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USE OF GNRH AND HCG HORMONES IN THE ARTIFICIAL PROPAGATION OF COMMON CARP FISH (*CYPRINUS CARPIO* L.)

Waleed Sami J. AL-Bachry and Ali Sami Amin AL-Tawash
Department of Animal Production, College of Agriculture, University of Al-Qadisiyah, Iraq
Corresponding author: waleed.albachry@qu.edu.iq, ali.amin@qu.edu.iq

Abstract

The study aims to using the Gonadotropin-releasing hormone (GnRH), Human Chorionic Gonadotropin (HCG) and Common Carp Pituitary Gland (CPG) in the artificial propagation for brooders of common carp fish (*Cyprinus carpio* L.) for the purpose of obtaining the products of sexual maturity (male and female). The study included two experiments on common carp fish, where each experiment was divided into two groups (control group and treatment group), each group included (males and females). The results of the study showed that the treatment of (1/4 pituitary gland + GnRH) was significantly excelled on all treatments of two experiments for females and for all studied traits (total number of egg, number of egg per 1 g, the amount of produced egg (g), Percentage of eggs to body weight (%), response period after last injection (hour) and the percentage of female response (%) amounted of (235130.00, 443.33, 561.67 g, 16.67%, 8.67 h, 100%), respectively. For males, the same treatment (1/4 pituitary gland + GnRH) was significantly excelled on the other treatments for the two experiments and for the studied traits (response period (min) and the quantity of Sperm (cm^3) which amounted of (8.00 min, 7.67 cm^3), respectively. As for the percentage of male response, no significant differences were observed between them for males of both the first and second experiment. Thus, the treatment (1/4 pituitary gland + GnRH) is excelled to all treatments in the response of brooders of each male and female.

***Keywords*:** Hormone, HCG, GnRH, Artificial multiplication, common carp, sperm, eggs.

Introduction

Fish is considered one of the most important animal used species in aquaculture globally (FAO, 2000). One of studies on the future demand for fish products showed a significant increase as a result of economic and demographic growth, which leads to a change in dietary habits (Dixon *et al.,* 2001; Alkhashali, 2009). Fish protein is the highest biological value of any other protein source as well as containing volatile fat that is not harmful to human health. A kilogram of it provides the human needs of iodine for 50 days, it is among the most widespread commodities in the world (Al-Mamouri, 2009). Many fish breeding projects have spreaded in Iraq, either through breeding in ponds or cages (Al-Akidi, 2008; Al-Salem, 2013). Therefore, the artificial propagation techniques were spreaded in Iraq, especially in Babylon province as in many countries of the world to provide the fingerlings necessary for such projects through the stimulation of hormonal (Woynarovich and Horvath, 1980; Zohar and Billard, 1979; Al-Hamyari, 2011; AL-gypori, 2011; Saleh, 2014). The hormone pituitary gland is the most common hormone and its use in the hormonal stimulation in the fish hatcheries for the whole of Iraq, but the price is high in the local markets and ranges from 250 to 700 dollars per gram and the difficulty of obtaining it because of the conditions of import or monopoly by some traders (Al-Hilli, 2013).

The study aims to use modern alternative materials not previously used and other substances that have been used but in a different way with or without the pituitary hormone at different levels to induce brooders and obtain eggs and sperm during the artificial propagation process in the hatchery without having a negative impact on important productive and studied traits on the brooders, and to reduce the cost of one injection and available locally in the Iraqi market and easy access to and preparation as well as being safe in terms of transmission of diseases for brooders herd.

Materials and Methods

The experiment was conducted for the period from 25/03/2018 to 30/04/2018 in Al-Hindia Barrier, northern of Babylon province. The appropriate fish were selected based on their sexual maturity and were pre-sorted by sex. The females were isolated from males and placed in the internal brooders ponds after being marked with different yarns in colors, it was fixed in the dorsal fin of (18 females and 18 males). The weights were taken by a hanged balance (for the nearest 10 g). The weighted range ranged for females from (2-5 kg) to males (1-2 kg). The stimulants for brooders of common carp fish were used to put up sexual Gamete as follows:

1) Pituitary gland for common carp (CPG) from American origin produced by Argent USA company.
2) Human Chorionic Gonadotropin (HCG): It is used to stimulate ovulation in women The package contains 5000 IU.
3) Gonadotropin-releasing hormone (GnRH): The commercial name (Gestar) is an Argentinean origin and contains 10 ml liquid solution. This hormone is used only for large farm animals such as cows and horses.
4) Medical marerial (Motilium): Dosage 10 mg.

