

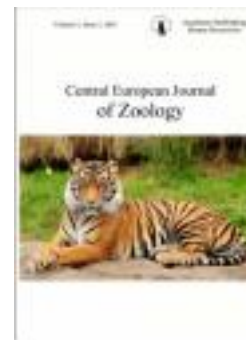
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## Biochemical Changes in Blood of African Catfish (*Clarias Gariepinus* Burchell, 1822) during Artificial Spawning Using Different Doses of Human Chorionic Gonadotropin (HCG) Hormone

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### Abstract

This study was conducted to investigate the effect of different doses injection of human chorionic gonadotropin (HCG) hormone on blood biochemical parameters during artificial spawning of African catfish (*Clarias gariepinus*). African catfish spawners were intermuscularly injected with different doses of HCG (500, 1500, 3000, 6000 IU/kg female), and group is not injected as a control; males were injected at half the female dose. In the present study, who observed that hormonal injection by HCG hormone in male and female African catfish led to disturbance in all biochemical parameters such as serum total protein, glucose level, liver enzymes (AST and ALT activates), cholesterol, and (creatinine and urea) and cortisol concentrations. Serum total protein that recorded in female decreased with increasing HCG dose than that in the control. The lower glucose levels in female were observed with fish injected by 3000 and 6000 IU/kg female. African catfish male that injected with HCG were recorded higher levels of glucose than those obtained in the control group. The levels of serum glucose in male groups were higher than those recorded in female groups. Cholesterol levels were showed relatively decreased in male groups treated with HCG than that in the control group. The levels of ALT in fish that treat with HCG significantly increased than that the control group. Serum cortisol levels were increased in females and males after hormonal injection with HCG as compared to the control group. Under this conditions (the disturbance in biochemical parameters) tested in this study, the highest numbers of fertilized eggs/ female and fertilization rate, number of larvae/female and hatching rate were observed with 6000 IU/ kg female. While the incubation egg with 500 IU/kg female don't showed any hatching larvae. It was observed, increased in reproductive performance with the increase in HCG dosage.

**Keywords:** African catfish, HCG hormone, serum total protein, glucose, liver enzymes (AST and ALT), cholesterol, creatinine, urea and cortisol, fertilization and hatching rate.

### 1. Introduction

African catfish (*Clarias gariepinus*) is one of the most widely produced food fish in the world (Al Dohail, 2005; Sutriana, 2007) and it considered a native fish in all freshwater bodies of Egypt (Saleh, 2007). The advantages that make this species a good choice to aquaculture including faster

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growth rate and its bigger maturity size, easy to reproduce, accepts artificial feeds, tolerates to high stocking densities, adapting with poor water quality, high resistance to disease, lucrative in local regional and international markets, and its economic feasibility in earthen pond culture systems, the most common culture system in East African Community (Teugels, 1986). However, lack of constantly available seed, low egg fertilization rate and low hatching rate are considered as the main obstacles (El-Sayed, 1999). In Africa, by 2004 Clariid catfish production recorded 32077 tonnes and then increased to reach into 208013 tonnes by 2010 meanwhile, Egyptian total production of African catfish increased from 459 tonnes in 2004 to 9719 tonnes in 2010 and then reached into 36487 tonnes in 2016 with 2.13 % of total fish production (FAO, 2012; GAFRD, 2017).

The illustration of the physiological pathway controlling reproduction in fish requires a basic conception of nutrient mobilization toward the gonads and hormonal changes that occur during the reproductive cycle. These fluctuations are affected by nutritional, environmental and social factors furthermore, the size and age of an individual teleost fish. During the reproductive cycle of fin fish, macronutrients such as lipid and protein with high amounts should be transferred to growing oocytes (Jerez et al., 2006).

Biochemical parameters are important for the enough monitoring and are reflection the physiological status of the fish, particularly during artificial spawning of fish (Suljevic et al., 2017). Furthermore, numerous studies have reported that the blood biochemical parameters in teleost fish are affected by sexual maturation, age, species, feeding regime, temperature, reproductive status, induction spawning, stress, diseases and anesthesia (Patriche et al., 2009; Yeganeh, 2012; Shokr, 2020).

