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STIMULATION SPAWNING OF COMMON CARP, GRASS CARP AND SILVER CARP BY CARP PITUITARY EXTRACT, HUMAN CHORIONIC GONADOTROPHIN, RECEPTAL AND OVAPRIM HORMONES FOR COMMERCIAL PURPOSES

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Abstract

One of the most important problems in commercial cyprinid aquaculture is difficulty of obtaining good quality gametes. For this purpose, hormonal treatment is used for stimulating of gametes maturation in commercial-cyprinid production. In the first week of April, common carp were spawned. Each tank contained 5 females and 5 males of common carp (*Cyprinus carpio).* In the second week of April, 4 females and males of silver carp (*Hypophthalmichthys molitrix*) were spawned and in the third week of April, grass carp (*Ctenopharyngodon idella*), 4 females and 4 males were spawned. Fish were divided into five trial groups for treatments. First group (G1) was induced by carp pituitary extract (CPE), second group (G2) was injected with mixture of human chorionic gonadotrophin (HCG) and (CPE), third group (G3) was injected with (HCG) alone, forth group (G4) was injected with Ovaprim hormone (OVP) and fifth group (G5) that was injected with (LH-RH) luteinizing hormone-releasing hormone analogues "Receptal" Buserelin Acetate (BA). The results showed that the hours of latency time in common carp were relatively shorter than both of grass and silver carp for all treated groups while, in silver carp the latency time was the longest. The hatching rate percentages of G1 showed the highest ratios; 50, 49 and 48% followed by G5 in common carp, grass carp and silver carp, respectively. The weight of eggs showed significance differences ($P<0.05$) among the five treatments. The highest masses of eggs were presented in G1, G4 and G5 and the lowest masses of eggs were presented in G3 for common, grass and silver carp, respectively.

However, a total cost was higher in G4 and G5 than G2 and G3 while, the lowest cost was found in G1 in common, grass and silver carp. Regarding the economic efficiency, CPE showed higher value followed by receptal for all carp species and common carp showed the highest value for carp pituitary extract.

Keywords: Stimulation spawning, Carp, Reproductive performances, Carp pituitary extract, Human chorionic gonadotrophin, Receptal (buserelin acetate), Ovaprim hormone.

INTRODUCTION

Carp is one of a few species of freshwater fish that can be considered as domesticated, but there is a considerable difference between the domesticated population and its wild relation with respect to reproduction capacity, growth, utilization of feeds, etc. The wild population is covered with scales and grows slowly while, the domesticated populations of both scaly and mirror varieties utilize artificially fed cereals and natural food well, giving rapid growth. Carps are highly resistant to handling, during harvesting, grading, transportation, etc., and to changes in water temperature and oxygen levels. The palatability of carp is high, and the fish still enjoys considerable demand in the marketplace in most east European countries, in near and far East Asia (Horváth *et al.*, 2002). 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. Usually referred to fish injection with crude fish pituitary glands to induction of ovulation in term "hypophysation". In practice, acetone-dried common carp (*Cyprinus carpio*) pituitary gland is the most commonly used agent to induce ovulation, which contains the active hormone (gonadotrophin), is collected from mixed populations of marketable carp in temperate climate (Brzuska, 2004; Szabó *et al*., 2014 and Horváth *et al.*, 2015). Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances. The

pituitary gland produces and stores gonadotrophin hormones (Gth), which play a decisive role in ovulation and spermiation. Injected pituitary material by passes the brain-pituitary link, acting directly on the ovaries and testes, providing the surge in blood (Gth) levels that normally precedes spawning (Rottmann *et al*., 1992; Brzuska and Bialowas, 2002). Artificial reproduction has been one of the bottlenecks because it has not been possible to reproduce wild cyprinids in hatchery conditions without hormonal stimulation (Żarski *et al.*, 2009). Not only was carp pituitary injection being one of the first important methods of inducing ovulation and spermiation in fish, but also it has stood the test of incubation time and is still the preferred methodology for many fish culturists. In some situations, it has been found to be the most efficient and reliable method of inducing final gamete maturation or spawning. The success many commercial aquaculture production programs are dependent upon its continued availability for use as an aid in spawning fish (Erdahl, 1996). Human chorionic gonadotrophin (HCG) is the most common purified gonadotropic hormone used for induced spawning. The fish injection gonadotrophin hormone, pituitary extracts and purified hormones such as (HCG) plus, mention, acting directly on the ovaries and testes. However, the comparatively large dose and frequent injection were required. The hormones last longer in the fish's system and have potent stimulatory effects on their ovulation and spermatizm (Rottmann *et al*., 1992). The beneficial traits of (HCG) gave the possibility of precise dosing without the necessity of weighing the preparation, a very simple method of preparing and storing injections and the elimination of an additional injection of the dopamine receptor blocker (Adamek, 1995). During the breeding season, Haque *et al*. (1995) injected the females silver carp and the female bighead carp with (HCG) and (CPE) together and found that a mixture of them was better than either (HCG) or (CPE) separately in induction spawning.

