

Effect of Recirculating Aquaculture System (RAS) on Growth Performance, Body Composition and Hematological Indicators of *Allogynogenetic crucian* Carp (*Carassius auratus gibelio*)

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Abstract: A Ditch Constructed Wetland unit (DCW) was integrated into an outdoor RAS with four fishponds. This study evaluated the performance of the wetland unit in treating the recirculating wastewater and examined the effect of Recirculating Aquaculture System (RAS) on growth performance, body composition and hematological indicators of the rearing fish. During a 165-days culture period, the DCW effectively reduced the influent concentrations of nutrients and can keep a good water quality at acceptable concentrations for growth of the fish. Growth performance, body composition and hematological indicators of *Allogynogenetic crucian* carp were closely related to water quality of their living environment. The RAS had improved the growth performance and quality of *Allogynogenetic crucian* carp.

Keywords: *Allogynogenetic crucian* carp (*Carassius auratus gibelio*), body composition, growth performance, hematological indicators, Recirculating Aquaculture System (RAS)

INTRODUCTION

FAO (2006) predicts that more than an additional 40 million tonnes of aquatic food will be necessary by 2030 to maintain the current per capita consumption. As a consequence of the over-exploitation and depletion of the world fish supplies from capture fisheries, aquaculture has undergone exceptional growth and is perceived as having the greatest potential to meet the growing demand for aquatic food. Aquaculture is now the fastest growing food-producing sector, accounting for almost 50% of the world's food fish (FAO, 2006). China is the world's largest producer of aquaculture products, accounting for 67% of the global production in terms of quantity and 49% of the global value in 2006 (FAO, 2008). Aquaculture in static ponds is conventionally and widely adopted in China. However, such a culture process typically requires a large amount of water resources and land area and produces polluted effluent, adversely impacting the environment. Additionally, the accumulated feed residue and fish excreta often cause the water quality in fishponds to deteriorate and further reduce the quantity and quality of aquaculture products. With the development of aquaculture and the growing public demand for healthy aquatic food, the latest aquaculture aim that named "good and fast" was formulated by

Chinese government in 2007. However, with the rapid expansion of aquaculture, China has also faced serious challenges to achieve the aim in recent years, including the limitations of finite land and water resources, the gradual deterioration of aquatic ecosystems, frequent disease outbreaks and difficulties with sediment and wastewater treatment (Cao *et al.*, 2007).

A solution to these ecological concerns is rearing fish in Recirculating Aquaculture Systems (RAS), i.e., land-based aquatic systems in which water is re-used after mechanical and biological treatment in an attempt to reduce the needs for water and energy and the emission of nutrients to the environment. As a kind of effective wastewater treatment process, constructed wetlands have been applied in the recirculating aquaculture. The recirculating marine shrimp and trout cultivation with constructed wetlands as treatment facilities were reported (Schulz *et al.*, 2003; Sindilariu *et al.*, 2008).

Fish is in direct contact with the water environment through its gills and skin. So the water quality not only influences the aquatic products but also impacts the fish quality. As the utilization of RAS is predicted to increase in the near future, it is imperative to evaluate the quality of fish originating from the RAS. The knowledge of fish quality in farmed fish is limited to a number of studies including comparing between farmed

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and wild caught fish, different ages (Geri *et al.*, 1995), different baits (Seon-Heui *et al.*, 2008), different densities (Rosalba *et al.*, 2004) and different transport systems (Lorenzon *et al.*, 2008). However, information about the effect of RAS on fish growth performance and quality remains scarce.

Crucian carp (*Carassius auratus*) as a kind of commercial fish because of its rapid growth and delicious flesh, has been farmed all over China, also was widely used in ecotoxicology, physiology and energy metabolism (Li *et al.*, 2003; Ohlhergger *et al.*, 2006).

The aim of this study is to investigate the performance of the wetland unit in treating the recirculating wastewater and examined the effect of Recirculating Aquaculture System (RAS) on growth performance, body composition and hematological indicators of the fish, in order to compare the physiological status under different rearing conditions and to validate the efficacy of RAS.

MATERIALS AND METHODS

System construction: The study site was located in the experimental base of the Research Center for Pond Ecological Engineering, Chinese Academy of Fishery Sciences, Jingzhou, Hubei Province and China. The RAS consisted of a Ditch Constructed Wetland unit (DCW) and 4 series-connected culture ponds.

