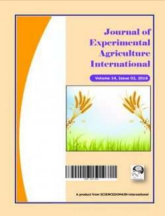

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Growth Rate and Disease Resistance of Inbreds and Novel Intra-specific Crossbreds Larva of *Clarias gariepinus* (Burchell, 1822) in Response to *Pseudomonas aeruginosa* Challenge

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OMA and SBS assisted in data collection and managed the analyses of the study. Author OMA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the differences in survival rate and Growth Rate (GR) in larva of novel inbred (CC) and crossbred (CS and SC) genotypes of *C. gariepinus* in response to *P. aeruginosa* disease challenge, in order to explore their potentials for improved aquaculture.
Study Design: Completely Randomized Block Design.
Place and Duration of Study: Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria, between June and November, 2015.
Methodology: Sixty individuals of four weeks old *C. gariepinus* inbred-CC (individuals not possessing serration at anterior portions of both sides' pectoral spine-C) and SS (Individuals possessing the serrations-S) and crossbred-SC (maternal-S x paternal-C) and CS (maternal-C x paternal-S) strains were immersed in 500 ml water containing 0.01ml of $2.56x10^7$ cfu/ml *P. aeruginosa* inoculums for 30 minutes. Challenged and control treatments were reared in

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freshwater for two weeks. Specimens were monitored for clinical signs, survival (%) and growth rate during 2weeks post challenge rearing period.
Results: At P= .05, values of survival and growth rates were significantly different across genotypes as well as across control and challenged treatments of each genotype. Fish from challenged treatments were sluggish, lost appetite, and had hemorrhage. 100% challenged-SC and 20.62±1.15% challenged-CC died within 72 hours; during this period, all control treatments, challenged-CS and challenged-SS survived. At 2weeks, survival rates (%) were higher in control than challenged SC (37.5±17.7:0.00) and CC(77.5±3.5:25.0±5.5), but similar (100%) in SS and CS. GR was 0.02±0.00 in control-SC. GR were significantly higher in challenged compared to control in CC, SS and CS: 0.20±0.01: 0.01±0.00, 0.46±0.03: 0.26±0.01 and 1.05±0.12: 0.33±0.02 respectively.
Conclusion: Paternal serrated crossbreed (CS) of *C. gariepinus* tolerated *P. aeruginosa* infection with superior survival and growth rate, thus indicating high potential for aquaculture in the face of the disease challenge, while maternal serrated crossbreed (SC) was highly susceptible.

Keywords: Clarias gariepinus; inbred and crossbred fish larva; resistance to pathogen.

1. INTRODUCTION

Food insecurity is a clear and serious threat to human security [1], challenging both developed and developing economies [2]. Achieving food security in sub-Saharan Africa remains a great challenge because, despite some advances, most of the region in Africa is not on track to reach the hunger target [3]. Meanwhile, living aquatic resources such as fish have provided livelihoods and revenue for populace especially in developing countries [4,5]. Fish has been proposed to be the cheapest and promising sources of animal protein [6], and it contributes positively towards eradication of hunger, food insecurity and malnutrition [7].

Aquaculture is one of the two main fish production sectors and it has become one of the most important sources of protein for the worlds' growing population; having major role in rural development, food security, source of foreign exchange earnings, man power development, income and employment generation. Aquaculture provides food at affordable prices to the poor segments of the community [8] as well as the rich. This sector has continued to show sustained growth, faster than all other food sectors [9,10,11]. In Nigeria, aquaculture production increased from 25.3 thousand metric tons to more than 85 thousand metric tons in 2007 [12] and to 200 thousand metric tons in 2012 [13], but fish production is still far below demand. Meanwhile, the other source of fish production, that is the capture fisheries, is globally perceived to be declining. This scenario has necessitated intensification of aquaculture with attendant challenge of disease management. The continued expansion of cultured fish and shell fish species has made aquaculture to become a key component of the animal health industry [14]; and aquatic diseases make the biggest constraint in aquaculture production [15]. Disease situation with its attendant challenges do set limit on the potentials of aquaculture sector in delivering the desired benefits to food security and to mankind in general. Disease often has negative impact on fish feeding behavior, growth and survival, thereby causing huge loss of investment in fisheries sector. Meanwhile, this would have relatively higher negative effects at early life stage (larva) because; fry would be tender at this stage.

