Effect of dose level of three hormones on egg production and thermal accumulated period during induced spawning of grass carp (*Ctenopharyngodon idella*)

Salman N.A.[1]; Al-Noor S.S.[2]; Khalaf K.A.[2]

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Abstract

Three present investigations aimed at determining the optimum doses of three hormones used for induced spawning of Grass Carp. PG extract, HCG, and Ovaprim Preparation). Egg production by females and acceleration of thermal accumulation period (TAP) are the two parameters used for evaluation. Results showed that the PG dose of 4.5 mg/kg was the best as it produced 1100 - 1500 g of eggs in fish of 4.5-6.75 kg in weight. The lowest TAP value (240°C.h) was recorded upon the use of 4 and 4.5 mg/kg doses of PG. Doses of HCG between 800–900 IU/kg produced 100-140 g of eggs form fish of 3.8-4.5 kg. TAP recorded for HCG hormone varied extensively between 265-453°C.h at the dose 900 IU/kg. Higher doses (1100 and 1200 IU/kg) produced similar TAP values (300-362°C.h). Response of grass carp females to HCG treatment was not promising despite changing the dose or incubation temperature. Positive response was recorded in grass carp females receiving doses of 0.4 and 0.5 mL/kg ovaprim preparation leading to the production of 100- 750 g of eggs weight. The best dose which produces the shortest TAP (297°C. h) and the shortest ovulation time (11 h) at 27°C was 0.4 mL/kg. The other dose 0.5 mL/kg also gave close values of (300-363°C. h) at incubation temperature of 24-25°C and ovulation time between 12 and 14.5 h. Relationship between Dose and egg production were calculated as: Egg wt.=377.04-53.89 ×Dose level (PG), Egg wt.=32.796–0.255×Dose level (HCG), Egg wt.=-81.90+270.59×Dose level (Ovaprim). Regression polynomial equations for relationships between the three parameters were calculated as: TAP(°C.h)= 282.1457+9.4933 Dose-0.1847 Egg wt. for PG, TAP (°C.h) = 256.4199+0.0744 Dose+ 1.2113 Egg wt. for HCG, TAP (°C.h)=142.3494+384.1948 Dose-0.1372 Egg wt. for Ovaprim .

Keywords: Hormone dose, Egg production, Thermal period, Grass carp

1- Al-Manara College for Medical Sciences, Maysan, Iraq

2- College of Agriculture, University of Basrah, Iraq

*Corresponding author's Email: naderabed @uomanara.edu.iq

Introduction

The success of many commercial aquaculture production programs is dependent upon success of fish spawning (Gupta and Gupta, 2006). Many years ago, fish farmers and scientists have been using hormone preparations for the artificial propagation of carps and other fish species for commercial and scientific purposes (Farag *et al*., 2017). Induced spawning has been one of the difficulties facing aquaculture of Cyprinid fish in many developing countries, because of necessities of hormonal stimulation for the reproduction under hatchery conditions (Żarski *et al*., 2009). Carp pituitary injection is one of the first important and preferred methods for inducing ovulation in fish and ensures a practical incubation time. The pituitary gland produces and stores gonadotrophin hormones (Gth), which play a decisive role in ovulationand spermiation. Injected pituitary material by passes the brain-pituitary link, acting directly on the ovaries and testes, providing the surge in blood (Gth) levelsthat normally precedes spawning (Brzuska and Bialowas, 2002). Chaudhuri, (2011) discussed attempts are being made to replace it by purified gonadotropins from piscine pituitary glands. Recent work on the purification of salmon and carp gonadotropin marks the beginning towards achievement of success in the field**.** Different ovaprim forms and their analogues are known to stimulate endogenous Gth release, are used with a dopamine receptor antagonist (DA) (Szabó *et al.,* 2014). Human chorionic gonadotrophin (HCG) is another common purified gonadotropic hormone used for induced spawning The induced breeding of carp was successfully carried out using LHRH-a by Jha' and Neupan (2019). The rate of breeding especially feitilization and hatching rate with LHRH-a was found higher than with the pituitary extracts. In the study of El-Gamal *et al*. (2019), the best dose level that give higher number of fertilized and hatching eggs were 5mg/kg of body weight of CPH, following 9 hours with 1500IU HCG per kg of body weight. No significance differences occurred in fertilized and hatching rates between HCG and CPH of treatment groups. According to Hu *et al*., (2020) optimal strategy for exogenous hormones administration could be acheived by using a mixture of LHRH/CPE/DOM to improve the rates of spawning success, weight of ovulated eggs and survival rate after spawning.

