

EFFECTS OF MATERNAL INJECTION WITH ORGANIC PHOSPHORUS AND VITAMIN B₁₂ ON REPRODUCTIVE PERFORMANCE AND NEWLY HATCHED OFFSPRING OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*).

AKRAM IBRAHIM ALKOBABY

Animal Production Department, Faculty of Agriculture, Cairo University
E-mail address: alkobaby@yahoo.com

Abstract

This study tested the effect of three doses (0.5, 1.0 and 1.5ml/kg body weight) of Tonozal 100 as a source of organic phosphorus and vitamin B₁₂ (each 1 ml of the injectable solution contains 100 mg Butaphosphan and 50 µg of vitamin B₁₂; The solution contains 17.3 mg of phosphorus in 1 ml) on the reproductive performance of Nile tilapia. Females were injected every week during the collection of fertilized eggs from the buccal cavity. The Relative fecundity, egg weight, egg weight index (as a percentage of female body weight), hatching percentage and incubation period were determined. The effect of the treatments on the yolk-sac fry weight, length, survival and the duration of yolk-sac absorption were also determined. The growth performance of the swim-up fry until 7-days post swimup was also determined. Generally, the best results were obtained after injection with phosphorus and vitamin B₁₂. Spawning rate and relative fecundity in Nile tilapia were affected significantly by the treatments ($P < 0.05$) with no significant differences between the three doses tested. It could be concluded that the reproductive performance of Nile tilapia may be improved by injecting Nile tilapia broodstock with a low dose of vitamin B₁₂ and organic phosphorus (25 µg and 50 mg, respectively) which enhance the metabolism of fish subsequently, enhance the egg production and reproductive performance of females.

Keywords: Nile tilapia – Reproduction – Phosphorus – Vitamin B₁₂

INTRODUCTION

Tilapia belong to the family Cichlidae, and is bred all over the world. Nile tilapia, *Oreochromis niloticus*, is among the most important species on account of its fast growth rate, adaptability to a wide range of culture conditions and high consumer acceptability. Nile tilapia is mouthbreeders and need a lot of energy during the spawning season (Bromage and Roberts, 1996), although their intensive culture remains constrained by poor spawning synchrony and low fecundity, adding significant cost to hatchery production. However, numerous fish species developed for aquaculture show high mortality during early development. For mass production of seedling, therefore, it is important to reduce mortality, to increase metabolic activity and to induce optimum growth in larva (Kang and Chang, 2004).

Phosphorus (P) is an essential macro-mineral required for growth, bone mineralization, reproduction and energy metabolism in fish (Roy and Lall, 2004). It is

directly involved in all energy-producing cellular reactions (NRC 1993). Phosphorus is very essential for vitellogenesis (yolk accumulation in the fish egg). Many studies were done to identify phosphorus content in the egg yolk of several fishes. It was 1.8% in tilapia (*Oreochromis mossambicus*) (Kishida and Specker, 1993).

Fish and other aquatic animals have the ability to absorb P from water; however, the concentration of this element is low in both freshwater (Boyd, 1971) and seawater (Lall, 1991). Although fish are able to absorb phosphorus from water, food is the major source of this mineral (Lall, 1991). However, a major portion of the organic P is undigestible and is excreted in the feces (Beveridge, 1987). Certainly the levels of phosphorus required are the highest of all the inorganic ions, with dietary inclusions of 0.4-0.9% of available phosphorus being required for most fish species (Shepherd and Bromage, 1992). Phosphorus requirements have been estimated for a variety of fish species (for a compilation of values see NRC 1993; Davis and Gatlin 1996; Chafez-Sanchez *et al.*, 2000; Lall, 2002).

The P requirement of tilapia (0.96% of diet; Roy and Lall, 2003), is similar to the P requirement of haddock (Robinson *et al.*, 1987) but higher than that for most salmonids (NRC, 1993).

However, excess of dietary phosphorus is responsible for the environmental impact caused by surplus phosphorus discharges in the effluents. Therefore, in recent years there has been a trend towards the reduction of dietary phosphorus to levels that satisfy, but do not exceed phosphorus requirements (Lall, 1991; Oliva-Teles *et al.*, 1998; Bureau and Cho 1999).

Vitamins are needed for normal growth, maintenance and reproduction in all animals (Shepherd and Bromage, 1992). The two B-vitamins, B₁ and B₁₂ are in fact complex phosphoric acid. Vitamin B₁₂ is one of the major vitamins essential to fish required to help the formation of erythrocytes and hemoglobin (Koenig, 1979). Also, it is required for growth and the metabolism of carbohydrates, lipids and proteins. Bhattacharyya *et al.* (2000) stated that vitamin B₁₂ is involved in the induction of some enzymes in fish brain.