Bacterial pathogens cause heavy mortality in both cultured and wild fish/shell species over the world [16]. Mean while, farmed fish are more susceptible to disease agents than fish in natural aquatic environments [17]. Intensification of fish culture has led to many problems, where bacterial diseases were indeed recognized as the main problem faced by local fish farmers [18]. Bacteria are responsible for high mortality in fish hatchery worldwide and *P. aeruginosa* is one of the commonest bacterium that can cause diseases in animals, including humans [14].

African catfish, *Clarias gariepinus*, is a major fishery in farm environments; especially in some developing countries, but bacterial infection threatens its intensive farming, resulting in colossal loss in huge investment. *P. aeruginosa* infection has been linked with mortality in *C. gariepinus* aquaculture [19,14,20]. Experimental infection of *C. gariepinus* with *P. aeruginosa* was associated with mortality rate of up to 40% [21].

Although there are arguments against the use of antibiotics in disease treatments; it is still the

most popular approach in treatments of bacterial infection in fish in developing countries. However, according to [20], majority of the *Pseudomonas aeruginosa* flora in *Clarias gariepinus* were resistant to common antibiotics such as ampicillin (63.6%), amoxycillin (54.5%), nalidixic acid (63.6%) and oxytetracycline (72.7%). The observations in the study [20] could have indicated that previous actions on management of the infection could have resulted in development of antibiotic resistance in *C. gariepinus* farming. Hence, there is the need to develop alternative approach.

The use of genetic approach proffers lasting effect to combat disease challenge as the gains will be heritable in subsequent generations. A feasible and sustainable alternative to prevent disease outbreaks may be represented by genetic improvement for disease resistance and this approach has potentials for helping in the control of disease problems [22]. This technique utilizes the intrinsic immune response of fish, which varies within and across genotypes.

Crossbreeding to produce intra-specific or inter-specific hybrids has potentials of exploring heterosis, which may be useful in evolution of genotypes that would resist disease challenge. However, the potentials must be tested in different fish genotypes using different pathogens. Development of genotypes that would resist disease challenge would be very relevant especially, for species that are of commercial values, and are widely farmed [22 and 23].

A previous study revealed genetic diversity within the *C. gariepinus* in a lake system in Nigeria [24]. The brooders of the genotypes had differences in certain morphologic traits, were available on farm, and their in breds and crossbreds were different in certain aquaculture production traits such as growth and survival [25]. Since most production traits are normally controlled by multiple genes and gene interactions, the reported phenotypes may co-vary with some other traits of importance such as disease resistance. However, this has to be ascertained for the purpose of utilisation.

Therefore, the inbreds and the intra-specific crossbred's fry of *C. gariepinus* were assessed for clinical symptoms, growth rate and survival rate in response to a disease (*P. aeruginosa)* challenge in a bid to evolve genotypes of improved disease resistance and growth productivity in the cases of disease challenge in aquaculture.

2. MATERIALS AND METHODS

2.1 Experimental Site

The research was conducted at the Fish Laboratory of the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria. Ibadan City is geographically situated in southwestern Nigeria, on the global coordinate- 7.3775°N, 3.9470°E.

2.2 Experimental Fish

The experimental fish were obtained from an on-going research at the experimental site. The fish specimens were produced from brooders of the pectoral spine strains of *Clarias gariepinus*. The strains were the group of individuals possessing smooth anterior portions of left and right side pectoral spines- C variant, and those with serration on the anterior portions of the left and right side pectoral spines- S variant. These variants were identified following earlier descriptions [25] and were then utilized to produce the inbred and crossbred progenies. The produced progenies were the CC inbred (Maternal-C x Paternal-C) and SS inbred (Maternal-S x Paternal-S); SC crossbred (Maternal-S x Paternal-C); and CS crossbred (Maternal-C x Paternal-S).

