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Effect of Carp Pituitary Extract, HCG and Mix of Them on Fecundity, Larvaes Production, Blood Sex Hormones and Biochemical Parameters of the African Catfish (*Clarias gariepinus*)

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ABSTRACT

The present work investigated the effects of carp pituitary extract (CPE), human chorionic gonadotropin (hCG) and CPE + hCG on blood sex hormones, fecundity, GSI, larval production and blood biochemical parameters. Females and males of African catfish were intramuscularly injected with one gland of CPE, 1500 IU hCG/kg and half CPE + 750 IU hCG/kg to be compared to a not injected group (a control group). A pair of broodstock was placed in a separate tank, equipped with palm leaves for 15 days after injecting until all experimental fish finished spawning. The results showed significant differences in all measured blood hormones (FSH, LH, progesterone, estrogen and testosterone) among treatments. In addition, significant differences were detected in ovaries weight, gonado somatic index (GSI) of females and males, egg diameter, absolute and relative fecundity and the number of larvae/ female. The injected brood stock with different hormones did not significantly differ in GSI, egg diameter, absolute and relative fecundity, but they were significantly higher in these indices than the control group. The treated group with hCG had the highest number of larvae/females. Meanwhile, the control group did not show any hatched larvae. The chemical composition of ovaries (moisture, protein and ash) showed significant differences ($P \leq 0.05$) among treatments except for fat content. While, the male chemical composition of the testis, insignificantly differed. Moreover, there were significant changes in biochemical parameters among all treatments. Finally, it was recommended that using CPE, hCG and the mixture of CPE+hCG has successfully induced the propagation of African catfish. Upon using hCG specifically, the highest number of larvae was obtained in the fish group injected with 1500 IU hCG/kg.

INTRODUCTION

The aquaculture sector's contribution to the supply of fish for human consumption surpassed that of fisheries for the first time in 2015 **(FAO, 2017)**. Since that time, aquaculture has continued to expand at a rapid rate, contributing more and more to food security and nutrition. Undoubtedly, the significant and unmistakable boom in

aquaculture together with the restrictions associated with food security qualified aquaculture as an alternative to fill the gap caused by the decline in fishing as a sector of food production. However, despite the continent of Africa's significant potential, development is still considerably hard to recognize. The absence of hatcheries that consistently generate large numbers of larvae throughout the year is one of the main issues that consistently impedes the promotion and growth of the aquaculture sector in the African continent **(FAO, 2022)**. African catfish (*Clarias gariepinus*) is native to Africa; it is regarded as one of the most crucial tropical catfish species for aquaculture. Since the 1940s, the sharp-tooth catfish *Clarias gariepinus* has been recognized in aquaculture **(Hey, 1941)**. According to **Al-Dohail (2005)** and **Sutriana (2007)** *C. gariepinus* is one of the most widely produced fish as human food in the world and is regarded as a native fish in all freshwater bodies in Egypt **(Saleh, 2007)**. Some of the benefits that make this species a good choice for aquaculture include the fast growth rates, large mature sizes, ease of reproduction, acceptance of artificial feeds, low production cost, tolerance of high stocking densities, adaptation to poor water quality, high resistance to diseases, profitability in local, regional and international markets in addition to the economic viability in earthen pond culture systems, the most popular culture system in the East African community **(Teugels, 1986; Mehrim *et al.*, 2014; Tyor & Pahwa, 2017)**.

Since the last sixty years, fish hatcheries have used hormonal manipulation to encourage spawning in fish to produce fry or fingerlings, which considerably increases the production of aquaculture as a whole **(Rahman *et al.*, 2011)**.

In commercial breeding of *C. gariepinus*, the hormonal manipulation is a very useful method to ensure a high percentage of spawning and the possibility to produce fish seed all year round. Numerous hormones have been used to successfully induce the spawning of African catfish such as pituitary gland **(Brzuske, 2003; Olaniyi & Akinbola, 2013; Saadony *et al.*, 2014; Ngueku, 2015; Moshaand & Mlingi, 2018**), hCG **(Sharaf, 2005; Mehrim *et al.*, 2014; Saadony *et al.*, 2014; El-Hawarry *et al.*, 2016; Ahmed, 2018; Zidan *et al.*, 2020)**, ovaprim **(Sharaf, 2012; Achionye-Nzeh & Obaroh, 2012; Olaniyi & Akinbola, 2013; Saadony *et al.*, 2014)** and gonadotropin releasing hormones GnRH **(Saadony *et al.*, 2014; Shourbela *et al.*, 2014; El-Hawarry *et al.*, 2016)**. The pituitary gland is the principal source of the main hormones responsible for fish reproduction **(Fagbenro *et al.*, 1998; Salami *et al.*, 2006)**. Ovulation and spermiation are significantly influenced by gonadotropin hormones (GtH), which are produced and stored by the pituitary gland. PG injection acting directly on the ovaries and testicles instead of being through the brain-pituitary axis provides the spike in blood GtH levels that often occurs before spawning **(Rottmann *et al.*, 1991)**. Pituitary gland extract as hormonal induction has a better rate of fertilization and hatching, as well as improved in larval growth and survival rate (**Oyeleye *et al.*, 2016**).