Three females and three males were selected for each of the control group and the hormonal treatment group for both experiments:

First: Control group (1): A (3 ml.kg/ femal of GnRH) hormone was used on two doses, the first one is 10% of the

total dose (0.3 ml.kg) and after 12 hours, the second dose was injected 90% of the hormone (2.7 ml.kg). The males were given half the female dose and injected once with the second dose for females.

Treatment group (T1): Motilium was used in a dose (10 mg/kg female) with (3 ml/kg female of GnRH) hormone and in two doses as in the control treatment and half dose of the female give to males.

Treatment group (T2): (3 ml of GnRH + 1/4 pituitary gland / kg female) used for female injection and in the two doses: The first one is 10% and the second 90%, The pituitary gland was injected with the first dose. Males injected with dose half of the female after 12 hours of the first dose for female.

Second: Control group (2): The hormone (HCG) was used with dose of (1000 IU/kg female) and in two doses 10% and 90% of the total dose and half the dose for males.

Treatment group (T1): It was used (1000 IU of HCG + 10 mg of Motilium / kg female) in two doses as control and half-dose treatment for males.

Treatment group (T2): It was used (1000 IU of HCG + 1/4/ pituitary gland / kg female) in two doses as in control treatment and half-dose for males.

A 0.5 cm^3 of the solution was used to inject a kilogram of fish weight and to dissolve the pituitary gland hormone. Water traits were recorded during the experiment period, The pH of the water was between 7.1 and 7.7. The water temperature was measured by a mercury thermometer ranging between 21-24 °C, the percentage of oxygen was between 5.2-6.7 kg/L. A sample was taken to determine the salinity of the water which ranged between (0.5-0.96 ppm) All were within the appropriate limits for the propagation of carp fish (Naif, 2005; Al-Ghazali, 2010; Saleh, 2014).

The studied traits recorded for the average of (the percentage of response %, response period (hour), total number of egg, number of eggs per 1 g, the amount of produced egg (g), Percentage of eggs to body weight (%),the quantity of Sperm (cm^3) for males. The data were analyzed statistically using SAS and Duncan to show the differences between the treatments.

Results and Discussion

Tables (1, 2) show for the first and second experiment for females, the treatment (1/4 pituitary gland + GnRH) is excelled by recording it significant differences ($P < 0.01$) for the studied traits (total number of eggs, number of eggs per 1 g, the amount of produced egg (g), Percentage of eggs to body weight (%), response period after last injection (hour) and Percentage of female response %) on female control and all studied traits (235130.00, 443.33, 561.67 g, 16.67%, 8.67 h and 100%), respectively. This may be due to the fact that GnRH is responsible for the release of Follicle-stimulating hormone (FSH) and (LH) hormone from the pituitary gland (Campbell *et al*., 2009; White *et al*., 2002; Comite *et al*., 1981), On the other hand is due to the mixing of the pituitary hormone with the hormone GnRH, which in turn raises the level of the Progesterone hormone in the body. Table (1, 2) shows that the excelling of (1/4 pituitary gland + HCG) treatment on the control treatment (HCG), with significant differences and for all studied traits (127190.33, 401.00, 308.00 g, 8.50%, 11.33 h, 100%). The female response to the control treatment (HCG), which is injected with HCG alone, does not appear, and this results agree with (AL-gypori, 2011; Assal, 2015; AL-Bachry, 2017). The treatment may be superior (1/4 pituitary gland + HCG) to contain the dose on a mixture of one quarter of the pituitary gland, While did not show its significant superiority on the control treatment (GnRH) and other treatments (10 mg Motilium + GnRH and 1/4 pituitary gland + GnRH) in the first experiment and for all previously studied traits.

Table (3) shows that the control treatment (GnRH) and the treatment (1/4 pituitary gland + GnRH) was significantly excelled ($P < 0.01$) for the trait of the quantity of Sperm which amounted of (7.67 cm^3) for each of them on the other treatments. The same treatment (1/4 pituitary gland + GnRH) was significantly excelled ($P < 0.05$) on the control treatment (GnRH) and other treatments in the traits of the response period (h) by recording it (8.00 h), while the control treatment (GnRH) and the treatment (1/4 pituitary gland + GnRH) gave the same average quantity of Sperm (cm^3) which amounted of (7.67 cm^3). In the second experiment, the superiority of the treatment (1/4 pituitary gland + HCG) showed significant differences for all the previously studied traits on the control treatment (HCG), while significant differences were recorded at the level of $P < 0.01$) in favor of the treatments (10 mg Motilium + HCG) and (1/4 pituitary gland + HCG) for the trait of the quantity of Sperm (cm^3) which amounted of (5.33, 6.96 cm^3), respectively. In the same trait, the treatment (1/4 pituitary gland + HCG) recorded their excelling on the treatment of (10 mg Motilium + HCG) which amounted of (6.96 cm^3). The males of the first and second experiments did not record any significant differences between them for the percentage of male response which amounted of (100%). This result agrees with (Mujer, 2011; Al-Hilli, 2013; Al-Bachry, 2017; 2018) in their study on common carp fish where the female response was low for HCG and was good for males with the same hormone and with half of the dose used for females.

