Variation of some hormones activity in wild and cultured common carp *Cyprinus carpio*

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Abstract

The activity of growth hormone GH and Thyroid Stimulating hormone TSH in the serum of cultured and wild common carp Cyprinus carpio were determined by ELISA technique. Wild common carp were obtained from Saleh river North Basrah and the cultured carp obtained from two farms, the first was the earth ponds of Marine Science Center (MSC) and the second was Seid Kamel (SK) farm in Qarmat Ali. Results showed that the highest values of GH obtained in fish of SK farm (0.78 \pm 0.13) ng/ml followed by wild fishes (0.37 \pm 0.03) ng /ml and in fish of MSC farm (0.25 ± 0.04) ng/ml. While the TSH showed the highest values in MSC farm (0.36 \pm 0.06) uIU/ml followed by SK farm (0.19 \pm 0.03) uIU/ml and then in the wild fishes (0.06 ± 0.02) uIU/ml. The study showed a clear effect of culture condition and food type on GH and TSH activities, the good management and nutrition in SK farm reflect a high activity of GH which responsible for somatic growth activities, and a medium activity of TSH which incorporated with physiological adaptations to stress which came from captive and high density condition. While the poor diet quality and bad management in MSC farm elevated TSH activity to highest levels.

Key words: Cyprinus capio, cultured, wild, GH, TSH.

Introduction

Carp, *Cyprinus carpio* L. (1758), is a freshwater fish species and has been known as the first cultured species all over the world due to its fast growth rate and easy cultivation

(Yeganeh *et al.*, 2012). Culture of carp back to at least the 5th century BC in China. It is one of the most abundant freshwater fish in the Iraq and the carp culture industry had developed only during the last thirty years (Kitto and Tabish, 2004; Mama, 2012). Many fish ponds and farms had increasingly established mainly in the middle and the northern part of south of Iraq (Mhaisen, 1993). Carp culture became the dominant cultured fish species in most fish farms and common in most inland water bodies of Iraq (Mama, 2012). The increased production of common carp has raised of the cultured fish in comparison with the wild fish (Yeganeh *et al.*, 2012; Kito and Tabish, 2004).

Growth hormone GH (also called somatotropin) is produced and secreted from cells in the anterior part of pituitary gland, somatotrophs called and it secretion under regulation of the hypothalamus (Jonsson and Bjornsson, 2002). The hormone form a 22 kDa protein consists of about 190 amino acid residues. and the number varies only slightly between species within the major vertebrate (Harvey et al., 1995). In fish. GH participates in almost all major physiological processes in the body including the regulation of ionic and osmotic balance, lipid, carbohydrate protein. and metabolism, skeletal and soft tissue growth, reproduction and immune function (Reinecke et al., 2005). It is involved in osmoregulation growth, and reproductive functions, as well as carbohydrate, lipid, in and metabolism protein and

behavioral functions including appetite, foraging behavior, aggression, and predator avoidance, which in turn has ecological consequences (Bjornsson, 1997; Bjornsson et al., 2004; Perez-Sanchez, 2000; Peter and Marchant, 1995). A clear relationship exists between nutritional status and GH levels (Le Bail and Boeuf, 1997). Himick and Peter (1995) found that in goldfish plasma GH increased after 30 min. from the beginning of meal.

Growth in fish is regulated by the growth hormone/insulin like growth factor I (GH/IGF I), GH secreted into Circulation system by the pituitary and binds its liver (GHR) receptor in to hepatic IGF-I stimulate production, which systemically drives somatic growth in normal and hyper anabolism and exerts negative feedback on GH secretion, lipolysis is an alternate function of GH during catabolism (Won and Borski, 2013).

Thyroid-stimulating hormone TSH (also called thyrotropin) is a 28- to 30-kDa glycoprotein synthesized and secreted from thyrotrophs of the anterior part of pituitary gland and stimulated by thyrotropin-releasing hormone (TRH) secreted from hypothalamus and inhibited by thyroid hormone in a classical endocrine negative-feedback loop (Qu et al., 2016). It is a key proteins in the control of thyroid

function by stimulating thyroid hormones production, Thyroxin Tri-iodothyronin and (Szkudlinski et al., 2002; Abbott and Volkoff, 2011). TSH is a kind of heterologous dimmers glycoprotein hormones, which is composed of two noncovalently associated subunits, α and β . The TSHα subunits is promoting follicular hormone (FSH) and gonadal hormone (CG). The TSHβ gene is specific to the gland pituitary and is significantly higher in immature fish than in mature fish (Li et al., 2011; Lema et al., 2008). the clinical detection of serum TSH level is mainly used to evaluate the function of hypothalamic pituitary thyroid axis (Yu et al., 2014).