Human Chorionic Gonadotropin (hCG) is purified gonadotropin hormone, the most commonly used to induce spawning in fish. The injected hCG mimics the natural GtH

produced and secreted from fish's pituitary, which acts directly in gonad, thus hCG acts much faster. It was shown that a mixture of pituitary gland (PG) and hCG was more effective at inducing female spawning than either hCG or pituitary gland alone **(Rottmann *et al.*, 1991)**.

Therefore, the objective of the study presented here was to evaluate the effects of carp pituitary extract (CPE), human chorionic gonadotropin (hCG) and CPE + hCG on blood sex hormones, fecundity, GSI, larval production and blood biochemical parameters in the African catfish.

MATERIALS AND METHODS

Ethics

This work was conducted with the strict recommendations and approval of the National Institute of oceanography and fisheries (NIOF, Egypt) Committee for ethical care and use of animals/aquatic animals (NIOF-IACUC, Code: NIOF-AQ5-F-22-R-029**).**

Brooders selection and site of work

Brooders selection of African catfish *Clarias gariepinus* (adult males and females) used in the present study were purchased from the private fish farm (Fayoum. Egypt) alive and in a good health; they were transferred to the National Institute of Oceanography and Fisheries (NIOF), Shakshouk Research Station, Fayoum Governorate, Egypt. The brood fish were placed in rectangular tanks, and females were kept separated from fish males after being disinfected with formaldehyde (15 ppm) for six hours.

The ripeness of females was determined through their external physical traits; the female had a soft, distended abdomen, round swollen and reddish genital papilla and readiness to spawn. A few egg samples were taken from the female broodfish's ovaries, using a catheter in order to examine them under a calibrated ocular micrometer. The egg diameter of more than 90% of the mature ovaries was greater than 900µm and homogenous in size. While, males were chosen based on their reddish and pointed genital papilla **(Sahoo *et al*., 2004; El-Hawarry *et al,* 2016; Gadisa, 2017)**.

Broodstock management

An 80 sexually mature brood-stock fish individuals (40 females and 40 males) with sex ratio (1:1 male: female),the females' body weight ranged from 920- 1050g, with total length values ranging from 49.5- 50.5cm. While, the males recorded a range of 850-1195g and 51-61cm/male for body weight and total length, respectively. The broodstock was held in rectangular tanks (1.75 x 1.75 x 1 m), with a water depth of 50cm; tanks were supplied with aerated water. Water quality during the experimental period recorded average values of 25.6°C, 7.5 and 6.4 mg/l for water temperature, pH and dissolved oxygen, respectively.

The brood fish were fed at a rate of 2% of body weight/day, with an artificial diet containing 30% crude protein during the experiment period that continued for 15 days after the hormonal injection. After the hormonal injection, each pair of brood fish (male x

female) were placed in a separate tank that were provided with nests of palm leaves as a substrate for females to lay their eggs on.

Hormones preparation

Human chorionic gonadotropin (hCG), acquired as chorionic gonadotrophin =5000 IU preparation, Egyptian International Pharmaceutical Industries Co., 10th of Carp pituitary extract: each pituitary gland was extracted with 1ml saline solution (sodium chloride 0.9 %) after being crushed in a pestle, then the suspension was transferred into a centrifuge to draw the supernatant fluid into a syringe for injection.

Chorionic gonadotrophin: Ramadan City, industrial area B1 box 149, Egypt.

Experimental design and hormonal injection

Experimental fish were divided into four treatments (each treatment containing 10 females and 10 males). Males and females were injected at the same time with a single dose as the following: the first group was injected with carp pituitary extract (CPE), the second group was injected with 1500 IU human chorionic gonadotropin (hCG)/kg, the third group was injected with a mixture of half CPE + 750 IU hCG/kg, and the fourth group was a control group.

The hormonal injection was done in a single dose in the morning (7-9 AM), and fishes were injected intramuscularly at an angle of 45 degrees above the lateral line toward the tail by using insulin syringe for hCG injection and 3ml syringe for CPE injection. After hormonal injection, the broodfish were returned to the tanks (3 couples for each treatment as one couple for each tank, and the other two couples in each treatment were used for blood samples, ovarian measurements and chemical composition of gonad for 24hrs after hormonal injection). Each tank was equipped with two nests of palm leaves as a substrate for incupating the eggs till hatching. Hatching larvae were collected and counted as soon as they appeared in tanks.

