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Test two sex hormone values with human clinical medicine Science for common carp (*Cyprinus carpio*) and crucian carp (*Carassius auratus auratus*)

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Abstract: The aims of this study were to detect the clinical chemical digital values of two sex hormones in common carp and crucian carp by treatment with the Chemiluminescent Immunoassay (CLIA) of human medicine. The digital values of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were detected.

Keyword: common carp, crucian carp, hormone.

INTRODUCTION

Test of Fish blood was prevalent used in fisheries management and disease diagnosis. Hormones play an important role in the regulation of metabolism, reproduction, growth, and development in vertebrates¹, including fish². Similar to mammals³, hormone synthesis of teleost is also regulated by feedback regulation through the hypothalamus–pituitary–thyroid (HPT) axis. The regulation of hormones homeostasis involves multiple steps, including iodine uptake, hormones synthesis, transport, deiodination, and binding to hormone receptors. Therefore, compounds that interact with any of these steps may interrupt the balance of hormones.

Common carp (*Cyprinus carpio*) was brought to the United States in 1831. In the late 19th century, they were distributed widely throughout the country by the government as a food-fish, but they are no longer prized as a food-fish. As in Australia, their introduction has been shown to have negative environmental consequences, and they are usually considered to be invasive species. Millions of dollars are spent

annually by natural resource agencies to control common carp populations in the world⁴. *Cyprinus carpio*, a species of *Cyprinus carpio*, which is native to the Eurasian continent but has been distributed in the waters of the world and has become an invasive species in many areas and has been listed as one of the world's most invasive species one. Cyprinidae (*Cyprinidae*) is named after its name. The fish is a primary freshwater fish, habitat in the middle and lower reaches of the river, lakes, reservoirs and other water still areas, especially like the bottom of the eutrophic or aquatic plants of the waters. Strong tolerance of the environment can survive in the low temperature and dissolved oxygen, lively and good-jump. There is omnivorous, with algae, aquatic plants and benthic animals for food⁵.

Common carp (*Cyprinus carpio*) originating in the size of the river in Asia, due to have delicious, disease resistance, rapid growth, easy to breed four major advantages, has been human breeding for more than two thousand years, the annual output of more than ten million tons, Is one of the important edible fish in Asia. Carp in the spring water temperature of 20 °C, the choice of plants on the river bank, in the aquatic plants under the sticky eggs, after a few days to hatch. Carp for the omnivorous fish, hilips stirring river bed soil, feeding algae, aquatic plants, aquatic insects and snails, clams and other benthic animals. Male more than 1 year old, more than 1 year old half of the female, you can breed the next generation, the beginning of only more than ten million eggs. Seven-year-old species of fish weighing about 10 kg the amount of fecundity up to one million or more. Carp for decades of life, coupled with sexual pleasure, there is the habit of carp leaping gantry, since ancient times as human favorite, is regarded as auspicious fish, Japan and even carp mutant "Koi" as national fish. The annual output of common carp (*Cyprinus carpio*) is more than 40 million tons in Taiwan⁶.

Crucian carp (*Carassius auratus auratus*) is omnivorous fish, but adult fish mainly to plant food. Because the plant feed is rich in water, the variety of each variety, for the wide range of food intake. The stems, leaves, buds and fruits of the weeds are the food of the Crucian carp. In the waters of the higher aquatic plants, the crucian carp is the most abundant nutrient. Diatoms and some algae are also carp food, shrimp, earthworms, young snails, insects and so they are also very fond of eating. Feeding time of crucian carp varies from season to season. Spring for the feeding season, day and night are constantly feeding; summer feeding time for the early, late and night; autumn all day intake; winter in the noon before and after feeding. Living in the river flowing water in crucian carp like the cluster line. Sometimes with water, sometimes against the water, to the lush vegetation, river, ditch branch, reeds in the search, spawning; encountered slow or static water flow, with rich food places, they temporarily habitat down. Living in lakes and large reservoirs of crucian carp, but also choose to eat and live. Especially in the shallow aquatic plants clustered, but also their concentration, even in the winter, they are eager to grass, most do not swim to the grass without deep water at the winter. Living in small rivers and ponds in the crucian carp, they are the flow of that line, no flow so far. crucian carp can dive into the depth of winter.

