A SYSTEM FOR NILE TILAPIA FRY SEX REVERSAL BASED ON PALM POLLEN GRAINS SUPPLEMENTS.

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ABSTRACT

Eighteen thousand Nile tilapia (*Oreochromis niloticus*) fry were allotted to 18 hapas situated in a commercial hatchery earthen pond at Fayoum Governorate. Each three hapas represented one of the experimental dietary treatments. Such treatments based on herring fishmeal and wheat flour. The control contained 17 α methyl testosterone (80mg/kg diet, MT) representing treatment one. The other diets contained palm pollen grains (PPG) at levels of 0.5, 1.0, 2.0, 5.0 and 12.5% respectively without the addition of MT. Palm pollen grains were added in the expense of wheat flour. Fry, after yolk sack absorption were maintained on each diet for 28 days for sex reversal. To obtain sex ratio such fry were further grown under commercial diet (30%CP) to 30 g in weight. Fry growth performance, feed conversion ratio (FCR) and sex reversal ratio were obtained.

Results revealed that fry specific growth rate %/d was the best when PPG represented 5-12.5% of the diet with better FCR and survival rate. However feed cost/ 1000 fry tended to increase as the level of PPG increased but with lower costs than the control (MT diet). However the results of sex reversal were the best with MT diet (the control, 96% males), the 12.5% PPG diet produced about 81% males with better survival rate and cost / 1000 fry than the MT diet, control.

In conclusion it seems that the use of PPG at level of 12.5 % in the diet for Nile tilapia sex reversal is accepted to avoid hormonal treatments hazards as it is a natural source beside the higher survival rate and price/ 1000 fry compared to the control (MT treatment).

Key words: Nile tilapia fry, sex reversal, growth rate, feed conversion, survival rate, costs.

INTRODUCTION

Tilapia is the most widely cultured freshwater fish in tropical and subtropical countries and cultured in more than 100 countries in the world (Borgeson *et al.,* 2006; El-Sayed, 2006; Tahoun, 2007 and Tsadik and Bar, 2007). Fry production is a vital factor and most farmers prefer to use all-male tilapia fry for its faster growth beside the elimination of the over-population problem (Samrongpan *et al.,* 2008).

Several commercially applicable techniques are used to produce progeny with a high percentage of males: hand sexing (Chervinsky and Rothbard, 1982), hormonal sex reversal using androgen (Vera-Cruz and Mair, 1994 and Gale *et al.*, 1999), interspecific hybridization (Mair *et al*., 1991), production of a YY super male line in *O. niloticus* (Beardmore_*et al.*, 2001). Oral administration of the synthetic androgen 17 α-methyltestosterone (MT)

and 17 α ethynyltestosterone (ET) are effective in producing all-male populations in tilapia (Varadaraj and Pandian, 1989; Guerrero, 1995). However, the use of such hormones is accepted in different countries and not allowed in the others, among them Egypt.

In recent years, Nemetallah *et al*., 2008a showed that treating tilapia fry with a combination of 9 mg 17 α MT plus 20 mg fadrozole (aromatase inhibitor)/ kg feed resulted in best growth. However the best survival rate was observed in fry group fed on 70 mg fadrozole/kg feed. The highest sex reversal (96 % males) was with 9 mg α 17 MT plus 20 mg fadrozole/kg feed and recommended to the possibility of lowering the commonly used dose of 17 MT (30-60 mg/Kg feed) to 9 mg/Kg feed only through combined addition of fadrozole at 20 mg/Kg feed. Also, Nemetallah *et al*., 2008b evaluated the probability of using some naturally occurring compounds to induce sex reversal in Nile tilapia treated with synthetic 17-α methyltestosterone (60 mg/Kg diet). They found that, the percentage of male fry in the treatments were 95.65, 76.28, 84.94, 59.52, 71.37, 78.41, 90.47 and 78.27 % for the treatment of 17-α methyl testosterone, sustanon, sustanon with nolvadex, testicular extract (TE), TE with nolvadex, fecal extract (FE), FE with nolvadex, and nolvadex, respectively.

Regarding palm pollens, Farag *et al*. (2008) demonstrated that the most predominant saturated and unsaturated fatty acids were 22:0, 18:1, also palm pollens contained the highest amounts of all essential amino acids. Hassan and Abou-Elwafa (1947), detected a non-crystalline estrogenic substance in extracts of dates palm pollen grain. Harraz *et al*. (2008) isolated estrone and cholesterol from date palm pollen. Others found sterols and flavonoids such as: β-amirin, β-sitosterol, rutin and quercetin, triterpenes and saponins (Mahran *et al.*, 1976). Wojcicki *et al*. (1987) obtained antioxidant and hypolipidemic effects of pollen extracts (cernitins) on male mongrel rabbits and Wister rats.