Measurements:

Weight and length of brood-stock, ovaries weight, gonadosomatic index (GSI), egg diameter, absolute and relative fecundity were determined according to **Nikolosky (1963)**, **Tseng and Chan (1982)** and **Saadony *et al.* (2014)**.

Sampling:

After 24 hrs of injection, a random sample of brood-stocks (3 male and 3 female)/ group was taken to draw the blood and collect the gonads to analyze the chemical composition.

Blood samples

Blood was collected at 24 hrs post-injection without the use of anticoagulants from the caudal vein (each male and female), which was then transferred to Wasserman tubes. To get a sample of serum, blood was centrifuged at 3500 rpm for 20 minutes after being allowed to clot at room temperature for 45 minutes **(Mehrim *et al.*, 2014)**. The serum samples were pipetted into Eppendorf tubes, labeled and kept in a deep freezer at a temperature of –20°C for further analysis.

Blood hormone:

CHROMATM Reader System was used to quantitatively examine the serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (P4) and testosterone. Estradiol was measured using an enzyme immunoassay using standard estradiol (0, 20, 100, 300, 800 and 3200 pg/ml) (biocheck, Inc. Foster City, CA 94404 U.S.A.).

Blood biochemical:

Serum glucose, albumin, cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea were determined by an enzymatic colorimetric method using BioSystem BTS-302 utilizing commercial kits. While, the cortisol level was quantitatively determined by using I-CHROMATM Reader System using commercially available kits.

Chemical composition of gonads:

At 24 hrs after injecting, ovaries and testes were chemically analyzed to estimate their contents of moisture, protein, ash and fat. Chemical analysis were done according to the standard methods of **AOAC (2005)**.

Statistical analysis:

Data were statistically analyzed using a one-way analysis of variance (ANOVA test) and SPSS Statistical Package Program (**SPSS, 2015**) version 23. Mean of treatments were compared by Duncan multiple range test when the differences were significant **(Duncan, 1955**). The level of significance in all tests was $P \leq 0.05$. The results were expressed as means ± standard error (SE)

RESULTS

Morphometric parameters of African catfish broodstock

The characteristics of females and males are summerized in Table (1), showing insignificant differences ($P \geq 0.05$) between treatments in weight (g) and length (cm) of brood-stock either males or females. Wherein, the body weight and length of female ranged from (920 to 1050g), (49.5 to 50.5 cm), respectively. While, in male broodfish, they ranged from 850 to1195g for body weight and from 51 to 61cm for length.

Table 1. Morphometric parameters of African catfish broodstock

Item	Control (not injected)	Hormonal treatments			*P*- value
		CPE	hCG	CPE+ hCG	
Female weight, g	960± 40[a]	965± 25[a]	1020± 30[a]	912± 27[a]	0.257
Female length, cm	50.25± 0.25[a]	50.25± 0.25[a]	51± 0.50[a]	50± 0.50[a]	0.929
Male weight, g	900± 50[a]	977± 22[a]	1097± 97[a]	975± 25[a]	0.252
Male length, cm	52± 1[a]	56± 2[a]	60± 1[a]	56± 3[a]	0.200

Values in the same row having similar superscript are not significantly different from one another (*P>0.05*)

Effect of hormonal treatments on female blood sex hormones

The results of blood sex hormones of female *C. gariepinus* that were recorded 24 hrs after the hormonal injection are presented in Table (2). It's obvious that in all measured hormones (FSH, LH, estrogen E_2, progesterone P_4 and testosterone T) were significantly variant among treatments at $P \leq 0.05$. The CPE- treated female had the highest value of FSH, LH and estrogen. Additionally, the highest level of P_4 and T was recorded with the hCG-treated female. While, the control group achieved the lowest level of P_4 and T hormones.

Table 2. Blood sex hormones changing 24 hours after the injection of African catfish female

Item	Control (not injected)	Hormonal treatments			*P*- value
		CPE	hCG	CPE+ hCG	
FSH, mIU/ml	0.46± 0.01^{b}	0.51± 0.02^{a}	0.29± 0.02^{c}	0.33± 0.01^{c}	< 0.001
LH, mIU/ml	0.23± 0.01^{ab}	0.26± 0.02^{a}	0.23± 0.02^{ab}	0.17± 0.01^{b}	0.051
Estrogen, pg/ml	580± 5^{b}	1162± 62^{a}	414± 15^{c}	490± 20^{bc}	< 0.001
Progesterone, ng/ml	0.02± 0.01^{c}	0.83± 0.02^{b}	0.97± 0.03^{a}	0.03± 0.01^{c}	< 0.001
Testosterone, ng/ml	0.25± 0.01^{c}	4.75± 0.25^{b}	7.42± 1.21^{a}	3.97± 0.26^{b}	0.006

Values in the same row having different superscripts differ significantly ($P \leq 0.05$).