Thyroid hormones of fish are small molecules very similar to other vertebrates. They are called Thyroxin T4 and Triiodothyronine T3. In fish T3 is the most effective biological thyroid hormone (Baldisserotto et al., 2007). Thyroid hormones are important to control development, reproduction, growth, metabolism and osmoregulation in fish and often do these activities in association with other hormones such as cortisol and growth hormone (Evans, 1997, Peyghan et al., 2013). Nutritional status has a strong influence on thyroid function in fish. The reduction of food ration, or fasting, decreases

the sensitivity of the thyroid tissue to TSH, and hepatic deiodinase activity. These two phenomena induce a decrease in circulating T3, and T4. (Le Bail and Boeuf, 1997; Darras et al., Leatherland 1995). (1994)suggested that T3 as an influence hormone in intermediate metabolism could act as а permissive factor which would facilitate the direct action of other anabolic hormones involved in food intake control. Pohlenz et al. (2013) inform that thyroid (TH) and growth (GH) hormones. and insulin-like (IGF-I) are growth factor I anabolic regulators in fish and responsive to nutrient intake.

Rearing under culture implies conditions radical modifications of the organism's environment and often deliberate manipulations of its biology, e.g. through breeding. selective Cultured fish thus enter a process of domestication, a process of developmental genetic and change in response to culture. Domestication gives rise to organisms that perform

better under culture conditions than their wild conspecifics and may express additional attributes desired by culturists (Lorenzen *et al.*, 2012).

On the other hand survival conditions and activities used during aquaculture practices cause stress (acute or chronic) and can involve a reduction of

welfare, the mainly relevant factors for the welfare reduction of farmed fish can be listed as following: genetic, environmental factors, stocking during growth, density starvation. malnutrition. deformities, transport, handing, selection, overcrowding (Conte, 2004, Elkaldi, 2010).

No reports have yet been published about the differences in hormones activity between the farmed and wild common carp. This study aim is to understanding the growth hormone and thyroid stimulating hormone activities differences wild and cultured between common carp C. carpio.

Materials and Methods Experimental groups

Three populations of common carp *Cyprinus carpio* have been studied. The farmed fish obtained from two farms, Marine Science Center (MSC) farm – University of Basrah and Seid Kamel (SK) farm in Qarmatt Ali and the wild fish obtained from Saleh River north Basrah.

The ponds area in MSC farm is about 2.5 - 7.5 Km², irrigated with tap water came from the desalination station in Qarmatt Ali, the farm use the monoculture system in fish cultivation, fish were fed a sink artificial diet twice daily by hand in a percentage of 4% from body weight. While the ponds area in SK farm is about $5 - 15 \text{ Km}^2$, irrigated from Al-Mishab River in Qarmatt Ali and they used the monoculture system, fish were fed a float commercial diet manufactured in Kuwait three times daily by hand in a percentage of 5% from body weight.

Fishes from MSC farm were collected at the morning of 25/10/2016 and fishes from Seid Kamel (SK) farm were collected at the morning of 4/11/2016. The wild fish collected from Saleh River north Basrah region at the morning of 6/11/2016. A total of 60 fish were collected from each site and were held alive in cooling boxes and transferred to laboratory.

Length and weight measurements

Length (cm) and weight (gm) were recorded after fish anesthetized by clove oil by putting fish in container and adding clove oil to the water (Durvill and Collet, 2001).

Blood sample collection

Blood samples were collected from the heart for collecting serum by a 3 ml syringe. The blood samples were immediately transferred to sterile tubes and left in room temperature for half an hour and then the serum was separated by centrifugation at 3500 rpm for 5 minutes to separate the serum from the clot using a universal centrifuge model (PLC-036).