The DCW as purification unit (i.e., a tank, three serial subsurface flow wetlands (SSFW 1, SSFW 2 and SSFW 3) (30 m in length, 3.1 m in width for each) and a Surface Flow Wetland (SFW) (30 m in length, 2.4 m in width)) was at the bank of the fish pond (Fig. 1). The frame of the DCW was built using bricks and mortar, whereas the bottom was reinforced with concrete as an impermeable liner fixed to a slope of 0.5%.

Each wetland cell was partitioned into three subareas along the direction of the flow, an inlet area, a wetland bed and an outlet area, which were all filled with different sizes of ceramic pellets to a depth of 0.8 m in SSFW and 0.4 m in SFW. At the inlet, a storing cistern was constructed with a serrated overflow weir on one side, which facilitated a horizontal flow of the water. In the bottom of every DCW near the outlet, a system of perforated 160 mm (in diameter) PVC pipes was placed to ensure efficient drainage. A PVC pipe vertically connected with water collecting pipe at the outlet of SFW which was used to keep water level at 0.4 m above the stroma.

After impoundment, *Thalia dealbata*, *Arundo donax*, *Var. versicolor* and *Phragmites australis* were planted respectively at SSFWs in order while *Myriophyllum* sp., was planted at SFW. All plants propagated rapidly and soon covered the entire surface of the DCW within a single growing season. These perennial aquatic or marsh plants have extended root

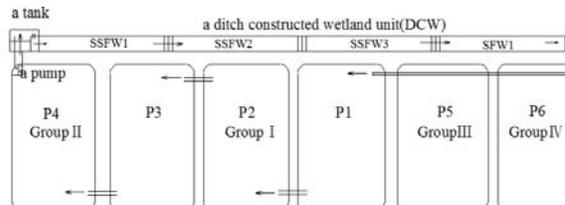


Fig. 1: Schematic diagram of integrated ditch wetland-pond aquaculture ecosystem

systems and large biomass and were easily obtained in this district.

After the construction and planting, the systems were allowed to acclimatize for 2 months to let the plants and microorganisms develop. During this period, the DCW were loaded with water from the fishpond.

The culture ponds included 4 series-connected recirculating ponds in the RAS and 2 static control ponds. Each of the culture ponds had an area of 660 m² and a mean water depth of approximately 1.5 m, with a mud bottom. The recirculating ponds, namely, the flow-through systems, were connected by culvert pipes that ensured a mixing of the upper stratum water with the lower one so as to enhance the passive aeration. The control pond without recirculation corresponded to the stagnant water conditions that are representative of traditional static aquaculture ponds.

Test fish: In this study, a polyculture strategy for the stocking of the fish was adopted. *Allogynogenetic crucian carp* (*Carassius auratus gibelio*) and grass carp (*Ctenopharyngodon idella*) as the main culture species mixed with a minor quantity of filter-feeding fish were raised. *Allogynogenetic crucian carp* were farmed in P2, P4, P5 and P6 (group I-IV) while grass carp were farmed in other ponds. The fry size and stocking density were respectively 25 g and 1500/667 m². All fry were disinfected with 3 g/L salt solution for 10 min before seeding. The result of trial was only about *Allogynogenetic crucian carp*.

Operation and management of the system: Before stocking the fish, all of the ponds were drained, the silt was removed and the bottom of the ponds was disinfected with lime. Water pumped from a nearby reservoir was then used to fill the culture pond to a depth of approximately 1.5 m; thereafter, the replacement of the water lost due to evaporation was mainly achieved from the groundwater and rainfall.

The fish stocking began on April 9th and the fish were harvested on November 7th, corresponding to a rearing period of 212 days. The ponds were harvested by complete drainage at the end of the rearing period. During the study, the fish were fed to satiation twice daily with a commercial diet and the amount fed was measured to determine the feeding efficiency. The feeds were Tongwei 103LP commercial floating feed. A

pump submerged in the pond 4 was used to recycle the RAS, which operated daily between 8:00 a.m. and 6:00 p.m., resulting in a Hydraulic Loading Rate (HLR) of 730 mm/day. The water exchange rate for each recirculating pond was maintained at 20-25% per day. The recirculating system was not in operation during rainy days.