On the other hand, the increasing of the cyprinid culture in the world caused the problem in the presenting of calibrated CPE to aquaculturists. This led to the development of a new approach in the inducing of spawning for

cyprinid fish. In this approach, different ovaprim forms and their analogues, stimulating of endogenous Gth release, are used with a dopamine receptor antagonist (DA), which potentate's response to the peptide (Peter *et al.,* 1988). The simple method of treating European catfish females with complex substances (Ovaprim) containing (Brzuska and Adamek, 1999). Ovaprim is one of these substances, satisfactory results have been obtained using Ovaprim as an ovulation stimulator in Carp (Amer *et al*., 2009), in African catfish females (Akar, 2008), in nase fish (Szabó *et al*., 2002) and in Northern pike fish (Szabó, 2003). Dopamine inhibits the release of hormones from the pituitary, effectively blocking the pituitary's positive response to injected (LHRHa) luteinizing hormone releasing hormone analogues (Receptal). There is a family of drugs that act as dopamine blockers, either by preventing the release or by inhibiting the binding of dopamine. Experimental results indicate that the use of dopamine blockers prevents this negative feedback, enhancing the effectiveness of LHRHa for induce spawning (Arabaci *et al.,* 2004).

The objectives of the present study to examine the effects of carp pituitary extract (CPE), human chorionic gonadotrophin (HCG), receptal/ buserelin acetate (BA) and ovaprim hormones (OVP) on induction of spawning in common carp, grass carp and silver carp species at commercial level as a comparative study in respect to the spawning performances.

MATERIALS AND METHODS

The present study was conducted at Abbassa Fish Hatchery, Central Laboratory for Aquaculture Research, Abbassa, Agricultural Research Center, Egypt.

Selection of Carp Spawners:

Selection of 130 healthy, disease free, fully mature ripe fishes aged of 2-4 years were selected; 25 females and 25 males' common carp (1.3-2.1 kg), 20 females and 20 males' grass carp (4.5-6.2 kg), 20 females and 20 males' silver carp (4.4-5.9 kg). On the first days of April, the spawners were caught, after

selection and sexing and transferred to a quarter feddan earthen pond with 1-meter depth. After acclimatization of fish for 24 hrs the water quality was measured twice a day (Table 1). At the spawning season (22-25 ^{o}C), female carp which showed spawning signs were randomly divided and distributed in five tanks of 3.5 m^3 volume filled with filtered Nile water supplied with aerator. All applied females were marked at different places of caudal fin. In the first week of April, common carp were spawned. Each tank contained 5 marked females as well as 5 males' common carp. In the second week of April, 4 marked females and males silver carp were spawned and in the third week of April, grass carp were spawned with 4 marked females 4 males. A sex ratio of 1 male : 1 female was applied.

Table 1. Physico-chemical characteristics of earthen water ponds of carp during climatic period before the experiment.

Items	Mean	Items	Mean
Temperature (^{o}C)	23.5	Nitrate (mg/l)	0.01
pH	8.7	Nitrite (mg/l)	0.02
Oxygen (mg/l)	8.1	Salinity (mg/l)*	0.3

*Water conductivity for salinity was calculated based on the relation (1000 micromhos per centimeter = 0.7g salinity) according to Dewis and Freitas (1970).