In recent years, the injectable phosphorous solutions became a very popular metabolism stimulators in several animals. Tonozal 100 is one of these solutions used widely in Egypt for many animals. Although the role of phosphorus in improving the reproductive performance and egg quality is well defined in mammals and birds, from the reviewing literatures there is no report dealing specifically with the organic phosphorus in the teleost. The objectives of this study were (1) to study the effect of this injectable phosphorous solution on the reproductive performance of Nile tilapia females and (2) to identify the best dose.

MATERIALS AND METHODS

The design of the experiment which lasted 62 days was to test the effect of three doses (0.5, 1.0 and 1.5ml/kg body weight) of Tonozal 100 as a source of organic phosphorus and vitamin B₁₂ on the reproductive performance of Nile tilapia. Each 1 ml of the injectable solution contains 100 mg of n-(Butylamino)-1-methylethyl phosphonous acid (Butaphosphan) and 50 µg of vitamin B₁₂. The solution contains 17.3 mg of phosphorus in 1 ml.

BROODSTOCK

Nile tilapia, *Oreochromis niloticus*, broodstock were collected a life from a commercial farm in Wady Alnotron, Egypt and transported to the fish research unit, Faculty of Agriculture, Cairo University. Thirty two females (70.0 - 116.0 gm) and eight males (99.8 – 128.7 gm) were assigned to eight outdoor concrete tanks (2.0 × 1.2 × 1.0 m). Stocking density was 5 fish/ tank with a sex ratio of one male to four females per each tank. The water depth was maintained at about 80 cm. the fresh water in concrete tanks was changed every third day. Fish were fed commercial diet (30% crude protein) twice a day at 3% broodstock body weight.

EXPERIMENTAL DESIGN

The females were divided into four groups of eight females each (Table 1). Each group was divided in two tanks (two replicates for each treatment). In group I (control) the females were not injected, in group II (low dosage) the females were injected with Tonozal 100 at a dose of 0.5 ml (8.65 mg of P)/ kg body weight, in group III (medium dosage) injected with 1.0 ml of Tonozal 100 (17.3 mg of P)/ kg body weight, and in group IV (High dosage) females were injected with 1.5 ml of Tonozal 100 (25.95 mg of P)/ kg body weight. Multiple intraperitoneally injections were given and the interval between injections was one week.

Table 1. Doses applied to Nile tilapia females and the number of females in each group.

Group	No. of females	No. of tanks	Dose of Tonozal 100 (ml/kg body weight)	Dose of Butaphosphan (mg/kg body weight)	Dose of vitamin B ₁₂ (µg/ kg body weight)	Dose of phosphorus (mg/ kg body weight)
I (control)	8	2	0.0	0.0	0.0	0.0
II	8	2	0.5	50	25	8.65
III	8	2	1.0	100	50	17.3
IV	8	2	1.5	150	75	25.95

The broodstock females were checked for fertilized eggs in the buccal cavity every third day. Females with broods were captured in a fine mesh net and transferred to a bucket containing fresh water; eggs were then removed and washed. Each spent female was weighed after egg collection, and then returned to the experimental tank and all data recorded. All females were weighed every week before the injection

Spawning rates during the spawning season in females were calculated in relation to the total number of females present in that treatment. The number of spawned eggs and the total egg weight per female were determined. Relative fecundity (the number of eggs produced per unit of post-spawned broodfish weight) was estimated by dividing the total number of eggs collected from each female/one spawn by the weight of the fish.

EGG AND FRY QUALITY

Eggs obtained from each female were weighed, counted and then transferred to the indoor rounded-bottomed plastic incubators in water at 26 – 30 °C. After hatching, yolk-sac fry were counted, weighed and body length was measured then returned to the same incubator. After yolk-sac absorption, swim-up fry were collected to record the count, body weight and body length. Then, transferred to 60-litre aquaria and fed commercial diet containing 30% crude protein during 7 days after yolk-sac absorption (7 days post swimup). These aquaria were continuously aerated and water exchanged four times a day. After seven days of feeding, fry were collected, counted, weighed and the body length was measured.

Hatching performance was checked including the determination of incubation period, hatching rate (%), and the duration of yolk-sac absorption. Hatching rate was calculated as a percentage of incubated eggs when higher than 90% of the embryos had hatched. Survival was recorded for yolk-sac fry, swim-up fry and 7 days post-swimup.

