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GROWTH AND PHYSIOLOGICAL PROFILES OF HYBRID CATFISH FED PRACTICAL DIET IN DIFFERENT WATER REGIMES

Kabir Mohammed ADAMU*[1] and Francis Obiora NWADUKWE[2]
[1]Department of Biology, Ibrahim Badamasi Babangida University, P.M.B. 11, Lapai, Niger State, Nigeria. [2]Department of Animal and Environmental Biology, Delta State University, P.M.B. 1, Abraka, Delta State, Nigeria.*

Corresponding author: kabrmoh@yahoo.com

The growth and physiological profiles as indices were used to evaluate the status of hybrid catfish (*Clarias gariepinus* (♀) and *Heterobranchus bidorsalis* (♂)) fed practical diet in Recirculating Aquaculture System (RAS) and Static Renewable Aquaculture System (SRAS). The study determined the suitability of growing the test fish in these systems. The trial was conducted for 12-week period with two replications. The physico-chemical parameters such as water temperature, dissolved oxygen, total alkalinity, pH, free carbon dioxide and conductivity were monitored. The result showed no significant difference (p>0.05) and were within acceptable range for hybrid catfish. The fish grown in SRAS showed insignificantly higher (p>0.05) biomass. However, all other determined growth profiles such as weight gain, daily growth rate, relative growth rate, specific growth rate, hepatosomatic index, viseromatic index, protein efficiency ratio, feed conversion ratio, gross efficiency of feed conversion, nitrogen metabolism, protein intake and survival rate were not significantly different (p>0.05). At the end of the 12-week experiment, serum, liver and kidney physiological profiles such as glucose, triglycerides, cholesterol, total protein, albumin, globulin, aspartate aminotrasferase, alanine aminotransferase, acid phosphatase, alkaline phosphatase, gamma glutamyltransferase, sodium, potassium, calcium, chloride, magnesium, inorganic phosphorous, urea, urea-nitrogen, uric acid and creatinine) were determined. There were slight differences in these profiles. This may be attributed to individual difference and the water exchange mechanism in the systems.

Keywords: Hybrid catfish, Water regimes, Practical diet, Growth and Physiological Profiles.

According to FAO (2004) aquaculture is increasingly becoming one of the fastest growing aspects of agricultural industry worldwide. One aspect of aquaculture in Nigeria that has gained momentous attention is fish production. As the aquaculture industry continues to grow in response to the demand for increased fish production, the need for environmentally conscious operational practices and facility designs becomes more important (Peachey, 2008). One of the fastest adapting environmental practices of aquaculture is the recirculating aquaculture system (RAS). The RAS is advantageous over other aquaculture systems in the reduction of incoming water volume (Verdegem *et al.*, 2006), reuse of more water within the culture system (Rosenthal *et al.*, 1986), reduction in the amount of water released and the effluent quality (Piedrahita, 2003), improved hygiene and disease management (Summerfelt *et al.*, 2009) and biological pollution control (Zoha *et al.*, 2005). Over the last decade, fish productions in Nigeria have been practiced in Static Renewable Aquaculture System (SRAS). The need to have high yield utilizing little space, reuse of water for better hygiene and disease management as well as biological pollution control has necessitated the use of RAS for this trial as comparative study.

The aquaculture productions of the catfishes such as *C. gariepinus, H. bidorsalis* and their hybrids have been practiced for a long time in Nigeria (Adewolu *et al.*, 2008). The hybrids are one of the commercially important species of fish for rapid aquaculture expansion in Nigeria (Adamu and Kori-Siakpere, 2011). They are being successfully produced in public as well as private hatcheries making them available for farming.

The growth profiles of fish are remarkable tool to ascertain the acceptability of the feed and the culture system in practice. The physiological profiles are biomarkers that interact with the fish and its environment. These profiles are used to determine the health status of the fish.

There are limited literatures on the comparison study of the growth and physiological profiles of hybrid catfish in RAS and SRAS fed commercial diet. Eding and Kamstra (2001) have reported the successful culturing of *C. gariepinus* in RAS at full commercial scale in Denmark while its biological performance was reported by Akinwole and Faturoti (2007) in Nigeria. On the other hand, Adewolu *et al.*, (2008) and Ataguba *et al.*, (2010) reported successful culture of hybrid catfish in SRAS fed commercial feed (Coppens). This feed is widely used and most preferred by fish farmers in Nigeria. The study is therefore aimed at evaluating the growth and physiological profiles of hybrid catfish fed Coppens in two different water regimes for 12 weeks in order test the efficiency of the feed in the two water regimes.