Growth and Thyroid hormones measurements

Growth hormone GH. Thyroid stimulating Hormone TSH were measured by enzyme linked immunosorbent assay (ELISA) method using a commercially available kits. manufactured bv (bioactive diagnostica, Germany, kit name hGH ELISA. number BDGH19-BA) for GH and (Foresight, kit number 1231-3011) for TSH. Absorbance was read at 450 nm in ELISA reader (Huma reader HS, Human, Germany).

Four environmental factors (temperature, dissolved oxygen, salinity, pH) were measured daily using the YSI instrument model (556 MPS).

Statistical analysis

One - way analysis of variance (ANOVA) and Revised Least Significant Difference (RLSD) were used. (P < 0.05) was set as the significance level using SPSS program. Values were expressed as the (mean \pm S.E.M.) (the standard error of the mean) (Alhakim, 2000).

Results

1: Experimental groups

The total length and weight of fishes were $(13.13 \pm 0.23 \text{ cm})$, $(32.56 \pm 1.8 \text{ gm})$ respectively for MSC farm; $(17.50 \pm 0.51 \text{ cm})$, $(115.15 \pm 9.61 \text{ gm})$ respectively for SK farm and $(17.96 \pm 0.56$ cm). (80.12) ± 4.87 gm) respectively for wild fish (Saleh River). Table (1) shows the environmental factors of water in the three stations such as temperature, salinity, pH and dissolved oxygen.

Environmental factor	MSC	SK	Wild
Temp. c ^o	25.67 ± 0.71	19.87 ± 0.76	18.6 ± 0.35
Sal. g/L	3.74 ± 0.07	2.73 ± 0.09	1.77 ± 0.09
рН	7.17 ± 0.03	7.83 ± 0.03	8.13 ± 0.09
D.O. mg/L	3.47 ± 0.15	6.23 ± 0.09	8.67 ± 0.12

Table (1): Environmental factors of water in MSC, SK cultures and wild (Mean \pm S.E.).

Fishes from MSC farm were fed on artificial diet consist of: fish meal 25%, soya been 25%, corn 10%, barley 10%, wheat bran 10%, wheat flour 20%, vitamins and minerals 0.5%.

Fishes from SK farm were fed on a float artificial commercial growing carp feed (Abdul Rahman Sultan and Sons Agricultural and Product animal Kuwait, Feed, Email: info@Keaaf.com) consist of following ingredients: Yellow Maize, Wheat, Soybean, Rab Seed, Animal Fat, Yeast, Sault,

Fish meal, Fish oil, Sorghum, Faba bean, Beans. Mono Decalcium Phosphate, Decalcium Phosphate.

Table (2)showed the chemical composition of the artificial diets used for common carp fed in MSC farm which were analyzed in the chemical analysis laboratory in the vertebrate department in marine science center. The chemical composition of the commercial diets used in SK farm were taken from the leaflet over the diet cover.

Nutritional factor	MSC	SK*, **, ***
Moisture %	8	9
Protein %	12	30
Fat %	5.1	3.5
Ash %	32	25
Carbohydrate %	42.9	32.5

Table (2) Chemical composition of fed used for MSC and SK farms.

* Trace elements for diet used in SK culture were: Iron (900 mg), Copper (300 mg), Manganese (1200 mg), Zinc (1400 mg), Iodium (40 mg), Selenium (8 mg).

** vitamins for diet used in SK culture were: Vit. A (160000 IU), Vit. D3 (3333.333 IU), Vit E (166.670 mg), Vit. K3 (26.671 mg), Vit. B1 (12.500 mg), Vit. B2 (66.667 mg), Vit B3 (200 mg), Vit B6 (20.00 mg), Vit. B12 (160 mg), Folic Acid (6.660 mg), Niacin (600 mg), cholin Chloride (6000 mg), Biotine (2 mg).