2.3 Disease Challenge Test

Four weeks old cohort of the inbred and crossbred genotypes was challenged with cultured *Psuedomonas aeruginosa* obtained from the Department of Microbiology, University of Ibadan, Nigeria. The colony of the *P. aeruginosa* used for the challenge was produced from an overnight growth on Biotel Nutrient Agar inoculated on 10 ml Biotel Nutrient broth. Cells were harvested by centrifugation at 3500 rpm at 4°C for 20 min and re-suspended in sterile saline. The bacteria suspensions were diluted with sterile saline to give a final concentration of 2.56×10^7 cfu/ml of inoculum. This concentration was utilized because 3.0×10^7 cfu/ml of *P. aeruginos*a inoculum was effective in causing infection in adult *C. gariepinus* [21]. A 0.01 ml of the inoculum was dispensed into each of the 500 ml rearing medium prepared for the challenge of each of the fry of CC, SS, SC and CS. These were prepared in triplicates. The challenge test followed earlier methods [26]. Sixty individuals comprising of twenty individuals each of the four weeks old cohort of the inbred and crossbred

genotypes were immersed in each of the *P. aeruginosa* inoculated 500ml rearing media and their replicates in a 30 minutes bath. Each treatment was then withdrawn into fresh rearing water of the same volume and reared for two weeks under two times *ad-libitum* feeding/day. Fry were fed with 0.8 mm Coppens feed (Coppens, USA). The non-challenged specimens (control) of each of the inbreds and the crossbreds were also prepared in triplicates and reared under the same water and feeding conditions alongside the challenged specimens.

2.4 Assessment of Response to Pathogens' Challenge

Clinical signs of *P. aeruginosa* infection, growth rate and survival rate of the challenged and the non-challenged treatments during the post challenge period were taken as indices of response to the *P. aeruginosa* challenge. The assessed clinical signs were haemorrhagic symptoms, sluggish movement, loss of appetite, mortality/survival and growth rate during the rearing period. Frequency of individuals with each clinical signs was estimated for each treatment. Growth rate was determined as the percentage of the difference between weights (g) of fry at two weeks of rearing, minus (-) the initial weight (g), (that is weight at the day zero of rearing of the four weeks old fry) divided by number of rearing days. Similar to this, survival rate was determined as the percentage of the difference between the survivors at two weeks of rearing and the initial number of stocked fry (number at day zero) of the experiment divided by the number of rearing days.

2.5 Statistical Analysis

Values were presented using descriptive statistics such as mean and standard deviation. Values of parameters taken from the four genotypes were analysed for differences through One-Way ANOVA. Differences between the challenged and control treatments in each genotype were analysed using student's *t- test.* Statistically significant differences were taken at P= *.05.* Data were analysed through the PAST statistical software [27].

3. RESULTS

3.1 Clinical Signs of *P. aeruginosa* Disease in *C. gariepinus* Strains

The challenged specimens showed symptoms of *P. aeruginosa* infection during 168 hours (7 days) post challenge period. The clinical signs were observed within 72 hours post challenge period (Table 1). The signs include sluggish movement, loss of appetite, head shaking behavior, haemorrhage and mortality. Percentage individuals that showed sluggish movement and loss of appetite were not significantly different (P>0.05) across the strains. Occurrence of sluggish movements ranged from 96.06±4.24% (CS) to 100.00±0.00% (SC) while loss of appetite ranged from 98.00±1.96% (CS) to 100.00±0.00% (SC and CC). Percentage individuals that showed head shaking behavior were significantly lowest (3.25±8.67) in CS, but highest (100.00±0.00) in SC. Meanwhile, SS and CS were statistically similar. Heamorrhage was only observed in mortal specimens. This symptom was observed at the ventral side of the body towards the head region.

3.2 Mortality of C*. gariepinus* Strains during 168 Hours (7 Days) Post *P. aeruginosa* Challenge Period

Challenged strains showed mortality within 72 hours post challenge period (Table 2). The result showed that 20.33±0.57% of SC crossbred died before 24 hours, and 100% within 72 hours while 20.62±1.15% CC inbred population died within the 72 hours. Mortality was not observed in challenged CS and SS. Also, mortality was not encountered in control treatments of all the strains (Table 2 footnote).

3.3 Survival of *C. gariepinus* Strains during 336 Hours (14 Days) Post *P. aeruginosa* Challenge Period

Result on percentage survival of the challenged and the control specimens at the end of the 14 days (336 hours post challenge period) of rearing are presented in Table 3. Percentage survival ranged from 0.0% (challenged) to 37.5±17.7% (control) in SC, 25.0% (challenged) to 77.5±3.5% (control) in CC and 100% (challenged and control) in SS and CS.