Carp pituitary extracts (CPE):

The carp pituitary extracts (CPE) was prepared from the pituitary glands of a live adult carps (1-3kg) collected in the pre-spawning season before the beginning of the experiment. Pituitaries were cleaned and conserved in acetone, and stored at room temperature in the dark bottle; the glands were cleaned in the dark with absolute acetone to remove fat remains, dead cells and any other impurities and then immersed in acetone for 6 hours, then with new acetone for another 6 hours. Cleaned glands were dried using filter paper completely and stored too in a dark bottle at room temperature. To prepare the extract, dry pituitary glands were grinded in a mortar into a fine powder and weighted in normal saline solution (0.7% NaCl) at ratio of 10 ml/1g pituitary powder. The

suspension was centrifuged at speed 3000 rpm then the supernatant was used for fish injection.

Human chorionic gonadotrophin (HCG):

Human chorionic gonadotrophin (HCG) has been found as an alternative for pituitary gland for broodstock injection (5000 I.U/ml) (Organon Company). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are glycoprotein hormones, called gonadotrophins that regulate gonadal functions. HCG is a glyco-protein, because of the carbohydrate molecules attached to the protein molecules. Its primary function is to maintain the production of estrogen and progesterone and can be used for early ripening of gonads. It is produced by the placenta and excreted through the urine during early stages of pregnancy (2-4 months).

Receptal (Buserelin Acetate):

Buserelin acetate (BA), luteinizing hormone-releasing hormone analogues, LH-RH is a potent synthetic analog of gonadotrophin-releasing hormone with D-serine substitution at residue 6, glycine10 deletion, and other modifications. Buserelin acetate is water soluble and readily absorbed after subcutaneous injection and it is marketed by Sanofi-Aventis under the brand name Suprefact. Buserelin binds to and stimulates the pituitary glands' gonadotrophin-releasing hormone receptor (GnRHR). The effects of buserelin on FSH and LH release are 20 to 170 times greater than those of LH-RH. Buserelin has a longer duration of action than natural LH-RH. Also it has compounds which increase the capacity to conceive (fertility and spawning) in females. Each 1ml of Receptal contains 0.004mg buserelin (Luteinizing Hormone-Releasing Hormone Analogue), Intervet Egypt Co., Egypt

Ovaprim hormone (OVP):

Ovaprim is a ready to use product and the solution is stable at ambient temperature. It contains an analogue of 20μg of Salmon gonadotrophin releasing hormone (sGnPHa) and a dopamine antagonsist, domperidone at 10

mg/ml (use at 0.5ml/Kg of fish by injection). The potency of ovaprim is uniform and contains sGnRHa which is known to be 17 times more potent than LH-RH. Ovaprim hormone (10 ml vial) made by Duopharma manufactured by Syndel Laboratories Ltd, V9K 1V5 BC, Canada.

Hormonal Injection:

Broodfish were sedated using MS-222 at a dilution of 1:10000 (1g/100 liter water). Each broodfish of females received one injection in an intraperitoneal (IP) at the base of the pectoral fin. The first group of females (G1) were injected with (CPE) alone at a rate of 3mg/kg common carp, 4 mg/kg grass carp and 5 mg/kg silver carp. The second group of females (G2) were injected with a mixture of (CBE) and human (HCG) at a rate of 1.5 mg+750 IU/kg for common carp, 2 mg+1000 IU/kg for grass carp and 2.5 mg+1250 IU /kg for silver carp. The third group of females (G3) were injected with (HCG) alone at a rate of 1500 IU /kg in common carp, 2000 IU /kg in grass carp and 2500 IU /kg in silver carp. The fourth group of females (G4) was injected with (OVP) at a rate of 0.5 ml /kg fish and the fifth group of females (G5) were injected with receptal (BA) at a rate of 10 microliter/1kg. Each male of the five groups was injected with 1/10 of the dose that was injected to females. Mature broodfish groups were separately incubated in their tanks which supplied with continuous aerated water and water exchange at optimal temperature of 22-25 ^{o}C during the hormonal treatment. Different latency times after treatment (12hrs of incubation) with hormonal preparations were estimated.

Fertilization:

After injection and appearance of signs of complete ovulation (eggs maturation), the males (stimulating factor) were left with the females to mate together and fertilize eggs during their spontaneously release from the mothers. Its meaning, male and female spawn side by side and released eggs are fertilized by males. Just the fertilized eggs to float up to the surface of water collect carefully into a dry plastic dish.