The present study aimed to investigate the effects of palm pollen grains as a feed supplement on growth performance and sex reversal ratio in Nile tilapia fry.

MATERIALS AND METHODS

The present study was carried out at a commercial hatchery (Fayoum Company for Development) at Wadi El-Rayan region during the period from 1 may to 1 September 2008 to evaluate the effect of palm pollen grains on sex reversal ratio of Nile tilapia fry. The first 28 days were used as a sex reversal period and the rest of the period was used for identifying sex at the end of the period.

Fry stocking: Nile tilapia, *Oreochromis niloticus*, fry after yolk sac absorption were stocked in hapas. The hapas were suspended from a wooden pier in a 0.25-feddan pond with a maximum depth of 1.2 m . The hapas measured 2.0 x 1.0 x 0.8 m , length x width x water column . Fry were distributed randomly in eighteen hapas (three hapas / each treatment) forming six dietary treatments. Fry were stocked into hapas at 1000 fry/hapa.

***Daily ration*:** Feed was prepared using herring fish meal mixed with wheat flour by the same quantity of each of them with the addition of 0.0, 0.5, 1.0, 2.0, 5.0 and 12.5 % from palm pollen grains in the expense of wheat flour forming six treatments. The first diet contained 80 mg 17-α methyl testosterone / 1kg diet.

Table (1): Formulation of the tested diets used in the study.

Item	Palm pollen concentration%					
	Control	0.5	1.0	2.0	5.0	12.5
Ingredients, % :						
Fish meal herring	50	50	50	50	50	50
Wheat flower	50	49.50	49.00	48.00	45.00	37.50
Palm pollen	0.0	0.50	1.0	2.0	5.0	12.50
Chemical composition , DM basis :						
DM%	89.75	89.79	89.82	89.9	90.12	90.67
CP , %.	41.9	42.02	42.13	42.37	43.07	44.81
EE , %.	5.90	5.92	5.95	5.99	6.13	6.48
CF , %.	0.55	0.55	0.54	0.54	0.52	0.46
Ash, %	5.6	5.67	5.74	5.87	6.28	7.3
NFE, %.	35.8	35.63	35.47	35.13	34.13	31.62
GE, M Cal/ kg	4.331	4.332	4.334	4.337	4.346	4.368
CP/GE, mg/kCal	96.7	97.0	97.2	97.7	99.1	102.6

Control contains 80 mg 17-α methyl testosterone / 1kg diet. Other treatments without such hormone.

Daily feed quantities were weighed and fed four times daily for 28 days (sex reversal period). The feeding rate was adjusted weekly by measuring 25 fish per hapa to the nearest milligram and estimating biomass per hapa. Feeding rates of body weight per day were 20% at the first two weeks and 10% at the second two weeks,at sex reversal period.

***Harvesting* :** After 28 days of tested treatment, fry were harvested and weighed to the nearest 0.1 g.. Five hundred fry were returned to the hapas and grown to a size of about 30 g (1 September 2008). After that the fingerlings were harvested and sexed manually by hand.

Feed Chemical analysis: Feeds chemical analyses were conducted after AOAC (2000).

Statistical analysis: Analysis of variance and Duncan's New Multiple Range test (SPSS,1997) were used to compare treatment means.

RESULTS AND DISCUSSIONS

Palm pollen grains (PPG) contained 6.14, 35.1, 6.4, 0.4 , 14.3 and 38.06 % moister, CP, EE, CF , Ash and NFE respectively. The corresponding values for wheat flour are 13.5, 11.8, 1.8 , 1.1, 0.7 and 51.1% for moister, CP, EE, CF , Ash and NFE respectively. The mean differences that PPG contained higher CP, EE, and Ash with lower NFE than wheat flower. Therefore, the diets in Table 1 tended to show increases in CP, EE, and Ash with a decrease in NFE as

the level of PPG increased in the diet. In the present study all diets were acceptable well by fry.

The effect of using PPG on growth performance of Nile tilapia fry is presented in Table 2. The presence of PPG in the diet improved fry growth performance (final weight, total gain & SGR%/d). Also as PPG level increases in the diet as growth performance increased. Accordingly the best growth performance was with the diet contained 12.5% PPG. These results may due to the increase in CP, EE and ash contents with the decrease in NFE content.

Table (2): Effect of using palm pollen grain on growth performance of Nile tilapia fry.