MATERIALS AND METHODS

The experiment was conducted for 12-week period with two replications. Fingerlings of hybrid catfish (*H. bidorsalis (♂) & C. gariepinus (♀)*) of the same brood stock and mixed sex of mean weight and length of 1.01±0.02g and 4.63±0.34cm were procured from Omu fish farm in Igbide, Isoko South L.G.A of Delta State, Nigeria. Twelve (12) fish were placed in each of the system aquarium. They were fed twice daily at 8.00 and 19.00 hours with Coppens produced by Coppens international, P.O. Box 543, 370AM Helmond, Holland at 5% rate of body biomass. The proximate composition of the diet is presented in Table 1. The ration was adjusted bi-weekly based on the sample total body biomass. The experiment was maintained under natural photoperiod which of 17/07 (light/dark) and 12/12 (light/dark) throughout the trial period. Some physico-chemical parameters such as water temperature, pH, conductivity, total alkalinity, dissolved oxygen and free carbon dioxide were monitored bi-weekly with exceptions of water temperature and pH that were monitored weekly as described by the method described in APHA (1998).

Table 1: Proximate composition of Coppens used as feed for hybrid catfish during the study period

Proximate compositions	Quantity (%)
Moisture	NP
Crude protein	45.00
Crude fiber	15.00
Carbohydrate	12.00
Ash	9.50
Sodium	0.40
Potassium	1.70
Crude lipids	NP
Total organic matter	NP

NP-not provided

In the determination of the growth profiles, all fishes were collected and measured bi-weekly to the nearest 0.10g for weight verification following the method described by Ufodike and Matty (1993) and returned into their respective tanks before the next feeding period. Mortalities were noted on a daily basis and percentage survival was calculated. The calculated growth profiles were in accordance to Metailler (1987), Iwama and Tantz (1981) and Goddard (1996). These profiles are:

- Weight gain (WG)g = (Wf – Wi) Where Wf = final weight and Wi = initial weight
- Daily growth rate (%) = × 100
- Hepato-somatic index (%) = × 100
- Visceromatic index (%) = × 100
- Protein efficiency ratio =
- Food conversion ratio (FCR) =

- Gross efficiency of food conversion (%) = × 100
- Specific growth rate (%) = x 100
- Relative growth rate (%) = x 100
  Where Wi is the initial weight, Wf is the final weight, t is trial periods and Log_e is natural logarithm
- Survival rate (%) = x 100
- Nitrogen metabolism = Where A = initial mean weight of fish, B = final mean weight of fish, H = trial periods in days
- Protein intake = Food supplied (g)/% crude protein of food

At the end of the 12-week trial period, twenty-four (24) fishes per set-up were sacrificed for the determination of the selected physiological profiles which are Glucose, Triglycerides, Cholesterol, Total protein, Albumin, Globulin, Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, Acid phospatase, Gamma-glutamyltransferase, Sodium, Potassium, Calcium, Magnesium, Chloride, Inorganic phosphorous, Urea, Urea-Nitrogen, Uric acid and Creatinine in the serum, liver and kidney. Blood collection was done as described in Kori-Siakpere (1998) and allowed to clot (Mahoba, 1987) before centrifuging for serum (Ogbu and Okechukwu, 2001). The liver and kidney samples were obtained as described by Mahoba (1987). The physiological profiles were determined using their respective commercial kits of either Cromatest Linear chemical Barcelona, Spain, Teco diagnostics, Anaheim, United State of America or Randox laboratory Ltd, Antrim, United Kingdom in accordance with the manufacturer's instruction.

All data were presented as mean and stardard error, the data were first analyzed using analysis of variance, after which the individual means were compared using Bonferoni multi-sample correction/test. All statistical analyses were performed using the software package (graphpad prism @ software version 6.0 San Diego, C.A).

RESULTS

The monitored physico-chemical parameters are presented in Table 2. SRAS recorded the higher values for water temperature and conductivity of 27.00(0.01) (^{O}C) and 147.50(0.01) (µs/cm). The higher values for pH, total alkalinity, dissolved oxygen and free carbon (iv) oxides were recorded in RAS to be 7.18(0.02), 0.50(0.01)mg/L, 7.80(0.01)mg/L and 1.95(0.02)mg/L respectively. There was no significant difference (p>0.05) in the monitored parameters amongst the two systems.

