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EVALUATION OF HAEMATOLOGICAL RESPONSES OF *CLARIAS GARIEPINUS* (Burchell 1822) TO AMMONIA AND NITRITE LEVELS IN SOME SELECTED FISH FARMS IN IBADAN, NIGERIA.

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ABSTRACT

This study evaluated the levels of ammonia and nitrite in *Clarias gariepinus* in some selected fish farms in Ibadan. Twelve active fish farms were selected on the basis of their cultural practices and environments: extensive 4 each from semi-intensive and intensive systems. Ammonia and nitrite levels and their sources to the farms were studied. Blood samples of randomly selected 20 *C. gariepinus* were collected in each of these farms and evaluated for primary and secondary stress indicators.

The mean PCV (37.1 ± 7.3%), WBC (3.4 ± 3.2l $10^3/mm^3$) of fish samples from farms with semi-intensive system were the highest ($P<0.05$) when compared with the other culture systems. The mean values of plasma cortisol (70.29 ± 8.9ng/dl), plasma sodium (134.6 ± 8.0mg/dl), plasma chloride (104.7 ± 4.0ng/dl) and plasma glucose (88.9 ± 33.6mM) recorded in semi-intensive system were the highest and were significantly different ($P<0.05$) from values obtained from the other culture systems. Haematological and hormonal balances of adult *C. gariepinus* were affected under short-term exposure to nitrite and ammonia toxicity in culture environment.

Keywords: Heamatology, *Clarias gariepinus*, nitrite, ammonia, toxicity

INTRODUCTION

In Nigeria, with increased level in intensity of fish farming, National Special Programme for Food Security (NSPFS, 2004), study of stress, especially water quality stress, in relation to the output is very important in order to ensure that optimal results are obtained. In view of recent advances in the culture methods, feed quality and quality of fish cultured, there is significant improvement in output from fish farms. However, the stress effects of these culture methods on mainly ammonia and nitrite build up as a result of various management practices (especially fish nutrition) need to be studied. These would provide useful information needed towards realization of optimal production capacity of fish farms.

C. gariepinus is one of the most commonly cultured fish species in Nigeria (NSPFS, 2004). The yield from *C. gariepinus* in fish farm contributed about 80% of the total aquaculture production in Nigeria. (Akinyemi, 1998; NSPFS, 2005). The study of responses of *C. gariepinus* to various water quality stresses would go a long way in improving the knowledge in the effective production of *C. gariepinus*.

This study therefore examines the physiological response of cultured *C. gariepinus* to ammonia and nitrite levels in some selected fish farms in Ibadan, Nigeria.

MATERIALS AND METHODS

Survey of twelve active fish farms in Ibadan was carried out. The farms visited were selected on the basis of cultural practices such as level of stocking, feeding method, feeding rate, feed types and operating system. Based on their cultural practices, the cultural systems were identified as described by Huet (1972) namely extensive, semi-intensive and intensive systems. Four fish farms were randomly selected covering each of the culture systems.

Through the use of structured questionnaire, the following information were obtained in all the fish farms surveyed:

(1) Types/species of fish stocked
(2) Stocking rate and density
(3) Stocking duration
(4) Feeding methods and type of feed fed to the fish
(5) Feeding frequency
(6) Management practices
(7) Source of water supply

Water samples in each of the farms were obtained in order to determine the water quality parameters (alkalinity, nitrite, ammonia, chloride, pH and dissolved oxygen) under study

systems are presented in Table 3. The variations in the mean values of each of the water quality parameters examined under different culture systems are stated below.
The ammonia and dissolved oxygen levels were significantly different ($P < 0.05$) among the culture systems while the nitrite, total dissolved solids and pH values obtained were not significantly different among the culture systems.

The Pearson correlation matrix of the culture systems with water quality parameters is presented in table 4. The culture systems have direct positive and significant ($p < 0.05$) correlations with ammonia ($r = 0.60$) and nitrite ($r = 0.4$) while the culture systems have negative correlation with dissolved oxygen ($r = -0.75$) and pH ($r = -0.03$). Ammonia had positive correlations with nitrite, total dissolved solid and pH with r-values of 0.66, 0.60 and 0.04 respectively. The dissolved oxygen level showed a negative but significant correlation ($p<0.05$) with ammonia and nitrite with r-values of -0.58 and –0.44 respectively.