Items	Palm pollen concentration%						SED
	Control	0.5	1.0	2.0	5.0	12.5	
Initial weight, mg	10	10	10	10	10	10	--
Final weight, mg	214^{e}	235^{d}	241^{d}	292^{c}	312^{b}	325^{a}	6.0
Total gain, mg	204^{e}	225^{d}	231^{d}	282^{c}	302^{b}	315^{a}	6.0
SGR %	10.21^{d}	10.52^{c}	10.61^{c}	11.25^{b}	11.47^{a}	11.60^{a}	0.10

Averages (a , b , …..) in the same row having different superscripts differ significantly , $p \leq .05$).

SGR%/d = 100(ln Wf – ln Wi) / period in days, where ln is the natural log, Wf is final weight , Wi is initial weight.

NRC (1993) reported that fish utilize protein and fat more than carbohydrates. Also Farag *et al*. (2008) demonstrated that palm pollens contained high amount of all essential amino acids. Besides, Mahmoud (2008) reviewed other effects for pollen grains in general such as antimicrobial, immunostimulatimg, antitumer activities. Earlier Wojcicki *et al*. (1987) obtained antioxidant effects of pollen extracts. Such findings may interpret the obtained results in Table 2.

Table 3 shows feed intake, feed conversion ratio (FCR), survival rate and feed cost/ 1000 fry as affected by palm pollen grains (PPG) inclusion level in the diet. As evident the presence of PPG improved FCR, survival rate and feed cost/ 1000 fry. Moreover as the level of PPG increased as FCR and survival rate improved but feed cost/ 1000 fry increased. However, the increase in feed cost/ 1000 fry was still lower than the control (MT diet). Such trend was expected as mentioned with growth performance data. Moreover, the increase in survival rate was due the increase in the viability due to the increase in PPG.

Table (3): Effect of using palm pollen grain on feed utilization of Nile tilapia fry.

Items	Palm pollen concentration%					
	Control	0.5	1.0	2.0	5.0	12.5
Feed intake, g	300	300	300	300	300	300
FCR	1.47	1.33	1.30	1.06	0.99	0.95
Survival rate%	78.70	78.78	79.3	79.5	80.30	80.52
Feed cost/1000 fry	223.5	129.4	131.3	135.15	146.6	165.7

FCR = feed intake/gain.

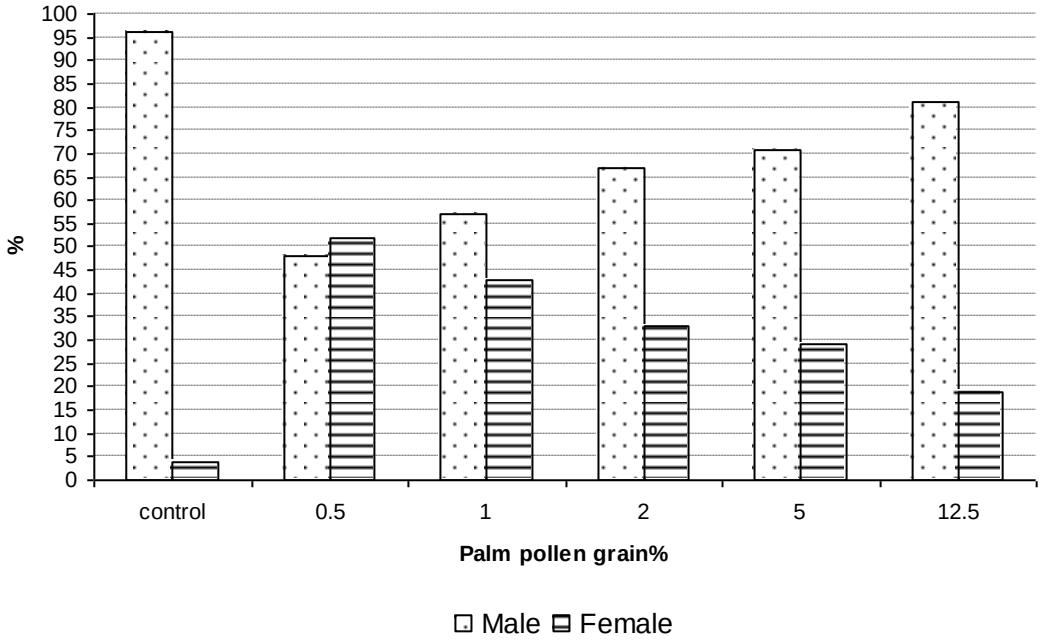
Table 4 and Fig 1 indicate the effect of the tested treatments on male and female percentages after sexing (30 g fry). Significant higher ratio of males was observed with the control (MT diet). But with PPG diets male percentages tended to increase up to the higher level tested (12.5 % PPG) to about 81% , with lower percentage than the control (96% males). In this regard, Nemetallah *et al*., 2008b found that, the percentage of male fry in their treatments were 95.65, 76.28, 84.94, 59.52, 71.37, 78.41, 90.47 and 78.27 % for the treatment of 17-α methyl testosterone, sustanon, sustanon with nolvadex, testicular extract (TE), TE with nolvadex, fecal extract (FE), FE with nolvadex, and nolvadex, respectively. They found with natural compounds lower males than obtained in the present study. This may suggest increasing the level of PPG since it is well known as folk medicine.