The cumulative biomass of hybrid catfish is presented in Table 3. It showed a progressive increase in biomass per sampling period. The cumulative biomass was higher in RAS fish from the first to the 3rdh sampling period, however, from the fourth to the 6th sampling periods the SRAS fish showed higher cumulative biomass. The final cumulative biomass for RAS and SRAS fishes was 16.45 (2.69)g and 17.01 (1.17)g respectively. There was no significant difference (p>0.05) in the final biomass between the fish grown in the two systems.

Table 2: Mean values of physico-chemical parameters of the water regimes where hybrid catfish was fed Coppens during the study period.

Water regimes	Physico-chemical parameters					
	Water Temperature (^{O}C)	pH	Conductivity (µs/cm)	Total Alkalinity (mg/l)	Dissolved oxygen (mg/l)	Free carbon dioxide (mg/l)
SRAS	27.00 (0.01)	7.10 (0.01)	147.50 (0.01)	4.50 (0.02)	7.65 (0.02)	1.85 (0.01)
RAS	26.50 (0.02)	7.18 (0.02)	146.00 (0.03)	5.00 (0.01)	7.80 (0.01)	1.95 (0.02)

Standard error in parenthesis

Table 3: Mean values of cumulative biomass of hybrid catfish fed Coppens in the water regimes for 12-week.

Water regimes		Sampling periods (weeks)					
	0	2	4	6	8	10	12
RAS	0.98 (0.04)	4.18 (0.44)	6.99 (1.44)	10.00 (0.79)	13.80 (3.43)	15.09 (2.53)	16.45 (2.69)
SRAS	0.98 (0.04)	3.92 (0.70)	6.43 (2.04)	9.92 (1.37)	14.36 (1.87)	15.22 (1.55)	17.34 (1.86)

Standard error in parenthesis

Table 4: Mean values of calculated growth profiles of hybrid catfish fed Coppens in the water regimes for 12 weeks.

Growth profiles	RAS	SRAS
Initial weight (g)	0.98 (0.02)	0.98 (0.02)
Final weight (g)	16.45 (1.55)	17.01 (1.19)
Weight gain (g)	15.81 (1.50)	16.03 (1.18)
Daily growth rate (%)	18.41 (1.88)	19.08 (1.41)
Relative growth rate (%)	158.12 (19.74)	163.15 (12.16)
Specific growth rate (%)	3.35 (0.13)	3.39 (0.08)
Hepato-somatic index (%)	1.35 (0.02)	1.30 (0.01)
Visceromatic index (%)	41.61 (0.73)	41.46 (0.64)
Protein efficiency ratio	0.39 (0.04)	0.40 (0.03)
Feed conversion ratio	1.09 (0.27)	0.96 (0.19)
Gross efficiency of feed conversion (%)	0.03 (0.00)	0.03 (0.00)
Nitrogen metabolism (g)	40.20 (3.45)	41.49 (2.74)
Protein intake	12.50	12.50
Survival rate (%)	90.00	80.00

Standard error in parenthesis

Table 5: Mean values of serum physiological profiles of hybrid catfish fed Coppens in the water regimes for 12 weeks.

Physiological profiles	RAS	SRAS
Glucose (mg/dl)	110.08 (0.14)	141.58 (0.14) *
Triglycerides (mg/dl)	164.12 (0.47)	186.84 (0.24)
Cholesterol (mg/dl)	123.95 (0.18)	146.04 (0.18) *
Total Protein (g/dl)	4.16 (0.01)	4.67 (0.01)
Albumin (g/dl)	3.05 (0.05)	3.54 (0.01)
Globulin (g/dl)	1.12 (0.01)	1.13 (0.01)
Aspartate aminotransferase (U/L)	24.55 (0.25)	15.85 (0.05)
Alanine aminotransferase (U/L)	40.95 (0.15)	42.50 (0.50)
Alkaline phosphatase (U/L)	5.52 (0.00)	5.52 (0.01)
Acid phosphatase (U/L)	1.28 (0.43)	1.28 (0.43)
Gamma glutamyltransferase (U/L)	5.00 (1.67)	1.07 (0.56)
Sodium (MEq/L)	86.65 (1.86)	88.73 (1.97)
Potassium (MEq/L)	4.13 (0.05)	4.06 (0.08)
Calcium (mg/dl)	10.29 (0.13)	10.26 (0.07)
Magnesium (MEq/L)	2.25 (0.17)	2.28 (0.03)
Chloride (MEq/L)	91.60 (0.26)	91.76 (0.34)
Inorganic Phosphorous (mg/dL)	2.37 (0.11)	2.34 (0.18)
Urea (mg/dL)	15.92 (0.06)	14.51 (0.06)
Urea-Nitrogen (mg/dL)	7.44 (0.03)	6.78 (0.01)
Uric acid (mg/dL)	6.59 (0.03)	6.35 (0.01)
Creatinine (mg/dL)	1.50 (0.05)	1.00 (0.00)