Analysis of the breeding of Crucian carp in 2015 is as follows: In 2015, the national carp into the fish out of the pond price, out of the pond, out of Tang income compared with the same period last year, are less volatile, but the price difference between the larger, the lowest 10.16 Yuan / kg and the highest 16.9 Yuan / kg. The overall increase in the prices of fish and poultry stocks increased significantly, feed prices decreased slightly, rents and labor costs increased, resulting in significant increase in the total cost of farming, but because the Crucian carp for large-scale, high-yielding breeding of fine varieties, so it is still aquaculture Important selection of 2016 production forecast. Combined with the above analysis, the current breeding of carp, although significantly affected by the impact of farming costs, farmers income is

weak, but the demand for stable prices did not appear large fluctuations, coupled with farmers farming habits, is expected to 2016 farming scale change is not Continue to maintain the current price.

Crucian carp (*Carassius auratus auratus*) was one fish with high economic value in Taiwan. Now, in freshwater fish market, crucian carp is the second to glass carp, it has become the second largest consumer species. Polyculture farming ponds and monoculture farming ponds all have many number in Taiwan, and the adult fish mainly support the domestic fish consumption that is very common thing⁷.

In 2015, collection points are the two carps breeding, mainly freshwater pond culture, and the country collected a total of freshwater pond culture an area of 21.9 million mus, which the two carps breeding area of 11.21 million mu, or 51.2% of freshwater ponds have the two carps breeding. The annual output of freshwater fish was 9.13 million tons, of which 13,000 tons of the two carps yielded 14.24% of the output of freshwater fish. According to farming fishery report data, combined with production research⁶.

CLIA instrumental analysis, is a high degree of wisdom of its operation, high sensitivity, specificity, precision and accuracy can be higher than the RIA, in particular, to detect high flexibility, fast and simple, stable and easy detection reagent indoor and compartment quality control, has tended to replace RIA immunoassay become widely used analytical techniques in hospital⁸. Use the principle of immunization as a means of analysis of the antigen and antibody interaction to achieve the goal. Most of the antibodies in the serum, the majority of a polymer protein. Subdivided, can be divided into IgG, IgM, IgG, IgD and so on. Antigens and antibodies usually have a strong ability to bind. For example: the body of antibodies against a certain kind of bacteria produced, the bacteria produced by the substance or the bacteria itself are the antigen. When the bacteria invade the body, leading to antibody hyperplasia in the body, it will be obvious antibody antigen binding reaction. We often use this reaction in the laboratory to detect very little material. With its sensitivity can reach $10^{-8} \sim 10^{-9}$ meter (\sim nM) concentration, can be checked out.

A chemiluminescence enzyme immunoassay based on magnetic microparticles (MmPs-CLIA) was developed to evaluate serum α -fetoprotein (AFP) in parallel with traditional colorimetric enzyme-linked immunosorbent assay (ELISA). A systematic comparison between the MmPs-CLEIA and colorimetric ELISA concluded that the MPs-CLEIA exhibited fewer dosages of immunoreagents, less total assay time, and better linearity, recovery, precision, sensitivity and validity. AFP was detected in forty human serum samples by the proposed MPs-CLEIA and ELISA, and the results were compared with commercial electrochemiluminescence immunoassay (ECLIA) kit. The correlation coefficient between MPs-CLEIA and ELISA was obtained with $R^2=0.6703$; however, the correlation between MPs-CLEIA and ECLIA ($R^2=0.9582$) was obviously better than that between colorimetric ELISA and ECLIA ($R^2=0.6866$)⁹. CLIA has more accuracy detected value than ELISA and RIA.

In the presence of complimentary antigen and antibody, the paratope of the antibody binds to the epitope of the antigen to form an antigen-antibody or an immune complex. Estimating the levels of such immune complex was used with labeled antibodies form the basis of CLIA (Chemiluminescent Immunoassay). It involves use of stationary solid particles coated either with the antigen or antibody of interest. Post incubation, which ensures intact immune complexes are formed, substrate is added. These results in generation of light, the intensity of which is directly proportional to the amount of labeled complexes present and which indirectly aids in quantification of the analysis of interest. The intensity of light is measured in terms of Relative Light Units (RLU). The main advantage of this technology includes sensitivity, specificity and its ability to be unaffected by background signals. Also, the analyzers working under this principle are simple in design and operation¹⁰.

