Comparative Study of Hormonal Stimulation of HCG, PG-extract and Ovaprim on Thermal Accumulation Period and Egg Production in Grass Carp *Ctenopharyngodon idella*

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Received: February 2022 Accepted: July 2022

Abstract

Three types of hormonal stimulation were tried using HCG, PG extract and Ovaprim to induce egg production and accelerate thermal accumulation period (TAP). Results showed that there is a 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. Values ranged between 258.97, 344.67 and 322.78 (C °-hour) in fish injected with PG extract, HCG and Ovaprim respectively. Results also 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 lowest weight of extracted eggs (7.3 g/kg) was in fish injected with HCG and the highest (139.7 g/kg) in fish receiving the PG hormonal extract.

Keywords: Grass carp, Hormonal-induced spawning, Egg production, Thermal accumulation period.

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Introduction

Grass carp *Ctenopharyngodon Idella* mature in 2-10 years of age depending on the geographical region and temperature. They mature when reaching 50-86 cm in length. The suitable temperature for sexual maturity is between 19-30°C and the optimum is 23°C (Embke *et al*., 2016). Reproduction and artificial propagation of grass carp *Ctenopharyngodon Idella* has been investigated by many researchers (Pankhurst *et al.*, 1996; Naeem *et al.*, 2011; Szabo *et al.*, 2019). Biochemical analysis of grass carp serum hormones was monitored by Metwally and Fouad (2008) after hormonal injection by Ovaprim and HCG. Testosterone, estradiol, and cortisol were affected by the hormone injection.

Fish differ in their response to hormonal induced spawning depending on weight of spawners, actual working fecundity and genetic and environmental factors. In this respect, Metwally and Fouad (2008) stated that in grass carp which gave wide range of egg weight (350-1700 g/kg) due to genetic variations among strains. Egg production per unit of weight varied among females due to dose concentration and number of doses (Rashid *et al*., 2014).

As for the effect of temperature on egg production, Dhillon and Fox (2007) hypothesized that short-term high temperatures associated with temperature fluctuation decreases development time, whereas reduced energy conversion efficiency at higher temperatures does not allow for increased egg production. There were three prominent peaks in egg production, each coinciding with increasing temperatures in Striped, bass *Morone saxatilis* (Secor and Houde, 1995).

Studies that search for the best hormonal stimulation of artificial insemination of fish have always relied upon egg and milt (semen) production as indicators for responses toward that stimulation. Results of such studies may improve the hatcheries technique to fix the appropriate types, doses and incubation temperature. The aim of the present investigation is to find out the best hormonal treatment for egg production and acceleration of thermal accumulation period (TAP).

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 Ovabrim hormone preparation (0.4, 0.5 and 0.6 mg/kg). Doses were accurately calculated according to fish weight. intramuscular 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 were tried as follows: 23, 24, 24.5, 25, 25.5, 26 , 26.5, 27°C. 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

Three types of hormones (Ovaprim, HCG and PG extract) were used to stimulate egg production in grass carp *Ctenophargedon idella* females. Results showed wide variation in the average egg weight produced by grass carp females treated with the above three hormones. The lowest egg weight was produced by females treated with HCG being 7.30 gm/kg and the highest weight of egg (139.6 g/kg) was produced by females treated with PG extract (Table 1 and Fig. 1).

Table 1: Effect of hormonal injection on egg production in grass carp

Hormone type	No. of fish	Average eggs weight (g/kg)
PG	21	139.6 ± 72.9 a
HCG	20	7.3 ± 11.4 b
Ovaprim	8	50.0 ± 57.5 b

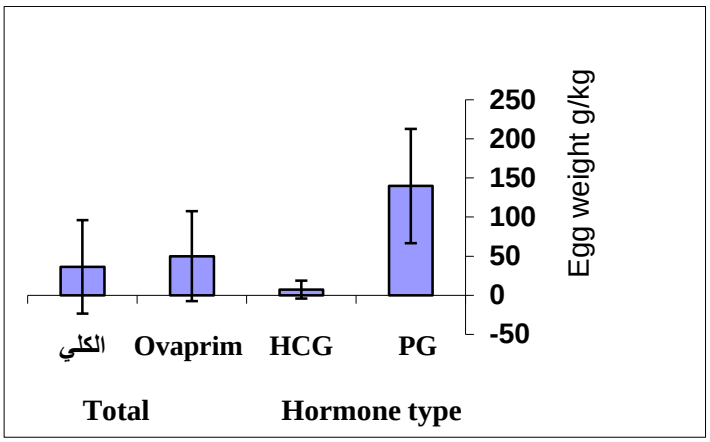
Differences in the average egg weight were significant (p<0.01) between fish treated with PG extract and the other two hormones. HCG and Ovaprim treatments, however, showed insignificant differences (p>0.05). Results of the present study proved the superiority of the PG treatment compared with the other two hormones (HCG, Ovaprim) in terms of its effect on egg production in grass carp.