Shokr (2020) showed that the increase in the creatinine, uric acid, AST and ALT activities, and level of plasma glucose due to stress in the fish as results of hormonal injection that used to induce spawning, and reported that disturbance in all biochemical parameters such glucose level, plasma total protein, AST and ALT activities and (creatinine and uric acid) concentrations as a result of stress of GnRH injection on African catfish that reflect the disturbance in all metabolic function.

The objective of the present study was, to evaluate the overall effects of different doses injection of human chorionic gonadotropin (HCG) hormone on blood biochemical parameters (serum total protein, albumin, glucose, cholesterol, liver enzymes (AST and ALT activates), creatinine and urea and cortisol) of African catfish (*Clarias gariepinus* Burchell, 1822).

## 2. Materials and methods

The present study was carried out at the Fish Farm in Agricultural Consulting Center, Faculty of Agriculture, El-Fayoum University, Egypt, in August 2019.

African catfish (*Clarias gariepinus*) broodstock used in this study were purchased alive and in good condition from private fish farm, El-Fayoum Governorate, Egypt and then stock and maintained the female fish separated from the male fish in rectangular tanks (3×2×1.2 m<sup>3</sup>), supplied with aerated water, where tanks water was continually replaced for 14 days for fish acclimatization to farm water conditions. Twenty five ripe females and Twenty five ripe males with sex ratio (1:1 male ♂: female ♀) were selected for the breeding experiment. Ripeness of females was determined by external morphological characteristics the females had a soft, distended abdomen and round swollen genital papilla and readiness to spawn. The females were ranged from 505-615 g/fish in body weight and 41.5-49.5 cm/fish in body length, while the males were ranged from 420-670 g/fish in body weight and 42.5-51 cm/fish in body length.

The male and female brooders were grouped into four treatments with five replicates each. African catfish (*Clarias garipains*) spawners were intermuscularly injected with different doses of human chorionic gonadotropin (HCG) hormone, the commercial name is (choriomon®). The doses were 500 (T<sub>1</sub>), 1500 (T<sub>2</sub>), 3000 (T<sub>3</sub>) and 6000 (T<sub>4</sub>) IU/Kg body weight of female and 250 (T<sub>1</sub>), 750 (T<sub>2</sub>), 1500 (T<sub>3</sub>) and 3000 (T<sub>4</sub>) IU/Kg body weight of male and group is not injected as a control. The injection was made in the evening between 5 pm and 6 pm, and after that, the injected females were returned into the containers until the checking for ovulation.

Water temperature around 29.5 °C, pH around 8.15 and dissolved oxygen concentration 6.37 mg/l approximately during the experimental period. Fish were held under natural photoperiod condition throughout the experimental period.

Blood samples were collected from the caudal vein (each female and male) at 12 hrs post injection without anticoagulant then transferred to Wasserman tubes. Blood was allowed to clot at room temperature for 45 min then centrifuged at 3500 rpm for 20 minute to obtain serum sample (Mehrim et al., 2014). The serum samples were pipetted into Eppendorf tube, labeled and stored in deep freeze at  $-20^{\circ}\text{C}$  till assayed. Serum total protein, albumin, glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine were determined enzymatic colorimetric using commercial kits by BioSystem BTS-302 device. Cortisol was quantitatively analyzed using commercial kits by *i*-CHROMA™ Reader System.

Blood biochemical parameters were analyzed as mean  $\pm$  standard error of the mean (S.E.M). The obtained data were subjected to one-way ANOVA. Differences between means were tested at the 5 % probability level using Waller Duncan's test. All the statistical analyses were done using Statistical Package for Social Sciences program (SPSS) for Windows (SPSS, 2015) 23, released version.