In both sexes, Luteinizing Hormone (LH) stimulates secretion of sex steroids from the gonads. In the testes, LH binds to receptors on Leyden cells, stimulating synthesis and secretion of testosterone¹¹. Theca cells in the ovary respond to LH stimulation by secretion of testosterone, which is converted into estrogen by adjacent granulosa cells. In females, ovulation of mature follicles on the ovary is induced by a large burst of LH secretion known as the preovulatory LH surge. Residual cells within ovulated follicles proliferate to form corpora lutea, which secrete the steroid hormones progesterone and estradiol. Progesterone is necessary for maintenance of pregnancy, and, in most mammals, LH is required for continued development and function of corpora lutea. The name luteinizing hormone derives from this effect of inducing luteinization of ovarian follicles.

As its name implies, Follicle-Stimulating Hormone (FSH) stimulates the maturation of ovarian follicles. Administration of FSH to humans and animals induces "superovulation", or development of more than the usual number of mature follicles and hence, an increased number of mature gametes. FSH is also critical for sperm production. It supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation. But the values of LH and FSH are not detected in fishery field till now. Fishery field can only be qualitative analysis of LH and FSH in the world, now.

Many hormones of human have been examined very precise with automatic digital recording instruments for many years, and a large number human blood tests in general hospitals every day in Taiwan. It is an extremely easy and convenient thing for mankind.

In this study, we wished to detect the two hormones value of Common carp and crucian carp blood with human medicine Chemiluminescent Immunoassay (CLIA) examining procedures.

Materials and methods Variety selection: In the choice of varieties, we can choose the kind of rapid growth, high yield, suitable for our region and a strong breeding capacity of high quality varieties.

Breeding conditions: Pond area of 3 acres -5 acres, water depth of 1.5 meters -2 meters, to ensure adequate water, stable, non-polluting, good water quality, pond irrigation and drainage convenient, impermeable not leak, the bottom of the mud to maintain the thickness of 10 cm -15 cm , Each pond is equipped with equipment such as aerator.

Acclimation

1. It is relatively easy to train domesticated species. Domesticated species in the adult fish when the floating snatching time is short, easy to manage.
2. Domestication in the feed to add the right amount of additives can shorten the acclimation time, increase the feeding area and the number of carp.
3. The effect of density on domestication. Large density number of groups will to be domestication. When the density has been determined cannot be changed, can reduce the pond water level, the relative increase in density, it is conducive to domestication and can improve the pond water temperature, promote fish growth.
4. With the silver carp, fish should be in the main raising carp floating after the formation of the habit of eating.
5. Domestication of the water should be "thin" should not "turbidity".
6. Domestication managers need to be patient.

EXPERIMENT

Breeding: 60 healthy crucian carp average weight about $(267.5 \pm 0.71\text{g})$, 60 healthy common carp average weight about $(825.6 \pm 0.75\text{g})$ had been raised for twenty-one days in 500 liters barrel, took a warm equipment in the barrel, fed with the commercial eel's fodder, and about 10 for every morning, collected fodder remained and fish's excrement of the twenty-one days artificially, to avoid pollution of water, and the water temperature was about 25°C in twenty -one days. Stopped feeding for more than 24 hours before the experiment begins^{12,13}. 120 fish (60 crucian carp average weight about $(267.5 \pm 0.71\text{g})$, 60 common carp average weight about $(825.6 \pm 0.75\text{g})$ and, had been raised for 21 days in 500 liters barrel, took a warm equipment in the barrel, fed with the commercial eel's fodder while raising and acclimatizing, and about 10 for every morning, collected fodder remained and fish's excrement of the last day artificially to avoid pollution of water, and the water temperature was about 25°C in 21 days. Stopped feed for more than 24 hours before the experiment.

Obtaining hormones value: 120 fish were grabbed from raising barrels at randomly. We collected blood and analyzed with the 120 fishes blood. To obtained LH&FSH values of 120 fish with Chemiluminescent Immunoassay (CLIA). Pick 60 fish from the barrel at random, and collected these blood samples were collected into heparin sodium injection apparatus (needles ($18\text{G} \times 1\frac{1}{2}''$) injection and syringe of 10ml). The bloodstains Acupuncture blood into the small artery blood of caudal vein in fish body. Poured into the adopt blood vessel that includes heparin solution injection and shake artificially about 5 min. Put the blood into a tube which centrifuge for separation. About 20 minutes later (but the Common carp blood was very sticky, must spend more than 100 minutes), collected the upper clear liquid to pack into the adopted blood vessel for CLIA. The CLIA instrument namely Siemens Immulite 2000.