Sample collection and chemical analysis:

Sample collection: Water sampling was conducted every 2 weeks, from June 3th to November 2th in 2010. Given the extreme daytime temperatures in the region, sampling was typically conducted in the early morning, between 7:00 and 8:00 a.m.

To investigate growth performances, fish (n = 30) from each pond were randomly collected on a monthly basis. Fish feeding was stopped 24 h prior to sampling. Fish sampling was performed using a casting net pulled up from the bottom to crowd the fish at the surface; Total and Standard Length (TL and SL) and wet Weight (W) were immediately recorded for each specimen.

To measure blood biochemical indicators, additional fish (n = 10) from each pond, were randomly sampled at the end of culture period. To avoid additional stress, fish for blood biochemical indicators measurement were captured before the fish used for growth performance determination. After capture, fish were immediately anaesthetized using a solution of 0.1 g/L MS222 (Ethyl 3-aminobenzoate methanesulfonate salt, Sigma-Aldrich, Milano, Italy). Careful netting and handling were implemented to minimize stress. Blood was withdrawn from the caudal vein by 2.5 mL syringe in less than 1 min for each fish. Blood samples were placed into 1.5 mL non-heparinisedependorf tube and after clotting were centrifuged at 3000 rpm for 10 min at 4°C. Serum samples were stored at -20°C and then determination with 24 h. A standardized handling procedure was applied in order to minimize the stress involved in the blood sampling.

Water analysis: The Total Suspended Solids (TSS), Chemical Oxygen Demand (COD_{Mn}), Total Ammonia Nitrogen (TAN), Nitrite (NO₂⁻-N), nitrate (NO₃⁻-N), TN and TP were analyzed following standard procedures (State EPA of China, 2002). The chlorophyll a (Chl-a) content was determined spectrophotometrically after filtration through Whatman GF/C glass filters and 24-h extraction with 90% acetone.

Growth performance analysis: The Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR) were calculated according to the following formulae: FCR = feed intake/weight gain, SGR = 100× [(Ln terminal BW-Ln initial BW) /test days], BW means body weight.

Body composition (nutrition constituent) analysis:

All chemical composition analyses of muscle and tissues were performed following the methods of the National Standard (GB/T5009-2003). Crude protein (N×6.25) was determined using the Kjeldahl method after an acid digestion using an Auto Kjeldahl System (2100-Auto-analyzer, Tecator, Hoganas, Sweden). Crude lipid content was determined using the ether extraction method. Moisture was determined using Atmospheric pressure constant temperature drying method. Crude ash was determined using method of burning of high temperature at 550°C in a muffle furnace.

Blood chemistry analysis: The T-CHO, TG, HDL-C, LDL-C, AKP, AST and ALT in the plasma were determined by using an automatic biochemistry analyzer (CHEMIX-180, SYSMEX and Japan).

Statistical analysis: All data are presented as mean±S.D., unless otherwise specified. Data were subjected to one-way ANOVA and if significant (p<0.05) differences were found, Duncan’s multiple range test (Duncan 1955) was used to rank the groups using the SPSS program Version 15.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

Purification efficiencies of the Ditch Constructed Wetland (DCW)

The DCW showed significant purification efficiencies for organic matter and nutrients, especial for removal of chlorophyll a (Chl-a) up to 90%. The purification efficiencies of nitrite (NO₂⁻-N), nitrate (NO₃⁻-N) content were not significant that might be related to the low level of the inlet water. Ammonia nitrogen (NH₄⁺-N), NO₂⁻-N, Total Nitrogen (TN), Total Phosphorus (TP) and 5-day Biochemical Oxygen Demand (BOD₅) content of final effluent were respectively 1.24, 0.048, 4.87, 0.24 and 4.16 mg/L, all satisfied the requirement of Fishery Water Quality Standard (GB11607-89). The pollutant removal effect of the Control Wetland (DCW) was as shown in Fig. 2.