STATISTICAL ANALYSIS

All statistical analysis were carried out using the SPSS program version 8.0. Data were tested for significant differences by one-way analysis of variance followed by the Duncan's multiple range test. P values < 0.05 were considered to be significant.

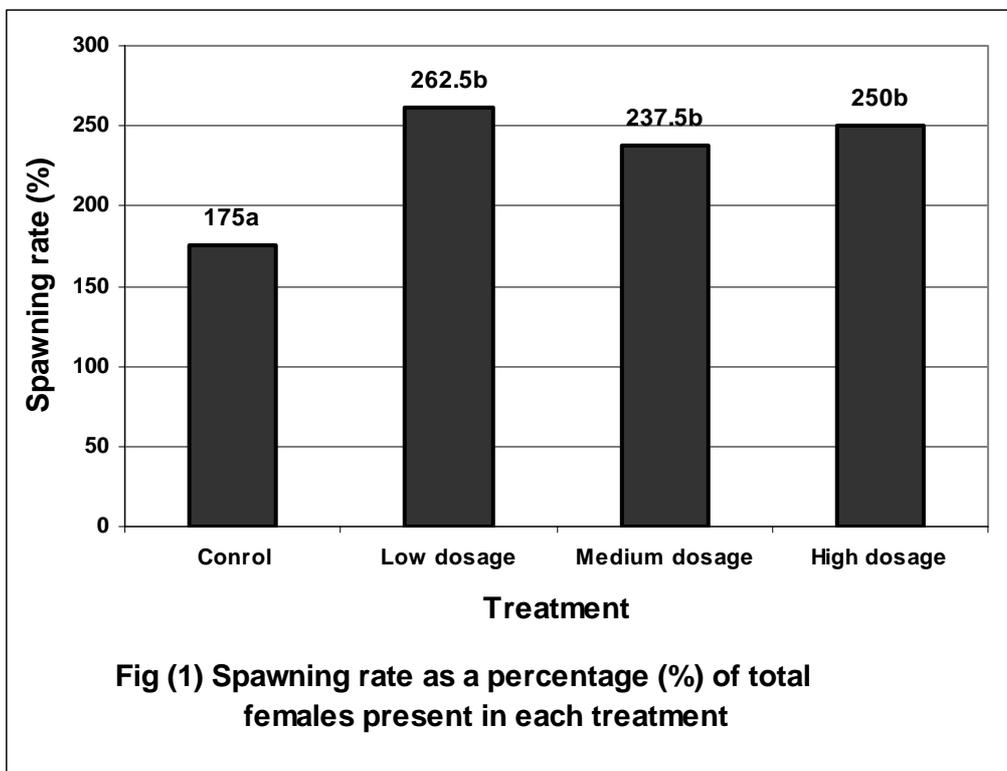
RESULTS AND DISCUSSION

SPAWNING PERFORMANCE

From the reviewing literatures, this is the first investigation to evaluate the effects of maternal injection of organic phosphorus and vitamin B₁₂ on the reproductive performance of Nile tilapia. The spawning rate (expressed as a % of the total number

of spawns obtained during the whole experimental period against the total number of females per treatment) was high in all treatments (175-262.5%). This may be due to the removing of eggs from the buccal cavity of females. Aizen *et al.* (2007) found that Plasma levels of both FSH and LH in Nile tilapia increased during the second day after the eggs had been removed, probably related to the vitellogenic phase. LH levels increased toward spawning. FSH levels also increased on day of cycle due to recruitment of a new generation of follicles for the successive spawning. The spawning rate (%) was 175% for the non injected fishes (control). EL-Naggar *et al.*, 2000 reported 171.4% at the same conditions. The spawning frequency rose to 237.5% -262.5% for the injected females.

Spawning rate in Nile tilapia (*Oreochromis niloticus*) was affected significantly ($P < 0.05$) by the injection with organic phosphorus and vitamin B₁₂ (Figure 1) with no significant differences between the three doses tested. Females were spawned 14 times in the control treatment while the total spawns was 21, 19 and 20 in the low, medium and high injection groups, respectively during the whole experimental period (62 days). This indicates that the injected females had a better metabolism and a good ability to accumulate the yolk in the eggs and complete the vitellogenesis and the maturation of ovaries faster than the non treated females. Subsequently, the spawning frequency was increased.