### 3. Results

**Blood biochemical parameters of African catfish (*Clarias gariepinus*) female.** The results of blood biochemical parameters that recorded when induced spawning of female African catfish *C. gariepinus* by different doses from HCG hormone were summered in Table 1. The results showed that the induced spawning by different doses of HCG for African catfish had significant effects ( $P \leq 0.05$ ) on blood biochemical parameters (serum total protein, glucose, cholesterol, AST, ALT and urea), while, its showed insignificant differences ( $P > 0.05$ ) between treatments in albumin, creatinine and cortisol.

The results in Table 1 showed that the highest level of serum total protein was observed in T<sub>1</sub> ( $4.5 \pm 0.11$  g/ dl), while the lowest level was recorded in T<sub>3</sub> and T<sub>4</sub> ( $4.1 \pm 0.08$ ,  $3.90 \pm 0.04$  g/dl), respectively, without significant differences ( $P > 0.05$ ) between T<sub>3</sub> and T<sub>4</sub>. In the other hand, treatment No. 2 reflected the highest level of serum albumin ( $1.77 \pm 0.04$  g/ dl) followed by T<sub>4</sub>, control and T<sub>1</sub>, respectively, while T<sub>3</sub> recorded the lowest level with ( $1.62 \pm 0.06$  g/ dl) which were insignificant differences ( $P > 0.05$ ) between treatments in serum albumin.

The control recorded the highest level of glucose with ( $144 \pm 3.05$  mg/ dl) followed by T<sub>2</sub>, T<sub>1</sub> and T<sub>4</sub> ( $102 \pm 2.08$ ,  $93 \pm 1$ ,  $93 \pm 1.15$  mg/dl), respectively, while T<sub>3</sub> reflected the lowest level of serum glucose ( $84 \pm 1$  mg/dl). There were no significant differences ( $P > 0.05$ ) in cholesterol level between all experimental treatments except treatment No. 3 which was recorded the highest cholesterol level with significant ( $P \leq 0.05$ ) differences compare other treatments with ( $325 \pm 1.15$  mg/ dl).

Liver enzymes are substances produced by the liver that can be measured with a blood test. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are two of the enzymes central to test the health of liver function. In this study, the lowest level of AST was observed in T<sub>3</sub> ( $13.8 \pm 1.53$  U/L), while T<sub>1</sub> and T<sub>4</sub> reflected the highest level of AST without significant differences between them ( $88.23 \pm 0.98$ ,  $86.1 \pm 2.35$  U/L). In the other hand, treatment No. 3 reflected the highest level of ALT followed by T<sub>2</sub>, T<sub>4</sub> and T<sub>1</sub> ( $108 \pm 1.52$ ,  $66.4 \pm 0.88$ ,  $49.6 \pm 1.36$  U/L), respectively, while the lowest level of ALT was observed in control ( $40.1 \pm 1.62$  U/L).

Serum creatinine showed the highest level in T<sub>1</sub> ( $0.40 \pm 0.07$  mg/ dl) followed by control, T<sub>3</sub> and T<sub>4</sub>, while the lowest level of serum creatinine was presented in T<sub>2</sub> ( $0.34 \pm 0.01$  mg/dl) without significant differences between treatments. The results from table (1) showed that the highest level of serum urea was observed in T<sub>2</sub> ( $4.8 \pm 0.29$  mg/dl) followed by control, T<sub>1</sub> and T<sub>3</sub> ( $4.3 \pm 0.21$ ,  $3.7 \pm 0.10$ ,  $3.1 \pm 0.24$  mg/dl), respectively, while T<sub>4</sub> reflected the lowest level of serum urea ( $2.5 \pm 0.15$  mg/dl). The results showed no significant differences between treatments in level of serum cortisol, but the highest level was recorded in T<sub>3</sub>, while the lowest level was observed in T<sub>1</sub>.

