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The Design of Prototype Recirculating Aquaculture System and its Use to Examine the Histology of Hybrid Catfish Fed Practical Diets

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ABSTRACT
The study designed and constructed a prototype recirculating aquaculture system (RAS) which was used to examine the liver and kidney histology of hybrid catfish (*Heterobranchus bidorsalis (♂)* X *Clarias gariepinus (♀)*) fed six practical diets after 12 weeks. Fingerlings of the test fish, mean weight and length of 1.01±0.02g and 4.63±0.34cm were used for the study. In the formulated diets fishmeal was replaced with 100% Fluted Pumpkin Leaf (FPL) as D1, 60% FPL and 40% Parboiled Fermented African Locust Bean (PFALB) as D2, 40% FPL and 60% PFALB as D3, 100% PFALB as D4 and 0% FPL and PFALB as D5 where Diet D6 was Coppens (commercial fish fed). At the end of the experimental period eight fish were sacrificed per tank for the histological examination of the liver and kidney. The designed RAS was efficient for the culture of catfish, however, the significant different in the constructed system and previously reported systems were the systematic operation mechanism, use of one pumping machine and the oxygenation method. The examined liver and kidney histology were normal with the slight difference observed in fish fed diet D2. This study revealed that the designed system can be used to culture hybrid catfish. Similarly, the formulated diets do not show an effect on the liver and kidney histology of the test fish.

Keywords: Recirculating Aquaculture System, Practical diets, Histology, Hybrid catfish.

1. Introduction
The decline in return from capture fisheries places the solution on aquaculture to bridge the widening gap between domestic fish demand and supply. The future of aquaculture in Africa lies in the increase production efficiencies and intensities as well as the use of less water and financial resources [1]. As the aquaculture industry continues to grow in response to the demand for increased fish products, the need for environmentally conscious operational practices and facility designs becomes more important [2]. The intensive aquaculture system culture employs intensive management of production system where culturist must provide for all the biological needs of the cultured organism [3]. This method is often adopted in Recirculating Aquaculture System.

Recirculating aquaculture system (RAS) is the newest form of the fish farming production system. RAS is typically an indoor system that allows farmers to control environmental conditions year round. The costs associated with constructing a RAS is typically higher than that of either pond or cage culture, however, when the system is managed properly, fish is produced on a year round basis where the economic returns can make it worth the increased investment. The RAS is advantageous over other aquaculture systems in the reduction of incoming water volume [4], reuse more water within the culture system [5], reduction in the amount of water released and the effluent quality [6] for better hygiene and disease management [7] and biological pollution control [1]. The design of a good RAS should focus on solids removal, system removal exchange, piping size and layout, filtration process, biofiltration unit, gas balancing and carbon (iv) oxide removal, oxygenation device and disinfection process [9]. The technology for the design of efficient and cost effective RAS is still on-going. Therefore, the slow adoption of RAS technology is partly due to the high initial capital investments, thus, high stocking densities and productions are required to be able to cover investment costs [10]. Studies have shown the successful culture of African catfish (*Clarias gariepinus*) in RAS at full commercial scale in Denmark and Netherlands [11] and Nigeria [12], however, that of Nigeria was faced with some challenges.

The choice of fish species to be cultured in RAS is very important for the success of the venture which largely depends on the culture fish conditions, its feeding habits, availability of seeds for stocking, acceptability of artificial feed and market value [13]. The catfishes are one of the most preferred fish species in Nigeria market, as they command positive patronage. The aquaculture production of catfishes such as *Clarias gariepinus, Heterobranchus bidorsalis* and their hybrids have been practiced over a long time in Nigeria [14]. The hybrid are reported to have faster growth rates in captivity, better environmental tolerance, hardiness to adverse environmental conditions, capacity to undergo aquatic and aerial respiration, resistance to parasites and diseases, as they also command a high consumer preference in the market [15, 16] which is *sine qua non* to ensuring fish food security in Africa.

