Gaunt *et al.*, 2010; Defoirdt *et al.*, 2011). Trace elements are needed for vitamin synthesis, hormone

*Corresponding author: Tel. : +201060465491

E-mail address: ftoth22@yahoo.com

production, enzyme activity, energy production and other physiological processes related to growth, reproduction and health (**Hayat *et al.*, 2010**).

The zinc and selenium results showed their importance for the production of the semen, as it is involved extensively in nucleic acid and protein metabolism (**Underwood and Somers, 1969; Surai, 2002**).

Zinc is a very important trace element that is involved in a wide range in metabolic activities and productive performances like growth, reproduction and humeral immune system (**Prasad and Kundu, 1995; Underwood and Suttle, 1999; Abdel Monem and El-Shahat, 2011**). Zinc plays important role in various biological activities in animals (**Swinkels *et al.*, 1994**). Zinc deficiency causes numerous physical and pathological changes such as growth retardation and decreased immunity to infection of several diseases (**Star *et al.*, 2012**). **Liu *et al.* (2011)** suggested that dietary zinc supplementation improved zinc status and resulted in promoting antioxidant ability and activities and gene expressions of fat metabolism-related enzymes of broilers.

Selenium (Se) is an essential ingredient that plays an important role in animal reproduction. Selenium is an integral component of glutathione peroxidase, an enzyme that protects the inner structures of cells from free radicals and is an antioxidant of cellular membrane lipids (**Bartle *et al.*, 1980; Gabryszuk, 1994**). Along with a number of functions, selenium plays an important role in the reproductive processes of both male and female livestock (**Heimann *et al.*, 1984; Marin-Guzman *et al.*, 2000**). **Bates *et al.* (2000)** found that Se deficiency has no significant effect on female reproduction. However, **Basini and Tamanini (2000)** reported that Se significantly stimulates the proliferation of cells from small follicles, and also enhances oestradiol production.

Vitamin E functions as a lipid-soluble antioxidant that protects biological membranes, lipoproteins and lipid stores against the damage induced by oxygen free radicals and reactive products of lipid peroxidation (**Bodas *et al.*, 2011**). Antioxidants play an important role in avian reproduction (**Bollengier-Lee *et al.*, 1999;**

Lin *et al.*, 2004 and 2005). The group of vitamin E consists of a mixture of vitamins in which the most important is the α -tocopherol that exists in nature accompanied by other compounds, β -, γ - and δ -tocopherols, and whose function is to inhibit or retard oxidation of animal tissue, especially unsaturated fatty acids and vitamin A (**Nascimento *et al.*, 2014**). The gonadal development and fecundity are affected by certain essential nutrients, especially in species with continuous spawning with short periods of vitellogenesis (**Izquierdo *et al.*, 2001**). In fish, vitamin E is used for diet supplementation with the purpose of improving growth, resistance to stress and immune system. It is efficient in the conservation of fish during processing and storage, inhibiting the degradation of lipids by oxidation (**Webster and Lim, 2001**) and also plays an important role in fish reproductive physiology, especially fertility and growth of crustaceans (**Conklin, 2000**). It is responsible for maintaining the permeability of capillaries and cardiac muscles, and also acts on cellular respiration and in the biosynthesis of DNA (**Pezzato *et al.*, 2004**). According to **Serezli *et al.* (2010)** the effect of vitamin E on female and male reproductive performance is not to be overemphasized.

The aim of the present work was to verify and compare the effect of a supplementation of selenium, zinc, and vitamin E on the gonad somatic index, fecundity, egg size, spawning efficiency and hatchability of Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

The study was conducted at the Department of Animal Production, Agriculture Faculty, Zagazig University and the practical work was carried out at Central Laboratory for Aquaculture Research (CLAR), Abbassa village, Abu-hammad district, Sharkia Governorate, Egypt. The experiment was conducted to study the effect of vitamin E with selenium or zinc as nutritional additives on the reproductive performance of female Nile tilapia and compare them with control for 144 days during the spawning season 2018.