**Table 1.** Effect of different doses of HCG on blood biochemical parameters of African catfish (*Clarias gariepinus*) female

	Control	Treatments			
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Serum total protein, g/ dl	4.2± 0.15 <sup>ab</sup>	4.5± 0.11 <sup>a</sup>	4.13± 0.03 <sup>ab</sup>	4.1± 0.08 <sup>b</sup>	3.90± 0.04 <sup>b</sup>
Albumin, g/ dl	1.71± 0.11	1.65± 0.11	1.77± 0.04	1.62± 0.06	1.75± 0.04
Glucose, mg/ dl	144± 3.05 <sup>a</sup>	93± 1 <sup>c</sup>	102± 2.08 <sup>b</sup>	84±1 <sup>d</sup>	93± 1.15 <sup>c</sup>
Cholesterol, mg/ dl	223± 3 <sup>b</sup>	226± 36.04 <sup>b</sup>	224± 2.64 <sup>b</sup>	325± 1.15 <sup>a</sup>	241± 1.73 <sup>b</sup>
AST, U/L	75.2± 2.37 <sup>b</sup>	88.23± 0.98 <sup>a</sup>	54.1± 2.09 <sup>c</sup>	13.8± 1.53 <sup>d</sup>	86.1± 2.35 <sup>a</sup>
ALT, U/L	40.1± 1.62 <sup>e</sup>	49.6± 1.36 <sup>d</sup>	108± 1.52 <sup>b</sup>	131± 1.53 <sup>a</sup>	66.4± 0.88 <sup>c</sup>
Creatinine, mg/ dl	0.39± 0.015	0.40± 0.07	0.34± 0.01	0.39± 0.02	0.37± 0.03
Urea, mg/ dl	4.3± 0.21 <sup>ab</sup>	3.7± 0.10 <sup>bc</sup>	4.8± 0.29 <sup>a</sup>	3.1± 0.24 <sup>cd</sup>	2.5± 0.15 <sup>d</sup>
Cortisol, ng/ dl	13± 0.45	12.5± 0.56	13.7± 0.98	14.6± 0.49	14.1± 0.26

- (a, b, c) Average in the same row having different superscripts are differ significantly ( $P \leq 0.05$ ).

Control: without hormonal injection, T<sub>1</sub>: female brood stock treat with 500 IU HCG/kg body weight, T<sub>2</sub>: female treat with 1500 IU HCG/kg body weight, T<sub>3</sub>: female treat with 3000 IU HCG/kg body weight, T<sub>4</sub>: female treat with 6000 IU HCG/kg body weight.

**Blood biochemical parameters of African catfish (*Clarias gariepinus*) male.** The results of blood biochemical parameters of male African catfish *C. gariepinus* that recorded 12 hrs after hormonal injection by different doses from HCG hormone were summered in Table 2. The results showed significantly effects ( $P \leq 0.05$ ) on blood biochemical parameters for male (serum total protein, albumin, glucose, AST, ALT, creatinine, urea and cortisol), while cholesterol showed insignificant differences ( $P > 0.05$ ) between treatments.

The results in Table 2 showed that serum total protein was the highest in each of T<sub>2</sub>, T<sub>1</sub> and T<sub>4</sub> (4.3±0.10, 4.2±0.10, 3.98±0.14 g/dl), respectively, without significantly differences ( $P > 0.05$ ) among these treatments which were significantly ( $P \leq 0.05$ ) higher than the control and T<sub>3</sub> (2.7±0.12, 3.4±0.05 g/ dl), respectively. Data of serum albumin showed that the highest level was recorded in T<sub>1</sub> and T<sub>4</sub> (1.81±0.07, 1.8±0.02 g/dl), respectively, followed by T<sub>2</sub> and T<sub>3</sub> while the

lowest level of serum albumin was recorded in control ( $1.53 \pm 0.03$  g/dl).

Serum glucose was the highest in T<sub>3</sub> ( $128 \pm 1.55$  mg/ dl) followed by T<sub>2</sub>, T<sub>4</sub> and T<sub>1</sub> ( $113 \pm 1.52$ ,  $107 \pm 1.0$ ,  $102 \pm 1.0$  mg/dl), respectively, while the serum glucose was the lowest in the control ( $82 \pm 2.08$  mg/dl). The results showed that no significant differences between treatments in serum cholesterol but the highest level was showed in the control ( $368.67 \pm 105.17$  mg/ dl), while the lowest level was observed in T<sub>1</sub> ( $227 \pm 1.155$  mg/dl).