A fresh fertilizing solution; [40g NaCl salt + 30g $CO(NH_2)_2$ Carbamide Urea + 10 liter H_2O]. To completely remove stickiness, 0.2 % fresh tannin solution (0.5g Tannic acid/1 liter of water) was used. Tannin solution should be used when eggs are fully swollen after fertilization. About 1 liter of tannin solution is used to treat 4–5 liters of swollen eggs. As soon as the solution is added, eggs should be quickly and gently stirred for 20-30 second and then diluted with 10 liters of clean, well-aerated hatchery water. After eggs have settled, the diluted tannin solution should be poured off and this process should be repeated 2–3 times in case of common carp because the eggs of common carp are more adhesion than eggs of both grass carp and silver carp. After these procedures, the eggs should be placed into cleaned incubation jars supplied with clean, well-aerated hatchery water. To prevent fungal and bacterial infections of carp eggs, a diluted solution of 10 ml of 36% formalin for 10 liters of hatchery water. Optimal temperature of the fertilizing solution should be 22 - 25°C, pH 8.5 – 9 and dissolved oxygen 5 - 8 mg/l (Table 1). In jars of incubation, a continuous flow from the bottom of the jar is needed. During incubation, the water flow should be adjusted to the actual development stages of the eggs. Both unfertilized eggs (white) and endanger fertilized eggs should be removed through siphoning carefully. Cleaning of the sieve of jars should only be done from outside not from inside. After hatching occur the latency time was calculated.

Spawning Parameters:

a. **Latency time** = the time (hour) between the injection and ovulation.

b. **Total No. Fertilized Eggs** = No. Eggs per g x Mean Female weight (g).

   The number of fertilized eggs of mature female broodfish was estimated based on the average number of eggs of individual fish for each species; (700, 450, and 500 eggs for common carp, grass carp and silver carp, respectively.

c. **Fry production number** = No. hatched eggs (larvae) X Hatching rate (%).

d. Hatching rate (%) = [(Number of hatched eggs (larvae) / Total number of fertilized eggs)] *100

e. Fry production price = Fry production No. (1000 fry) X Current price of fish species (EP).

The one thousand fry is priced in 50 Egyptian pounds (EP) for common carp, 187.5 EP for grass carp and 110 EP for silver carp.

f. Injection Cost (Total) = Injection cost per kg fish X No. Female broodfish X Mean female weight (kg).

g. Return (EP) = Total Fry production price – Total Injection cost.

h. Economic Efficiency = [Money return / Total Injection cost] X 100

Statistical Analysis:

Data analysis was performed using the analysis of variance (ANOVA) one-way classification and Duncan's multiple range test to evaluate the significant differences between treatments means. The standard deviation of treatment means was also estimated. The software Co-Stat program version 6.311Win (Co-Stat, CoHort Software, USA) was performed for Statistical analyses. A probability at level of 0.05 or less was considered significant (Bailey, 1981).

RESULTS AND DISCUSSION

Common carp:

The reproductive performances in common carp showed that latency time between the injection and ovulation in group (G1) which were injected with CPE was 12 hrs, while G2 which were injected with mixture of CPE and HCG was 15 hrs. Group G3 which were injected with HCG alone was 16 hrs. Group G4 which were injected with OVH was 14 hrs and that of females in group G5 which were injected with receptal was 13 hrs. Meanwhile, hatching rate recorded 50, 44, 42, 46 and 48% among responded females in groups G1, G2, G3, G4 and G5, respectively as shown in Table 2. These results were in

agreement with the findings of Akar *et al*. (2010) reported that latency time of females' common carp were spawned in group CPE double dose and CPE & HCG was 10 hours, wherever CPE single dose and HCG was 18 hours. On the contrary, Mahmoud (2006) showed that splitting of the CPE dose (double dose) did not affect the ovulation time and ovulation response in climbing perch (*Anabas testudineus*). Also, Kouril *et al.* (2003) showed that higher percentage of tench females *(Tinca tinca)* spawned after lecirelin treatment than after CPE (89 % and 23 %, respectively). This might be due to species differences and water temperature.