Haematological parameters of fish samples examined under different culture systems

The mean haematological parameters of the fish samples in all the culture systems examined are given in Table 5.
The mean PCV values of *C. gariepinus* varied from 32.50 $\pm$ 3.53% to 37.14 $\pm$ 7.52%. Analysis of variance on the PCV values of *C. gariepinus* under the different culture systems showed that there was no significant variation ($P > 0.05$) in the PCV values among the different culture systems.

The mean haemoglobin values of the *C. gariepinus* sampled range from 10.83 $\pm$ 4.04g/dl (extensive system) to 10.93 $\pm$ 1.60g/dl (semi – intensive). Mean values of white blood cell counts of *C. gariepinus* ranged from 13.73 $\pm$ 2.23 $10^3/mm^3$ to 34.16 $\pm$ 3.23 $10^3/mm^3$. The mean value of white blood cells of *C. gariepinus* samples in semi – intensive system was the highest with mean white blood cell value of 34.16 $\pm$ 3.23 $10^3/mm^3$ while the *C. gariepinus* examined in intensive system had the lowest mean WBC values of 13.73 $\pm$ 2.23 $10^3/mm^3$. The mean white blood cell values of *C. gariepinus* sampled under different culture systems were significantly different ($p<0.05$) among the culture systems. The mean values of red blood cell counts in all *C. gariepinus* sampled under different culture systems are significantly different ($P<0.05$) among the culture systems. The mean values of platelets varied from 1.11 $\pm$ 1.98 $10^3/mm^3$ to 2.34 $\pm$ 1.56 $10^3/mm^3$. The mean platelets value exhibited a significant variation ($P<0.05$) among the culture systems.

Plasma biochemistry of fish samples examined under different culture systems

The mean plasma biochemistry of *C. gariepinus* under different culture systems is presented in Table 6.

The mean plasma sodium level varies from 129.67 $\pm$ 4.5mg/dl to 134.57 + 8.00mg/dl. The mean plasma sodium level in *C. gariepinus* (129.67 $\pm$ 4.5mg/dl) sampled under extensive system is significantly different ($P<0.05$) from the other culture systems with values of 133.00 $\pm$ 11.31mg/dl and 134.57 + 8.00mg/dl recorded for intensive and semi – intensive systems respectively. The mean plasma potassium level in *C. gariepinus* ranges from 2.70 $\pm$ 0.57mg/dl in intensive system to 9.23 $\pm$ 4.66mg/dl (extensive system). The mean plasma potassium value in *C. gariepinus* is significantly different ($P <0.05$) among the different culture systems.

The value of plasma chloride varied from 67.50 $\pm$ 49.30mg/dl to 104.50 $\pm$ 6.30mg/dl. The mean plasma chloride values of 67.50 $\pm$ 49.30mg/dl was recorded for intensive system, this was significantly different ($P<0.05$) from the other two systems. The plasma chloride values of 104.50 $\pm$ 3.99mg/dl and 104.50 $\pm$ 6.30 mg/dl were obtained for semi-intensive and extensive systems, respectively. The lowest mean total protein value of 4.19 $\pm$ 0.82g/dl was obtained in *Clarias gariepinus* sampled under extensive system while the highest mean value for total protein of 5.13 $\pm$ 1.54g/dl is recorded for *C. gariepinus* samples under semi-intensive systems. The mean total protein value for *C. gariepinus* sampled under semi – intensive system is significantly different ($P<0.05$) from the other systems.

The mean plasma glucose of the fish sampled ranged from 48.33 $\pm$ 38.0mM in extensive system to 88.86 $\pm$ 16.90mM for semi – intensive system. The mean plasma glucose values of all the fish sampled were significantly different ($P<0.05$) among the culture systems. The mean values of plasma cortisol ranged from 61.50 $\pm$ 10.61ng/d to 70.29 $\pm$ 8.9ng/dl. The highest value of 70.29 $\pm$ 8.9ng/dl was obtained under semi – intensive system followed by the values of 61.67 $\pm$ 1.53ng/dl and 61.50 $\pm$ 10.61 ngldl for extensive and semi intensive systems respectively. The plasma cortisol value obtained for fish samples in semi intensive system was significantly higher ($P<0.05$) than those of the other systems.

DISCUSSION

The management and cultural practices are relatively advanced in farms with intensive systems with consequent advantage of improvement in the yield recorded when compared with other culture systems. This agreed with the observation of Huet (1972) that intensive system is an advanced system of fish culture and with high level of stocking and good quality artificial feeding.

The water quality parameters varied with each culture system. The relatively low level of pH in intensive system may be attributed to the management practices, which encourage introduction of pollutant due to high level of feeding rate, interval and increase in stocking density (Huet, 1972). Ammonia and nitrite levels were the highest under intensive system while lowest values were obtained with extensive system.