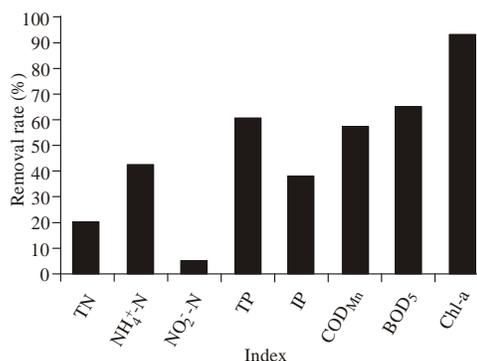


Fig. 2: Pollutant removal effect of the Ditch Constructed Wetlands (DCWs)

Table 1: The growth performance and feed utilization of *Allogynogenetic crucian* carp in the culture ponds of the Recirculating Aquaculture System (RAS) and Control Aquaculture System (CAS) (n = 10)

| Item | Test group | Control group | Group I | Group II | Group III | Group IV |
|-------------------------------|-------------|---------------|---------|----------|-----------|----------|
| Total output (kg) | 633.25±2.05 | 574.85±9.97* | 634.70 | 631.80 | 567.80 | 581.90 |
| Yield per unit area (kg/ha) | 9595±31.11 | 8710±151.32* | 9617 | 9573 | 8603 | 8817 |
| Total net weight (kg) | 586.85±2.05 | 528.45±9.97* | 588.30 | 585.40 | 521.40 | 535.50 |
| Food consumption (kg) | 830±42.43 | 860±0.00 | 860 | 800 | 860 | 860 |
| Feed Conversion Rate (FCR) | 1.42±0.06* | 1.63±0.03 | 1.46 | 1.37 | 1.65 | 1.61 |
| Specific Growth Rate (SGR, %) | 1.06±0.00 | 1.05±0.01 | 1.06 | 1.06 | 1.04 | 1.05 |
| Survival rate (%) | 99.30±0.14 | 94.40±6.51 | 99.4 | 99.20 | 89.80 | 99.00 |

*: Significant differences (p<0.05) between different treatment groups by t-test

Table 2: The muscle and liver composition and organ/body weight ratio of *Allogynogenetic crucian* carp in the culture ponds of the Recirculating Aquaculture System (RAS) and Control Aquaculture System (CAS) (n = 10)

| Item | | Group I | Group II | Group III | Group IV |
|-----------------------------|---------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Muscle | Moisture (%) | 70.56±0.33 ^a | 70.48±6.44 ^a | 73.45±0.73 ^b | 72.99±2.13 ^b |
| | Crude protein (CP, %, DM) | 73.60±2.07 ^b | 72.89±1.28 ^b | 65.42±3.43 ^a | 66.24±0.60 ^a |
| | Crude fat (EE, %, DM) | 10.51±1.03 ^a | 13.73±2.06 ^b | 16.09±1.94 ^b | 14.60±1.10 ^b |
| | Crude ash (Ash, %, DM) | 4.53±0.49 | 4.39±0.17 | 4.25±0.19 | 4.31±0.23 |
| Liver | Moisture (%) | 64.96±1.59 | 65.07±1.09 | 68.08±4.40 | 67.27±1.95 |
| | Crude protein (CP, %, DM) | 17.00±1.33 ^b | 17.12±1.57 ^b | 13.97±0.80 ^a | 13.45±0.55 ^a |
| | Crude fat (EE, %, DM) | 2.87±0.64 | 2.71±0.67 | 3.16±1.11 | 2.81±0.28 |
| | Crude ash (Ash, %, DM) | 2.38±0.14 | 2.26±0.09 | 2.34±0.16 | 2.57±0.31 |
| Organ/body weight ratio (%) | | 12.04±0.91 ^a | 12.72±0.68 ^a | 14.04±1.06 ^b | 13.86±0.32 ^b |

Organ/body weight ratio (%) = 100×terminal gut weight/terminal BW; Mean values denoted with various letters in the same row for a given body part are statistically significantly different (p<0.05)