3.4 Growth of *C. gariepinus* Strains during 336 Hour (14 Days) Post *P. aeruginosa* Challenge Period

Table 4 showed the results on growth rates of the challenged and control specimens of the *C. gariepinus* strains at the end of the 336 hours (14 days) rearing period. The mean initial weights of the age cohort of the strains were: 0.25±0.07 g (SS), 0.11±0.03 g (SC), 0.31±0.07 g (CS), and 0.17±0.04 g (CC).

Table 1. Disease Symptom Score (%) of *P. aeruginosa* challenged Age Cohort Larva of Inbred and Crossbred Strains of *C. gariepinus* during 168 hours post challenge period

Symptoms	Percentage individuals that showed symptom in Strains			
	SS	SC	CS	CC
Sluggish movement	98.35±2.00	100.00±0.00	96.06±4.24	99.00±1.00
Loss of appetite	99.00±0.89	100.00±0.00	98.00±1.96	100.00±0.00
Head shaking behavior	5.26±5.34[a]	100.00±0.00[c]	3.25±8.67[a]	25.48±7.29[b]

** Symptoms occurred within 72 hours post challenge period; *Heamorrhage was observed in mortal specimens; *clinical signs were not encountered in control specimens of all strains*

**SS=Inbred (egg from smooth X milt from smooth pectoral spine strain); SC=Crossbred (egg from serrated X milt from smooth pectoral spine strain); CS=Crossbred (milt from serrated X eggs from smooth pectoral spine strain); CC= Inbred (egg from serrated X milt from serrated pectoral spine strain)*

Table 2. Percentage mortality of the *P. aeruginosa* challenged Age Cohort Larva of Inbred and Crossbred Strains of *C. gariepinus* during 168 hours (7days) post challenge period

Parameters	Percentage (%) Mortality in Challenged Strains			
	SS	SC	CS	CC
24 hours PC	Nil	20.33±0.57	Nil	Nil
48 hours PC	Nil	100.00	Nil	Nil
72 hours PC	Nil	Nil	Nil	20.62±1.15
96 hours PC	Nil	Nil	Nil	Nil
120 hours PC	Nil	Nil	Nil	Nil
144 hours PC	Nil	Nil	Nil	Nil
168 hours PC	Nil	Nil	Nil	Nil

**Note: Mortality was not encountered in control specimens of all strains; All mortal specimens showed haemorrhagic symptoms; PC indicate post-disease challenge; Descriptions of SS, SC, CS and CC is the same as presented in Table 1*

Table 3. Percentage survival (%) of the *P. aeruginosa* challenged and control treatments of Age Cohort of Inbred and Crossbred Strains of *C. gariepinus* at 336 hours (14 days) rearing period

Parameters	Percentage survival in Strains			
	SS	SC	CS	CC
Challenged	100±0.00	0.00[a]	100±0.00	25±0.00[a]
Control	100±0.00	37.5±17.68[b]	100±0.00	77.5±3.54[b]

**Note: Mean with different superscript along the same column are significantly different at P= .05; Descriptions of SS, SC, CS and CC is the same as presented in Table 1*

Table 4. Initial weight, final weight and growth rate of *P. aeruginosa* challenged and Control Age Cohort of Inbred and Crossbred Strains of *C. gariepinus* at 336 hours (14 Days) of rearing

Parameters	Strains			
	SS	SC	CS	CC
Initial weight (g)	0.25±0.07[a]	0.11±0.03[b]	0.31±0.07[a]	0.17±0.04[c]
Final weight (g)				
Challenged	6.75±0.61[a]	Nil	15.01±1.12[a]	2.98±0.60[a]
Control	3.83±0.60[b]	1.26±0.01	4.95±0.64[b]	0.37±0.01[b]
Growth rate (%)				
Challenged	0.46±0.03[a]	Nil	1.05±0.12[a]	0.20±0.01[a]
Control	0.26±0.01[b]	0.02±0.00	0.33±0.02[b]	0.01±0.00[b]

