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Detection the two sex hormone digital values with human clinical medicine science in milk fish (*Chanos chanos*)

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

Keyword: *Chemiluminescecent immunoassay, follicle stimulating hormone, luteinizing hormone, milk fish, qualitative analysis, quantitative analysis*.

INTRODUCTION

Milkfish is a warm-water marine species, and its culture is widespread in the Asian-Pacific region; it is a unique species in the family Chanidae.[1]Currently milkfish is an important brackish water aquaculture species in Southeast Asia and represents an important component of the fisheries sector and national economy in Indonesia, Philippines and Taiwan.[2]

Milkfish (*Chanos chanos*) belongs to the order Gonorynchiformes and is the only species of the family Chanidae.[3] Milkfish are widely distributed throughout the tropical and subtropical Indo-Pacific oceans, being found on the coast of Africa, as far east as the Pacific waters of Central America, and as far south as Hawaii, Mexico, southern-Australia and New Zealand.[4, 5]

Chanos chanos (English common name: milk fish) is an important fish catch in Southeast Asia, warm water fish, distributed in the subtropical or tropical waters. This is evidenced by the fact that the eastern Pacific

extends from northern South America to southern Central California, where the whereabouts of the Pacific Ocean and the Indian Ocean have been more frequency, particularly as they have crossed the Strait of Baskets to the Indian Archipelago from the southern coast of Taiwan.1 In addition to the culture in Taiwan, Philippines and Indonesia and other Southeast Asian countries also have milkfish farming. And Taiwan's milkfish breeding technique is considered to be the first by the Dutch from Indonesia to Taiwan.[6]

In the life history of milkfish, different stages can be described based on their morphological characteristics and ecological requirements.[4]. The larvae and juveniles spend their lives in the inshore estuarine areas and then migrated into rivers in the direction of fresh water.[7, 8]

Body features: milkfish fish jaw suture has a prominent bulge, no teeth in the two jaws, the left and right gill film attached to the abdomen, but with the isthmus off, the scales of body surface is smaller, no scales on the head. The dorsal fin is above the posterior part of the pelvic fins, and the basal scaly sheath.[9]

Breeding and growth Ecology: Milkfish can live in fresh water, the most appropriate salinity 12-30. Living temperature is 16-39℃. When water temperature dropped to 15℃, milkfish fish ability become weaker, less than 9 ℃ within a few minutes of death. Because the milkfish is not hardy, so the pool water temperature is too low will cause a large number of milkfish deaths. Milkfish like to sport and group tour, is a plant-based omnivorous fish, you can feed with feed. The breeding methods are extensive and intensive farming. Milkfish raised in northern China could use the fry of these areas before and after the Chin Ming Festival from Taiwan, Guangdong, Guangxi, and Hainan. The milkfish larvae had to breed in the Indoor-pool, till grew to the appropriate size then emigrate into the outdoor pond farming; each fish body weight could grow up to 500g. Milkfish had to live in the indoor pool at winter. Milkfish body weight could be up to 1000-2000g after 2- 3 years, and fish production shall be up to 100-500kg per mu.[10]

Economic value: Taiwan's milkfish food widely popular preferences, the whole body of milkfish could be fully utilized 100%, milkfish fish fresh meat, high nutritional value, suitable for fried, roasted, boiled, steamed, fried, pickled, burning, etc. There were "champion fish" honor. In addition to normal fish cooking methods, and according to the physiological characteristics of milkfish, the development of a very native flavor.[11]

Nutritional value: Milkfish EPA and DHA content higher than the eel, EPA and DHA is the key of health care of human, can lower cholesterol, prevent blood embolism to improve brain qualifications to prevent the occurrence of Alzheimer's disease. Milkfish bone can boil into a delicious soup, the contents which had 17 kinds amino acids and rich of glia, calcium, phosphorus, amino acids on the human liver has a very good protective effect, glial, calcium and phosphorus on child development, women, the bones of the old people have a complementary role.[12]

Milkfish annual total production has surpassed 450,000 metric tons since 2000 in Taiwan. However, milkfish production is faced with limitations, the most significant of which is the unpredictable and limited supply of fry. Milkfish production comes mostly from aquaculture, and the availability of fry for stocking plays an important role in the success and development of the industry. Traditionally milkfish production relied on the capture of fry from the wild; but important attempts have been made to develop milkfish brood stock-hatchery technology, and significant successes have been achieved in the artificial propagation of this species.[13]

Taiwan is known as the reputation of the kingdom of milk fish, the annual production capacity of more than 58.453 tons. Tainan has about 7900 hectares of milkfish area and about 14,000 tons annual product. Tainan is

the largest milk fish producer country in Taiwan. [1] Continue to develop easy breeding and fast growth, meat delicious varieties of efforts to move forward.[14]