AST level was the highest in T<sub>2</sub> ( $106 \pm 1.73$  U/L) followed by T<sub>3</sub> and T<sub>4</sub> ( $86.3 \pm 1.82$ ,  $82.2 \pm 1.05$  U/L), respectively, while the lowest was observed in each of the control and T<sub>1</sub> ( $81.1 \pm 1.01$ ,  $78.5 \pm 0.82$  U/L), respectively. ALT was the highest in T<sub>1</sub> ( $75.5 \pm 1.50$  U/L) followed by T<sub>4</sub> ( $58.3 \pm 1.30$  U/ L), while the lowest level of serum ALT was observed in each of T<sub>2</sub>, T<sub>3</sub> and the control ( $40.4 \pm 0.83$ ,  $35.8 \pm 3.10$ ,  $34.6 \pm 1.83$  U/L), respectively, without significant differences between these treatments.

The results in Table 2 showed that the highest level of serum creatinine was showed in T<sub>2</sub> (0.51±0.01 mg/dl) followed by T<sub>3</sub>, T<sub>1</sub> and T<sub>4</sub> (0.48±0.03, 0.47±0.02, 0.43±0.02 mg/dl), respectively, while the serum creatinine was the lowest in the control (0.41±0.02 mg/dl). The highest level of serum urea was recorded in T<sub>3</sub> (6.4±0.10 mg/dl) followed by T<sub>2</sub>, control and T<sub>1</sub> (5.1±0.36, 4.2±0.35, 3.7±0.15 mg/dl), respectively, while the lowest level was showed in T<sub>4</sub> (0.53±0.01 mg/dl). Serum cortisol was the highest in T<sub>3</sub> (14.6±0.47 ng/ dl), while the lowest level was observed in the control (12.3±0.41 ng/dl).

**Table 2.** Effect of different doses of HCG on blood biochemical parameters of African catfish (*Clarias gariepinus*) male

	Control	Treatments			
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Serum total protein, g/ dl	2.7±0.12 <sup>c</sup>	4.2±0.10 <sup>a</sup>	4.3±0.10 <sup>a</sup>	3.4±0.05 <sup>b</sup>	3.98±0.14 <sup>a</sup>
Albumin, g/dl	1.53±0.03 <sup>c</sup>	1.81±0.07 <sup>a</sup>	1.74±0.06 <sup>ab</sup>	1.58±0.02 <sup>bc</sup>	1.8±0.02 <sup>a</sup>
Glucose, mg/ dl	82±2.08 <sup>e</sup>	102±1.00 <sup>d</sup>	113±1.52 <sup>b</sup>	128±1.55 <sup>a</sup>	107±1.00 <sup>c</sup>
Cholesterol, mg/dl	368.67±105.17	227±1.155	248±2.51	238±2.64	257.67±34.86
AST, U/L	81.1±1.01 <sup>c</sup>	78.5±0.82 <sup>c</sup>	106±1.73 <sup>a</sup>	86.3±1.82 <sup>b</sup>	82.2±1.05 <sup>bc</sup>
ALT, U/L	34.6±1.83 <sup>c</sup>	75.5±1.50 <sup>a</sup>	40.4±0.83 <sup>c</sup>	35.8±3.10 <sup>c</sup>	58.3±1.30 <sup>b</sup>
Creatinine, mg/dl	0.41±0.02 <sup>b</sup>	0.47±0.02 <sup>ab</sup>	0.51±0.01 <sup>a</sup>	0.48±0.03 <sup>ab</sup>	0.43±0.02 <sup>ab</sup>
Urea, mg/ dl	4.2±0.35 <sup>c</sup>	3.7±0.15 <sup>c</sup>	5.1±0.36 <sup>b</sup>	6.4±0.10 <sup>a</sup>	0.53±0.01 <sup>d</sup>
Cortisol, n g/dl	12.3±0.41 <sup>d</sup>	14±0.36 <sup>ab</sup>	13.3±0.20 <sup>bc</sup>	14.6±0.47 <sup>a</sup>	13.2±0.26 <sup>bc</sup>

- (a, b, c) Average in the same row having different superscripts are differ significantly ( $P \leq 0.05$ ).

