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Evaluation of pathogenicity and growth rate among the genetic strains of rivers Benue and donga *Clarias gariepinus* fingerlings in response to a disease (*Aeromonas hydrophila*) Challenge

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Abstract
Pathogenicity and growth rate among the genetic strains of *Clarias gariepinus* fingerlings in the inbred and the intra-specific crossbred's population of were evaluated to reveal haplotypes of growth productivity and improved disease resistance in disease challenge cases in fish culture. The fish samples were created from parent stock of the *C. gariepinus* Benue and Donga Haplotypes then used to create inbred and crossbred fingerlings. The bacteria suspensions were diluted with sterile saline to achieve a final concentration of 1.0 x 10^7 cfu/ml of inoculum which was then prorated into each 500 ml rearing medium. A total of two hundred and forty individuals comprising of ten (10) from each of the eight (8) weeks old cohort of the inbred and crossbred haplotypes, were immersed in each of the *Aeromonas hydrophila* inoculated 500 ml rearing media and their replicates in a 30 minutes' bath. The non-infected specimens (control) of each of the inbreds and the crossbreds of Benue and Donga haplotype were cultured in triplicates alongside the infected specimens in the identical freshwater and feeding condition. Specimens were observed for clinical signs and growth rate during 14 days post challenge cultured period. During the 14-day post-challenge culture phase, specimen were monitored for clinical signs symtoms and growth rate. During the 14-day post infection period, the infected specimens displayed symptoms of *A. hydrophila.* The disease symptoms include inappetence, swimming abnormalities, pale gill, bloat and skin ulceration and death. The survivors of the *A. hydrophila* challenge *C. gariepinus* had significantly higher growth rate than their respective control in both haplotypes. In response to *A. hydrophila* infection, *C. gariepinus* haplotypes from Benue and Donga River have the potential for high survival and growth rate. This knowledge is critical for enhanced productivity, brood stock selection, and profitable culture *C. gariepinus.*

Keywords: *Aeromonas hydrophila*, *Clarias gariepinus*, genetic, growth and haplotype

1. Introduction
Aquaculture is one of the fastest expanding food-producing industries, serving as a viable supplement to and substitute for wild fish [1]. Infectious illnesses of cultured fish are the most notable restriction to aquaculture spread and realization of its full potential [2]. Bacterial infection are the most serious illness concern in fish farming, accounting for approximately 80% of fatalities [3].

African catfish are teleosts having skin made up of living non-keratinized stratified squamous epithelial cells covering their entire body surfaces, barbells, and fins [4]. Skin is the organ of interface with the environment and it acts as the first site of attachment for a variety of microorganisms in the aquatic environment [5]. Microorganisms attachment to fish skin frequently causes skin sores, which, regardless of size, result in colonization by many opportunistic pathogens, life threatening osmotic stress increased energy cost from locomotion due to impaired mucus production, swimming imbalance, increase predation due to color change, and oxygen deficiency. Thus, skin lesions have a negative impact on the performance and productivity of the affected fish. Infectious illnesses induced by aerobic bacteria generate significant financial losses to fish producers worldwide [6].

Among the aerobic bacterial organisms that inhibit African catfish production, motile Aeromonas species (MAS), specifically *Aeromonas hydrophila* have been found to be indigenous (authochtonous) in the aquatic environment, and hence are linked with the fish as normal commensal flora [7]. MAS, on the other hand, has been linked to a skin illness known as motile aeromonad septicaemia known as red sore, red pest, haemorrhagic septicaemia or epizootic ulcerative disease (EUD) or syndrome (EUS) [8]. This illness is economically significant because it mostly affects juvenile fish and causes epidemic outbreaks that results in large mortality in many parts of the world [9].

The genetic method provides a long-term effect in combating illness challenges since the improvement are heritable in the following generations. Genetic enhancement for disease resistance may represent a practical and sustainable option to preventing disease outbreaks, and this technique has the potential to aid in disease control [10]. This method makes use of fish innate immunological response, which differs within and between haplotypes.

Crossbreeding to develop intra-specific hybrids has the potential to investigate heterosis, which could aid in the evolution of disease resistant haplotypes. However, the creation of haplotypes that are resistant to disease challenge would be highly important, especially for commercial valuable and widely farm species [10].

A recent study found genetic diversity in *C. gariepinus* from Benue and the Donga River in Nigeria. The haplotypes brooders differed in certain morphologic features, were farm-available, and their inbred and crossbred differed in certain aquaculture production traits such as growth and survival. Because most production features are generally controlled by many gene to gene interactions, the observes phenotypes may co-vary with other important qualities like disease resistance. This must, however, be determined in this investigation.

As a result, fingerlings of *C. gariepinus* inbred and the intra-specific crossbred were observed for symptoms and growth rate in response to a disease (*Aeromonas hydrophila*) challenge to reveal haplotypes of improved disease resistance and growth productivity in the situation of disease challenge in fish culture.

Materials and Methods

Site of Experimentation

The research was carried out at the Federal University Wukari, Central Laboratory in Nigeria. Wukari is located in North East Nigeria, on the global coordinates 7.9303°N, 9.8125°E.

Fish for Experimentation

The fish specimens were created from brooders of the *Clarias gariepinus* Benue and Donga Haplotypes and then used to create inbred and crossbred fingerlings.