A total of 108 healthy female Nile tilapia were brought in weight (70-75 g) and length (14-15 cm), and the number of 108 healthy male Nile tilapia weight between (90-100 g) and length ranging from (17-18 cm) at ratio male-female 1:1 from the hatchery of the Central Laboratory for Fish Research Abbassa to one of the docks research of the laboratory. The fish were divided into three treatments. The first treatment fed diet without any treatment as (control group), the second was supplemented with 350 mg vitamin E + 120 mg zinc/kg diet and the third was supplemented with 350 mg vitamin E + 0.3 mg selenium/kg diet. Water quality was measured every 12 days during the trial period, as the water temperature ranged from (12-31°C), dissolved oxygen ranged from (5.4 - 7.4 mg / l) and pH (6.9-8).

All fish groups were fed on basal pelleted diet consistent of fish meal 30.0%, soybean meal 15.0%, corn 12.0%, wheat bran 19.0%, rice bran 20.0% molasses 2.75%, minerals mixture 0.75% and vitamin mixture 0.5%. The chemical composition of the diet was crude protein 32.6%, ether extract 5.9%, and crude fiber 5.7% and gross energy 4119.0 Kcal/Kg.

The fish of the experiment were distributed on 9 hapas (4 X 2 X 1 m) with water level of 80 cm, held on earthen pond (1000 m²) where from 30 -40% of the water level was changed with fresh water every 4 days. The hapas were divided into four treatments each treatment contained three replicates, each replicate was stoked at density of 24 fish (12 females: 12 males). Fish were fed on experimental diets with 4% of body weight for the first 23 days of the trial period, and the remaining trial period (24-144 days). Fish were fed 3% of body weight for the end of the experiment and meals were given daily in two batches at 9, 12 in the morning. Fish were weighed at 24-day intervals during the trial period and feed quantities were reset according to changes in live body weight. The body weight was determined by using electrical balance and the survival rate.

Eggs were removed each 12 days from brooder's buccal cavity and number of eggs in each spawn was reported. The total number of eggs per individual fish was estimated by taking total weight of eggs and the mean weight of

subsamples containing 100 eggs into account. The mean weight of egg was determined to the nearest 0.001 g by taking random samples. Eggs are taken from the oral cavity of mothers after each spawn are placed in incubation units (hapa 1 X 1 X 1 m = 1 m³). The fertilization rate refers to the ratio of fertilized eggs to the total number of eggs as a percentage. The percentage of egg hatchability is the ratio of number of eggs hatched to the total number of fertilized eggs as a percentage. Larval survival rate is the ratio of number of larvae survived until completely yolk sac absorption to the total number of eggs hatch as percentage. Variables were estimated from the data included the number of spawned eggs and the total egg weight per female were determined. Absolute fecundity equals the number of seeds per spawning female. Relative fecundity equals the number of seeds per weight of female (g). Hatchability performance was checked including the survival yolk-sac fry and swim-up fry after 15 days post-swim up. Hatching rate (%) was calculated as a percentage of incubated eggs when higher than 90% of the embryos had hatched. Survival rate percent of larvae after 15 days of hatching = number of live fries / total number.

The data were statistically analyzed with SAS (2004) according to the following model: $Y_{ij} = \mu + T_i + e_{ij}$, Where, μ is the overall mean, T_i is the fixed effect of treatments and e_{ij} is random error. Significant differences between treatments were tested with Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth Performances of Female Nile Tilapia

The results of growth performances on female Nile tilapia (*O. niloticus*) feeding on diet combined with vitamin E, selenium and zinc (vitam E + Zn and vitam E + Se) and compared with control diet for 144 days during the spawning season, showed that the increases in the final body weight with vitam E + Zn and vitam E+Se treatments were 141.80±0.92 and 142.00 ±1.15 g also body length increased and valued 20.60±0.06 and 20.60±0.06 cm compared to the control groups (136.00±0.92g,