The aim of the present study is to determine the best dose of three hormones (PG extract, ovaprim and HCG) used for the induced spawning of grass carp in terms of egg production and best thermal accumulation period.

Materials and methods

Thirty-four Grass carp brood stock ranging in length between 35-78.5 cm, were obtained from private hatchery in Basra and kept in incubation tanks for 12 hours before hormonal injection with Pituitary gland extract PG (4.0, 4.5, 5.0 and 6.0 mg/kg), Human Chorionic Gonadotropin HCG (700, 800, 900,

1100 and 1200 IU/kg) and Ovaprim hormone preparation (0.4, 0.5 and 0.6 mg/kg). Doses were accurately calculated according to fish weight. Intra-muscular injection was done under the dorsal fin, Fish were returned to the tanks for adaptation. Eight hours after injection, fish were examined on hour basis to insure full maturation and ready status for egg stripping. Stripping was done by hand for egg collection. Eggs were weighed and then fertilized with the collected semen as soon as possible.

For calculating the thermal accumulation period TAP as C°.hr, fish were kept at different constant temperature as follows: 23, 24, 24.5, 25, 25.5, 26, 26.5, 27C°. Egg collection time after injection was also calculated. TAP values were then calculated by multiplying time of collection in hours by the incubation temperature.

Results

Effect of Hormone Doses on Egg Production (PG Extract)

Data of Table 1 showed the relationship between various doses of PG extract on egg production in female grass carp. It can be seen that the dose of 4.5 mg/kg was the best as it produced 1100 - 1500 g of eggs in fish of 4.5-6.75 kg in weight. Fish of less weight (3.5 – 4.0 kg) produced 250 -800 g of eggs. The dose of 4 mg/kg produced a smaller number of eggs (500-1000 g) in large sized fish. These data are comparatively different (p<0.05) from those obtained by using higher doses which produced 200 – 475 g for the 5 mg/kg dose and only 50 g of eggs for the 6 mg/kg dose (Table 1).

Table 1: Data of using various doses of PG extract for induce spawning of grass carp.

Dose (mg/kg)	Fish wt. (Kg)	Egg wt. (g)	Incubation Temp. (°C)	TAP (°C.h)	Ovulation time (h)
4.0	3.2	610	24.5	329	16
4.0	6.7	1000	24	240	10
4.0	6.7	1000	24	240	10
4.0	6.5	950	24	240	10
4.0	6.0	900	24	240	10
4.0	6.0	900	24	240	10
4.0	5.0	750	24	240	10
4.0	4.5	650	24	240	10
4.0	4.0	500	24	252	10.5
4.5	6.7	1500	24	240	10
4.5	5.5	1400	24	240	10
4.5	4.5	1100	24	240	10
4.5	4.0	800	24	240	10
4.5	3.7	600	24	240	10
4.5	3.5	550	24	240	10
4.5	3.5	250	24	240	10
5.0	5.0	200	27	297	11
5.0	5.5	475	27	297	11
6.0	4	50	26.5	371	14

HCG Hormone

Responses of grass carp females varied according to the treated HCG dose. Injecting a dose of 700 IU/kg produced 0-25 g of eggs. With increasing the dose to 800–900 IU/kg improved egg production to 100 g of eggs. Doses above 1000 IU/kg again produced little or no response in grass carp females, with fewer number of eggs (0-20 g of eggs). Differences in egg production between HCG doses were significant (p<0.05) with superiority for doses ranging from 800- 900 IU/kg (Table 2).

Table 2: Data of using various doses of HCG for induce spawning of grass carp.