Control; without hormonal injection, T<sub>1</sub>: male treat with 250 IU HCG/ kg body weight, T<sub>2</sub>: male treat with 750 IU HCG/ kg body weight, T<sub>3</sub>: male treat with 1500 IU HCG/ kg body weight, T<sub>4</sub>: male treat with 3000 IU HCG/ kg body weight).

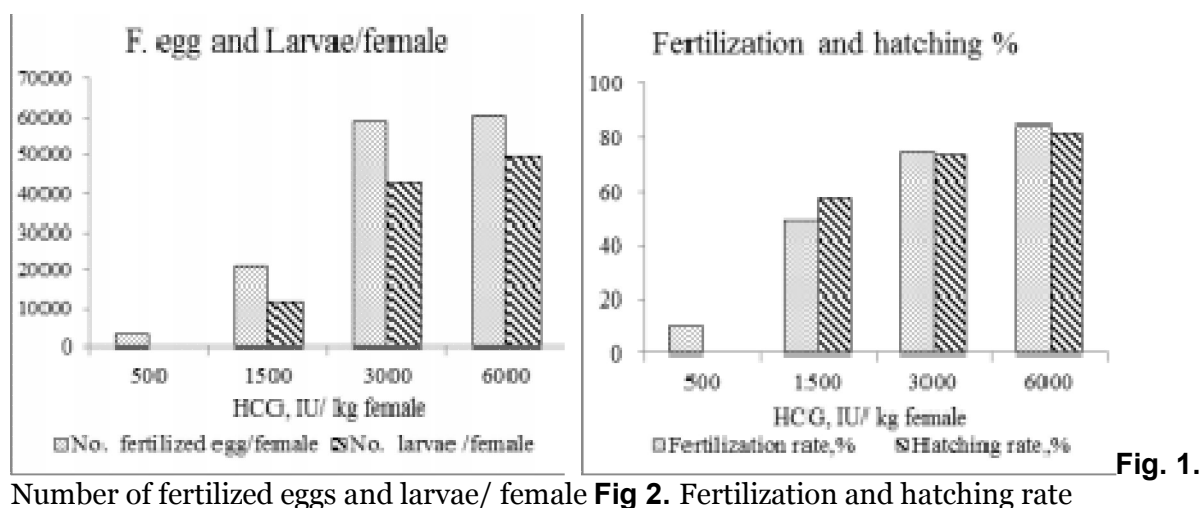
### Reproductive performance

Under the conditions tested for biochemical parameters in this study, the highest number of fertilized eggs/ female was observed with fish group injected by 6000 IU HCG/kg female (60848 fertilized eggs) and the lowest number of fertilized eggs/ female was presented in 500 IU HCG/ kg female (3372 fertilized eggs). The highest number of larvae was observed with 6000 IU HCG/ kg female (49657 larvae) followed by those 3000 IU HCG/ kg female (43177 larvae), 1500 IU HCG/ kg

female (12099 larvae), while the incubation egg in 500 IU HCG/kg female don't showed any hatching larvae are presented in [Figure 1](#).

From the result in [Figure 2](#) the highest fertilization rate was observed with 6000 IU HCG/kg female (84.45 %) and the lowest fertilization rate was presented in 500 IU HCG/kg female (10.15 %). The highest hatching rate was observed with 6000 IU HCG/kg female (81.45 %) followed by those 3000 IU HCG/kg female (73.65 %), 1500 IU HCG/kg female (57.9 %), then 500 IU HCG/kg female (0 %).