In aquaculture, the cost of feeding is one of the major challenges that impede the amount of profit made. The use of fish as a protein source in the fish diet as resulted to the high cost of fish production. This has necessitated researchers to research for alternate protein source for fish. Researcher thereof focused on the under-utilized plants and their products to produce protein for the growth of fish. There are two vital organs that detect the health status of an organism, these are the kidney which performs an important function related to electrolyte and water balance and the maintenance of a stable internal environmental and the liver which is the metabolic centre for the detoxification and conversions of chemicals. In the replacement of the fish in diet with less expensive raw materials of plant origin, there is the need to monitor histological structures of the fish liver and kidney in order to assess the effect of a nutrient mixture on the test fish health. Several authors have used the histological alteration to determine the effect of feed on animal such as *Clarias batrachus* [17, 18], *Channa striatus* [19], *Solea senegalensis* [20], *Cyprinus carpio* [21] and rats [22]. Therefore the study had two aims; to design and construct a prototype RAS for the culture of catfishes and to evaluate diet-related adaptive changes in the liver and kidney of hybrid catfish (*Heterobranchus bidorsalis (♂)* X *Clarias gariepinus (♀)*) fed Parboiled Fermented African Locust Bean (PFALB) and Fluted Pumpkin Leaf (FPL) for a 12-week trial periods.

2. Materials and Methods

The RAS was constructed in the Animal House of the Department of Animal and Environmental Biology, Delta State University, Abraka. The RAS consists of culture units, flow lines, solid removal tanks, pump station, sand bed biofilters, degassing/carbon (iv) oxide removal and disinfection column as presented in Fig 1. The system was designed such that the flow system was regulated, to recirculate water within 7.00 – 10.00 and 18.00 – 22.00 hours daily. The culture unit/holding tanks consists of six rows of six circular tanks each tank has a diameter of 2.1ft and depth of 1.8ft as shown in the sketch (Fig 2).

Mixed sex of hybrid catfish fingerlings of mean weight and length, 1.01±0.02 g and 4.63±0.34 cm respectively from the same brood stock were procured from Omu fish farm in Igbide, Isoko South L.G.A of Delta State, Nigeria. The fish were acclimatized for 14 days in 60 litre-rectangular plastic tanks before been transferred to the experimental tanks (twelve (12) fish per aquarium).Six (6) experimental diets were used for the study as their fish meal component was replaced with 100% FPL(D1), 60% FPL and 40% PFALB(D2), 40% FPL and 60% PFALB(D3), 100% PFALB(D4), 0% FPL and PFALB(D5) while D6 was the commercial fish feed (Coppens) produced by coppens International, P.O. Box 543, 370AM Helmond, Holland. The diet ingredients and proximate composition are listed in Table 1.

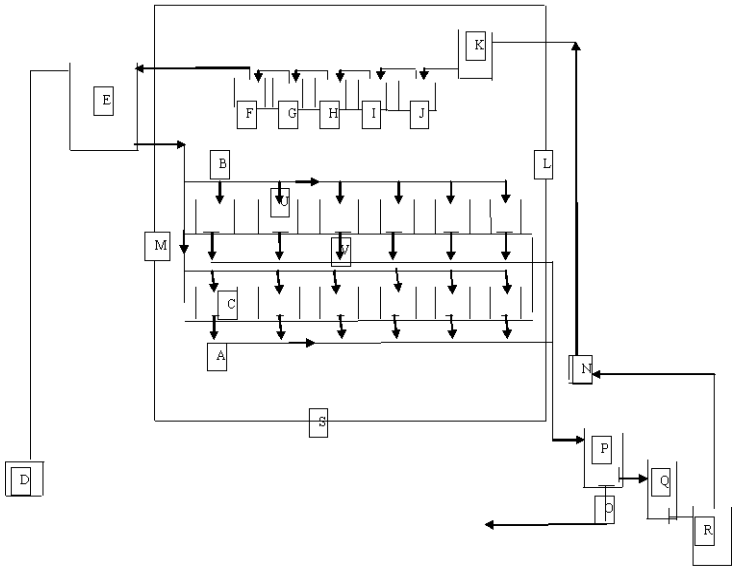


Fig 1: Schematic representation of prototype RAS used for the culture of hybrid catfish.