Effect of hormonal treatments on male blood sex hormones

Table (3) shows the results of blood sex hormone of male *C. gariepinus* obtained 24 hrs after hormonal treatments. There were significant differences ($P \leq 0.05$) between treatments in all estimated hormones (FSH, LH, E_2, P_4 and T). FSH level was the highest in males treated with CPE, followed by hCG with insignificant difference ($P \geq 0.05$) between them; however, these groups were significantly higher in FSH level than CPE+ hCG and the control group.= Males that were treated with CPE+hCG or hCG had the highest value of E_2, and the lowest value was found with the control group. The CPE-treated males recorded the highest level of P_4, while the lowest level of P_4 was observed in each of the control group and hCG group. T level did not significantly change among the hormonal–treated males (CPE, hCG or CPE+hCG), and they were significantly higher than the control group in these parameters.

Table 3. Blood sex hormones changing 24 hours after the injection of African catfish male

Item	Control (not injected)	Hormonal treatments			*P*- value
		CPE	hCG	CPE+ hCG	
FSH, mIU/ml	0.31± 0.01^{c}	0.55± 0.02^{a}	0.51± 0.01^{a}	0.41± 0.02^{b}	0.002
LH, mIU/ml	0.23± 0.02^{b}	0.27± 0.01^{ab}	0.30± 0.01^{a}	0.24± 0.02^{b}	0.053
Estrogen, pg/ml	113± 6^{c}	177± 7^{b}	211± 8^{a}	224± 8^{a}	0.002
Progesterone, ng/ml	0.03± 0.02^{c}	0.41± 0.02^{a}	0.03± 0.01^{c}	0.23± 0.01^{b}	< 0.001
Testosterone, ng/ml	1.57± 0.05^{b}	9.98± 0.28^{a}	11.02± 0.67^{a}	11.34± 0.56^{a}	< 0.001

Values in the same row having different superscripts differ significantly ($P \leq 0.05$).

Effect of hormone type on fecundity, GSI and egg diameter of African catfish

The differences in ovaries weight, female GSI, egg diameter, absolute and relative fecundity, and male GSI among treatments (control, CPE, hCG or CPE+hCG) were significant (*P≤0.05*) and presented in Table (4). Female of CPE and hCG group did not significantly alter in weight and recorded the highest weight of ovaries (124.08± 2.59g, 123.37± 3.16g, respectively), while the lowest ovaries' weight was observed in the control group. Females and males treated with CPE, hCG or CPE+hCG achieved the highest GSI, and these groups were significantly higher, compared to the control group. Furthermore, the hormonal treatments did not show significant differences in egg diameters, absolute and relative fecundity; however, these treatments were significantly (*P≤0.05*) higher than the control in these indicators.

Table 4. Effect of hormone type on fecundity, GSI and egg diameter of African catfish

Item	Control (not injected)	Hormonal treatments			*P*- value
		CPE	hCG	CPE+ hCG	
Ovaries weight, g	93.27± 2.59^{b}	123.37± 3.16^{a}	124.08± 2.59^{a}	110.57± 10.08^{ab}	0.049
Female GSI, %	9.73± 0.14^{b}	12.80± 0.66^{a}	12.17± 0.11^{a}	12.10± 0.74^{a}	0.043
Egg diameter, mm	0.97± 0.08^{b}	1.18± 0.03^{a}	1.17± 0.02^{a}	1.15± 0.03^{ab}	0.048
Absolute fecundity	60281± 819^{b}	126299± 8045^{a}	143875± 1325^{a}	126681± 8364^{a}	0.002
Relative fecundity	62.87± 1.77^{b}	130.75± 4.95^{a}	141.14± 2.85^{a}	138.68± 4.99^{a}	< 0.001
Male GSI, %	0.46± 0.03^{b}	0.81± 0.06^{a}	0.67± 0.04^{a}	0.69± 0.01^{a}	0.013

Values in the same row having different superscripts differ significantly (*P≤0.05*).

Effect of hormone type on larval production of African catfish

Larval production is presented in Table (5). Significant changes were detected among treatments in number of larvae/female, number of larvae/kg brood-stock and hormonal dose cost (L.E/1000 larvae). The group of hCG had the highest number of larvae/female. Conversely, the control group didn't show any hatched larvae. The lowest hormonal dose cost (L.E/1000 larvae) was recorded by the treated fish with CPE.

Table 5. Production of larvae per female and per kg of brood-stock (male and female together)

Values in the same row having different superscripts differ significantly (*P≤0.05*).