Standard error in parenthesis, value with asterisk (*) showed significant difference (p<0.05)

Table 6: Mean values of liver physiological profiles of hybrid catfish fed Coppens in the water regimes for 12 weeks.

Physiological profiles	RAS	SRAS
Glucose (mg/dl)	100.00 (0.28)	98.62 (0.28)
Triglycerides (mg/dl)	125.17 (0.24)	188.84 (0.24) *
Cholesterol (mg/dl)	115.29 (0.37)	122.84 (0.18)
Total Protein (g/dl)	4.42 (0.01)	5.41 (0.01) *
Albumin (g/dl)	3.10 (0.01)	4.52 (0.01) *
Globulin (g/dl)	1.32 (0.01)	1.01 (0.01)
Aspartate aminotransferase (U/L)	44.10 (1.10)	24.10 (0.10) *
Alanine aminotransferase (U/L)	50.25 (0.05)	42.55 (0.05)
Alkaline phosphatase (U/L)	2.76 (0.00)	4.14 (1.38)
Acid phosphatase (U/L)	0.85 (0.00)	0.85 (0.00)
Gamma glutamyltransferase (U/L)	1.67 (0.56)	3.33 (0.00)
Sodium (MEq/L)	54.83 (0.88)	53.87 (1.30)
Potassium (MEq/L)	3.00 (0.01)	3.37 (0.36)
Calcium (mg/dl)	6.78 (0.01)	5.12 (0.01) *
Magnesium (MEq/L)	1.95 (0.02)	2.00 (0.01)
Chloride (MEq/L)	60.45 (0.15)	54.29 (0.03) *
Inorganic Phosphorous (mg/dL)	3.09 (0.19)	3.00 (0.04)
Urea (mg/dL)	12.53 (0.01)	14.60 (0.01) *
Urea-Nitrogen (mg/dL)	5.86 (0.01)	6.82 (0.01) *
Uric acid (mg/dL)	7.26 (0.02)	6.47 (0.01) *
Creatinine (mg/dL)	2.50 (0.50)	1.50 (0.50)

Standard error in parenthesis, value with asterisk (*) showed significant difference (p<0.05)

The calculated growth profiles are presented in Table 4. SRAS fishes showed higher final biomass of 17.01(1.19)g, nitrogen metabolism of 41.49(2.74)g, weight gain of 16.03(1.18)g, relative growth rate of 163.15(12.16)%, specific growth rate of 3.39 (0.08)%, visceromatic index of 41.46(0.64)% and protein efficiency ratio of 0.40 (0.03) while RAS fishes recorded the higher hepato-somatic index of 1.35(0.02)%, feed conversion ratio of 1.09(0.27) and survival rate of 90% respectively. There was no significant difference (p>0.05) in the calculated growth profiles between the fishes grown in the different water regimes.

The determined serum physiological profiles are presented in Table 5. All the determined profiles were not significantly different (p>0.05) except glucose and cholesterol that were significantly higher (p<0.05) in SRAS fishes.

The liver physiological profiles are presented in Table 6. There was significant difference (p<0.05) in all the determined profiles with exceptions of glucose, globulin, alanine aminotransferase, alkaline phosphatase, acid phosphatase, glutamyltransferase, sodium, potassium, magnesium and creatinine.

The kidney physiological profiles are presented in Table 7. Profiles such as glucose, alanine aminotransferase and calcium showed significant difference (p<0.05) between in RAS and SRAS fishes.