Keys: A= drainage pipe, B= water supply pipe C= fish tanks, each row of six tanks in duplicated, D= pumping machine, E= head tank, F= ultra violet light filter, G= 0.5µm filter, H= 1.0µm filter, I= 5.0µm filter, J= carbon filter, K=bio-filtration tank, L= outer wall of the building, M= inner wall of the building, N= pumping machine, O= waste water released outside, P= settleable solid control tank, Q= sedimentation tank, R= sump tank, S= floor of the building, T= roof of the building, U= inlet pipe, V= outlet pipe. The cross bars are 1mm mesh size nets.

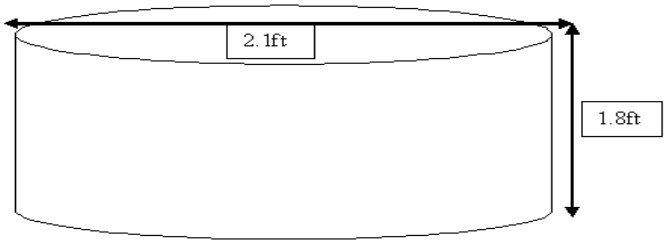


Fig: 2: Sketch of culture unit used in the experiment

Table 1: The quantity of feed ingredients and proximate compositions of prepared of diets for hybrid catfish in RAS during the 12-week experiment

Ingredients (g)	Experimental diets					
	D1	**D2**	**D3**	**D4**	**D5**	**D6***
Fishmeal	0.00	0.00	0.00	0.00	55.79	
Maize flour	29.67	30.32	30.32	29.23	37.71	
Cassava flour	5.00	5.00	5.00	5.00	5.00	
Vitalyte premix	1.00	1.00	1.00	1.00	1.00	
Chromide oxide	0.50	0.50	0.50	0.50	0.50	
Fluted pumpkin leaf	63.83	37.91	25.27	0.00	0.00	
PFALB	0.00	25.27	37.91	64.27	0.00	
Proximate composition						
Moisture (%)	7.40 (0.09)	8.50 (0.10)	8.30 (0.05)	9.80 (0.12)	8.10 (0.23)	NP
Crude protein (%)	36.70 (0.12)	37.62 (0.23)	38.80 (0.21)	37.85 (0.14)	42.90 (0.04)	45.00
Crude fiber (%)	14.00 (0.03)	8.48 (0.04)	8.00 (0.03)	6.70 (0.01)	6.70 (0.05)	15.00
Carbohydrate (%)	35.90 (0.05)	36.90 (0.07)	34.90 (0.02)	40.65 (0.07)	28.00 (0.43)	12.00
Ash (%)	6.00 (0.06)	7.50 (0.02)	7.00 (0.04)	5.00 (0.30)	14.00 (0.33)	9.50 (0.54)
Sodium (%)	16.50 (0.08)	15.00 (1.12)	10.0 (0.12)	12.00 (0.03)	40.00 (0.23)	0.40
Potassium (%)	5.00 (0.02)	16.09 (4.12)	12.34 (3.45)	40.44 (0.89)	47.26 (2.65)	1.70
Crude lipids (%)	16.50 (0.08)	2.60 (0.11)	20.00 (0.06)	10.00 (0.04)	23.00 (0.13)	NP
Total organic matter (%)	94.00 (0.01)	95.00 (0.02)	94.00 (0.01)	95.00 (0.01)	90.00 (0.01)	NP

coppens: compositions are fishmeal, soya dehulled, extracted toasted wheat, wheat gluten, fish oil, maize gluten, rape oil and premix. NP- not provided, standard error in parenthesis; PFALB – parboiled fermented African locust bean

At the end of the experiment, a section of the liver and kidney from eight fishes per tank was sampled, fixed in 10% physiological saline, dehydrated in a graded ethanol series and embedded in paraffin. Histological sections of 4µm were stained with haematoxylin and eosin (H & E).

3. Results

The lay-out of the culture unit and holding tanks designed for this experiment are presented in Plate 1. Three types of tanks were used. These were two 500-litres tanks, one used as an overhead tank/water reservoir and the other as underground/sump tank; two 300-litres tanks were used as sedimentation tanks and thirty-six 30-litres circular culture units that were used for the culture of the fish.