The results of Table 2 showed the highest significance ($P<0.05$) in weight of eggs and the high values were 1575±585.2g; 1340±77.8g and 1150±69.7g in G1, G5 and G4, respectively, while the low values were presented in G3 (HCG) and G2 (CPE+HCG); 250±7g and 440±88.3g, respectively. The present results are in agreement with Yaron *et al.* (2009) reported that in the technology of induced breeding of common carp hCG is only marginally effective in inducing ovulation requiring repeated injections of relatively large doses. In this context, Akar *et al*. (2010) reported that the mass of eggs was significantly higher percentage in CPE with HCG (362g & 11.94 %) and CPE double doses (250 g & 10.2 %) than in CPE single dose (238 g & 8.7 %) and HCG (172 g & 5.62 %), respectively. While, Mahmood (2006) showed that splitting of the CPE double dose did not affect the ovulation time and ovulation response in climbing perch *(Anabas testudineus).* The recent study of Szabó *et al.* (2014) reported that the relative amounts of stripped eggs, mean fertilization rate values, ovulation rates and different reproductive traits were similar between Northern pike fish (*Esox lucius*) and common carp (*Cyprinus carpio*) treated with common carp or silver carp pituitary. Herein, data of total costs were higher in group 4 and 5 (25 and 21.25 EP/kg, respectively) than group 1 and 2 (8.5 and 11 EP/kg fish) and group 3 was 13.5 EP/ kg fish. Consequently, the economic efficiency showed higher values in G1, G5 and G4 which recorded 307.8, 114.2 and 106.3, respectively than in group 2 and 3 which recorded 74.6 and 24.9, respectively as shown in Table 2.

In this regard, Akar *et al.* (2010) showed that return was higher significantly (P<0.05) in groups CPE with HCG & CPE double doses than groups CPE single dose and HCG, respectively.

Grass carp:

Latency time of G1 treatment CPE was 16 hrs, wherever G2 treatment which were injected with mixture of CPG and HCG was 19 hrs. Group (G3) which was injected with HCG was 20 hrs. Group G4 which were injected with OVP was 18 hrs and that of females in G5 (receptal) was 17 hrs. Thus, hatching rate in groups recorded 49, 43, 41, 45 and 47% of spawned females in groups G1, G2, G3, G4 and G5, respectively as shown in Table 3. In this context, Akar *et al.* (2010) found that latency time in both CPE double doses and CPE with HCG was 8 hours, while, in both CPE single dose and HCG was 15 hours. The latter author found also, the spawning percentages recorded 80, 70, 30 and 90% in groups CPE double doses, CPE single dose, HCG and CPE with HCG, respectively.

The results of Table 3 showed that the highest significance (P<0.05) in weight of eggs and high values were 2996.7±1339.2; 2100±249.4g and 1130±160.03 in G1, G5 and G4, respectively. The low values were presented in G3 and G2; 1010±376.7g and 1029.1±137.2g, respectively. These results were contrary with the findings of Akar *et al.* (2010) showed that the mass of eggs was significantly higher in both CPE double doses and CPE with HCG (530 ±88.7g and 440 ±119.4g) than in CPE single dose and HCG (200 ±79.1 & 30 ±9.4g), respectively. Also, the increase in the ovulation activities of the common carp and grass carp may be due to the Oogenesis which is controlled by stimulating hormone (FSH) and Luteinizing hormone (LH). Ovulation activity also needs to participation of several paracrine autocrine mechanisms of regulation as reported by Kouril *et al.* (2003). In addition, the increase in ovulation activities may be due to HCG is increase the speed of eggs maturation in fish as reported by Hodson and Sulivan (1993). On the other hand, total costs in Table 3 showed higher values in groups 4 and 3 (25 and 22.50 EP/kg, respectively), while the lower values of total costs were observed in G1

followed by G2 (14.17 and 18.33 EP/kg fish) and G5 recorded 21.25 EP/kg fish. Moreover, the economic efficiency value was higher in G1 also and G5 (127.989 and 46.498) than other groups as shown in Table 3. Besides, Akar *et al.* (2010) showed that return were higher significantly ($P<0.05$) in both CPE with HCG & CPE double doses (3733.7 and 3588.7 EP) than in CPE single dose and HCG (1749.4 & 162.9 EP), respectively. Not significantly difference was observed between CPE double doses and CPE with HCG. Additionally, Rashid *et al.* (2014) conducted single dose for female/male grass carp and silver carp in Kashmir and found that the fertilization percentage of grass carp was higher than silver carp and the hatching percentage of grass carp was slightly higher than silver carp. The fertilization percentage of grass carp and silver carp were recorded as 80.03% and 78.12%, respectively. The hatching percentage of grass carp and silver carp were recorded as 70.10% and 69.71%, respectively. Also the fry survival percentage of grass carp and silver carp were recorded as 15.21% and 14.56%, respectively. The latter authors also reported that economically important and fast growing food fishes grass and silver carp were successfully spawned with Ovatide (combination of GnRH analogue with dopamine antagonist pimozide).