DISCUSSION

The monitored physico-chemical parameters are paramount to the many factors that affect fish health, growth and physiology (Camus *et al.,* 1998). According to Boyd and Lichthoppler (1979) the water temperature range for freshwater fishes is 24.5°C – 29.5°C and is inversely proportional to dissolved oxygen level. The level of water temperature monitored are within the reported range for hybrid catfish as supported by Abu *et al.,* (2009), Nwabueze and Agbogidi (2006) and Oguguah *et al.,* (2011). The pH value of 7.10(0.01) – 7.18(0.02) obtained in this study is in consistence with that reported for freshwater fishes as 7.40 – 7.55 by Belarin (1979) and Boyd (1982).

Table 7: Mean values of kidney physiological profiles of hybrid catfish fed Coppens in the water regimes for 12 weeks.

Physiological profiles	RAS	SRAS
Glucose (mg/dl)	129.97 (0.14)	151.94 (0.28) *
Triglycerides (mg/dl)	154.79 (0.24)	139.95 (0.24)
Cholesterol (mg/dl)	106.14 (0.74)	107.80 (0.19)
Total Protein (g/dl)	4.30 (0.03)	4.53 (0.16)
Albumin (g/dl)	3.38 (0.03)	3.88 (0.02)
Globulin (g/dl)	0.92 (0.01)	0.65 (0.14)
Aspartate aminotransferase (U/L)	50.10 (0.10)	55.20 (0.10)
Alanine aminotransferase (U/L)	68.10 (0.10)	58.10 (0.10) *
Alkaline phosphatase (U/L)	4.14 (1.38)	12.42 (1.38) *
Acid phosphatase (U/L)	0.85 (0.00)	1.71 (0.00)
Gamma glutamyltransferase (U/L)	4.44 (0.00)	6.66 (0.00)
Sodium (MEq/L)	210.61 (0.44)	248.11 (0.44)
Potassium (MEq/L)	4.15 (0.01)	4.56 (0.05)
Calcium (mg/dl)	5.69 (0.01)	6.28 (0.01) *
Magnesium (MEq/L)	1.87 (0.01)	1.88 (0.04)
Chloride (MEq/L)	52.36 (0.03)	56.56 (0.13)
Inorganic Phosphorous (mg/dL)	2.53 (0.11)	2.51 (0.03)
Urea (mg/dL)	15.72 (0.01)	15.42 (0.02)
Urea-Nitrogen (mg/dL)	7.34 (0.01)	7.20 (0.01)
Uric acid (mg/dL)	6.39 (0.01)	6.61 (0.01)
Creatinine (mg/dL)	2.00 (0.00)	2.50 (0.50)

Standard error in parenthesis, value with asterisk (*) showed significant difference (p<0.05)

It is particularly in accord with that of Adamu and Kori-Siakpere (2011) for hybrid catfish. Therefore, the pH recorded in the two systems is within the acceptable range for hybrid catfish. The dissolved oxygen was noticed to be higher in RAS than SRAS. This may be due to the water exchange rate and oxygenation mechanism in RAS. This therefore, implies that there was good quality of dissolved oxygen for fish utilization as supported by Akinwole and Faturoti (2007). The value of free carbon dioxide in this study was noticed to be inversely proportional to the value of dissolved oxygen signifying proper and adequate supply of oxygen and water exchange. The total alkalinity and conductivity were all within acceptable range for hybrid catfish (Adamu and Kori-Siakpere, 2011 and Gabriel *et al.*, 2010).

The survival rate was insignificantly higher (p>0.05) in RAS than SRAS. However, the recorded mortality may be due to natural condition as there was no significant water quality fluctuation, no disease problem nor any evidence of handling stress after weighing. The study revealed that hybrid catfish grown in RAS showed lower weight gain compared to SRAS of but accommodates higher survival rate and stocking density as supported by Oso *et al.*, (2011). The relative growth rate and specific growth rate were better than that reported by Omoruwou and Edema (2011) and (Akinwande *et al.*, 2002 and Odedeyi, 2007) respectively. The feed conversion ratio recorded was inversely proportional to weight gain, specific growth rate, protein intake, gross efficiency of feed conversion and nitrogen metabolism. The percentages are within the range reported by Fagbenro *et al.*, (1992) and Ndimele and Owodeinde (2012) but above the value for culturing *Clarias gariepinus* in RAS (Eding and Kamstra, 2001). According to Cowey *et al.*, (1974) the metabolic rate of fish is higher at young stage and much of the ingested protein is used as a source of energy. Thus the level of protein efficiency ratio recorded in the study. The study of Kiriratnikom and Kiriratnikom (2002) and Ndimele and Owodeinde (2012) supported this finding.