EGG PRODUCTION AND HATCHABILITY

Total weight of egg production, as a percentage of female body weight, in Nile tilapia ranged from 4.13 to 4.80% per one spawn for all treatments (Table 2). The average weight of egg production per one spawn ranged from 3.28 to 3.93 g for all treatments. The capability of egg production per one spawn per female was affected significantly ($P < 0.05$) by the injection with organic phosphorus and vitamin B₁₂ with no significant differences between the doses tested. Relative fecundity (number of eggs produced per gram of female live body weight per one spawn) in individual females followed the same trend as those of egg production. These results indicated that the injection with mixture of organic phosphorus and vitamin B₁₂ may enhance the metabolism of brood females which activate vitellogenesis and increase the egg produced from ovaries per one spawn and the relative fecundity (Table 2, Figure 2).

Table 2. Effect of different doses of organic phosphorus and vitamin B₁₂ on egg production and hatchability in Nile tilapia, *Oreochromis niloticus*, females.

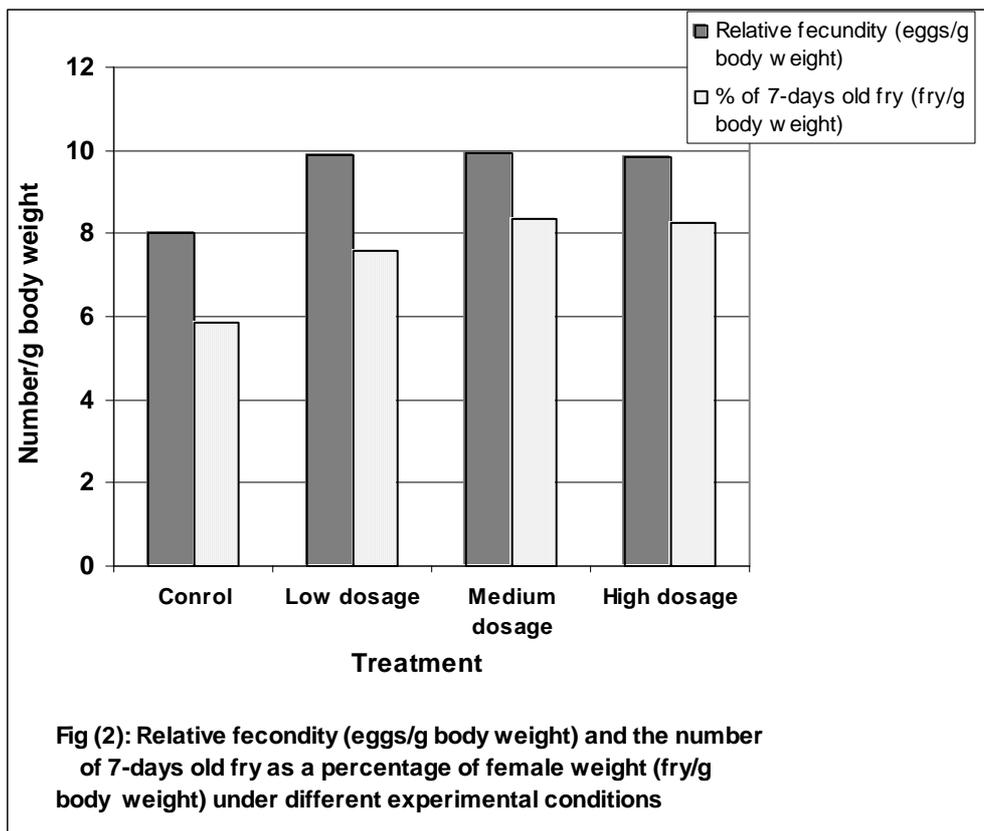
Treatment	Control	Low dosage	Medium dosage	High dosage
Average number of eggs per female per one spawn	636±109 ^a	843±170 ^b	906±169 ^b	857±146 ^b
Relative fecundity (eggs / g body weight)	8.03±0.82 ^a	9.91±1.5 ^b	9.93±1.17 ^b	9.86±0.76 ^b
Average egg weight (mg)	5.17±0.61 ^b	4.85±0.66 ^{ab}	4.83±0.69 ^{ab}	4.41±0.61 ^a
Average weight of egg production per female per one spawn (g)	3.28±0.51 ^a	3.69±0.86 ^{ab}	3.93±0.81 ^b	3.46±0.60 ^{ab}
Egg weight index (%)	4.13±0.55 ^a	4.79±0.89 ^b	4.80±0.92 ^b	4.35±0.69 ^{ab}
Incubation period (days)	3.66±0.49 ^a	3.43±0.62 ^a	3.35±0.63 ^a	3.45±0.51 ^a
Hatching rate (%)	90.8±4.8 ^a	91.0±4.8 ^a	93.0±2.5 ^a	93.0±3.3 ^a