Silver carp:

In G1 treatment (CPE), latency time was 21 hrs, wherever G2 which were injected with combination of CPE and HCG was 24 hrs. Group G3 which were injected with HCG was 20 hrs. Group G4 which were injected with OVP was 23 hrs and that of females in G5 (receptal) was 22 hrs. Hence, hatching rate in treated groups were 48, 42, 40, 44 and 46% of responded females which spawned in groups G1, G2, G3, G4 and G5, respectively as shown in Table 4. A total cost data was higher in groups 4 and 5 (25 and 21.25 EP/kg, respectively) than in groups 1 and 2 (11.33 and 14.67 EP/kg fish) and G3 recorded 18 EP/kg fish. Therefore, the economic efficiency values were higher in G1 and G5 which were 87.1 and 44.9 than in G2 and G3 which were 27.2 and 13.6, respectively as shown in Table 4. The results are in agreements with the results

obtained by study of Makeyeva *et al.* (1996) who recorded that a latency time of more than 20 hrs upon using LHRH-analogue as ovulation stimulator in silver carp. On the contrary, El-Hawarry *et al.* (2012) explained the success of spawning induction with reduced doses of all the hormones used in combination with dopamine antagonist. Concerning the latency period, all silver carp broodfish began spawning 7-12hrs after hormones injection with or without dopamine antagonists' injection. Also, in opposite to this study, Haque *et al*. (1995) found that a suspended mixture of HCG and pituitary gland was better than either HCG or pituitary gland separately in induction spawning of females in the silver carp and the bighead carp during the breeding season. Additionally, the fact that the silver carp females' eggs yield in a short time interval in case of LHRH-a is very important on account of the short period in which the optimum spawning occurs in herbivorous fish at temperatures between 20°C and 26°C (Brzuska, 1999). Furthermore, it seems to be worth stressing that the females of silver carp began spawning more than 9 hrs after the LHRH-a and pimozide injection at a temperature of 20–24°C (Peter *et al.*, 1988), an equal latency of 8–12 hrs was recorded for these species of herbivorous fish at the temperature of 18–30°C, irrespective of the application of mammalian or salmon analogues.

The results of Table 4 showed the highest significance ($P<0.05$) in weight of eggs and high values were 1600.8±248.3g; 1509.1±175.5g and 1419.2±276.2g in G1, G5 and G4, respectively, while the low weight of eggs in G3 and G2 were 509.5±107.7g and 919.2±389.2g, respectively. These results were contrary with the findings of Brzuska (2004) and Akar (2006) showed that synchronization of ovulation silver carp was observed in all the females after the injection by Aquaspawn. The results are in agreements with the results obtained by several studies (Żarski *et al.*, 2009; El-Hawarry, *et al.* 2012 and Szabó *et al.*, 2014) they reported that successful induction of spawning of silver carp using different spawning agents; CPE, HCG or luteinizing hormone releasing hormone (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 stimulating hormone (FSH) and luteinizing hormone (LH) but need also participation of several paracrine autocrine mechanisms of regulation as reported by Koural *et al.* (2003). Moreover, the increase in ovulation may be due to that the HCG increases speed the maturation of the eggs in fish as reported by Hodson and Sulivan (1993). Hence, the differences in reproductive performances in common carp; grass carp and silver carp might be due to species differences. Accordingly, the current work studied the effects of CPE, HCG, mixed from them, luteinizing hormone releasing hormone analogues (receptal / buserelin acetate) and ovaprim hormones (OVP) and described changes in reproductive performances during induced spawning of common carp, grass carp and silver carp. From the previous results could be concluded that the injection of CPE and receptal followed by ovaprim hormone (OVP) were the most effective treatment in economic efficiency possessed the most effective results in the artificial spawning of common carp, grass carp and sliver carp for commercial practices. Thus, gametes quality is associated with larvae quality and is a requirement not only in production but also in several research fields such as embryology, genetics, biotechnology and, etc. Egg quality is associated with state of broodfish and also is a requirement for fertilization success and several biomarkers such as egg morphological characteristics, enzymatic and metabolic markers and novel molecular markers.