In Taiwan, milk fish annual total production has surpassed 450,000 metric tons since 2000. [1]However, milkfish production is faced with limitations, the most significant of which is the unpredictable and limited supply of fry. Milkfish production comes mostly from aquaculture, and the availability of fry for stocking plays an important role in the success and development of the industry. Traditionally milkfish production relied on the capture of fry from the wild; but important attempts have been made to develop milkfish brood stock-hatchery technology, and significant successes have been achieved in the artificial propagation of this species. In Taiwan, annual total production has surpassed 450,000 metric tons since 2000.[1] However; the milkfish production had faced with limitations, the most significant of which is the unpredictable and limited supply of fry. Milkfish production comes mostly from aquaculture, and the availability of fry for stocking plays an important role in the success and development of the industry. Traditionally milkfish production relied on the capture of fry from the wild; but important attempts have been made to develop milkfish brood stock-hatchery technology, and significant successes have been achieved in the artificial propagation of this species.

Milkfish artificial reproduction, have been the pursued goal for a long time by industry and researchers. The latitude is higher in Taiwan, in addition to coastal Pingtung found large species of milkfish propagation. Taiwan people had captured some adult fish near Dongsha Island in 1947. In 1929, the former Japan Governor General Aquatic Experimental Field had carried out milkfish propagation cultivation test, stocking more than six years, seven fish were weighing about four kilograms, made a check after five months, the ovary diameter was 13.5 cm, but not yet mature. In 1970, had catch milkfish propagation for artificial insemination and incubation hatch in the Nanren Bay by Fisheries Research Institute, COA.[1]

1978, Dr Liao Yikuu, director of the East Port Division of the Water Testing Laboratory, was invited to go to the Philippines to host milkfish for artificial breeding and hatch 36,000 fry. In 1979, Pingtung Xinli farms in Zeng Leiqiang, Xiao Shimin, Lu Fenggang and other efforts, the first breeding of milkfish propagation, injection of human chorionic gonadotropin (HCG) ripening and successful breeding.[15]

Nature scientific research methods: Qualitative analysis: Qualitative analysis method is only made a total amount analysis of the research object. Specifically, the various things obtained are summed up, deduced, analyzed and synthesized in an abstract and generalized way. The researchers need to further imagine and try to figure out what they can think by themselves, in order to achieve understanding of the nature of things. However, this scientific method does not have real data to support, and this method is more difficult to identify and accept.

Quantitative analysis: In scientific research, quantitative analysis can make researchers further quantify the object for understanding, in order to reveal the law more scientifically, grasp the essence, to clarify the relationship and predict the development trend of the thing. Anyway, this scientific method is indeed being expressed with real data. And even more scientific and clear mean narration of the obtained data.

Test of fish blood was prevalently used in fisheries management and disease diagnosis. Hormones play an important role in the regulation of metabolism, reproduction, growth, and development in vertebrates[16,] including fish[17]. Similar to mammals[18], 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.

CLIA instrumental analysis is a high level and smart technology, 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 hospita[l9]. 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 are 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 an 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} ~ 10^{-9} meter (~ nm) concentration, can be checked out.[19, 20]

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 immune-reagents, 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). CLIA has more accuracy detected value than ELISA and RIA.

In the presence of complimentary antigen and antibody, the paratrooper 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 the use of stationary solid particles coated either with the antigen or antibody of interest. Post incubation, which ensures intact immune complexes are formed, the substrate is added. These results in the generation of light, the intensity of which is directly proportional to a number of labeled complexes present and which indirectly aids in the 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 operation.[21]

Fish maturation is usually stimulated by local ecological factors change such as sunshine time, water temperature…etc. Must to be controlled from the gonadotropin hormones (GTH) of pituitary secretion, and milk fish known GTH were only follicle-stimulating hormone (FSH) and luteinizing hormone (LH), currently. The two hormones could help fish gametes to achieve growth and maturity.[22]

In fact, many hormones of human have been examined very precise with automatic digital recording instruments for many years, and a large number human blood samples were tested in general hospitals every day in Taiwan. It is an extremely easy and convenient thing for mankind. In this study, the author wished to detect FSH and LH digital numbers of milk fish with the human CLIA medicine technique.

MATERIALS AND METHODS

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

Breeding conditions: Pond area of 12 acres -- 15 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 10cm -- 15cm. Each pond is equipped with equipment such as aerator.