Table 3: The hematological indicators of *Allogynogenetic crucian* carp in the culture ponds of the Recirculating Aquaculture System (RAS) and Control Aquaculture System (CAS) (n = 10)

| Index | Group I | Group II | Group III | Group IV |
|----------------|-------------------------|-------------------------|-------------------------|-------------------------|
| T-CHO (mmol/L) | 7.92±0.31 ^a | 9.78±0.32 ^c | 8.89±0.32 ^b | 8.86±0.37 ^b |
| TG (mmol/L) | 4.41±0.29 ^a | 5.61±0.76 ^b | 5.68±0.33 ^b | 5.78±0.19 ^b |
| HDL-C (mmol/L) | 1.37±0.07 ^a | 1.55±0.06 ^b | 1.59±0.14 ^b | 1.56±0.10 ^b |
| LDL-C (mmol/L) | 0.48±0.18 | 0.67±0.10 | 0.64±0.06 | 0.61±0.09 |
| AKP (U/L) | 35.33±3.21 ^a | 40.00±1.63 ^b | 43.00±2.16 ^b | 39.25±2.06 ^b |
| AST (U/L) | 381.25±10.90 | 360.00±5.00 | 338.50±42.15 | 338.25±38.53 |
| ALT (U/L) | 21.33±2.89 | 20.50±2.65 | 23.25±5.62 | 22.00±3.16 |

The growth performance and feed utilization of *Allogynogenetic crucian* carp: Base the date in Table 1, at the condition that the feedings for test groups were less than the controls, the total output, yield per unit area, net gain were significantly (p<0.05) higher, while the feed conversion rate was significantly (p<0.05) lower than those in the controls. The specific growth rate, survival rate showed a tendency of increase, but the difference was not significant (p>0.05).

The muscle and liver composition and organ/body weight ratio of *Allogynogenetic crucian* carp: At the aspect of muscle composition, the moisture content was significantly (p<0.05) lower and the crude protein content was significantly (p<0.05) higher than control group, the crude fat content in group I was significantly (p<0.05) lower than others, the crude ash did not change significantly (p>0.05). At the aspect of liver composition, there were no significant (p>0.05) differences except that the crude protein content was significantly (p<0.05) higher than control group. The organ/body weight ratio was significantly (p<0.05) higher than control group (Table 2).

Hematological indicators of *Allogynogenetic crucian* carp: At the aspect of hematological biochemical indicators, the general trend was that the test groups were lower than the control group. The Total plasma Cholesterol (T-CHO), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C) in group I were significantly (p<0.05) lower than control group, Low Density Lipoprotein Cholesterol (LDL-C) was also lower, but no significant (p>0.05) difference. There were no significant (p>0.05) differences between group II and control group except that the total cholesterol was significantly (p<0.05) higher. At the aspect of hematological biochemical indicators, the Alkaline Phosphatase (AKP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) had no significant (p>0.05) differences between test group and control group except that AKP in group I was significantly (p<0.05) lower than control group. Overall, growth performance, body composition, hematological indicators of *Allogynogenetic crucian* carp are closely related to water quality of their living environment, the RAS has improvement on growth performance and quality of *Allogynogenetic crucian* carp (Table 3).

DISCUSSION

Effect of RAS on growth performance and feed utilization of *Allogynogenetic crucian carp*: The fish growth performance was affected by combined of heredity, bait and water environment. In the present study, *Allogynogenetic crucian carp* fry were the same group of which the standard and quality were all the same, while the bait feed in the test group and the control group both were Tong Wei 103LP commercial floating feed. Therefore, the only reason which caused the difference of fish growth performance and efficiency of feed utilization between the test group and the control group was water environment. In the factory breeding production process, the temperature, pH, DO, TAN and NO_2^- -N content were the main intimidation factors (Hong and Zhang, 2004). It was reported that the survival rate, growth performance and the immunologic function of fish would be affected if the concentration of TAN and NO_2^- -N were too high (Huchette *et al.*, 2003; Wang *et al.*, 2007; Xu *et al.*, 2006). In the present study, the harmful nutrients of effluent water were remarkable decreased after treatment by the DCW and water quality of outlet was better than the inlet. So the total output, net gain of the test group were significantly ($p < 0.05$) higher while the feed conversion rate was significantly ($p < 0.05$) lower than control group. The result was in accordance with the existing research. The research result about charrs from Pickering (1989) showed environmental intimidation had an important influence on fish survival rate. The research result about *Paralichthys olivaceus* (Seon-Heui *et al.*, 2008) that the average growth of the test group increased, accumulation of mortality was reduced, as compared to those of controls might be induced from an improved fish tank environment, as shown by the decreased Chemical Oxygen Demand (COD_{Mn}) and the Total Suspended Solids (TSS) levels and enhanced non-specific immune response by chitosan. All of these were accordance with the study.