Number of fertilized eggs and larvae/ female **Fig 2.** Fertilization and hatching rate

#### 4. Discussion

Blood biochemical analyses give important information related the health of fish in hatcheries in which, strongly correlation between metabolism and reproductive strategies of fish. From this point, it is important prepare the broodstock nutritionally before starting the spawning season. Fish could use carbohydrates for metabolic requires, however lipids are the most important source of energy for the different stages of maturation, and eventually, in the maturation phase, proteins are active participants (Suljevic et al., 2017).

Serum total protein that recorded in female African catfish decreased in this study with increasing in hormonal dose of HCG than that in the control, this similarly with Shokr (2020) who reported decrease in plasma proteins under effect of GnRH injection in male and female of *C. gariepinus* than that in the control group. This decrease in serum total protein may be attributed to the effect of HCG injections on female African catfish to increase growing and development gonads.

In the present study, levels of serum glucose in female groups showed significantly effected and the lower levels were observed with fish injected at 6000 IU HCG/ kg female and 3000 IU HCG/kg female with (93 and 84 mg/dl) respectively, this decrease as compared to control, may be due to the collection samples time. Cahyono et al. (2019) reported that under hormonal stimulation increased glucose level is indicated release of glucose into circulation where, the fish need to elevated energy requirements for oocyte maturation in gonad fish. However, treatments of male African catfish that injected with HCG hormone were recorded higher levels of serum glucose than those obtained in the control group. These results are similar to that recorded in grass carp by Mousavi and Yousefian (2012) and African catfish by Shokr (2020) who reported that there were relatively increased in levels of glucose under hormonal injection as compared to the control. Furthermore, the levels of serum glucose in male groups were higher than those recorded in female groups in this study. These results are in agreement with Mousavi and Yousefian (2012) and Shokr (2020).

The levels of blood glucose have long been used in fish as indicators of stress as recorded by Wedemeyer and Mcleay (1981) and Gross and Wood (1988) who recorded that under status of stress, hyperglycemia may supply additional energy during times need to high metabolic (like "fight or flight" response).

Cholesterol is a main component of formulation steroid hormones, such as progesterone, estrogen and testosterone, which work as the major components of the basic constitutive of cell membranes and energy reserves for reproductive activity (Cahyono et al., 2019). In the present study, there were insignificantly differences in cholesterol levels among female treatments expect T<sub>3</sub>. While, cholesterol levels were showed relatively decreased in male groups treated with HCG hormone than that in the control group.

Cholesterol acts as a precursor to synthesis of steroid hormones in ovarian follicles. Sex steroid hormones play a vital role in vitellogenesis and maturation. So, increase growing the size of the eggs caused reduced in level of cholesterol at vitellogenesis, but cholesterol level increased



again during the maturation process, this fluctuation occur because of use cholesterol in steroidogenesis (Reading, Sullivan, 2017).

In this study, the levels of serum alanine aminotransferase (ALT) in fish that treat with HCG hormone significantly increased than that the control group. In addition, there was significantly change in the levels of Aspartate aminotransferase (AST) in fish injected with HCG hormone as compared to the control group. These results similar to Shokr (2020) who reported that injection of GnRH alone at 4, 8 and 12 µg/kg was effective to induce ovulation in (*C. gariepinus*) and observed that enzymes liver function (AST and ALT) had gradually increased with increase in dose of GnRH injection also, as compared to the control group.

In teleost fish, cortisol is a major hormone that regarding with stress and its level increasing in response to stress (Billard, Gillet, 1981; Sumpter et al., 1986). In the field of endocrinology, working with fish and taking sample of blood to determine the biochemical composition is very important because any handling or even using anesthesia induce stress in fish and affect hormonal level in blood. In The present study, serum cortisol levels were increased in females and males (*Clarias gariepinus*) 12 h after hormonal injection with HCG at 500, 1500, 3000 and 6000 IU/kg female; 250, 750, 1500, and 3000 IU/kg male) as compared to the control group. These results agree with Mousavi and Yousefian (2012) who reported that both male and female grass carp injected with HCG recorded significantly increased in the serum cortisol levels as compared to the control group during the induction period and the high level in female was recorded at 10 h with 13.2 ng/ml while, in male the high level was recorded at 8 h with 12.6 ng/ml. Furthermore, the authors reported that the cortisol levels recorded in females were higher than those observed in males. Tanck et al. (2001) also reported that the level of cortisol increased during the spawning time and stated that in fish the primary stress hormones is cortisol.