*** Minerals and Amino acids for diet used in SK culture were: Phosphorus: 4.150%, Calcium: 6.00 5, Sodium: 2.6%, Chlorine: 3.58 %, Lysine: 2.41%,, Methionine: 5.20%, Methionin + Cystine: 5.51%, Threonine : 1.24%, Arginine: 1.76%, Valine: 3000.150 Kcal/kg

2: Growth Hormone (GH) level

Table (3) and figure (1) shows the concentration of GH (ng/ml) in the serum of the cultured and wild *C. carpio*. Results showed that the highest values of GH obtained from fish from SK farm $(0.78 \pm 0.13 \text{ ng/ml})$, followed by the Wild fish from Sale River $(0.37 \pm 0.03 \text{ ng/ml})$ and the lowest activity in GH showed in fish from MSC farm $(0.25 \pm 0.04 \text{ ng/ml})$.

Statistical analysis showed a significant differences (P < 0.05) between SK culture and other groups in GH activity, but there were no significant differences (P > 0.05) between wild fish and fish in MSC farm.

Table (3): Growth hormone GH (ng/ml) and thyroid stimulating hormone TSH (uIU/ml) concentrations in the serum of wild and cultured *C. carpio* (mean \pm S.E.)

Station	GH	TSH
MSC	0.25 ± 0.04	0.36 ± 0.06
	В	a
SK	0.78 ± 0.13	0.19 ± 0.03
	А	b
Wild	0.37 ± 0.03	0.06 ± 0.02
	В	b

*The capital letters (A,B,C) means a significant differences in GH activity among the groups of wild and cultured *C. carpio*.

**The small letters (a,b,c) means a significant differences in TSH activity among the groups of wild and cultured *C. carpio*.



Figure (1): Growth hormone GH concentration (ng/ml) in the serum of *wild* and cultured *C. carpio*.

2: Thyroid stimulating hormone (TSH) level

Table (3) and figure (2) shows TSH concentration (uIU/ml) in the serum of the cultured and wild *C. carpio*. Results showed that the highest values of GH obtained from MSC culture (0.36 \pm 0.06 uIU/ml), followed by SK culture (0.19 \pm 0.03 uIU/ml) and the lowest concentration in TSH obtained in the wild fish (0.06 \pm 0.02 uIU/ml).

Statistical analysis showed a significant differences (P < 0.05) between MSC culture and other groups in GH activity, but there were no significant differences (P > 0.05) between wild fish and SK culture.



Figure (2): Thyroid stimulating hormone TSH concentration (uIU/ml) in the serum of *wild* and cultured *C. carpio*

Discussion

Results of our study indicated that the good quality of the artificial diet and the good managements in SK culture had a clear positive effect on *C. carpio* welfare by showing the highest values of GH activity ($0.78 \pm$ 0.13 ng/ml) which have a clear relationship with the nutritional status at the fish (Le Bail and Boeuf, 1997), so the cultured *C. carpio* in SK culture showed better GH activity from the wild fish because of the use of special float diet for growing carp. While the poor quality of diets in MSC culture caused the lowest value obtained of GH activity ($0.25 \pm$ 0.04 ng/ml). Our result was in agreement with the study of Le Bail and Boeuf (1997) which found that hormones like (GH, TH, and leptin) had long term effect on bettering food intake behavior and growth in rainbow trout. Himick and Peter (1995) found that GH increased 30 min after the beginning of the meal in plasma of goldfish.

Conversely, many studies like (Sumpter et al., 1991; Farbridge and Leatherland, 1992; Pérez-Sanchez et al., 1994; Peter and Marchant, 1995; Toguyeni et al., 1996).found that plasma GH increased when food ration size decreased and reached its highest values when fish were starved after a prolonged treatment. The study of Small et al. (2002) confirm that short term fasting in striped bass increases their circulating levels of GH. They also demonstrates that pituitary GH mRNA expression increases during fasting, which suggests a corresponding increase in pituitary GH synthesis.

The high levels of GH may act as an appetite stimulatory factor and the peripheral injections of GH stimulate food intake in several teleosts species (Donaldson *et al.*, 1979; Le Bail *et al.*, 1993, Peter and Marchant, 1995). Johnsson *et al.* (1996) suggested that, in rainbow trout, the increase of food intake was as a result of the GH stimulation of appetite and competitive ability.