Data of Figure 2 showed that values of the weight of eggs produced after PG and ovaprim treatments were comparable to the theoretical values of the normal distribution curve, while those produced by HCG treatment have deviated from the theoretical values.

Data of Table 2 and Figure 3 showed the thermal accumulation period (TAP) values (°C-h) which express the period of collecting eggs from grass carp females incubated at a constant temperature after treatment with the three types of hormones. It appeared that the lowest values were recorded in females injected with PG extract and were significantly differ (p<0.01) from those injected with HCG and Ovaprim. Again, no significant difference was noted in TAP values of females injected with HCG and Ovaprim (p>0.05). The recorded values of TAP for PG, HCG and Ovaprim treatments were 258.97, 344.67, 322.78 °C-h respectively. The results clearly showed the effect of hormone type on the period necessary to produce eggs after the last injection at a constant female incubation temperature.

By comparing the actual results of the thermal accumulation period in response to hormonal treatments with

the expected values it appeared that only data of HCG hormone synchronized with the normal distribution curve with peak observations within 300–350°C-h (Figure 4-lower). On contrast, actual values of TAP related to PG extract injection showed no kind of synchronization with the expected distribution as they concentrated at one side between 220 -240°C-h (Figure 4-above). Ovaprim injection however, revealed clear deviation as date were distributed among wide range of TAP values (290-380°C-h (Figure 4-middle).



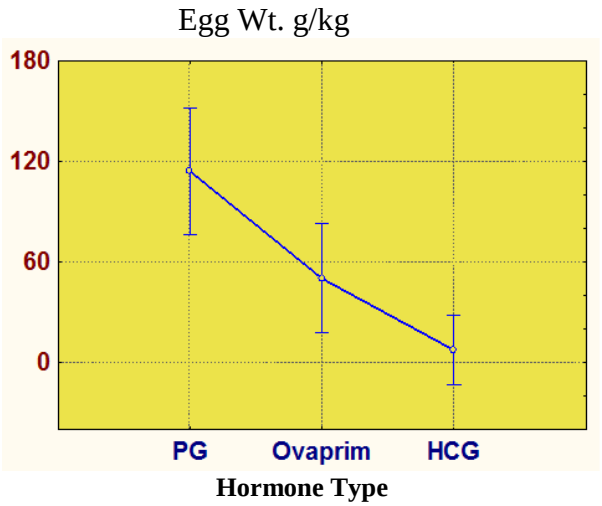


Figure1: Effect of hormone type on eggs weight in grass carp.