20.30±0.05cm respectively) as shown in Tables 1 and 2. This increase in growth is a result of the fact that vitamin E and selenium (*via* glutathione peroxidase) act as parts of a multi-component antioxidant defense system. This system protects cells from the adverse effects of reactive oxidation of polyunsaturated membranous phospholipids. **Raederstorff *et al.* (2015)** reported that vitamin E is one of the major antioxidants in the body and plays a major role in protecting different organs against oxidative stress. Also, the obtained results are agreed with **James *et al.* (2008)** who found that feeding golden fish with 300mg of vitamin E/kg diets recorded a significant increase in fish body weight. **Le and Fotedar (2013 and 2014)** found that weight gain and feed intake were significantly reduced in the yellow squash fish diet that feeds on 3.35 mg of selenium/kg diet. As well, **Li and Huang (2016)** found that Juvenile hybrid tilapia fed on diet containing 31 mg zinc/kg diet showed the lowest growth rates. On the other hand, weight gain increased in fish fed on 127 mg zinc/kg diet.

Reproductive Performances of Female Nile Tilapia

The results of spawning efficiency of female Nile tilapia (*O. niloticus*) feeding on diet combined vitamin E with zinc and selenium (vitam E + Zn and vitam E + Se) and compared with control diet for 144 days during the spawning season showed an increase in average weight of eggs with vitam. E + Zn and vitam. E + Se treatments, the increment valued 2.613± 0.085 and 2.643± 0.018 g and the number of eggs produced 453.33±18.05 and 475.67±4.05 egg/female/ spawning compared to the control group (2.043± 0.074 g and 365.67±15.81 egg/ female/spawning, respectively), as shown in Tables 3 and 4. While, no significant effect was observed among the experimental groups on egg weight per female as shown in Table 5. That makes clear that there is a positive correlation between the supply of nutrients during vitellogenesis in the broodstock diet and reproductive related factors such as a better oocyte development and maturation, higher rate of vitellogenesis, larger egg size and better embryonic development (**Ghosh *et al.*, 2007**). In a study of the effects of vitamin E and

growth hormone on gonadal maturity in the common carp (*Cyprinus carpio*), dietary vitamin E resulted in a higher gonadosomatic index, larger ova, and more eggs with higher hatchability than the control (**Gupta *et al.*, 1987**).

Selenium represents an integral component of glutathione peroxidase, an enzyme which, protects cell internal structures against free radicals and is an antioxidant for cellular membrane lipids (**Bartle *et al.*, 1980 and Gabryszuk, 1994**). Besides a number of functions, selenium plays an important role in reproductive processes of both males and females of livestock animals (**Heimann *et al.*, 1984 and Marin-Guzman *et al.*, 2000**). **James *et al.* (2008)** found that females fed the 300 mg vitamin E/kg diet had significantly heavier gonads and a higher number of eggs with better hatchability than those fed other diets, Egg weight and diameter and larvae weight and length were significantly higher in fish fed 300 mg vitamin E than in fish fed the control diet but did not differ from other vitamin E treatments. **Pamungkas *et al.* (2014)** found that supplementation of α -tocopherol on the diets of female Nile tilapia with level 225 mg/kg diet can be useful for improving gonadal development, the number of mature female, percentage of maturation and value of spawning frequency on female tilapia.