-Mean ± SD

-Means in the same row with different letters are significantly different ($P < 0.05$)

Two forms of vitellogenin (Vg), the yolk-precursor protein (HA I and HA II), were purified from the plasma of Indian major carp, mrigal (*Cirrhinus mrigala*), HA II was phosphorous rich. Annual profiles of plasma Vg and gonadotropin levels presented a good correlation with gonadosomatic index (GSI) and mean number of vitellogenic oocytes during different reproductive phases (Maitra *et al.*, 2007). Nagler *et al.* (1987)

found a linear relationship of serum concentrations of total phosphoprotein phosphorus (TPP) and alkali-labile phosphoprotein phosphorus (ALPP) with the serum concentration of the vitellogenin (Vg) in mature female; rainbow trout (*Salmo gairdneri*) during active vitellogenesis. Kishida and Specker (1993) isolated two forms of vitellogenin from plasma of the tilapia, *Oreochromis mossambicus*, the total amount of phosphorus in these forms was 1.7 and 0.1%, respectively.



The average egg weight in the high dosage treatment was significantly ($P < 0.05$) smaller than the control treatment with no significant differences occurred with the other doses. The average egg weight of *Oreochromis niloticus* in milligrams ranged from 4.41 to 5.17 mg/egg with an overall average of 4.78 mg/egg. However, no work had been reported on the influence of organic phosphorus and vitamin B₁₂ on egg size of *Oreochromis niloticus*. Although the high values of hatching rate in the injected treatments, statistical analyses did not show any significant difference among all means of hatching rates and incubation period (Table 2). Lahnsteiner and Patarnello (2003) investigated that the phospholipid levels were significantly lower in nonviable eggs, and the inorganic phosphate levels were lower in nonviable than in viable gilthead sea bream eggs.

FRY QUALITY

Broodstock exposed to the control treatment and the medium dosage of vitamin B₁₂ and phosphorus during spawning season produced heavier yolk-sac fry than that of the low and high dosage treatments ($P < 0.05$). The overall mean wet weight of yolk-sac fry produced from the broodstock of Nile tilapia under all treatments was around 5 mg. Except the low dosage treatment, the injection of tonozal 100 did not have an effect on total body length of yolk-sac fry after hatching. Yolk-sac absorption in the medium and high dose treatments was faster than the non injected and the low dose treatment.

Survival rate has the same in yolk-sac fry, swim-up fry during 7 days and the overall survival from hatching to 7 days post-swimup. Generally, the lower survival rate occurred in fry produced from the non treated females (control) while, the higher survival rate was in fry produced from the high dose treated females (group IV) which indicated that the maternal injection of organic phosphorus and vitamin B₁₂ increased survival rate of newly hatching fry. Wet weight of swim-up fry did not affected by the treatments, the same trend was found after 7 days of yolk-sac absorption.

Table 3. Effect of different doses of organic phosphorus and vitamin B₁₂ on the quality of yolk-sac fry, swim-up fry and 7 days post-swimup fry* in Nile tilapia, *Oreochromis niloticus*, females.

Treatment	Control	Low dosage	Medium dosage	High dosage
Wet weight of yolk-sac fry (mg)	5.99±0.61 ^b	5.10±0.69 ^a	5.70±0.89 ^b	5.12±0.61 ^a
Yolk sac fry length (mm)	6.51±0.06 ^{ab}	6.37±0.05 ^a	6.77±0.03 ^b	6.47±0.04 ^{ab}
Duration of yolk-sac absorption (days)	4.72±0.90 ^b	4.75±0.85 ^b	4.00±0.87 ^a	3.55±0.51 ^a
Survival rate of yolk-sac fry (%)	88.9±5.4 ^a	90.1±6.5 ^a	93.9±2.6 ^b	94.4±3.1 ^b
Wet weight of swim-up fry (mg)	12.5±1.2 ^a	11.6±1.9 ^a	11.9±3.3 ^a	11.7±2.9 ^a
Swim-up fry length (mm)	8.0±0.52 ^b	7.9±0.86 ^b	7.17±1.32 ^a	7.19±0.78 ^a
Survival rate of swim-up fry (%)	91.5±2.3 ^a	92.1±6.4 ^{ab}	94.4±2.5 ^{ab}	95.0±3.7 ^b
*Wet weight of 7 days post-swimup fry (mg)	16.1±1.2 ^a	15.8±3.1 ^a	14.8±3.1 ^a	14.9±2.6 ^a
*Length of 7 days post-swimup fry (mm)	11.1±0.52 ^b	10.7±0.62 ^b	10.8±1.21 ^b	9.3±0.40 ^a
*Survival rate from hatching to 7 days post-swimup (%)	81.4±5.1 ^a	83.1±8.4 ^a	88.7±3.1 ^b	89.8±5.1 ^b
*Number of 7 days post-swimup fry as percentage of female weight	5.88±0.8 ^a	7.60±1.7 ^b	8.36±1.1 ^b	8.25±0.9 ^b