Generally, the water quality parameters examined in all the culture systems studied fall within the acceptable range as reported by Boyd (1979). The culture systems had a positive correlation with ammonia and nitrite, this show that the impacts of ammonia and nitrite are felt more as the culture system or management practice advances. This may be due to the pollution effects of increasing waste feed and higher density stocking from extensive system to intensive system.

The PCV values in all the systems examined fell within the baseline value of 37.0 $\pm$ 0.5% as reported by Adedeji *et. al.*, (2000). However, relatively low mean values obtained for extensive and intensive systems may be linked to stress due to high stocking density and intensity of feeding with consequent problem of pollution.

The mean haemoglobin level and the mean red blood cell count obtained in all the culture systems are in accord with the baseline values of 10.0 $\pm$ 0.10mg/dl and 2.4 $\pm$ 0.10 10^6/ml for haemoglobin and red blood cell count respectively by Adedeji *et. al.*, (2000). Low red blood cell count in extensive system may be attributed to management practices where the nutrient quality of the feed fed is very poor.

The mean value of white blood cell count, which is 13.73 $\pm$ 2.33 $10^3/mm^3$ under the intensive culture system, is the lowest. This may be as a result of stressors in the water body due to management practices in this system. A good proportion of the cells may have been released to maintain homeostatis. It was noted that in all the culture systems, the white blood cell count is lower compared to the 40, 400.0 $\pm$ 5,096.6µl as the baseline recorded by Adedeji *et. al.*, (2000). According to Hussein *et. al.*, (1996), decreases in leukocyte (white blood cell) numbers commonly occur during the stress response.

The low value of plasma chloride (67.50 $\pm$ 49.30mg/dl) recorded for intensive system may have resulted from stress as corroborated by Wedemeyer *et al.*, (1990) when he stipulated that the value that falls below 90mEq/^{1} often result from stress and can become life threatening. The kind of stress may be rationalized to be connected with nitrite since nitrite appears to enter the fish through the same route as chloride by being a competitive inhibitor of the active chloride uptake mechanism in the gills as noted by William and Eddy, (1986). Nitrite's entry and accumulation may be due to the low level of chloride. The mean values of total protein obtained in all the systems are in line with the baseline total protein. The value of 4.19 $\pm$ 0.82g/dl obtained under extensive culture system was the lowest and this may be due to nutritional imbalance under this system as a result of poor quality feed and irregular feeding interval (Wedemeyer and Yasutake, 1977; Coles, 1986; Jain, 1986). The mean value of plasma glucose (48.33 $\pm$ 38.0mM) that was obtained for extensive system may be due to the type of cultural practice done. With little feeding done apart from liming and fertilization and the fish being left to nature to take care of them, the glucose level will be low. In other words, nutritional imbalance may be a very strong factor.

The mean values obtained for the hormone, plasma cortisol in the different systems range between 60ng/dl and slightly over 70ng/dl.

Although, there is scanty information on response level of plasma cortisol level of *C. gariepinus* in relation to stress but from available information, (Schreck *et. al.*, 2001) the three systems can be classified as been stressed even though, the severity differs among them with lowest stress level observed in fish under extensive system.

CONCLUSION

It therefore concluded through this study that the toxic effects of nitrite and ammonia were more pronounced with increase in intensity of fish production technique especially under intensive system. Therefore, it is very important that these water quality stressors i.e. nitrite and ammonia levels be monitored regularly and level should be controlled through various management practices when necessary. Uncontrolled levels of ammonia and nitrite in culture environment may not only lead to mortality but may prevent the fish from achieving its full genetic potential in terms of growth and reproductive capability.

Recommendation

- Feed quality, feeding interval and rate must be well monitored in culture system. Practices that allow wastages of feed in culture systems should be discouraged as these

Table 1: Operational mechanisms in the fish farms surveyed in Ibadan

Culture system/ Operations Mechanism	Intensive (4 farms)	Semi – Intensive (4 farms)	Extensive (4 farms)
Year of operations:			
a) > 10 years	50%	75%	75%
b) < 10 years	50%	25%	25%
Culture type			
a) Polyculture	Nil	Nil	30%
b) Monoculture	100%	100%	70%
Stocking rate (No./m^2)	30 – 60 fish/ m^2	5 – 10 fish/ m^2	1 –2fish/ m^2
Stocking duration	6 – 9 months	6 – 10 months	≥ 12months
Time of stocking	Variable	Seasonal	Seasonal
Integrated farming	50%	100%	--
Yield / ha	7 – 12 tonnes	4 – 6 tonnes	< 2 tonnes

Table 2: Management practices in fish farms surveyed in Ibadan.