The flow lines were of varying diameter, ranging from 1.0 to 3.0 inch pipes, depending on the location and the function/carrying capacity. Each of the tanks or chambers had two types of flow lines (the outlet and the inlet flow lines) as illustrated in Plate 2. The supply line operates at a maximum system flow rate of 0.8 m/s with a minimal slope of 0.20%. The slope was to allow the draining of all the water inside the culture tanks when required. Each tank had an inlet and outlet lines. The inlet line to the culture tank had a diameter of 1.0 inch with a spray bar/tap designed as an inverted L of 0.3 m away from the tank to promote the water distribution in the tank as well as the introduction of oxygen. The outline was equipped with a 1.0inch drain pipe at the bottom, which empties water/effluent to a larger 2.0 inch flow line that empties into the sedimentation tank. A 3.0 inch line conveys water from the borehole to the overhead tank, while a 2.0 inch

line brings/takes the water from the overhead tank to a distributing point to the tanks. Each of the six rows of tanks had a recirculation line that runs parallel.

All the effluent lines were designed with an average water velocity of 0.8 m/s for a proper solid transport. The tank flow rate was adjusted to ensure stable inflow and outflow.

The walkway (Plate 3) was designed between the rows of culture tanks to provide for easy access to each tank. It was 2.0 m apart. A 1.0 Hp pumping machine was installed (Plate 4) to pump water from the submerged tank to the bio bed that flows gradually through a series of filtration points and degassing chamber to the over head tank to continue the 're-circulation'. There were varying methods of filtration units (Plate 5) adopted in this set up, the sedimentation tanks which aid in the removal of solid wastes as it serves in the purification process. The bio-bed which contains graded levels of gravel, coarse and sharp sands, helps in the break-down of toxic ammonia and nitrite components of the used water into non-toxic nitrate before the gases were removed by the carbon filter which was located after the bio-bed. The 1.0 and 5.0 µm filters aid in the removal of particles that were not removed in the earlier stages. These were located after the carbon filter in sequential order. The installed ultra-violet light ray (Plate 6) was located at the last chamber that returns water into the overhead tank which aid in the destruction of escaped microorganisms from other chambers as oxygenation in the units was achieved by the distance of water fall and splash from the tape into the tanks (from a height of about 0.8 m).

The examined liver and kidney histology were presented in Plate 7 and 8. The liver showed normal hepatic parenchyma arranged in cord like fashion with clear visible intact nuclei and nucleoli, well packed sinusoidal spaces and no vacuolization. The kidney also revealed normal tubules and cells. However, the fish fed diet D2 recorded little dense and prominent nuclei.

Plate 1: The different types of tanks used in RAS design, A=500L tanks (over head and submerged), B = 350L tanks (sedimentation tanks), C = 30L tanks (culture/rearing tanks).

Plate 2: The different flow lines used in the designed RAS: a- outlet water from the culture tank, b-inlet water from overhead tank to culture tanks, c- water line from submerged tank into the overhead tank, d- effluent water from culture tank into first sedimentation tank, e- water line moving water from first to second sedimentation tank, f- water line bringing water to each culture tank, g- inlet distributing point, h-water line taking water from submerged tank via filter chambers to the overhead tank, i- outlet control point for culture tank, j-outlet water line from the overhead tank, k- inlet distribution point, l- outlet water converging point, m- water line from the borehole into the overhead tank.