CONCLUSION

The results obtained in this study indicate the possibility of increasing the effectiveness of controlled reproduction in case of the species studied by applying the appropriate type of hormonal stimulation. It also confirms suitability of carp pituitary extract (CPE), in fish reproduction stimulation for aquaculture requirements.

Table 2. Means of weights, spawning performances and economic efficiency of Common carp (*Cyprinus carpio*).

Group/ Treatment	Latency time (hour)	Female Wt (kg)	Male Wt (kg)	Wt of Eggs (g)	No. Fry production	Hatching rate	Total Fry price	Inject. costs/fish	Total return	Economic efficiency
		Mean±SD	Mean±SD	Mean±SD	Mean	%	*EP.	EP/kg	EP.	
1/ *CPE	12	2.10 ±0.8	1.52 ±0.3	1575±585.2[a]	472.5	**50**	23625	8.5	23548.5	307.8
2/CPE+HCG	15	1.63 ±0.3	1.28 ±0.1	440±88.3 [c]	135.5	44	6776	11.0	6686.4	74.6
3/ HCG	16	2.10 ±0.6	1.51 ±0.3	250±70.4 [c]	73.5	42	3675	13.5.0	3533.3	24.9
4/ OVP	14	1.38 ±0.1	1.29 ±0.2	1150±69.7[b]	370.3	46	18515	25.0	18342.5	106.3
5/ BA	13	1.84 ±0.1	1.26 ±0.1	1340±77.8[ab]	450.2	48	22512	21.3	22316.5	114.2
F – Value				22.8						
Probability				0.0001						
Significant				***						

Means with the same letter in the same column were not significantly different (P > 0.05).
*CPE (carp pituitary extract), HCG (human chorionic gonadotrophin), OVP (ovaprim), BA (buserelin acetate or receptal), Wt (weight), EP (Egyptian Pound)

Table 3. Means of weights, spawning performances and economic efficiency of Grass carp (*Ctenopharyngodon idella*).

Group/ Treatment	Latency time (hour)	Female Wt (kg)	Male Wt (kg)	Wt of Eggs (g)	No. Fry production	Hatching rate	Total Fry price	Inject. costs/fish	Total return	Economic efficiency
		Mean±SD	Mean±SD	Mean±SD	Mean	%	*EP.	EP/kg	EP.	
1/ *CPE	16	4.53±2.02	6.10 ±0.8	2996.7±1339.2[a]	661.5	**49**	33075	14.2	32818.6	127.9
2/CPE+HCG	19	5.53 ±0.7	6.20 ±1	1029.1±137.2[b]	199.3	43	9965.3	18.3	9561.9	23.6
3/ HCG	20	4. 70 ±1.8	6.13 ±1.03	1010±376.7 [b]	186.3	41	9317.3	22.5	8822.3	20.9
4/ OVP	18	5.50 ±0.8	6.08 ±1.4	1130±160.03[b]	228.8	45	11441.3	25.0	10971.3	19.9
5/ BA	17	5.50 ±0.7	6.15 ±1.5	2100±249.4[a]	444.2	47	22207.5	21.3	21737.9	46.5
F – Valu e				7.5						
Probability				0.0015						
Significant				**						

Means with the same letter in the same column were not significantly different (P > 0.05).
*CPE (carp pituitary extract), HCG (human chorionic gonadotrophin), OVP (ovaprim), BA (buserelin acetate or receptal), Wt (weight), EP (Egyptian Pound)

Table 4. Means of weights, spawning performances and economic efficiency of Silver carp (*Hypophthalmichthys molitrix*).