The results are of relative fecundity of females (*O. niloticus*) feeding on diet combined vitamin E with zinc and selenium (vitam. E+Zn and vitam. E+Se) and compared with control diet for 144 days during the spawning season are shown in the Table 6. Recorded highest value (3.20± 0.12 and 3.35±0.03 egg/ g of body weight) with vitam. E+Zn and vitam. E+Se treatments compared to the control group (2.70± 0.12 egg/ g of body weight) respectively. On the other hand, the hatching percentage of eggs in treatment fed on supplementation diet with vitamin E+Zn group was higher (87.13±0.08%) followed by the group of vitamin E+Se (86.40±0.23) compared to the control group (85.02±0.07) as shown in Table 7. The results of this study are in line with, **Nascimento *et al.* (2014)** study on the effect of nutrition for several

Table 1. Effect of dietary vitamin E with Zn or Se supplementation during the spawning season on weight (g) of females (*O. niloticus*)

Treatment	Initial	24 day	48 day	72 day	96 day	120 day	144 day
Control	72.33±1.20	88.067 ^b ±0.03	96.50 ^b ±0.35	105.50 ^b ±0.46	115.03 ^b ±0.72	125.03 ^b ±0.84	136.00 ^b ±0.92
Vitamin E+ Zn	72.33±0.66	88.63 ^a ±0.08	97.66 ^a ±0.20	107.60 ^a ±0.40	117.80 ^a ±0.30	129.43 ^a ±0.37	141.80 ^a ±0.92
Vitamin E+Se	72.33±1.20	88.56 ^a ±0.12	97.76 ^a ±0.31	107.80 ^a ±0.11	118.46 ^a ±0.86	129.73 ^a ±1.03	142.00 ^a ±1.15
Significance	NS	**	*	**	*	**	**

Where: NS=Not significant * = p<0.05 ** p < 0.01

Table 2. Effect of dietary vitamin E with Zn or Se supplementation during the spawning season on the length (cm) of females (*O. niloticus*)

Treatment	Initial	24day	48day	72day	96day	120day	144day
Control	14.46±0.26	16.53±0.03	17.33±0.16	18.50 ^b ±0.05	19.16 ^b ±0.03	19.70 ^b ±0.05	20.30 ^b ±0.05
Vitamin E+ Zn	14.43±0.12	16.40±0.20	17.70±0.05	18.70 ^a ±0.05	19.33 ^a ±0.03	19.96 ^a ±0.03	20.60 ^a ±0.06
Vitamin E+Se	14.46±0.20	16.76±0.09	17.66±0.03	18.73 ^a ±0.03	19.40 ^a ±0.06	20.00 ^a ±0.05	20.60 ^a ±0.06
Significance	NS	NS	NS	*	*	*	*

Where: NS=Not significant * = p<0.05

Table 3. Effect of dietary vitamin E with Zn or Se supplementation during the spawning season on Eggs weight (g) per female (*O. niloticus*)

Treatment	24 day	48 day	72 day	96 day	120 day	144 day
Control	0.818±0.029	1.032±0.027	1.247±0.014	1.450±0.045	1.873±0.037	2.043±0.074
Vitamin E+ Zn	0.787±0.008	1.037±0.035	1.363±0.022	1.597±0.035	2.167±0.060	2.613±0.085
Vitamin E+Se	0.774±0.020	0.975±0.020	1.283±0.042	1.533±0.068	2.127±0.067	2.643±0.018
Significance	NS	NS	NS	NS	NS	NS

Where: NS=Not significant

Table 4. Effect of dietary vitamin E with Zn or Se supplementation during the spawning season on Eggs No per femal per hapa per spawn of (*O. niloticus*)

Treatment	24 day	48 day	72 day	96 day	120 day	144 day
Control	176.00±7.50	211.66±6.69	242.33±3.84	276.00±10.78	346.67±9.21	365.67 ^b ±15.81
Vitamin E+ Zn	168.67±2.60	210.00±6.08	263.33±6.69	298.00±9.29	389.67±13.74	453.33 ^a ±18.05
Vitamin E+Se	168.33±5.21	205.00±3.78	258.33±6.36	296.33±11.41	397.33±15.26	475.67 ^a ±4.05
Significance	NS	NS	NS	NS	NS	**