Dose (IU/kg)	Fish wt. (Kg)	Egg wt. (g)	Incubation Temp. (°C)	TAP (°C.h)	Ovulation time (h)
700	4.250	-	-	-	-
700	4.250	-	-	-	-
800	4.500	-	26.5	344	13
800	4.250	100	26.5	344	13
900	4.000	140	24.5	453	18
900	3.800	100	26.5	265	10
900	4.500	100	26.5	318	12
1100	5.500	10	25	362	14
1100	5.000	10	25	362	14
1100	7.000	20	24	312	13
1100	4.000	10	24	312	13
1200	5.250	20	25	362	14
1200	6.750	20	24	423	18
1200	4.200	20	24	312	13
1200	5.500	20	25	300	12

Ovaprim

Positive responses were recorded in grass carp females receiving 0.4 and 0.5 mL/kg ovaprim hormone leading to the production of 100-750 g of eggs weight. Higher doses (0.6 ml/kg) produced fewer number of eggs (150 g) (Table 3). Differences were not significant between ovaprim hormone doses (p>0.05).

Table 3: Data of using various doses of Ovaprim for induce spawning of grass carp.

Dose (mL/kg)	Fish wt. (Kg)	Egg wt. (g)	Incubation Temp. (°C)	TAP (°C.h)	Ovulation time (h)
0.4	4.5	100	27	297	11
0.5	2.0	-	24	312	13
0.5	5.7	220	24	312	13
0.5	4.7	200	25	363	14
0.5	4.2	750	25	300	12
0.5	3.7	300	24.5	331	13
0.6	3.0	150	26.5	371	14

Comparison between hormones

Grass carp females showed higher response to treatment with PG extract in terms of egg production (610-1500 g) more than Ovaprim (100-750) and HCG (20-140 g) treatments. Differences in

egg production between various hormones were significant (p<0.05). HCG hormone produced the lowest response in stimulating egg production in the females of grass carp.

Effect of Doses on Thermal Accumulated Period (TAP)

PG Extract

As seen in Table 1, values of TAP varied according to PG doses and incubation temperatures. The lowest Tap value (240°C. h) was recorded upon the use of 4 and 4.5 mg/kg doses and the highest (297 and 371°C. h) was recorded at 5 and 6 mg/kg doses respectively. It was noticed that TAP values for the same dose may occurred due to different incubation temperature which affect the ovulation time. At dose 4 mg/kg, a difference of 0.5°C in incubation temperature (from 24 to 24.5°C) caused a delay in ovulation time to 16 h instead of the 10 h period recorded for the same dose at 24°C (Table 1). Differences in TAP between all doses were significant (p<0.05), but differences between 4 and 4.5 mg/kg doses were not significant (p>0.05).

HCG Hormone

Thermal accumulated period recorded for the use of HCG hormone varied extensively between 265-453°C.h at the dose 900 IU/kg. For higher doses (1100 and 1200 IU/kg) the TAP period s was more closure ranging between 300-362°C.h (Table 2). Differences in TAP values among various doses, however, was not significant (p>0.05). Impact of incubation temperature which ranged between 24–26.5°C, however, was noticeable on ovulation period and furthermore on TAP values. Apparently increasing incubation temperature, even slightly within the optimum limits, has an effect on ovulation time within the same dose. As seen in Table 2, the response of grass carp females to HCG treatment was not promising despite changing the dose or incubation temperature.

Ovaprim

Table 3 summarized the effect of ovaprim doses (0.4–0.6 mL/kg) on thermal accumulated period TAP in grass carp. The best dose which produces the shortest TAP (297°C. h) and the shortest ovulation time (11 h) at 27°C was 0.4 ml/kg. The other dose 0.5 ml/kg also gave close values of (300-363°C. h) at incubation temperature of 24-25°C and ovulation time between 12 and 14.5 h. Differences between these two doses are not significant (p>0.05). The shortest ovulation time coincides with the warmer temperature within the optimum limits.