Shokr (2020) reported that disturbance in plasma total protein, (AST, ALT activities), glucose level and (uric acid and creatinine) concentrations as a result of stress resulting from hormonal stimulation by GnRH hormone on female and male *Clarias garepinus* reflect the disturbance in whole metabolic function. This similar to the present study, who observed that hormonal injection by HCG hormone in male and female African catfish led to disturbance in all biochemical parameters such as serum total protein, glucose level, (AST and ALT activates), cholesterol, and (creatinine and urea) concentrations. Under this conditions (the disturbance in biochemical parameters) tested in this study, the highest numbers of fertilized eggs/female and fertilization rate, number of larvae/female and hatching rate were observed with 6000 IU/kg female. While the incubation egg with 500 IU/ kg female don't showed any hatching larvae. It was observed, HCG hormone has successfully and accelerate induced spawning in African catfish (*Clarias gariepinus*) and increased in reproductive performance with the increase in HCG dosage and as compared to group not injected.

Biochemical parameters are affected with hormone manipulation that used to stimulate spawning in numerous fish species where, Shokr (2015) reported that administration of follicular stimulating hormone (FSH) and luteinizing hormone (LH) (10, 20 and 40 µg/ kg body weight) in *Oreochromis niloticus* increase blood constituents, plasma total protein, glucose level, creatinine and uric acid level and AST, ALT activities, and increasing their white blood cells. Okoye et al. (2019) reported that induction of spawning in *Clarias gariepinus* by using salmon gonadotropin releasing hormone analogue (GnRH<sub>a</sub>) and ovaprim led to significantly higher serum levels of cholesterol, testosterone, as well as significantly higher ALT and AST activities at different time points after injection as compared to the control group.

Yeganeh (2012) illustrated that cholesterol, protein and 17β-estradiol increased significantly during the period from autumn to spring in common carp fish and indicated that sexual maturation can affect biochemical parameters of blood serum. Chatzifotis et al. (2004) reported maximum levels of serum protein, lipid, triglyceride, and cholesterol during the spawning season in comparison with vitellogenesis and the pre-spawning stage of *Dentex dentex* maturation.

#### 4. Conclusion

From this results the hormonal injection by HCG hormone in male and female African catfish led to disturbance in all biochemical parameters such as serum total protein, glucose level, liver enzymes (AST and ALT activates), cholesterol, and (creatinine and urea) and cortisol

concentrations. Serum total protein that recorded in female African catfish decreased with increasing hormonal dose of HCG than that in the control. Levels of serum glucose in female groups showed significantly effected and the lower levels were observed with fish injected by 3000 and 6000 IU/kg female, this decrease as compared to control. African catfish male that injected with HCG hormone were recorded higher levels of serum glucose than those obtained in the control group. The levels of serum glucose in male groups were higher than those recorded in female groups. Cholesterol levels were showed relatively decreased in male groups treated with HCG hormone than that in the control group. The levels of serum ALT in fish that treat with HCG hormone significantly increased than that the control group. In addition, there was significantly change in the levels of AST in fish injected with HCG hormone as compared to the control group. Serum cortisol levels were increased in females and males after hormonal injection with HCG as compared to the control group. Under this conditions (the disturbance in biochemical parameters) tested in this study, the highest numbers of fertilized eggs/female and fertilization rate, number of larvae/female and hatching rate were observed with 6000 IU/kg female. While the incubation egg with 500 IU/kg female don't showed any hatching larvae. It was observed, HCG hormone has successfully and accelerate induced spawning in African catfish (*Clarias gariepinus*) and increased in reproductive performance with the increase in HCG dosage.

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