Item	Control (not injected)	Hormonal treatments			*P*- value
		CPE	hCG	CPE+ hCG	
No. of larvae/female	0.0^{c}	38557± 1442^{b}	51800± 700^{a}	36150± 1150^{b}	< 0.001
No. of larvae/kg brood-stock	0.0^{c}	19854±302^{b}	24468±295^{a}	19157±550^{b}	0.025
DC*, L.E**/1000 larvae	-	0.75±0.025^{b}	1.36±0.090^{a}	1.10±0.100^{ab}	0.028

*, Dose cost (DC)= cost of the hormone dose used to produce 1000 larvae. Note:- doses costs / kg brood stock of CPE, 1500 IU hCG, and (750 IU hCG+ CPE) were 15, 27, and 21 L.E, respectively. **, Egyptian pound.

Effect of hormonal treatments on ovaries' chemical composition of African catfish

Chemical composition of ovaries are presented in Table (6). There were significant variations among groups in moisture content which was the lowest with hCG group, while the control group had the highest content in each of moisture and protein. Meanwhile, the lowest protein % was found in the CPE+ hCG-injected group.

Table 6. Ovary chemical composition changing 24 hours after the injection of African catfish

Item	Control (not inject)	Hormonal treatments			*P* value
		CPE	HCG	CPE+ HCG	
Moisture, %	76.59± 0.48^{a}	65.75± 0.74^{bc}	62.55± 1.41^{c}	66.81± 0.64^{b}	0.001
Protein, %	79.78± 0.26^{a}	78.29± 0.35^{ab}	78.09± 0.17^{b}	76.27± 0.63^{c}	0.015
Fat, %	9.79± 0.33^{a}	9.08± 0.47^{a}	8.62± 0.37^{a}	9.05± 0.16^{a}	0.267
Ash, %	7.44± 0.93^{b}	10.14± 0.32^{a}	11.12± 0.37^{a}	12.18± 0.29^{a}	0.014

Values in the same row having different superscripts are differ significantly ($P \leq 0.05$).

Effect of hormonal treatments on testis chemical composition of African catfish

Chemical compositions of testis were presented in Table (7). Contents of moisture, protein, fat and ash were insignificantly ($P \geq 0.05$) affected by different hormonal treatments.

Table 7. Chemical composition of testis 24 hours after the injection of African catfish

Item	Control (not injected)	Hormonal treatments			*P*- value
		CPE	hCG	CPE+ hCG	
Moisture, %	86.96± 1.05^{a}	88.49± 1.30^{a}	85.46± 0.55^{a}	88.37± 0.92^{a}	0.247
Protein, %	69.95± 0.07^{a}	69.11± 1.54^{a}	70.63± 1.17^{a}	71.83± 1.04^{a}	0.448
Fat, %	17.21± 0.71^{a}	17.54± 0.95^{a}	16.85± 1.30^{a}	15.41± 1.59^{a}	0.636
Ash, %	9.85± 0.37^{a}	11.01± 0.25^{a}	10.02± 0.37^{a}	9.77± 0.45^{a}	0.199

Values in the same row having similar superscript are not significantly different ($P>0.05$).

Effect of hormonal treatments on female blood biochemical parameters

Blood biochemical changes 24 hrs after the injection of female were summerized in Table (8). There were significant changes among treatments in the levels of cortisol, cholesterol, triglycerides, liver and kidney function. While, the level of glucose and albumin did not significantly differ between treatments. The highest cortisol level was recorded for the injected female with hCG, CPE, respectively. While, the lowest level was observed in groups of CPE+ hCG and the control. Cholesterol level decreased upon using the hormonal treatments compared to the control, which had the highest cholesterol level. The control group had significantly higher AST than the hormonal injected females. In contrast, the control group had the lowest level of ALT, whereas the highest level of ALT was observed in fish treated with CPE+ hCG and hCG.

Effect of hormonal treatments on male blood biochemical parameters

The change in blood biochemical 24hrs after the injection of male African catfish is recorded in Table (9). Parameters of cortisol, glucose, cholesterol, albumin, triglycerides,

AST and ALT were significantly ($P \leq 0.05$) affected by hormonal injection among groups, while the levels of creatinine and urea were not significantly ($P > 0.05$) affected. The CPE-injected male recorded the highest level of cortisol, while the lowest level was observed in the injected male, with the combination of CPE+ hCG. Values of cholesterol and albumin was not affected by hormonal treatments (CPE, hCG or CPE+ hCG) and these groups were significantly lower than the control.