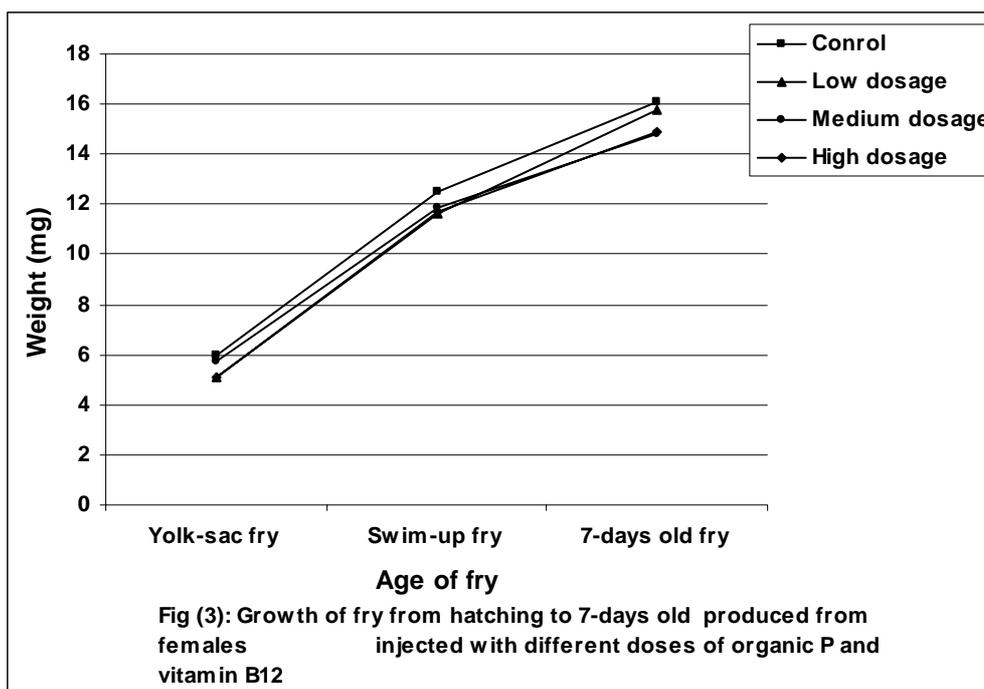
-Mean ± SD

-Means in the same row with different letters are significantly different ($P < 0.05$)

* 7-days old from yolk-sac absorption

The results of the present study indicated that the number of fry after 7 days of yolk-sac absorption as a percentage of gram female body weight was affected significantly by the injection with the mixture of phosphorus and vitamin B₁₂. The

mean number of fry after 7 days of yolk-sac absorption per one gram of female body weight was 5.88 fry/g body weight raised to 7.6-8.36 fry/ g body weight by injection treatments (Table 2; Figure 3). Figure (3) showed the growth of offspring produced from broodstock injected with organic phosphorus and vitamin B₁₂ and control broodstock. However, data in table (3) indicated that no significant differences occurred in the weight of fry produced from the control treatment (non injected) and the fry produced from the Tonozal100 injection treatments ($P>0.05$).



CONCLUSION

It could be concluded that there might be a possible potential for enhancing the reproductive performance of Nile tilapia by injecting Nile tilapia broodstock with a low dose of organic phosphorus and vitamin B₁₂ (25 μ g and 50 mg, respectively) which enhance the metabolism of fish subsequently, enhance the egg production and reproductive performance of females. It is wise to say that this issue still subject for further investigation.

ACKNOWLEDGMENTS

The author would like to thank Dr. M. A. Elnady for valuable comments on an earlier version of this manuscript. The author also is very grateful to Prof. M. M. Shafei for his support and helpful comments.

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