Interaction between foses, egg production and TAP

Figures 1, 2, and 3, explained the three imensional interaction between the three studied parameters (Hormone dose, Egg production and Thermal accumulated period) for PG extract, HCG and ovaprim inducers of grass carp ovulation respectively. For PG extract the dose 4 mg/kg seemed to be the best in terms of

egg produced and short TAP. The response to the other dose (6 mg/kg), however, was not suitable because of the lower egg production and relatively long thermal accumulated period (Fig. 1). Upon the usage of HCG hormone grass carp females did not show positive response to the doses of 700.1100 and 1200 IU/kg as they produce little or no eggs at suitable TAP. The best response was recorded upon using the dose 900 IU/kg in terms of producing 100-140 g of eggs although TAP was relatively long (Fig. 2). Ovaprim inducer produced the best response at 0.5 ml/kg by giving the highest egg weight (175 g) and the shortest TAP (<300°C.h). Doses> 0.5 or <0.45 mL/k gave lower responses in grass carp females then the dose 0.5 mL/kg (Fig. 3).

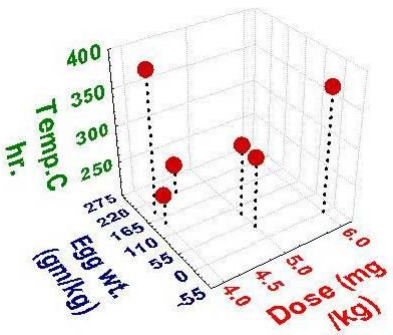

Figure 1: 3D relationship between PG extract dose, egg weight and thermal accumulated period in grass carp.

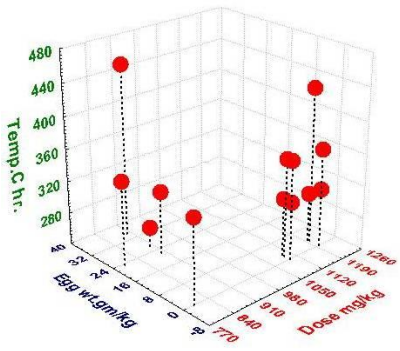

Figure 2: 3D relationship between HCG hormone doses, egg weight and thermal accumulated period in grass carp.

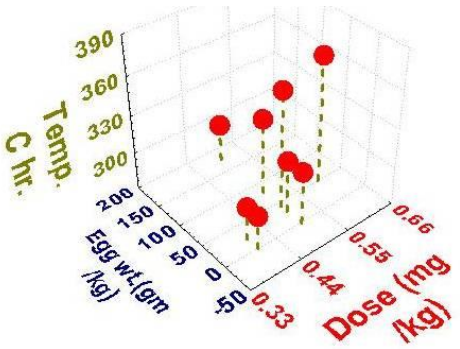

Figure 3: 3D relationship between Ovaprim doses, egg weight and thermal accumulated period in grass carp.

The polynomial regression equations

Regression equations for the relationship that correlate the three parameters (Thermal accumulated period, Egg production and the Hormonal doses) were calculated as follows:

For PG extract: TAP (°C.h) = 282.1457 + 9.4933 Dose - 0.1847 Egg weight

For HCG hormone: TAP (°C.h)= 256.4199 + 0.0744 Dose + 1.2113 Egg weight

For Ovaprime: TAP (°C. h) = 142.3494 + 384.1948 Dose - 0.1372 Egg weight

The three-dimensional graphic representations for these relationships are shown in Figure 4. for the three inducers (PG, HCG and ovaprim).