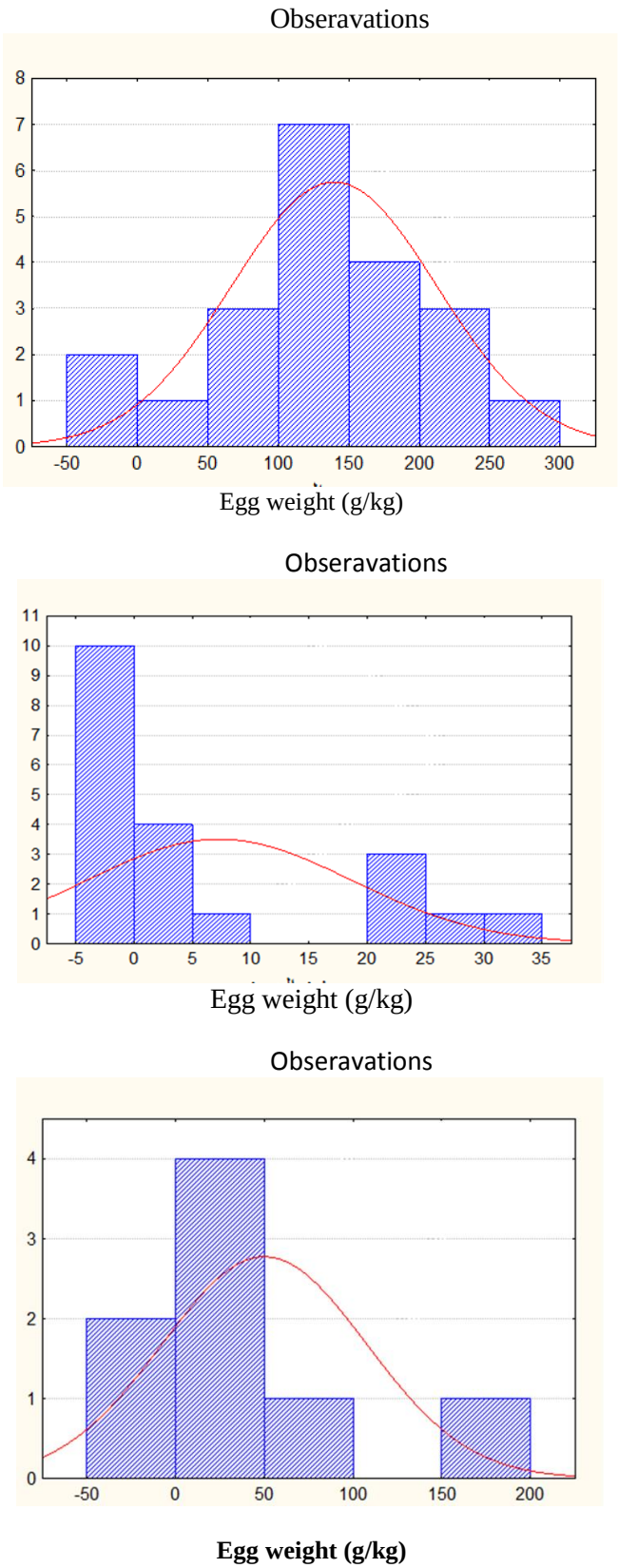


Figure 2: Comparisons of egg production values with the normal distribution curve after hormonal treatment with PG (above), HCG (middle) and Ovaprim (lower).

Table 2: Values of thermal accumulation period after hormonal treatment.

Hormone Type	No. of Fish	Average Thermal Accumulation Period (C °-h)
PG	21	45.66 ±258.9 a
HCG	20	52.14 ±344.6 b
Ovaprim	8	29.39 ±322.7 b

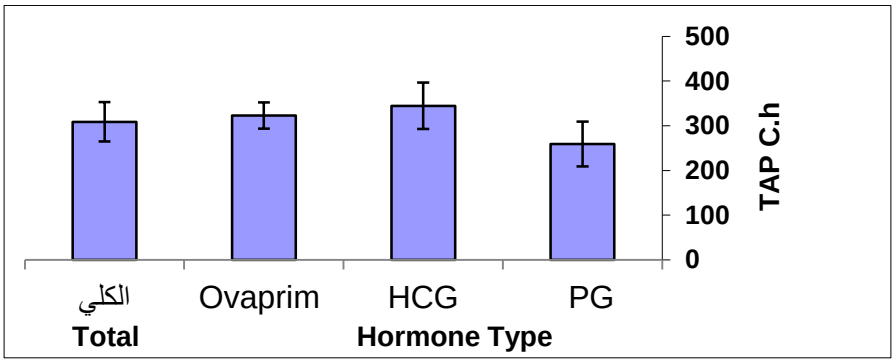
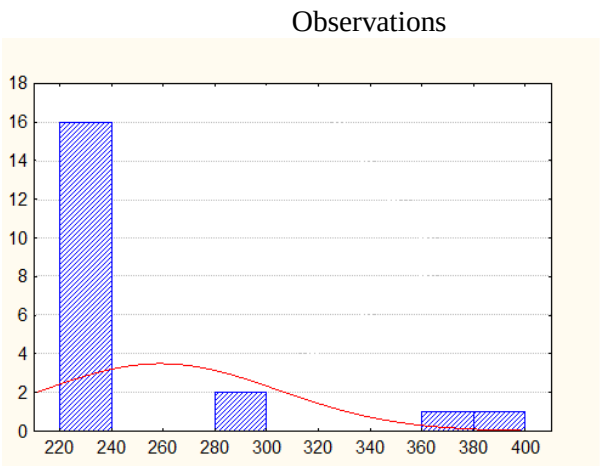
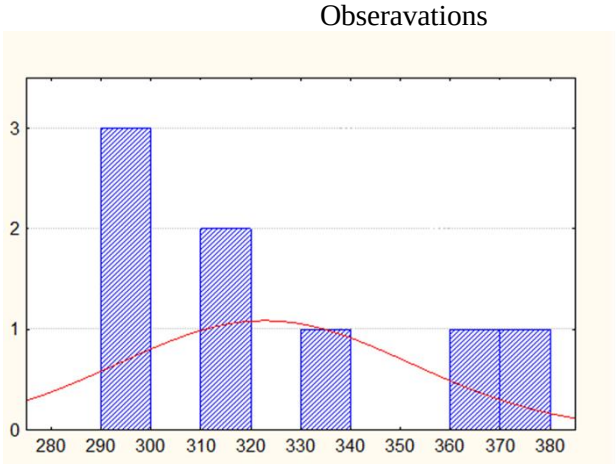