**For initial weight, mean with different superscript along the same row are significantly different at P=.05; for final weight and growth rate, mean with different superscript along the same column are significantly different at P=.05; Descriptions of SS, SC, CS and CC is the same as presented in Table 1*

The SS and CS had significantly higher weight compared to the CC and SC despite of their age bracket. The mean final weight (g) was lowest in control of CC (0.37±0.01) and highest in the

challenged CS (15.01±1.12).Growth rate was 0.02% in the survivors of the SC control. The survivors of the *P. aeruginosa* challenged *C. gariepinus* had significantly higher growth rates than their respective controls in the CC, SS and CS: 0.01±0.00% (control):0.20±0.01 (challenged) in CC, 0.26±0.01% (control): 0.46±0.03% (challenged) in SS and 0.33±0.02% (control): 1.05±0.12% (challenged) in CS.

4. DISCUSSION

4.1 Clinical Signs of *P. aeruginosa* Disease in *C. gariepinus* Strains

Pseudomonas aeruginosa is one of the major pathogen of fish and have been isolated from skin, gills and stomach content of cultured *Clarias gariepinus* fingerlings [28]. The fact that all the challenged treatments showed sluggish movement, loss of appetite and head shaking behavior within 72 hour after the *P. aeruginosa* challenge while all the control treatments did not show these clinical signs implies that the *P. aeruginosa* infected the challenged specimens.

The significant differences in the number of individuals per treatment that showed head shaking symptom (in which all SC individuals had the symptoms, and the lowest percentage individuals that showed the symptom occurred in CS, while SS and CS were statistically similar) despite the similarity in the number per treatment that showed other symptoms could indicate the possibility of different level of reaction in the genotypes as conditioned by extent of the pathogen effects on the strains. All mortal specimens showed heamorrhage at the ventral side of the head region. Heamorrhagic symptom and mortality did not occur in all the control and the challenged SS and CS, while the challenged CC and SC showed the symptoms. This may indicate that the challenged CC and SC were relatively more susceptible to the *P. aeruginosa* challenge, while the SS and the CS were likely to have tolerated the challenge from the pathogen.

In general, clinical signs of *Psuedomonas aeruginosa* infection were observed in all the challenged treatments of the studied *C. gariepinus* strains. The observed symptoms were similar to those reported in an earlier study [29]. Meanwhile, mortality occurred in some genotypes, and the mortal specimens showed external haemorrhage. The haemorrhage was observed to be prominent at the ventral side of the body towards the head region. It is of note that Heamorrhagic symptom was observed only in the mortal specimens. The nature of the heamorrhage in the *P. aeruginosa* challenged *C. gariepinus* specimens is in agreement with the report in [30], who claimed that the characteristic symptom of the disease produced by *Pseudomonas* bacteria is a remarkable septicemic hemorrhage in the skin of the mouth region, opercula and ventral side of the body. It has been reported that *P. aeruginosa* infected fishes showed irregular hemorrhages on body surface, especially at the ventral part of abdomen [31] and it has been associated with external haemorrhage with mortality rate of 40% [21].

Presence of heamorrhage on only the mortal specimens could indicate that haemorrhagic symptom was the most severe and death causing among the investigated pathogenic signs. The co-incidence of mortality and this heamorrhage occurring at the ventral head region of the fishes could be linked with the possible damages on the sensitive organs of the infected specimens. This is possible because haemorrhagic condition occurs as a result of production of proteolytic enzymes [32] and *P. aeruginosa* infection induced haemorhagic condition with necrosis of fish organs such as gills [33] and liver damage [34].

It is therefore opined that the *P. aeruginosa* infection could have caused damages to these vital organs in the challenged and mortal *C. gariepinus* samples. This could be responsible for the observed heamorrhagic condition and mortality in the challenged populations.