The protein intake and gross conversion of feed efficiency were all within the reported values of Akinwande *et al.,* (2002) and Omoruwou and Edema (2011).

Glucose provides the major energy source for fish. It is derived from the breakdown of carbohydrate obtained from daily diet that is regulated through the process of glycogenolysis and gluconeogenesis. The glucose level reported in this study is higher in kidney, less in serum and least in liver which are all within the acceptable range for catfish (Yilmaz *et al.,* 2006). The high kidney glucose may be attributed to its role in homeostasis by filtration and re-absorption. The triglycerides level is reported to be higher in serum, less in kidney and least in liver which thereby showed that the fish are not responding to any challenge as supported by Ogueji and Auta (2007). The cholesterol level was within the acceptable range for hybrid catfish. The least cholesterol level was recorded in the kidney and less in the liver signifying its function in the synthesis and excretion. The high serum cholesterol content is linked to lipid metabolism. The total protein value in the study is supported by Abu *et al.,* (2007). Total protein is important constituent of all cells and tissues thus play vital role in the physiology of living organism. That is reflected in its high value in the liver, less in serum and least in kidney. According to Grant (1987) albumin is the most abundant serum protein representing 55-65% of the total protein thus the value reported in the study is consistent with that reported by Adamu and Kori-Siakpere (2011) for hybrid catfish. This has been proven by the globulin values obtained which is involved in the transport of varieties of substance.

Aminotransferases are gainfully used in the diagnosis of disease and damage tissue where it functions as a link between carbohydrate and protein metabolism by catalyzing the inter-conversion of strategic compounds. The activity of aspartate aminotransferase is generally higher than that of alanine aminotransferase as supported by Gabriel *et al.,* (2009, 2010 and 2012). The lowest activity of aminotransferase in serum is adequate as they only exist in small amount (Wells *et al.,* 1986). The higher liver aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase inferred the intermediary metabolism as supported by Begum (2004). Phosphatases are important enzymes of animal metabolism which plays important role in the transport of metabolites across membrane (Vorbrodt, 1959). This suggests for its high serum value and least kidney values. The activity of gamma glutamyltransferase was recorded to be high liver and least in serum as supported by the report of Adamu (2009) for hybrid catfish because it catalyses the transfer of the gamma-glutamymoiety of glutathione to an acceptor that may be an amino acid, a peptide or water.

Sodium is the major cation of extracellular fluid; potassium is the principal cation of the intracellular fluid and calcium plays important role in bone formation, reproduction (Pang *et al.,* 1980), in mitochondrial function (Lehninger, 1975) and facilitates neuromuscular excitability (Aurbach *et al.*, 1985) showing their activity to be higher in kidney, less in serum and least in liver thus that provides their ability to perform their respective functions. Chloride is a major anion in the maintenance of the cation/anion balance intra- and extra-cellular fluids. Magnesium is important for physiological processes. It is an indicator of many enzymes. Sodium, chloride, potassium and calcium are primarily for osmoregulation of fishes. These electrolytes also serve electrochemical, enzymatic and structural functions. The level of sodium shown to high in kidney, less in serum and least in liver is an indication of normal physiological status of the fish and within range reported by Fagbenro *et al.,* (1992) and Yilmaz *et al.,* (2006).

The determined metabolic waste products (urea, urea-nitrogen, uric acid and creatinine) in serum, liver and kidney were within the range reported by Adamu and Kori-Siakpere (2011). The amount of urea-nitrogen in the serum is an indication of protein metabolism. The determined metabolic waste products are important in understanding the protein metabolic activity of the fish. These parameters are recorded to high in the kidney for their excretive function, less in the liver for their metabolic activity and least in the serum for the transportation function.

CONCLUSION

Producing more food from the same area of land while reducing the environmental impact requires what has been called sustainable intensification (Godfrey *et al.*, 2010). Considering all aquaculture production systems in use today, RAS offers the possibility of achieving a high production, maintaining optimal environmental condition and creating a minimal ecological impact. The slight difference in the determined physiological profiles observed within the two systems may be attributed to individual difference on one hand and the lower water renewal/exchange of RAS on the other hand.

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