Table 8. Blood biochemical changing 24 hours after the injection of African catfish female

Item	Control (not injected)	Hormonal treatments			*P*- value
		CPE	hCG	CPE+ hCG	
Cortisol, µg/dl	17.65± 0.45^{b}	58.40± 3.40^{a}	67.00± 4.00^{a}	21.85± 3.55^{b}	0.001
Glucose, mg/dl	127± 3^{a}	122± 2^{a}	120± 2^{a}	116± 3^{a}	0.223
Cholesterol, mg/dl	737± 17^{a}	715± 25^{a}	595± 14^{b}	570± 16^{b}	0.007
Triglycerides, mg/dl	256± 8^{a}	190± 20^{b}	160± 6^{b}	155± 7^{b}	0.011
Albumin, g/dl	6.25± 0.26^{a}	6.80± 0.30^{a}	6.70± 32^{a}	7.10± 0.29^{a}	0.343
AST, U/L	266± 13^{a}	211± 4^{b}	208± 7^{b}	232± 3^{b}	0.016
ALT, U/L	30± 1^{c}	51± 2^{b}	61± 2^{a}	62± 3^{a}	< 0.001
Creatinine, mg/dl	0.39± 0.03^{b}	0.43± 0.03^{b}	0.47± 0.02^{ab}	0.52± 0.02^{a}	0.050
Urea, mg/dl	16.95± 0.83^{b}	20.17± 1.27^{b}	32± 2.90^{a}	27.79± 1.98^{a}	0.016

Average in the same row having different superscripts differ significantly ($P \leq 0.05$).

On the contrary, the highest level of glucose was observed in the control group and was significantly higher than the fish injected with CPE, hCG or CPE+ hCG. No significant differences were noted in triglycerides level between the control and CPE group, and they were higher than the fish injected with hCG or CPE+ hCG. Moreover, the highest level of ALT was recorded for groups injected with hCG or CPE+ hCG, and they were significantly higher compared to the control group and CPE. On the other hand, the hCG-injected males had the lowest level of AST.

Table 9. Blood biochemical changing 24 hours after the injection of African catfish male

Item	Control (not injected)	Hormonal treatments			*P*- value
		CPE	HCG	CPE+ HCG	
Cortisol, µg/dl	33.57± 2.48^{bc}	52.30± 3.20^{a}	39.50± 5.50^{ab}	21.65± 3.95^{c}	0.023
Glucose, mg/dl	138± 4^{a}	121± 3^{b}	123± 3^{b}	129± 2^{ab}	0.036
Cholesterol, mg/dl	657± 13^{b}	751± 20^{a}	768± 13^{a}	782± 17^{a}	0.017
Triglycerides, mg/dl	219± 2^{a}	206± 4^{a}	157± 8^{b}	178± 10^{b}	0.008
Albumin, g/dl	7.05± 0.15^{a}	6.10± 0.16^{b}	6.25± 0.25^{b}	6.27± 0.15^{b}	0.054
AST, U/L	218± 2^{a}	201± 6^{a}	137± 5^{b}	209± 12^{a}	0.004
ALT, U/L	46± 2^{b}	51± 3^{b}	61± 4^{a}	62± 4^{a}	0.006
Creatinine, mg/dl	0.62± 0.01^{a}	0.60± 0.02^{a}	0.61± 0.02^{a}	0.64± 0.03^{a}	0.688
Urea, mg/dl	23.00± 1.00^{a}	20.08± 1.56^{a}	26.83± 1.71^{a}	23.98± 2.56^{a}	0.208

Values in the same row having different superscripts differ significantly ($P \leq 0.05$).

DISCUSSION

In the current study, it was observed that the use of CPE, hCG, and CPE+hCG had successfully induced propagation of African catfish. Moreover, many researchers observed good results when using hormonal induction for the spawning of many fish species especially catfish **(Sharaf, 2005; Mehrim *et al.*, 2014; Saadony *et al*, 2014; El-Hawarry *et al*, 2016; Ahmed, 2018; Moshaand & Mlingi, 2018; Saleh *et al.*, 2020; Zaki & Abd El-Ghaffar, 2020; Zidan *et al.*, 2020)**. The control group didn't spawn or spermiate during the study period in experimental tanks, while fish injected with CPE, 1500 IU hCG/kg or half of CPE+ 750 IU hCG/kg completely spawned and spermiated. Additionally, these results agree with those of **Mehrim *et al.* (2014)**, **Okoye *et al.* (2020)** and **Shokr (2020)** who reported that, using hormonal injection to induce spawning in fish increased blood sex hormones' concentration. In this context, **Biran and Levavi-Sivan (2018)** reported that ,FSH and LH hormones were stored and released from pituitary gland serving as the main regulators of gonadal growth and spawning by stimulating the synthesis of three main sex steroids: estrogen E_2, 11 ketotestosterone (11-KT) and 17α-20β dihydroxy-4-pregnen-3-one (DHP). E_2 acts as the main estrogen and promotes germ-cell proliferation and growth and vitellogenesis.

In the current study, the CPE-treated females showed the highest value of blood FSH, LH and E_2. In the opposite trend, **Mehrim *et al.* (2014)** observed that a high level of blood FSH and LH was found in female African catfish that were injected with (1700 IU hCG/ kg b.w) comparable to the injected female with (import carp pituitary gland, carp pituitary gland and catfish pituitary gland). In addition, **Mahsoub *et al*. (2017)** reported no differences in estradiol concentration among female African catfish injected with CPE, 1200 IU hCG/kg and 0.15ml CPE+ 600 IUhCG/ kg.