4.2 Mortality of C*. gariepinus* Strains during 168 Hours (7 Days) Post Challenge Rearing Period

Mortality rates in the current study were at variant with an earlier report [21]. Mortality in the challenged specimens ranged from 0 (zero) to 100%, whereas 40% was reported in the earlier study. The result indicates a total eradication of one of the genotypic groups (SC strain), while all individuals in some of the other groups (SS and CS) survived the challenge. The higher level of mortality in the current study may be due to the stage of life being studied, while the variation in mortality rate across the genotypes could indicate the latent role of genetic factors in the susceptibility and resistance of *C. gariepinus* to *P. aeruginosa* infection. The occurrence of clinical signs in all challenged treatments during the first 72 hours post challenge period indicates

that *P. aeruginosa* is pathogenic to the studied genotypes of *C. gariepinus*. However, the varied frequency and time of mortality, in which the crossbreds and the inbreds showed variation in mortality rates indicate a possible genetic influence on the susceptibility to infection of *C. gariepinus*. Grading the susceptibility pattern, one could say the SC crossbreds were highly susceptible; the inbred CC was mildly susceptible, while the inbred SS and the crossbred CS withstood the challenge. The advent of the resistant and susceptible genotypes in the current study is possibly revealing a trend for genetic management of *P. aeruginosa* infection in early life stage of *C. gariepinus*. However, it is important to clarify whether these genotypes were resistant or tolerant to other pathogens of fish.

Disease resistance refers to a situation in which the host fish is not infected by a pathogen; while situation where the host is infected by the pathogen, but suffers little adverse effect is referred to as tolerance [35]. Mechanism of resistance could involve blocking the reproduction of pathogen within a host while tolerance would involve putting limit on impacts of pathogens on the infected host [36,37]. In the current study, all the *C. gariepinus* genotypes showed symptoms of infection, but SC showed severe infection and mortality of the whole populations, CC showed symptom of infection and little mortality, while the SS and CS showed symptom of infection but did not have mortality. Hence, the survivors of the CC, and the whole of the inbred SS and the crossbred CS strains could be conceived to have tolerated the *P. aeruginosa* infection.

Generally, resistance/tolerance to disease is mediated by immune systems which includes the component of innate and acquired immunity and their interplay. Meanwhile, genes play major roles in immunity and a number of candidate genes / loci associated with disease resistance have been identified in livestock [38,39,40]. The major histopathological complex (MHC) genes have been well discussed in respect to immunity to diseases in fish [41-48].

It is opined that the survivors in the inbred CC, the whole of the inbred SS, and the crossbred CS strains may have taken advantage of their genetic endowment in this regard. However, this would need a future molecular analysis to unravel the perceived endowment for resistance to the *P. aeruginosa* pathogen.

4.3 Survival Rates of *C. gariepinus* Strains during 336 Hour (14 Days) Post *P. aeruginosa* Challenge Period

The result on survival rate of *C. gariepinus* strains during the 14 days post *P. aeruginosa* challenge revealed that the lowest survival among the challenged and the control occurred in SC. This implies that the genotype seems to be the weakest in terms of survival with or without pathogenic challenge. In SC and CC, the controls had higher survivals than the challenged, thus indicating that the *P. aeruginosa* challenge could have created extra stress resulting in more mortality.

The similar survival rates in the challenged and control specimens of the SS inbred and the CS crossbred indicate that these genotypes maintained their earlier pattern of survival (observed during the first 72 hours after the challenge). The similar survival in the control and challenged specimens of the two genotypes confirms the strength of their challenged specimen to withstand the pathogen challenge without mortality. Experimental infection of *C. gariepinus* with *P. aeruginosa* was associated with mortality rate of up to 40% [21] However, higher mortality (100% in SC crossbred and 75% in inbred CC) and no mortality (zero mortality in crossbred CS and inbred SS) were observed in the current study. The differences in the mortality rates in *P. aeruginosa* challenged *C. gariepinus* in the current study and the report in [21] could be attributed to differences in the stage of life of the studied infected fish, the separation of the *C. gariepinus* population to the genotypes and the production of crossbreds of the genotypes. Therefore, it is important to consider these factors in pathogenic studies and their interpretations in *C. gariepinus*.