The results of the study showed the possibility of the use of new hormones such as (GnRH), which is used for large animals as an attempt to reduce the use of pituitary to obtain the outputs of sexual maturity of male and female during the process of artificial propagation in the hatcheries, it is worth noting that this hormone is less expensive than the pituitary gland and available locally and It does not require preparation. We recommend extensive studies and the use of modern scientific techniques for GnRH hormone, with different doses and for different types of fish. This is the first use of GnRH hormone for common carp fish in Babylon Province.

Table 1: Effect of studied hormonal treatments on egg production for fish females

Experiment number		Type of hormone and treatment	Average ± standard error			
			Total number of eggs	Number of egg with 1 g	The amount of produced egg (g)	Percentage of eggs to body weight
First	Control	GnRH	43066.67 21033.33±bc	107.67 52.67 ± bc	133.33 67.33 ± bc	4.33 2.16 ± bc
	T_1	10 mg Motilium + GnRH	133638.33 67472.00±ab	265.67 132.89 ± ab	335.00 168.55 ± ab	7.67 3.84 ± bc
	T_2	1/4 pituitary gland + GnRH	235130.00 16596 ± a	443.33 22.00 ± a	561.67 57.32 ± a	16.67 0.33 ± a
Second	Control	HCG	0.00 0.00 ± c	0.00 0.00 ± c	0.00 0.00 ± c	0.00 0.00 ± c
	T_1	10 mg Motilium + HCG	0.00 0.00 ± c	0.00 0.00 ± c	0.00 0.00 ± c	0.00 0.00 ± c
	T_2	1/4 pituitary gland + HCG	127190.33 11281.74 ± ab	401.00 40.12 ±	308.00 38.89 ± ab	8.50 0.76 ±
LSD			**	**	**	**
** (P <0.01) The averages with different letters within the same column vary significantly between them.						

Table 2: Effect of studied hormonal treatments on weight and responsiveness for female fish

Experiment number		Type of hormone and treatment	Average ± standard error		
			Weight females (kg)	Response period after last injection (h)	percentage of response for females (%)
First	Control	GnRH	0.33 ± 3.33 a	0.00 ± 11.00 c	16.67 ± 33.33 bc
	T_1	10 mg Motilium + GnRH	0.57 ± 4.00 a	0.33 ± 9.50 ab	66.67 ± 33.33 bc
	T_2	1/4 pituitary gland + GnRH	0.33 ± 3.33 a	0.33 ± 8.67 ab	0.00 ± 100.00 a
Second	Control	HCG	0.33 ± 3.67 a	0.00 ± 0.00 c	0.00 ± 0.00 c
	T_1	10 mg Motilium + HCG	0.58 ± 4.00 a	0.00 ± 0.00 c	0.00 ± 0.00 c
	T_2	1/4 pituitary gland + HCG	0.33 ± 3.67 a	0.33 ± 11.33 a	0.0 ± 100.00 a
LSD			**	NS	**
** (P <0.01) The averages with different letters within the same column vary significantly between them.					

Table 3: Effect of Hormonal treatments on Male Fish

Experiment number		Type of hormone and treatment	Average ± standard error			
			Weight males (kg)	Response period after last injection (h)	The quantity of Sperm (cm^3)	percentage of response for males (%)
First	Control	GnRH	0.17 ± 1.67 a	0.33 ± 8.67 ab	7.67 1.45 ± a	0.00 ± 100
	T_1	10 mg Motilium + GnRH	0.09 ± 1.83 a	0.33 ± 8.67 ab	4.090.57 ±ab	0.00 ± 100
	T_2	1/4 pituitary gland + GnRH	0.23 ± 1.56 a	0.57 ± 8.00 b	7.672.40 ± a	0.00 ± 100
Second	Control	HCG	0.11 ± 1.70 a	0.33 ± 9.33 ab	4.57 2.72 ± b	0.00 ± 100
	T_1	10 mg Motilium + HCG	0.10 ± 1.90 a	0.67 ± 9.67 a	0.88 ± 5.33 b	0.00 ± 100
	T_2	1/4 pituitary gland + HCG	0.08 ± 1.46 a	0.33 ± 8.67 ab	6.96 1.73 ± a	0.00 ± 100
LSD			**	NS	*	**
** (P <0.01), * (P <0.05), NS no significant The averages with different letters within the same column vary significantly between them.						

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