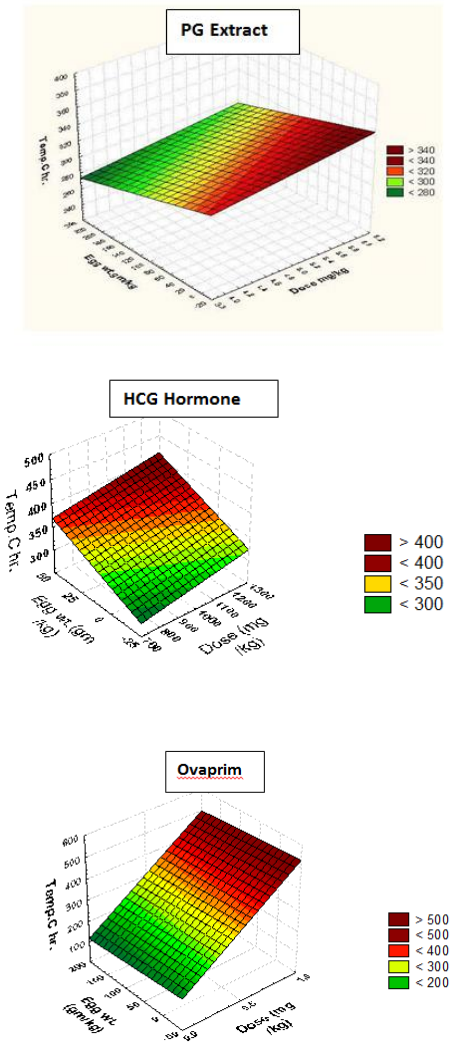

Figure 4: The3D graphic representations for the relationship between TAP and egg production with the doses of PG, HCG and ovaprim used for induce ovulation in grass carp.

Statistical relationship between dose and egg production

To study the statistical relationship between the dose level and egg weight in grass carp treated with PG Extract the following equation was calculated to describe the regression line:

Egg weight=377.04-53.89×Dose level

Figure 5 shows the graphic representation of the regression line along with the confidence limits which represent the enclosing and dispensing points from the regression line. The lowest value of confidence limit was at 4.5 mg/kg dose level and the highest was at 6.0 mg/kg dose level.

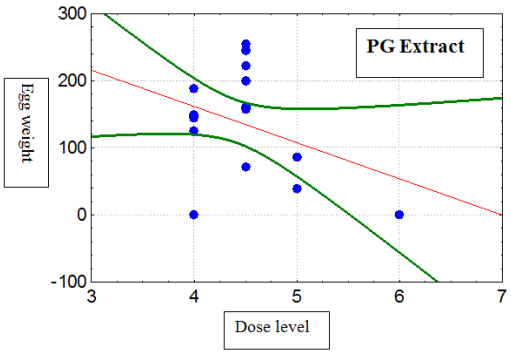

Figure 5: Regression line for the relationship between egg weight and the dose level of PG extract used for induce ovulation in grass carp.

Relationship between egg weight and dose level for HCG hormone was represented by a regression line with the following equation:

Egg weight = 32.796 – 0.255 x Dose level

The lowest confident limits were found at the dose level 900 IU/kg and the highest at dose level of 700 IU/kg (Fig. 6). They represent the enclosing and dispensing points from the regression line.

For ovaprim the relationship was represented by the equation:

Egg weight= -81.90+270.59×Dose level

The dose level 0.5 ml/kg was the best dose with the lowest confident limits which represent the enclosing point, while the highest confident limits values which represent the dispensing points were distributed among lower and higher dose levels from the 0.5 mL/kg dose (Fig. 6).

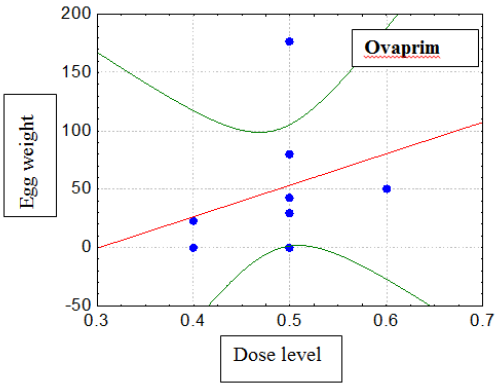

Figure 6: Regression line for the relationship between egg weight and the dose level of Ovaprim used for induce ovulation in grass carp.