Group/ Treatment	Latency time (hour)	Female Wt (kg)	Male Wt (kg)	Wt of Eggs (g)	No. Fry production	Hatching rate	Total Fry price	Inject. costs/fish	Total return	Economic efficiency
		Mean±SD	Mean±SD	Mean±SD	Mean	%	*EP.	EP/kg	EP.	
1/ *CPE	21	4.81 ±0.8	5.2 ±0.5	1600.8±248.3[a]	384	**48**	19200	11.3	18981.9	87.1
2/CPE+HCG	24	5.88 ±2.5	5.2 ±0.8	919.2±389.2 [b]	193.2	42	9660	14.7	9374	27.2
3/ HCG	20	4.88 ±1.03	5.1 ±0.8	509.5±107.7[c]	102	40	5100	18.0	4781.9	13.6
4/ OVP	23	4.59 ±0.9	5.2 ±0.5	1419.2±276.2[a]	312.4	44	15620	25.0	15161.3	33.03
5/ BA	22	4.42 ±0.5	5.1 ±0.6	1509.1±175.5[a]	347.3	46	17365	21.3	16865.6	44.9
F – Value				12.95						
Probability				0.0001						
Significant				***						

Means with the same letter in the same column were not significantly different (P > 0.05).
*CPE (carp pituitary extract), HCG (human chorionic gonadotrophin), OVP (ovaprim), BA (buserelin acetate or receptal), Wt (weight), EP (Egyptian Pound).

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التفريخ التحفيزى لأسماك المبروك العادى والحشائش والفضى بمستخلص الغدة النخامية للمبروك وهرمون الجونادوتروفين البشرى والريسيبتال والأوفبريم للأغراض التجارية

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الملخص العربى

استهدفت هذه الدراسة مقارنة تأثير الحث لكل من الحقن بمستخلص الغدة النخامية للمبروك وهرمون الجونادوتروفين البشرى وبهما معا والأوفبريم والريسيبتال علي الكفاءة التناسلية للمبروك العادى والحشائش والفضى على نطاق تجارى مع شرح عملى مبسط، وقد استخدمت لهذه الدراسة أسماك ناضجة (أمهات) سليمة خالية من الأمراض (٢٥ سمكة مبروك عادى ذكور و ٢٥ إناث) و(٢٠ سمكة مبروك حشائش ذكور و٢٠ إناث) و(٢٠ سمكة مبروك فضى ذكور و٢٠ إناث) وقد قسمت الأسماك المستخدمة إلى خمسة مجموعات. الأولى تم حقنها بمستخلص الغدة النخامية للمبروك العادي (٣، ٤، ٥ ملجم لكل ١ كجم من أسماك المبروك العادى والحشائش والفضى، على التوالى). والمجموعة الثانية تم حقنها بخليط من الغدة النخامية للمبروك العادى وهرمون الجونادوتروفين البشرى بمعدل (١.٥ ملجم + ٧٥٠ وحدة لكل كجم مبروك عادى، ٢ ملجم + ١٠٠٠ وحدة لكل كجم مبروك حشائش، ٢.٥ ملجم + ١٢٥٠ وحدة لكل كجم مبروك فضى)، والمجموعة الثالثة تم حقنها بهرمون الجونادوتروفين البشرى منفردا بمعدل (١٥٠٠، ٢٠٠٠ ، ٢٥٠٠ وحدة لكل كجم من أسماك المبروك العادى والحشائش

والفضى، على التوالى)، أما المجموعة الرابعة فتم حقنها بهرمون الأوفبريم بمعدل (٠.٥مل لكل كجم من وزن الأنثى)، والمجموعة الخامسة تم حقنها بالريسيبتال بمعدل (٢٠ ميكروليتر لكل كجم من وزن الأنثى) وكان الزمن بين الحقنة الأولي والتبويض أسرع وأفضل في المجموعة الأولي والخامسة. وأظهرت النتائج إختلافات معنوية (نسبة الاحتمالية أقل من ٠.٥) فيما بين المعاملات الخمسة، حيث وجدت زيادة لكمية البيض الناتج في المجموعة الأولي والخامسة وأقل كمية في وزن البيض لوحظت في المجموعة الثالثة فى الأنواع الثلاثة لأسماك المبروك، وأيضا وجدت هناك زيادة في قيمة العائد الاقتصادى فى المجموعة الأولي والخامسة عن باقي المجاميع فى المبروك العادى والحشائش والفضى. ومما سبق نوصي باستخدام كل من مستخلص الغدة النخامية أولاً لانخفاض تكلفته وارتفاع قيمة العائد الاقتصادى منه ثم الريسيبتال عند تفريخ أسماك المبروك.