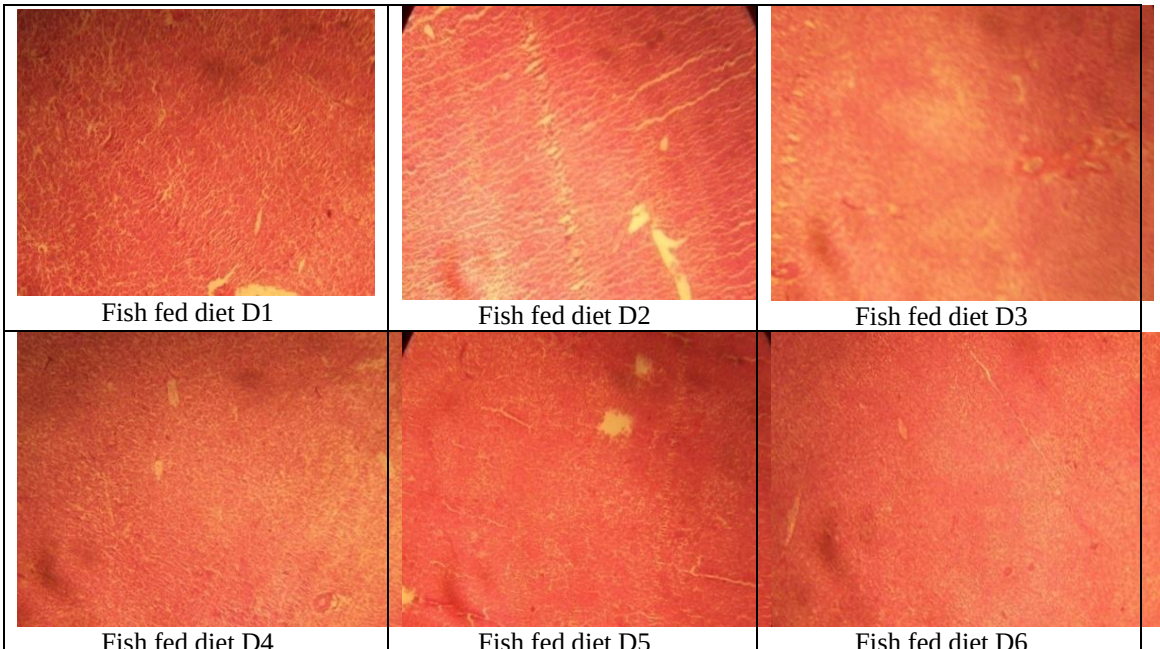
Plate 3: Walkway in the designed recirculating aquaculture system.

Plate 4: Pumping machine used in the designed recirculating aquaculture system.

Plate 5: Different filtration mechanism adopted in the designed recirculating aquaculture system. A- Bio-bed chamber filled with layers of sand (fine & coarse) and gravel, B- encased carbon filter, C- encased 1.0 µm filter, D- encased 5.0 µm filter.

Plate 6: Encased ultra-violet light used in the designed recirculating aquaculture system.

Plate 7: Adopted oxygenation method used in the designed recirculating aquaculture system



Fish fed diet D1 | Fish fed diet D2 | Fish fed diet D3

Fish fed diet D4 | Fish fed diet D5 | Fish fed diet D6

Plate 8: Photographic representation of hybrid catfish liver fed prepared diets in RAS during the second trial for 12 weeks.