Discussion

Effect of hormone doses on egg production

Results of the present investigation revealed the superiority of PG extract at 4.5 mg/kg in terms of egg production (1500 g) over the other two doses such as 3.5 and 6.0 mg/kg which produced negative response. Ovaprim at 0.5 ml/kg produced only 750 g which is significantly higher than egg production by the other two doses (0.6 and 0.4 mL/g). Fish response to HCG hormone was weak, the highest number of eggs was only 140 g at 900 IU/kg and lower than that at other doses. The present results are a follow up for our previous findings (Salman *et al*., 2023) which showed noticeable variation in egg production in fish receiving various hormones with clear superiority for fish injected with PG-extract compared with those receiving HCG and Ovaprim. The PG extract results are comparable to recent studies on grass carp such as that of Szabó *et al*., (2023) which investigate the efficacy of an extremely long-stored batch of pituitary gland. The results are also in agreement with those obtained by severalstudies (Żarski *et al*., 2009; El-Hawarry *et al*., 2012; Szabó *et al*., 2014) who reported successful spawning of silver carp using CPE, HCG or LHRH analogues (buserelin) with or without dopamine antagonist, as indicated by the breeding response. The increase in the ovulation of the common carp and grass carp may be due to that the oogenesis is controlled by follicle stimulating hormone (FSH) and luteinizing hormone (LH) but need also participation of several paracrineautocrine mechanisms of regulation as reported by Kouril *et al*. (2003).

According to Jamróz *et al*., (2008), the greatest degree of ovulation synchronization was obtained after 36 h of the application of Ovopel. Tthe best results of controlled reproduction were obtained after using Ovaprim and a combination of Ovopel and Ovaprim . More recently, grass carp and silver carp were successfully spawned with Ovatide (combination of GnRH analogue with dopamine antagonist pimozide) in Kashmir, using injection of single dose of 0.7 and 0.8-0.9 mL/kg body weight for female grass carp and silver carp (Rashid *et al*., 2014). Authors recorded a fecundity of grass carp and silver carp as 70000- 80000 and 1-1.10 lac eggs/kg body wt. of fish respectively. Grass carp (Ctenopharyngodon idella).were spawned successfully following a single dose of injection of ovaprim -C (LH releasing hormone analogue) with 0.6 ml/kg for female and 0.2 mL/kg for male (Naeem *et al*., 2011). Regression analysis was applied to assess the body

weight dependence of absolute fecundity and relative fecundity. Body weight has positive influence on absolute fecundity (r=0.926) and equations were developed to describe these relationships.

Relationship of dose to thermal accumulation period (TAP)

TEP varoied in the present study with hormonal doses and incubation temperatures. TEP with Ovaprim decreased from 331 to 297 h.C with the change of dose from 0.5 to 0.4 mL/kg. With PG extract TEP varied slightly with increasing the dose from 0.4 to 0.6 mg/kg and changing incubation temperature. The same trend was shawn when using HCG, as TEP decreased with increasing dose level. Therefore, the choice of the best lower TEP (240 h.C) occurred with 0.4 mg/kg PG at 24 C and 1200 IU HCG (TEP=300 h.C). With Ovaprim the best TEP (300 h.C) occurred at 0.4 ml/kg. The present results are comparable with other findings which revealed different TEP with different doses. These findings confirmed our previous results using the same hormones (Salman *et al.*, 2023), where direct relationship between the type of hormones and TAP. Fish receiving PG-extract had the shortest TAP which was significantly different than those receiving HCG and Ovaprim treatments. Makeyeva *et al*. (1996) recorded a latency time of more than 20 hrs upon using LHRH-analogue as ovulation stimulator in silver carp. Naeem *et al*., (2005) used Ovaprim on grass carp at 0.6 ml/kg which produced eggs in 11 h at 25 C. Concerning the latency period, all silver carp broodfish beganspawning 7-12hrs after hormones injection with or without dopamineantagonists' injection. Brzuska (1999) pointed out that silver carp females, eggs produced in a short time interval by using LHRH- at temperatures between 20°C and 26°C. Spawning began more than 9 hrs after the LHRH-a and pimozide injection at 20–24°C, an equal latency of 8–12 hrs was recorded for this species at 18–30°C, HCG was also found to increase the speed of eggs maturation in fish which may explain the increase in ovulation activities.

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