Where: NS=Not significant ** p < 0.01

Table 5. Effect of dietary vitamin E with Zn or Se supplementation during the spawning season on weight of one Egg (mg) /female (*O. niloticus*)

Treatment	24 day	48 day	72 day	96 day	120 day	144 day
Control	4.65±0.029	4.88±0.051	5.14±0.023	5.26±0.069	5.41±0.063	5.59±0.042
vitamE +Zn	4.67±0.033	4.93±0.032	5.18±0.056	5.36±0.049	5.56±0.047	5.77±0.045
vitamE +Se	4.60±0.057	4.72±0.088	4.97±0.086	5.17±0.052	5.35±0.072	5.56±0.084
Significance	NS	NS	NS	NS	NS	NS

Where: NS=Not significant

Table 6. Effect of dietary vitamin E with Zn or Se supplementation during the spawning season on relative fecundity of (*O. niloticus*)

Treatment	24 day	48 day	72 day	96 day	120 day	144 day
Control	2.00±0.09	2.21±0.0	2.30±0.03	2.40±0.09	2.55±0.06	2.70 ^b ±0.12
Vitamin E+ Zn	1.90±0.03	2.15±0.06	2.45±0.06	2.52±0.07	2.75±0.09	3.20 ^a ±0.12
Vitamin E+Se	1.90±0.06	2.07±0.02	2.40±0.06	2.50±0.09	2.80±0.12	3.35 ^a ±0.03
Significance	NS	NS	NS	NS	NS	**

Where: NS=Not significant ** p < 0.01

Table 7. Effect of dietary vitamin E with Zn or Se supplementation during the spawning season on hatching percentage of (*O. niloticus*)

Treatment	24 day	48 day	72 day	96 day	120 day	144 day
Control	79.02 ^b ±0.09	84.00 ^c ±0.23	88.15 ^b ±0.21	88.05 ^b ±0.29	85.05 ^b ±0.15	85.02 ^c ±0.07
Vitamin E+ Zn	78.99 ^b ±0.00	86.03 ^a ±0.03	88.05 ^b ±0.24	88.38 ^b ±0.08	86.25 ^a ±0.06	87.13 ^a ±0.08
Vitamin E+Se	80.00 ^a ±0.01	85.03 ^b ±0.03	90.15 ^a ±0.20	89.33 ^a ±0.12	86.10 ^a ±0.15	86.40 ^b ±0.23
Significance	***	***	***	**	***	***

Where: ** p < 0.01 *** p < 0.001

different levels of vitamin E (200, 300, 400, and 500 mg kg⁻¹) on the reproductive of female Nile tilapia for a period of 90 days. Note an increase in the rate of hatching by 62% with a level of 400 mg/kg compared to the lowest supplement level (200 mg).

Sex steroids hormones as affected by feeding on vitamin E with Zn or Se supplementation during the spawning season on follicular stimulating (FSH) and luteinizing hormones (LH) of female. In Table 8, the results of FSH

hormone in serum of Nile tilapia females fed diet supplemented with studded feed additives increased significantly, while the concentration of LH insignificantly affected. Female Nile tilapia fed diet supplemented with vitamin E and zinc recorded higher concentration of FSH than these females group. Sex steroids are implicated in many important physiological processes in all vertebrates, and measurement of blood sexual hormones seems to be a valuable tool for assessing the reproductive cycle of fish (Guerrero *et al.*, 2009).

Table 8. Effect of dietary vitamin E with Zn or Se supplementation during the spawning season on follicular stimulating (FSH) and luteinizing hormones (LH) of *O. niloticus* females

Treatment	FSH	LH
Control	0.056 ^c ±0.003	0.166±0.033
Vitam. E +Zn	0.100 ^a ±0.006	0.133±0.033
vitamE +Se	0.073 ^b ±0.003	0.166±0.033
Significance	***	NS

Where: NS=Not significant *** p < 0.001

Conclusion

That is to say, female Nile tilapia (*O. niloticus*) feeding on diet combined with vitamin E, selenium and zinc had the positive effect on growth and reproductive performances in spawning season.