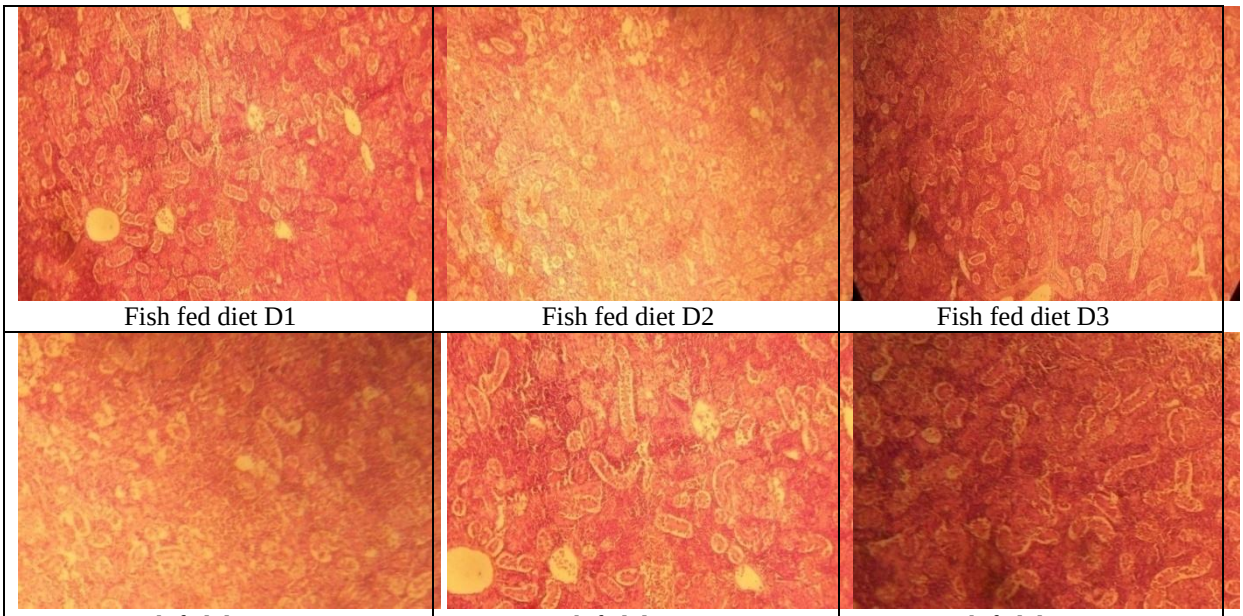


Plate 9: Photographic representation of hybrid catfish kidney fed prepared diets in RAS during the second trial for 12 weeks.

4. Discussion

Data on RAS production are generally difficult to evaluate, as there is no compiled dataset available for this type of production system in Nigeria. The designed RAS was typically in-door and closed system. The water flow in the components was by gravitational flow, as the components are placed at the appropriate elevation relative to one another to enhance water flowing. The pumping machine moves water within a complete unit as earlier reported [12]. The designed RAS gives an appropriate tank diameter to depth ratio of 1.8 ft/2.1 ft which enhanced an effective flow injection mechanism. The circular tanks used to enhance the removal of settleable solids and gave the fishes the impression of living in the wild. The use of the circular tank has been supported [23, 24]. Since the water was 'recirculated' the systems requires relatively small additions of new water of the total volume daily. In this study, about 8% of the water was added to replace the lost ones due to evaporation and discharged. The volume was within the recommendation value [3]. The use of different filtration mechanism in this study as encased in a framed enclosure was similar to that reported [12] were the filtration chambers were wrapped with tarpaulin. The microorganisms that were not able to be eliminated in the fore-filtration stages were removed by ultraviolet light. The use of ultraviolet light was an essential component in the RAS design [9]. Water aeration was achieved by a simple mechanism of water flowing into the culturing tank from a height. These methods of disinfection and oxygenation of water have been used and recommended [24].Thus, this design was similar in mechanism to that earlier designed [25]. Therefore, considering the design of water flow from the water inlet into the culture tank down to the effluent pipeline as well as the water filtration unit suggested that the designed system was effective and flexible [9, 26]. This was because adequate filtration is critical to maintaining a healthy water suitable healthy fish. The significant of sedimentation and filtration mechanism is the management of the amount of feed going into the system and wastes coming out to maintain optimal water quality.

However, the significant difference in this system design and previously reported systems are the systematic operation mechanism, use of one pumping machine and the oxygenation method. Therefore, the adopted designed in this study for the culture of hybrid catfish showed no performance difference with that reported [9, 12]. The designed RAS revealed that it can support high stocking density with a relatively less mortality rate of hybrid catfish [27].

Histology of the liver and kidney showed no significant difference in the size of the hepatocytes, cells and tubules of fish fed the prepared diets as earlier reported [28] revealing no significant change or damage to the histology of the test fish thus the formulated diet can be used to culture the test fish.

Producing more food from the same area of land while reducing the environmental impacts requires what has been called *sustainable intensification* [29]. The RAS described in this work provides a lot of flexibility in operation, putting into consideration the non-constant power supply in the country. The engineering process of the design was very useful which had provided a new vision on the design and culture process. *P. biglobosa* and *T. occidentalis* in the prepared proportions can be successfully used to replace foreign fish feed in diet of hybrid catfish, for optimum growth and nutrient utilization diet D3 and D4 is recommended.

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