Medical laboratory shop has the same level testing equipment equal to hospital, and all over the streets of the city and county.

RESULTS AND DISCUSSION

Security analysis: All fishes resumed balance and there was no death to appear within 5 minutes in water. Analysis of these digital values of the two sex hormones LH&FSH in fish blood Hormones would be metabolized and dissolution continuously that hormone must lose its activation by itself, must separate the serum to use as soon as possible after obtaining fish's body blood. The average values and standard-error of luteinizing hormone and follicle-stimulating- hormone of each 60 fish were listed (**Table 1, and Table 4**)

Table 1: The luteinizing hormone digital values in Common carp blood

Sample	Common carp
Results (mIU/mL)	0.34 ± 0.02

Table 2: The luteinizing hormone digital values in Crusium carp blood

Sample	Crucium carp
Results (mIU/mL)	0.39±0.05

Table 3: The follicle stimulating hormone digital values in Common carp blood

Sample	Common carp
Results (mIU/mL)	0.07±0.01

Table 4: The follicle stimulating hormone digital values in Crusium carp blood

Sample	Crucium carp
Results (mIU/mL)	0.39±0.05

CONCLUSION

Known by this research, the digital values of LH&FSH in common carp and crucian carp were be detected and automatic record with human medicine CLIA.

The inspection of examining fish blood with human medical instrument and procedure is the innovation, usability and first in the world. These data will be a clinical biochemistry blood repository of fish and build a connecting channel of both fishery medicine and human medicine. It is very practical and innovation adding for fishery science and human medicine. And all these data must be considered to be a fish clinical chemistry database for common carp (*Cyprinus carpio*) and crucian carp (*Carassius auratus auratus*).

REFERENCES

1. C.N. Walpita, A.D. Crawford, E.D. Janssens, S. Van der Geyten, V.M. Darras,. Type 2 iodothyronine deiodinase is essential for thyroid hormone-dependent embryonic development and pigmentation in zebrafish. *Endocrinology*. 2009, 150, 530–539.
2. Y.W. Liu, L.J. Lo, W.K. Chan, Temporal expression and T3 induction of thyroid hormone receptors alpha 1 and beta 1 during early embryonic and larval development in zebrafish, *Dania rerio*. *Mol. Cell. Endocrinol*. 2000, 159, 187–195.
3. L.Q. Yu, J. Deng, X.J. Shi, C.S. Liu, K. Yu, B.S. Zhou, Exposure to DE-71 alters thyroid hormone levels and gene transcription in the hypothalamic–pituitary– thyroid axis of zebrafish larvae. *Aquat. Toxicol*. 2010, 97, 226–233.
4. Echuca Moama Fishing - European Carp. Echuca.ws. Retrieved 2011-12-03.
5. Jian Feng Zhou, Qing Jiang Wu, Yu Zhen Ye & Jin Gou Tong (). Genetic divergence between *Cyprinus carpio carpio* and *Cyprinus carpio haematopterus* as assessed by mitochondrial DNA analysis, with emphasis on origin of European domestic carp *Genetica* 2003, 119, 93–97

6. Fishery Statistics Annual Report of Taiwan, 2015.
7. M. Ahmed, M.H. Lorca. Improving developing country food security through aquaculture development-lessons from Asia Food policy. 2002, 27:125-141
8. Ha MF. Hematology, Ho-Chi Book Publishing CO. Ltd., 2008, 701-703.
9. Qian-Yun Zhang., Hui Chen., Zhen Lin., and Jin-Ming Lin. Comparison of chemiluminescence enzyme immunoassay based on magnetic microparticles with traditional colorimetric ELISA for the detection of serum α -fetoprotein. Journal of Pharmaceutical Analysis.2012, 2(2), 130-135
10. Ha MF, Wang BL. Development of the instrument with automatic calculate blood count. Measurement issue vol. 2002, 17, 1-12.
11. R.R. Costa, WA Varanda, CR Franci. A calcium-induced calcium release mechanism supports luteinizing hormone-induced testosterone secretion in mouse Leydig cells. Am J Physiol Cell. 2010, 299,316-323.
12. P. A. Gilderhus, C. A. Lemm, and L. C. Wods, Benzocaine as an anesthetic for striped bass. Prog. Fish. Cult., 1991, 53,105-107.
13. P. A. Gilderhus, C. A. Lemm, and L. C. Wods. Benzocaine as an anesthetic for striped bass. Prog. Fish.Cult., 1991, 53,105-107.

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