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تأثير التغذية على مخلوط من فيتامين هـ والسيلينيوم والزنك على الكفاءة التناسلية لأسماك البلطي النيلي (*Oreochromis niloticus*)

السيد محمد أبو الفتوح^١ - جمال الدين على عبدالرحمن^٢
محمد السيد فرج^١ - بكرى عبد الغنى خليل^٢ - محمد صلاح الدين عياط^٢

- ١- قسم فسيولوجيا الأسماك- المعمل المركزي لبحوث الأسماك- مركز البحوث الزراعية- العباسية أبو حماد- شرقية - مصر
٢- قسم الإنتاج الحيواني - كلية الزراعة - جامعة الزقازيق - مصر

أجريت التجربة لدراسة تأثير التغذية على مخلوط من ٣٥٠ ملجم من فيتامين E مع ١٢٠ ملجم من الزنك أو ٠,٣ ملجم من السيلينيوم/كجم علف كإضافات غذائية على الأداء التناسلي لإناث أسماك البلطي النيلي (*Oreochromis niloticus*) لمدة ١٤٤ يوماً، تم قياس كل من معدل النمو، متوسط وزن وعدد البيض، ووزن البيضة، الخصوبة النسبية، نسبة الفقس، معدل البقاء على قيد الحياة للزريعة وتركيز الهرمون المنبه لنشاط الحويصلات المبيضية (FSH) والهرمون المحرر للبيوضات من المبيض (LH) في مصل الدم في المعاملات المختلفة مقارنة مع المجموعة القياسية، أظهرت النتائج أن الزيادة في وزن الجسم النهائي كانت مع الأسماك المغذاه بمكمل غذائي من فيتامين E مع السيلينيوم ويليها المجموعة المكمله من فيتامين (E) مع الزنك وذلك مقارنة بالمجموعة القياسية، كما لوحظ زيادة في وزن وعدد البيض لكل انثى لكل تقريخة مع مجموعة الاسماك المغذاه بمكمل غذائي من فيتامين E مع السيلينيوم والمجموعة المكمله من فيتامين (E) مع الزنك وذلك مقارنة بالمجموعة القياسية، أيضا سجلت الخصوبة النسبية أعلى قيمة للإناث للأسماك المغذاه بمكمل غذائي من فيتامين E مع السيلينيوم، و أعلى نسبة فقس مع المجموعة المكمله من فيتامين (E) مع الزنك وذلك مقارنة بالمجموعة القياسية، وتركيز الهرمون المنبه لنشاط حويصلات المبيض كان أعلى تركيز له بالدم للأسماك المغذاه بمكمل غذائي من فيتامين E مع الزنك، بخلاف الهرمون المحرر والذي زاد تركيزه بدم الأسماك في كل المعاملات، كما سجل أعلى معدل زيادة في وزن الجسم ليرقات الأسماك المغذاه بمكمل غذائي من فيتامين E مع الزنك، وأعلى معدل بقاء مع مجموعة الأسماك المغذاه بمكمل غذائي من فيتامين E مع السيلينيوم، وهذا يعني أن أنثى البلطي النيلي (*O. niloticus*) التي تتغذى على النظام الغذائي مع فيتامين E والسيلينيوم والزنك كان لها تأثير إيجابي على النمو والأداء الإيجابي في موسم التقريخ.

المحكمون:

١- أ.د. هاني إبراهيم المراكبي
أستاذ تغذية الأسماك بالمعمل المركزي لبحوث الأسماك بالعباسية- أبو حماد - شرقية.
٢- د. أدهم عبدالله الصغير
أستاذ تغذية الحيوان المساعد- قسم الإنتاج الحيواني- كلية الزراعة- جامعة الزقازيق.