Culture systems/Management practices	Intensive(4 farms)	Semi – Intensive (4 farms)	Extensive (4 farms)
Feeding			
a) Regular	100%	100%	75%
b) Not regular	-	-	25%
Feed production			
a) Internally	8%	50%	100%
b) External sources	92%	50%	-
Feed quality			
Crude protein	35% - 45%	30% - 40%	20% - 28%
Feeding rate	3 – 5% body weight	2 – 5% body weight	N/A
Feeding interval	3 – 5 times daily	2 times daily	1 –2 times daily
Liming	-	100%	100%
Fertilization	-	100%	100%
Record of Mortality	100%	75%	N/A
Record of diseases			
a) Regular	25%	-	N/A
b) Occasional	75%	25	N/A
c) Rarely	-	75	N/A
d) None	-	-	N/A

N/A – Not Available

Table 3: Mean values of Water quality parameters in the farms sampled under different culture systems

Culture systems	pH	Total Dissolved solid (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Dissolved Oxygen(mg/l)
Extensive	7.72± 0.42[a]	262.30±170.56[a]	53.13±13.57[a]	17.68±14.30[a]	7.30± 1.09[a]
Semi-intensive	7.40±0.18[a]	256.78±265.53[a]	55.18±86.56[a]	18.87±16.01[a]	8.40± 4.01[b]
Intensive	7.48±0.36[a]	334.95±193.18[b]	95.71±58.01[b]	21.36±20.54[b]	6.50± 2.23[c]

Means followed by the same superscript in each column are not significantly different ($P > 0.05$)

Table 4: Pearson Correlation matrix for the culture systems and water quality parameters

	Culture systems	Ammonia	Nitrite	Dissolved Oxygen	Total Dissolved Solid	PH
Culture systems	1.00	0.60*	0.43*	-0.75*	0.10	-0.03
Ammonia	0.60*	1.00	0.66*	-0.58*	0.60*	0.04
Nitrite	0.43*	0.66*	1.00	-0.44*	0.65*	0.09
Dissolved Oxygen	-0.75*	-0.58*	-0.44*	1.00	-0.20	-0.08
Total Dissolved Solid	0.10	0.60*	0.65*	-0.20	1.00	-0.31
PH	-0.03	0.04	0.09	-0.08	-0.31	1.00

Marked correlations are significant at $P< 0.05$

Table 5: Mean values of some Haematological parameters of *Clarias gariepinus* cultivated under different culture systems

Parameters/ Culture system	Packed Cell Volume (%)	Heamoglobin (g/dl)	White Blood Cell (10^9/l)	Red Blood Cell (10^6/l)	Platelets (10^9/l)	Lymphocytes
Extensive	34.33±6.34[a]	10.83±4.04[a]	1.48±3.96[a]	2.43±0.25[a]	1.11±1.78[a]	61.67±1.53[a]
Semi intensive	37.14±7.52[a]	10.93±1.60[a]	3.42±3.23[b]	2.83±0.47[a]	2.04±0.98[b]	70.29±8.71[b]
Intensive	32.50±3.53[a]	10.90±0.71[a]	1.37±2.22[a]	2.61±0.32[a]	2.34±1.56[b]	61.50±10.61[a]

Means followed by the same superscript in each column are not significantly different (P > 0.05)

Table 6: Mean Plasma Biochemistry parameters of *Clarias gariepinus* cultivated under different culture systems

Parameters/ Culture system	Plasma Sodium (mg/dl)	Plasma Potassium (mg/dl)	Plasma Chloride (mg/dl)	Total protein (g/dl)	Plasma glucose (mM)	Plasma cortisol (ng/dl)
Extensive	129.67±4.51[a]	9.23±4.66[a]	104.50±6.30[a]	4.19±0.82[a]	48.33±38.00[a]	61.67±1.53[a]
Semi intensive	134.57±8.00[b]	4.40±0.57[b]	104.50±3.99[a]	5.13±1.54[b]	88.86±16.90[b]	70.29±8.71[b]
Intensive	133.00±11.31[b]	2.70±0.57[c]	67.50±49.30[b]	4.70±0.71[a]	82.00±16.90[c]	61.50±10.61[a]

Means followed by the same superscript in each column are not significantly different (P > 0.05)