During the reproductive cycle in nature, the levels of FSH raise in the blood during (first phase) the gonadal development step and then decrease during the second phase final oocyte maturation and ovulation. Conversely, the LH hormone increases during the second phase and stimulates the ovarian follicle to produce the maturation-inducing hormone, Maturation Inducing hormone (MIH) and17α20β-dihydroxy-4 pregnen-3-one (17α20βP) (DHP) that regulate final maturation and ovulation **(Mylonas *et al.*, 2010; Biran & Levavi-Sivan, 2018)**.

Results of male blood hormone 24 hrs after injecting the hormonal levels in the table (3) showed significantly defferences ($P \leq 0.05$) between treatments in all tested hormone (FSH, LH, E_2, P_4 and T) and the results of serum hormones in fish injected with CPE, 1500 IU hCG/kg or half of CPE+ 750 IU hCG/kg were higher than that observed in the control group. Similar results were observed by **Shokr (2020)** who observed serum levels of FSH, T and E_2 in fish treated with (4, 8 and 12 µg GnRH/kg) were relatively higher than those of control group (non treated fish). In addition, a similar trend was reported by **Saleh *et al*. (2020)** who used hCG at levels of 250, 750, 1500 and 3000 IU/ kg to induce

the spermatozoon of African catfish male. In the same context, this was affirmed in other studies **(Seifi *et al*., 2011; Mousavi & Yousefian, 2012)**.

Testosterone T level was higher in males treated with CPE, hCG or CPE + hCG than in the control group. Males treated with CPE had the highest level of P4, while the lowest level of P4 was found in both the control and hCG group. This may be attributed to differences between individuals, or male catfish being more responsive to CPE injection than hCG or the dose of hCG used may have led to a significant increase in serum steroid hormones and then a feeding-back occurred 24h prior to blood sampling leading to a decrease in P4.

Since sex steroid hormones play a major role in controlling spermiogenesis, E_2 regulates spermatogonial renewal, and 11-KT are produced from Leydig cells under the stimulation of gonadotropin (mainly FSH), and they have a major role in initiating the proliferation of spermatogonia toward meiosis **(Biran & Levavi-Sivan, 2018)**. Our findings revealed that hormonal treatments in female lead to an increase in the blood sex hormone level, fecundity and GSI as compared to the control group. These results concur with those of **Saleh *et al*. (2020)** and **Saadony *et al*. (2014)** who observed that, 3000 IU hCG /kg was the best dose to induce spermiation in *C. gariepinus*. In addition, hormonal induction improved GSI, sperm volume and spermatocrit % **(Shokr, 2020)**. Additionally, **Mahsoub *et al*. (2017)** reported that, GSI were 14.75 - 14.9 and 14.69% with injected females by using CPE- 1200 IUhCG/kg and 0.15ml CPE+ 600 IUhCG/kg, respectively, compared to the control. Furthermore, **Saleh *et al.* (2020)** observed that, the lowest GSI was detected in the control, compared to groups treated with 500, 1500, 3000 and 6000 IU hCG/kg b.w, respectively. On the contrary, **Mehrim *et al*. (2014)** reported insignificant variation in GSI of the African catfish female treated with 1700 IU hCG/kg, imported Argent CP, native CP and catfish PG, respectively.

For the weight of ovaries per female, the control group had the lowest ovaries weight per female, while the highest weight was observed with the hCG-injected females and CPE, respectively. In the opposite trend, **Mehrim *et al.*(2014)** reported that, the females of catfish injected with 1700 IU hCG/kg, imported Argent CP, native CP and catfish PG recorded values for their weight of ovaries with 175 – 205 – 189 and 143 gram, respectively.

In the current study, the highest egg diameter, absolute and relative fecundity were achieved with hormonal treatments (CPE, hCG or CPE+hCG) in comparison with the control. These agreed with **Mahsoub *et al*. (2017)** who reported egg diameter of 1.46, 0.94 and 1.07 mm for female African catfish injected with CPE, 1200 IUhCG/kg and 0.15ml CPE+ 600 IUhCG/kg, respectively. Moreover, **Saadony *et al.* (2014)** found that egg diameter increased from 1.20 to1.43 mm, with increasing the dose of hCG from 1000 IU to 3000 IU. Conversely, **Zidan *et al.* (2020)** noted that, the diameter of the eggs (1.44, 1.31, 1.33 and 1.17mm) was gradually decreased with increasing hCG doses (500, 1500, 3000 and 6000 IU hCG/kg body weight, respectively). During the study period, the

females of the control group did not ovulate. This may be due to an insufficient gonadal hormone that needed to initiate the final maturation and ovulation (**El Hawary *et al.*, 2016)**.