Figure 3: Effect of hormonal treatment on the thermal accumulation period (TAP) in grass carp females





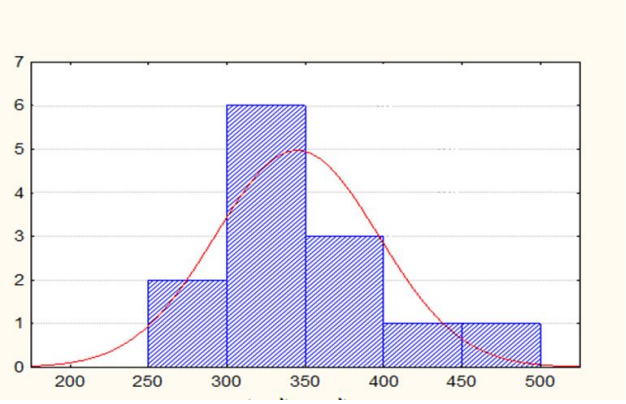


Figure 4: Comparison of actual values of TAP and the assumed values of the normal distribution curve for PG (above), ovaprim (middle) and HCG (lower) treatments.

Discussion

Effect of hormonal treatment on egg production

By comparing the efficiency of the hormones used in the present study to promote egg production in grass carp, it was found that females injected by PG extract produced the highest number of eggs (40 g/kg) followed by Ovaprim and the lowest was in female received HCG (7.3 g/kg). These findings are comparable to other studies which proved PG extract as the best hormonal treatment for egg production in grass carp (Szabo *et al.*, 2019).

Ovaprim is characterized by the presence of Domperidone which is Dopamine inhibitor and hence it could stimulate gonadotropins. It was first used in Iraq by Al-Mukhtar *et al.*, (2004). According to Nandeesha *et al*. (1990) Indian carp receiving PG and Ovaprim may increase their egg production. These observations were supported by the findings of Dhawan and Kaur (2004) who proved the superiority of using Ovaprim and Ovapel together. The Ovaprim treatment was used also by Metwally and Fouad (2008) to increase the gonado-somatic index (GSI) in grass carp.

As for the HCG hormone, the present results are in agreement with previous reports by Peter *et al*. (1988) that using HCG alone is not effective for grass carp unless used in high dose or mixed with PG extract as mentioned by Opuszyhskiok and Shireman (1995). The same authors stated that the activation level by HCG increase in grass carp after 6h then dropped sharply after 12 h due to reduction in steroid hormones. According to Naeem *et al*. (2011), HCG usage as a substitute for PG extract may be effective for certain types of fish species such as silver carp but not for grass carp.

Effect of hormonal treatment on thermal accumulation period

It is well known that pre-spawning thermal regime appears to be an

important environmental factor for normal ovarian development in cultured fish. Previous studies on grass carp reproduction proved that sexual maturity and egg production occur when temperature reached 20°C and the best period ranged between 20-22°C (Embke *et al*., 2016) Grass carp needs 1500-2000°C-day yearly to develop gonadal maturation and egg production at maturity length of 58-67 cm (Chilton and Muoneke, 1992).

In fish hatcheries, ovulation depends upon use of stimulated hormones and incubation temperature. Ovulation period is considered as °C-hr or °C-day after the last dose of hormonal treatment. According to Zonneveld (1983) ovulation in grass carp needs >27°C and around 1500-2000 C°-day in a year. Grass carp incubated at 24-27°C in the present study had a positive response to hormonal stimulation and egg production. Lower incubation temperature reflected negative responses. Similar observations were recorded by Pankhurst *et al*. (1996). In common carp, accumulated thermal values of 1000-2000°C-day were observed (Rothbard and Yaron, 1995) or 1800-2000 °C-day (Horuath, 1986) for both common and grass carp. The two species showed similar thermal requirements.

Results of the present study also showed that TAP is affected by the type of hormone used for stimulation of egg production. The lowest TAP (260°C-h) needed by grass carp females injected with PG extract compared with Ovaprim (322 °C-h) and HCG (345°C-h). The present findings are comparable with those obtained by Dorafshan *et al*. (2003) on common carp stimulated by PG extract or GnRH. Contradictory results, however, were recorded by Brzaska (1999) who used LH-RH and stated that the response of grass carp to both PG and GnRH was similar.

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