Results for TSH in our study showed an arise of TSH activities in MSC farm (0.36 \pm 0.06 uIU/ml), followed by SK farm (0.19 \pm 0.03 uIU/ml) and the lowest concentration obtained in

the wild fish (0.06) \pm 0.02 This uIU/ml). indicate the multipurpose physiological functions of TSH in fish body. So the cultured fish in the tow farms gave higher TSH concentrations from the wild fish, this may be contributed the to many aquaculture practices that don in the farm managements causing higher stress on farmed fish than the wild Our results in agreement with the study of Qu et al. (2016) they mentioned that Cultured fish often respond to environmental stresses and the factors of this stress have a main effect on the growth of fish by changing the physiological and biochemical status of fish body for a long time, resulting a neuroendocrine changing. Results of them showed that there are differences in TH and TSH concentration among the three sturgeon populations. And they suggested that the sturgeon thyroid endocrine system is relatively complex the and environment factors could interfere with the normal physiological function of the thyroid, and have direct or indirect influence on the regulation of growth, development, reproduction of fish.

Many studies showed a relationship between food intake and thyroid hormones activity. The study of Setkow *et al.* (1996)

demonstrated that secretion of T3 from thyroid was increased in starved trout in percentage of 75-81% in comparison with fed fish. Eales and Brown (1993) found an opposite situation in the same fish, however, the drop in thyroid normally hormones occurs several days after the beginning of the starvation. While some studies found no relationship between appetite, time of meal or level of food ration and T3 during a daily period (Farbridge and Leatherland, 1993; Gomez et 1997). All of al.. these observations identify TH as don't having a short-term role in the control of food intake but may be had a long term effect . Lastly, as most aspects of the thyroid hormones influence intermediary metabolism, Leatherland (1994) suggested that T3 could act as a permissive factor which would facilitate the direct action of anabolic other hormones involved in food intake control. That may be explain the elevated TSH activity in the MSC culture which had a poor diet quality and bad management resulting low levels of environmental factors to water quality in the ponds.

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التغيرات في فعالية بعض الهرمونات في مصل دم اسماك الكارب الشائع .Cyprinus carpio L النهرية و المستزرعة.

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المستخلص

تم قياس فعالية هرمون النمو GH والهرمون المحفز للثايرويد TSH في مصل دم اسماك الكارب الشائع Cyprinus carpio النهرية والمستزرعة بتقنية الالايزا. جلبت اسماك الكارب الشائع النهرية من محطة نهر صالح في شمال البصرة, بينما استحصلت الاسماك المستزرعة من مزرعتين الاولى هي مزرعة مركز علوم البحار MSC والثانية هي مزرعة سيد كامل SK الواقعة في مدينة الكرمة.

اظهرت النتائج ان القيم الأعلى لهرمون النمو كانت في مزرعة سيد كامل ng/ml (0.13 ± 0.13) الظهرت النتائج ان القيم الأعلى لهرمون النمو كانت في مزرعة مركز علوم البحار (0.06 ± 0.06) تبعتها الأسماك النهرية ng/ml (0.06 ± 0.03) ومن ثم مزرعة مركز علوم البحار امتلكت القيم ng/ml. بينما اظهرت نتائج الهرمون المحفز للثايرويد ان مزرعة مركز علوم البحار امتلكت القيم ng/ml (0.06 ± 0.05) والأخيرة هي الأعلى nJ/ml (0.06 ± 0.06) ومن ثم مزرعة سيد كامل nJ/ml (0.06 ± 0.06) والأخيرة هي الأسماك النهرية السرائي النهرية 10.00 الأعلى الأسماك النهرية الأسماك النهرية المراحين المحفز الثايرويد ان مزرعة مركز علوم البحار المتلكت القيم ng/ml

اظهرت الدراسة تاثيرا واضحا لظروف التربية ونوع العليقة المقدمة على فعاليات هرموني النمو والمحفز للثايرويد فالادارة والتغذية الجيدة في مزرعة سيد كامل عكست فعالية عالية لهرمون النمو المسؤل عن فعاليات النمو الجسمي وفعالية متوسطة لهرمون المحفز للثايرويد الذي يشارك بفعاليات التكيف الفسلجي للجهد الناتج عن عمليات الحجز وكثافات الاستزراع العالية. بينما نوعية العليقة الفقيرة والادارة السيئة في مزرعة مركز علوم البحار رفعت فعالية الهرمون المحفز للثايرويد الى اعلى مستوى مقانة مع المحطات الاخرى.