4.4 Growth Rates of *C. gariepinus* Strains during 336 Hour (14 Days) Post *P. aeruginosa* Challenge Period

Results on growth rates of the challenged and controlled *C. gariepinus* genotypes showed that the initial mean weight of the samples of the genotypes showed differences in spite of their age similarity and having been raised under the same culture condition. The lowest weight occurred in the crossbred SC while and the highest occurred in the crossbred CS. Meanwhile, SS and CS populations had significantly higher weight compared to the rest genotypes. It is noteworthy that the lowest and

the highest mean weight at same age occurred among the crossbreds (SC and CS). Also, the crossbred SC which was highly susceptible had the lowest mean weight, while the highest value occurred in crossbred, CS. The differences in mean weight of the two crossbreds in which one had the lowest values while the other had the highest value among the genotypes indicates that not all crossbreeding trials will result in positive heterosis; that of the CS was positive while that of the SC was negative. Since the size disparity across the genotypes cannot be removed, it is practically considered that the growth rate of the genotypes would better be measured based on differences between the initial and the final weight of each of the genotypes in the reared challenged and control treatments. At the end of the two weeks of rearing, the mean final weight (g) was lowest in control of CC and highest in the challenged CS. The complete mortality of the challenged SC crossbred genotype did not allow a comparison between the challenged and control in this treatment. However, survivors of the *P. aeruginosa* challenged *C. gariepinus* had significantly higher growth rates than their respective controls in the CC, SS and CS. This result indicate that the lowest growth rate occurred in the control of the CC, while the highest growth rate and the highest difference between the growth rate in the infected and the control occurred in the crossbred CS. Although, the challenged CS and SS had similar desirable pattern of survival, the final mean weight and the growth rate of the challenged SS was lower than the values in CS. The comparatively higher growth rate in the challenged specimen in all the CC, SS, and the CS genotypes indicates that the challenged specimens had responded to the challenge by increase in growth. More so, the challenged treatments had significantly higher growth than the controls. This could reflect the superiority in the challenged CS over that of the SS. This pattern could indicate the coping mechanism of the *C. gariepinus* genotypes in response to the *P. aeruginosa* infection.

Although, little is known about this mechanism as it relates to the *C. gariepinus* genotypes under *P. aeruginosa* infection, more investigation is needed to unravel the possible mechanism for this observation. However, [21] reported that pathogenicity of *P. aeruginosa* in *C. gariepinus* correlates with hypertrophy of the columnar epithelium of the intestinal villi and crypts, as well as increased secretory activity with vacuolated clear cytoplasm and infiltrated inflammatory cells. Certain secretory proteins, antigen 5, and pathogenesis-related proteins super family members are found in a remarkable range of organisms spanning each of the animal kingdoms; and majority of these have notable expression bias to the reproductive tract and immune tissues [49]. Meanwhile, pathogenesis related proteins can accumulate to levels from 0.3 up to 1% of the total protein content [50]. The superior growth rates in the challenged treatments over the control and especially that of the challenged CS and SS over other genotypes may be as a result of relatively higher mobilization for this immune response which could have ultimately resulted in their relatively higher growth rates. More so, superiority in growth rate had been demonstrated in the significantly superior initial weight of the CS and SS genotypes despite their age similarity with other genotypes. The *P. aeruginosa* challenge may have triggered a signal for this immune response, to which all the challenged specimens reacted to at different extent, causing higher growth rates than that of their respective controls. Therefore, the differences across the challenged genotypes could be based on the differences in their intrinsic endowment for the immune response.

Individuals can vary in their level of resistance to a certain pathogen, with some individuals being fully resistant, described by a gene-for-gene relationship [51]. The challenged specimens of the studied genotypes could have demonstrated superiority in growth and survival rates relative to their control, due to their genetic endowment to mobilize for increased cell growth and immune related protein production in response to *P. aeruginosa* disease challenge. Meanwhile, the CS crossbred showed superiority in this attribute while the crossbred SC was inferior having being highly susceptible to the pathogen. The high susceptibility of the SC compared to the CS agreed with the fact that hybridization may produce relatively less fit hybrid genotypes which may suffer decreased performance and reduced ability to cope with emergent pathogens; and hybrid response to disease may vary according to degree of genetic admixture [52].

5. CONCLUSION

The crossbreeding of the milt from serrated pectoral spine strains of *C. gariepinus* with eggs from the smooth pectoral spine strains to produce CS crossbred has potentials of high survival and growth rate in response to *P.*

aeruginosa infection, while the reciprocal crossbreed SC will be highly susceptible to the challenge. This knowledge is essential for broodstock selection, improved production and profitable culture of *C. gariepinus* in the face of *P. aeruginosa* infection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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