Table (5) reveals the number of larvae/ femeal or per kg of broodstock showing the highest with hCG. This may be due to the significantly longer half-life of HCG, compared to the pituitary extract both in fish and humans **(Ludwig *et al.*, 2002)**. Besides, the pituitary extract contains other non-sex hormones such as prolactin cells and adrenaline cortex, and it was found that some of these hormones have opposite effects on sex gland stimulants such as prolactin and thyroid stimulants. Additonally, our results partially coincide with those of **Mahsoub *et al*. (2017)** who found that, the highest hatching rate was recorded for African catfish injected with 1200 IU hCG/kg, compared to those injected with CPE or 0.15 ml CPE+ 600 IU hCG/kg. However, these results contradict with those of **Zaki and Abd El-Ghaffar (2020)** who demonstrated that, the treated female with 3000 IU of hCG had the lowest number of larvae, compared to those injected with 4mg of CPE. This may be attributed to the increase in the used dose of hCG which led to negative effects and thus decreasing the number of larvae/female. On the other hand, the lowest cost (L.E/1000 larvae) was recorded for the treated fish with CPE.

During fish artificial spawning, biochemical parameters are crucial for adequate monitoring and serve as a representation of the physiological status of the fish (**Suljevic *et al*., 2017)**. Blood biochemical changed 24 hrs after injecting the female and male. In female, there were significantly effects among treatments in level of cortisol, cholesterol, triglycerides, liver and kidney function. While, the level of glucose and albumin showed insignificant differences between treatments. In male, blood biochemical significantly ($P \leq 0.05$) differed among treatments in the levels of cortisol, glucose, cholesterol, albumin, triglycerides, AST and ALT, while the levels of creatinine and urea showed insignificantly ($P > 0.05$) differences among treatments. A study of **Shokr (2020**) showed disturbance in biochemical parameters (glucose, plasma total protein and liver and kidney function (AST, ALT, creatinine and uric acid) levels as a result of using GnRH to induce the propagation of African catfish, whereas he observed an increase in plasma glucose and liver and kidney function, compared to the control group. This finding conforms the results of **Zidan *et al.* (2019)**.

The analysis of serum did not show significant differences among treatments in glucose level of female fish. While in male, control group reflected the highest level in serum glucose compared to the hormonal treated males. These results agree with those of **Zidan *et al*. (2019)** who postulated that, the level of serum glucose decreased in the injected females with 6000 IU hCG/ kg and injected males with 3000 IU hCG/kg (93 and 84 mg/dl), respectively, compared to the control group, when inducing the propagation of African catfish

Notably, cortisol is a main indicator of stress in fish, according to **Tanck *et al*. (2001)** who noted that the amount of cortisol increased during the spawning season. Our results

showed that cortisol level in female was at its highest with hCG and CPE injected groups, respectively, while the lowest level was observed in CPE+ hCG and the control. The injected males with CPE showed the highest level of cortisol, while the lowest level was observed in fish injected with the combination of CPE+ hCG. This was interpreted by **Zidan *et al*. (2019)** who cleared that, cortisol level in males and females of the African catfish increased as a result of inducing the propagation by hCG hormone, compared to the control group. On the other hand, **Mahsoub *et al*. (2017)** observed insignificant differences in cortisol level among females of the African catfish that were injected with CPE, 1200 IUhCG/kg and 0.15ml CPE+ 600 IU hCG/kg to induce the propagation.

With regard to cholesterol level, it decreased in the treated females with hormones compared to the control. In males, the levels of cholesterol and albumin of the injected males with CPE, hCG or CPE+ hCG were significantly lower than those recorded for the control fish. This finding corroborates that observed by **Zidan *et al*. (2019)** who confirmed that cholesterol concentration relatively decreased in the injected males with hCG (250, 750, 1500, 3000 IU/kg), compared to the control group when inducing the spawning of African catfish. Furthermore, **Reading and Sullivan (2017)** reported that, during the reproductive season, the swing in cholesterol concentration occurs due to the use of cholesterol in steroidgenesis, which acts as a precursor to synthesis of steroid hormones. During vitellogenesis and maturation, sex steroid hormones play a vital role. Thus, increasing the size of the eggs leads to a decrease in cholesterol concentration during vitellogenesis. Nevertheless, cholesterol concentration increases again during maturation.

CONCLUSION

Conclusively, hormonal induction is a useful tool to successfully ensure the propagation of the African catfish and producing high percentage of larvae. The present study concluded that, using CPE, hCG, CPE+hCG had successfully induced propagation of African catfish. In addition, the highest number of larvae was obtained in fish group injected with 1500 IU hCG/kg, but inducion with CPE was more economically efficient.

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