Water quality: Aquaculture is actually to mean the fish pond water-quality management, water quality should be handled well, so the most of the milkfish farming that smooth growth, until to the harvest. Water quality is divided into freshwater and saltwater, freshwater milkfish grow faster than saltwater milkfish, the premise is to handle fresh water, this farming method, more power consumption, suitable for inland, away from the coast farther fishpond breeding. The saltwater milkfish is suitable for use in the coastal areas, with gutter and gate equipment, the use of mobile circulating water, so that the fish of the sea water constantly interflow, into the outflow, the fish into the water as clean water, this farming method, sea water is free, on the other hand, electricity is more provincial. In general raise milk fish farmers breeding experience, such as fishing near the beach and near the coast environment, the use of mobile circulating water is the best way and treatment, milkfish growth was normal. Salt water aquaculture will not grow slower than the freshwater aquaculture, water quality treatment technology, is an important process affecting the growth of milkfish.

Acclimation:

- It is relatively easy to train domesticated species. Domesticated species in the adult fish when the floating snatching time is short, easy to manage.
- Domestication in the feed to add the right amount of additives can shorten the acclimation time; increase the feeding area and the number of milk fish.
- The effect of density on domestication. Large density number of milk fish will 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 milk fish growth.
- With the large milk fish, fish should be in the main raising milk fish floating after the formation of the habit of eating.
- Domestication of the water should be "thin" should not "turbidity".
- Domestication managers need to be patient.

EXPERIMENT

Breeding: 60 healthy milk fish with average body weight about (1125.5±7.11g), had been raised for twenty-one days in 5000 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℃ in twenty -one day. Stop feeding for more than 24 hours before the experiment begins12. 60 milk fish average weight about (1125.5±7.11g), and had been raised for 21 days in 5000 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℃ in 21 days. Stopped feed for more than 24 hours before the experiment. Obtaining hormones value: 60 milk fish were grabbed from raising barrels at randomly. We collected blood and analyzed with the 60 fish blood. To obtained LH and FSH values of 60 fish with Chemiluminescent Immunoassay (CLIA). Selected 60 fish from the barrel at random, and pumped these blood samples were collected into heparin sodium injection apparatus〔needles（18G ×1$\frac{1}{2}$"）injection and syringe of 10ml〕.

The bloodstains acupuncture blood into the small artery blood of caudal vein in the fish body. Poured into the adopt blood vessel that includes heparin solution injection and shakes artificially about 5 min. Put the blood into a tube which centrifuges for separation. About 20 minutes later, collected the upper clear liquid to pack into the adopted blood vessel for CLIA. The CLIA instrument namely Siemens Immulite 2000.In Taiwan, general medical laboratory shop had the same level testing equipments equal to the hospital, and all over the streets in the city. Analysis of these digital values of the two sex hormones LH and FSH in milk fish blood. Hormones would be metabolized and dissolution continuously that the obtained hormone must lose its activation by itself, the author must separate the serum as soon as possible after obtaining fish's body blood.

RESULTS AND DISCUSSION

Known by this research, the digital values of LH and FSH in milk fish were detected and be automatic recorded with human medicine CLIA.Security analysis: All fish resumed balance and there were no death to appear within 5 minutes in water. The average ± standard deviation digital values of LH and FSH of 60 milk fish were listed. **(Table 1 and Table 2)** Special note is: mu is the same as miu. The middle i is an international unit, it is often used as an inspection unit for test human hormones. That is to say 1u/ml=1000mu/ml=1000miu/ml.

Table 1: The average ± standard deviation digital values of luteinizing hormone in 60 milk fish

Sample	Milk fish
Results (mIU／mL)	2.19±0.14

Table 2: The average ± standard deviation digital values of follicle stimulating hormone in 60 milk fish

Sample	Milk fish
Results (mIU／mL)	0.96±0.11

CONCLUSION

In Taiwan, the milkfish farmers must culture larvae for some time, fed larvae hormones preparations then milkfish will self-maturity. Feed the immature milkfish with hormonal preparations by throw feed. This is a qualitative analysis and lack of precise feeding method. This method will vary large difference of each

farmed milkfish from each other according to the individual experience of fish farmers. So the author apply human blood hormone test technology to detect milkfish hormones, and actually extract milkfish blood from fish body for quantitative analysis and test fish hormone. This is a quantitative analysis and more scientific and clear mean narration of the obtained data. This technology should be a great help to academic research and industrial progress of milkfish in the future. Currently milkfish is the most important inland farm-raised fish cultured in Taiwan.[23]

The inspection of examining milk fish blood with human medicine CLIA examining technique is the first innovation and usability in the world. These data will be a clinical biochemistry blood repository of milk fish and will build a connecting channel for fishery science and human medicine. It will be very practical and innovation adding to fishery science and human medicine. And all these data must be considered to be a clinical chemistry database of milk fish. This technology will be very effective benefit for milkfish industry in the future. The practical technique in this study should used to be an advanced clinical research to milk fish. And it is a very useful technique for the future study for physiology and biochemistry of milk fish. The method was powerful suggested as a useful means of assessing fish health and there was a need for strong body indicator values in health. Milk fish disease and various stress conditions before their value in diagnosis can be evaluated.

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