Effect of RAS on muscle and liver composition and organ/body weight ratio of *Allogynogenetic crucian carp*: The main nutritional composition of food contained protein, fat and ash. In nutrition, it is generally accepted that the higher the content of dry matter in food, the higher total nutrition composition content. The result that the moisture content in the test group was significantly ($p < 0.05$) lower showed trophic value of fish in test group was higher than that in the control group. Protein content in food was not only the indicator evaluating food quality but also related to the health of human body. As a kind of food, the more of protein content in fish muscle, the higher nutritional value (Bing and Zhang, 2006). The result that the crude protein content in the test group was significantly ($p < 0.05$) higher also showed trophic value of fish in test group was higher than that in the control group. In the

present study, the crude protein content in the test group was significantly ($p < 0.05$) higher and the crude fat content was significantly ($p < 0.05$) lower, these trends was accordance with Cenjianwei (Cen *et al.*, 2008; Liang *et al.*, 2010; Miao *et al.*, 2011; Wu *et al.*, 2007) research.

The fat and protein of liver were significant influenced by the feed protein (Gao *et al.*, 2010). Martin *et al.* (2003) found 30% of soybean protein in feed could change the structure of HSPs of the liver, which indicated that bait induced the physical stress reactions. In addition, lesion would influence the physiological function and metabolism and then caused its liver protein content below the normal. There was little report about impact of environment on liver composition. In this study, crude protein content in liver was significantly ($p < 0.05$) higher than control group declared that water quality affected the liver composition, but the mechanism of action need further study.

Muscle content was an important indicator which estimated the quality and production performance. It was affected by breed, environment and bait. In this work, the organ/body weight ratio was significantly ($p < 0.05$) lower than control group explain muscle content of fish in recirculating ponds was significantly ($p < 0.05$) higher than the control group.

It is report that the muscle composition had close relationship with the living environment, diet and growth period (Song *et al.*, 2007). In this trial, juvenile *Allogynogenetic crucian carp* were obtained from the same fish farm, hatched at the same time and fed the same kind of fodder. Therefore the difference of living environment in two farming models was the major factor which caused the difference in tissue proximate compositions of fish.

Effect of RAS on blood biochemistry and immune indices of *Allogynogenetic crucian carp*: Fish blood hematological indicators not only had closed relationship with the body metabolism, nutritional status and disease, but also they were usually used to evaluate the physiology and ecology status of fish, monitor the water ecological environment change, assess ecological stress caused by water environment change and so on (Ruane *et al.*, 2001; Weil *et al.*, 2001; Harikrishnan *et al.*, 2003). They would be changing when the growth and survival of fish were affected by deteriorating environment (Mugnier *et al.*, 2008). The change of the blood hematological indicators could be the reflection of the degree of environment stress (Paterson *et al.*, 2003; Foss *et al.*, 2007).

The Total Cholesterol (T-CHO) and Triglycerides (TRIG) were import components of blood lipids. Lipid level was an important biochemistry indicator reflecting the carbohydrate metabolism and fat metabolism level of the organism (Tong, 2007). In the research that the

influences of illumination on growth, haematological and biochemical indices of juvenile Chinese sturgeon (*Acipenser sinensis*), Zhang *et al.* (2010) found there were significant difference in values of TRIG between all-dark group and natural light group, so they thought the change of light caused the difference of fish metabolism. In this study, the TRIG values were significant lower than the values of control group and the result revealed that fish metabolism were different between two culture models.

Cholesterol mainly synthesized by the liver (Shen and Wang, 1998) that also was an indicator of ammonia nitrogen stress to body (Yin *et al.*, 2011). In our study, the value of total-CHO in test group I was significant lower and test group II was significant higher than the value in the control groups, which was in conformity with the result that the value of ammonia nitrogen in test group I was lower and test group II was higher than the value in the control groups.

Wedemeyer and McLeay (1981) research found that the pressure could lead to high CHO in teleost fishes. In this study, the value of T-CHO of fish in group I was significant lower than controls while the value in group II was significant higher than controls. The result suggested that the stress caused by water quality was little in group I while environmental stress to fish from water quality deterioration in the last pond of the recirculating agriculture system increased.