Table 4. The percentage of male and female

Sex %	Palm pollen concentration%						SED
	Control	0.5	1.0	2.0	5.0	12.5	
Male %	96[a]	48[f]	57[e]	67[d]	71[c]	81[b]	0.816
Female %	4.0[f]	52[a]	43[b]	33[c]	29[d]	19[e]	0.816

Averages (a , b , …..) in the same row having different superscripts differ significantly , p ≤ .05).

Fig. 1 Effect of palm pollen grains on sex reversal ratio in Nile tilapia.



These results may be due to that palm pollen grains contains quercetin which play active role in sex reversal as reported by Taepongsorat *et al.* (2008) who indicate that quercetin might indirectly affect sperm quality through the stimulation of the sex organs, both at the cellular and organ levels, depending on the dose and the duration of treatment. Moreover Kassen *et al*. 2000 reported that beta-sitosterol, are useful for male hormone balance and prostate health and it can inhibit the function of the

enzyme 5 alpha-reductase, which works to convert testosterone to a metabolite known as 5 hydroxytestosterone. By inhibiting the 5 alpha-reductase enzyme, beta sitosterol helps to maintain testosterone levels and prevent loss to testosterone metabolites that contribute to negative prostate health.

In conclusion it seems that the use of PPG at level of 12.5 % in the diet for Nile tilapia sex reversal is accepted to avoid hormonal treatments hazards as it is a natural source beside the higher survival rate and price/ 1000 fry compared to the control (MT treatment). However further investigation is needed to increase the level of PPG in the diet aiming to increase male percentage.

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نظام للتحول الجنسى لزريعة البلطى النيلى عن طريق اضافة حبوب طلع بالنخيل

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تم توزيع 18000 يرقة بلطى نيلى فى 18 هابة موضوعة فى حوض ترابى بمفرخ تجارى فى محافظة الفيوم . ومثلت المعاملة فى ثلاثة هابات ، وتتكون هذه المعاملات من مسحوق السمك هرنج مع دقيق القمح اساسا ، وتحتوى عليقة الكنترول على هرمون 17 الفا ميثيل تستوستيرون (80 ملج/ كجم علف) فى المعاملة الاولى. احتوت باقى العلائق على بودرة طلع النخيل بمستويات 0,5 ، 1,0، 2,0 ، 5,0 ، 12,5 % بدون اضافة هرمون ، وتم احلال بودرة طلع النخيل محل دقيق القمح . وغذيت اليرقات بعد امتصاص كيس المح على هذه العلائق لمدة 28 يوم (فترة التحول الجنسى) ثم غذيت الزريعة بعد فترة التحول الجنسى على عليقة تجارية (30% بروتين خام) حتى وزن 30 جم وذلك لتمييز الجنس . تم الحصول على مظاهر نمو الزريعة ، معدل التحول الغذائى ، نسبة التحول الجنسى.

اظهرت النتائج ان معدل النمو النوعى للزريعة كان الافضل مع طلع النخيل بمستويات 5,0 و 12,5 %، وكذلك معدل التحول الغذائى والاعاشة ، وزادت تكلفة انتاج 1000 يرقة بزيادة مستويات طلع النخيل وان كانت اقل من عليقة الكنترول. وكان التحول الجنسى افضل مع عليقة الكنترول التى احتوت الهرمون (96% ذكور) ، وانتجت العليقة التى احتوت 12,5 % طلع نخيل 81% ذكور مع تحسن 12,5 % الاعاشة وتكلفة انتاج 1000 زريعة عن الكنترول.

ويبدو ان استخدام بودرة طلع النخيل بمستوى 12,5 % فى العليقة ادى الى تحول جنسى مقبول لتفادى الآثار الضارة للمعاملة الهرمونية بكونه مصدر طبيعى بجانب ارتفاع معدل الاعاشة وانخفاض تكلفة انتاج الف زريعة مقارنة بالكنترول والتى تحتوى الهرمون.