In recent years, there were many efforts to reduce the CHO of fish based on the study of feed formulation and density (Seon-Heui *et al.*, 2008; Di Marco *et al.*, 2008), but no study about impact of culture model on the CHO of fish had been reported. In this study, the value of T-CHO and LDL-C in fish in recirculating ponds were all lower than the value in control ponds, the result could provide important reference to the *Carassius auratus gibelio* culture model.

AKP was an important kind of enzyme to regulate and control the animal metabolism which had great significance to animal survival (Miao *et al.*, 2011). In our study, the value of AKP in fish in test group I was significant lower than the value in the control group. The result explained the fish in test group I suffer smaller irritate (Hong and Zhang, 2004) and within which condition fish was more relaxed and could grow faster.

As referent of liver function, AST and ALT were usually used to monitor fish health condition (Jyothi and Naran, 1997). ALT also could be used as an index enzyme in aquatic ecological toxicology because it could reflect the impact of water pollution on fish health (Hong and Zhang, 2004). In this study, there were no significant difference in the value of ALT and AST between the test and the control groups, the result showed fish were health and fish liver were normal and that the differences in water quality between two agricultural modes had no marked impact. In Vedel

et al. (1998) study found that the value of AST and ALT in *Oncorhynchus mykiss* had no significant change after nitrate nitrogen treatment, which result also appeared in our study.

CONCLUSION

In order to study the effects of RAS on *Allogynogenetic crucian* carp (*Carassius auratus gibelio*), a Ditch Constructed Wetland unit (DCW), consisting of Subsurface Flow (SSF) and Surface Flow (SF) constructed wetlands arranged in series, was integrated into an outdoor RAS with four fishponds. Two fishponds (I and II) in the RAS were compared with the other two traditional static fishponds (III and IV) as the Control Aquaculture System (CAS).

This study evaluated the performance of the wetland unit in treating the recirculating wastewater and examined the effect of Recirculating Aquaculture System (RAS) on growth performance, body composition and hematological indicators of the rearing fish. During an 165-days culture period, the DCW operated at a mean quantity of circulating water of 240 m³/day and a mean hydraulic loading rate of 730 mm/day and effectively reduced the influent concentrations of 5-day Biochemical Oxygen Demand (BOD₅), Chemical Oxygen Demand (COD_{Mn}), Suspended Solids (SS), chlorophyll *a* (chl-*a*), Total Ammonium (TAN), Total Nitrogen (TN) and Total Phosphorus (TP). The DCW can keep a good water quality with NH₄⁺-N (1.24 mg/L), NO₂⁻-N (0.048 mg/L), TN (4.87 mg/L), TP (0.24 mg/L) and BOD₅ (4.16 mg/L) at acceptable concentrations for growth of the fish. Results of the study showed that the total output, yield per unit area and net gain were significantly (*p*<0.05) higher, while the feed conversion rate was significantly (*p*<0.05) lower than the control groups. The specific growth rate and survival rate showed a tendency of increase, but the difference was not significant (*p*>0.05). At the aspect of muscle composition, the moisture content of fish in the test groups were significantly (*p*<0.05) lower and the crude protein content was significantly (*p*<0.05) higher than the value in the control groups; the crude fat content in group I were significantly (*p*<0.05) lower than the others, the crude ash content did not significantly (*p*>0.05) change. At the aspect of liver composition, there were no significant (*p*>0.05) differences except that the crude protein content were significantly (*p*<0.05) higher than the value in the control groups. The organ/body weight ratio was significantly (*p*<0.05) lower than the value in the control groups. At the aspect of hematological biochemical indicators, the values of the total plasma cholesterol, triglycerides and H-CHL of fish in group I were significantly (*p*<0.05) lower than those in the control groups, the value of L-CHL was also lower, but no significant (*p*>0.05) difference

observed. There were no significant ($p>0.05$) differences between the group II and the control groups except that the value of the total cholesterol was significantly ($p<0.05$) higher. At the aspect of hematological biochemical indicators, the values of AKP, AST and ALT had no significant ($p>0.05$) differences between the test and the control groups except that the value of AKP of fish in group I was significantly ($p<0.05$) lower than that in the control groups.

Overall, growth performance, body composition and hematological indicators of *Allogynogenetic crucian* carp were closely related to water quality of their living environment. The RAS had improved the growth performance and quality of *Allogynogenetic crucian* carp.

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