Table 1: 2x2x2 Factorial Design for the Breeding, growth ETC

Location		Generic Groups	
Inbred hap 1- Gen1	Inbred hap3-Gen 2	Crossbred hap1xHap3-Gen3	Recip Crossbred hap1xHap3-gen-4
Benue Hap1 MB X Hap1FB	Hap3 MB X hap3FB	Hap1 MB X Hap3FB	Hap1FB X hap3MB
Donga Hap1 MD X hap1FD	Hap3 MD X hap3FD	Hap 1MD X Hap3FD	Hap1FD X Hap3MD

Hap = Haplotype; Gen= Generic Group; RECIP = Reciprocal; MB = Male of Benue; FB= Female of Benue, MD = Male of Donga, FD = Female of Donga

Bacterial cultures and preparation of inocula

Agar preparation: 40 g of MacConkey agar was poured into a conical flask and 100ml of distilled water was then added and mixture was allowed to dissolved. The mouth of the conical flask was plugged and autoclaved at 121 ^{o}C. The sterilized agar was cool to about 45^{0}C. 15 to 20 ml of the molten agar was swoosh into pre – sterilized dishes was allowed to solidify. The prepared agar plates were stored in the refrigerator at 6-7 ^{o}C.

Biochemical test: Growth from a blood agar subculture in a drop of sterile distilled water on one end of the slide and on another loopful of growth in a drop of peptone water on the other end of the slide, each preparation was covered with a slide slip and inspected microscopically at x40 objective.

Oxidase test: A drop of dye solution was place on a portion of culture containing the colonies of *Aeromonas hydrophila* a positive reaction is indicated by the pink color of the colonies turning maroon progressing to dark red.

Disease Challenge Test: A post-fingerlings batch of the inbred and crossbred Haplotype was challenged with cultured *Aeromonas hydrophila* procured from Central laboratory, Benue State University Makurdi, Nigeria at the age of eight (8) weeks. The bacteria suspensions were diluted with sterile saline to a final concentration of 1.0 x 10^{7} cfu/ml of inoculum. This concentration was chosen because *Aeromonas hydrophila* at 1.0 x10^{7} cfu/ml was effective in infecting in adult *C. gariepinus* [4].

A 1.0mL of the *Aeromonas hydrophila* was added to each 500 mL raising media prepared for the challenge of each post fingerlings of Benue and Donga Haplotype of *Clarias gariepinus* as stated by [11].

Two hundred and forty fingerlings, ten (10) from each of the eight (8) weeks old cohort of the inbred and crossbred haplotype were immersed in each of the *Aeromonas hydrophila* inoculated 500mL rearing media and their replicates for 30 minutes. Each treatment was then transferred to fresh rearing water of the same amount and reared for two weeks with two ad-libitum feeding/day of 2mm Coppens feed (Coppens International by 5700 AM Helmond, Holland). Non-challenged specimens (control) of each inbreds and the crossbreds Benue and Donga haplotype were created in duplicate and reared alongside the challenged fish in the identical water and feeding condition.

Response to Pathogens' Challenge Evaluation

As markers of response to the *A. hydrophila* infection, clinical indications of infection and growth rate of the challenged and the non-challenged treatments throughout the post challenge period were used. During the rearing stage, the clinical indications detected were inappetence, swimming abnormalities, pale gills and bloat skin ulcer, mortality/survival and growth rate. For each haplotype, the frequency of specimen with each clinical symptom was

assessed.

Growth rate was calculaed as the percentage of the difference between post fingerlings weights (g) at eight (8) weeks of raising, minus (-) the initial weight (g), (that is weight at the day zero of rearing of the eight (8) weeks old post fingerlings) divided by number of rearing days [10].

Statistical Analysis

The results were reported using descriptive statistics, and the values of parameters drawn from the four genotypes were compared using One-Way ANOVA. The student's t- test was used to compare the differences between challenged and control treatment in each haplotype.

Results

Symptoms of *A. hydrophila* infection in *Clarias gariepinus* Haplotypes

During 14-day post-infection period, the infected *C. gariepinus* fingerlings showed evidence of *A. hydrophila*. Benue haplotype (figure 1) and Donga haplotype (figure 2). The clinical symptoms were detected 3 days after infection. Inappetence, swimming abnormalities, pale gill, bloat and skin ulceration and mortality are all symptoms of the condition.

Among all the Benue haplotype (figure 1) percentage of individual that show symptom of inappetence ranges from 3. 33% in Hap1 ♀B x Hap3 ♂B, 26.66% in Hap3 ♀B x Hap3 ♂B, 26.66% in Hap1 ♂B x Hap3 ♀B and 33.33 in (Hap1 ♀B x Hap1 ♂B). Higher percentage of swimming abnormalities were observed in the inbreed haplotype. Pale gill, Bloat and skin ulceration were absent in inbred (Hap1 ♀B x Hap1 ♂B) and crossbred (Hap1 ♀B x Hap3 ♂B) while Percentage of individuals that showed swimming abnormalities, pale gill, bloat and skin ulceration were significantly low.

The chart (figure 2) below shows the percentage disease symptom of the challenge inbreed and crossbreed *C. gariepinus* of Donga haplotype. All the haplotype recorded higher percentage of inappetence and swimming abnormalities among all the disease symptoms. Inappetence ranges from 13.33% in Hap3 ♀B x Hap3 ♂B, 13.33% in Hap1 ♀B x Hap3 ♂B, 20.0% in Hap1 ♀D x Hap1 ♂D and 26.66% in Hap1 ♂B x Hap3 ♀B while bloat and skin ulceration were absent in inbreed (Hap1 ♀D x Hap1 ♂D). Among all the Donga haplotype only the inbreed (Hap1 ♀D x Hap1 ♂D) do not show sign of bloat and skin ulceration. The crossbreed (Hap1 ♀B x Hap3 ♂B) however recorded higher percentage of pale gill symptom.

In both Benue and Donga haplotype, haemorrhage was only found in mortal specimens on the ventral side of the body, towards the skull. Bacterial specimen obtained from artificially infected fish lesions were confirmed to be identical to those utilized in the infection Table 1.

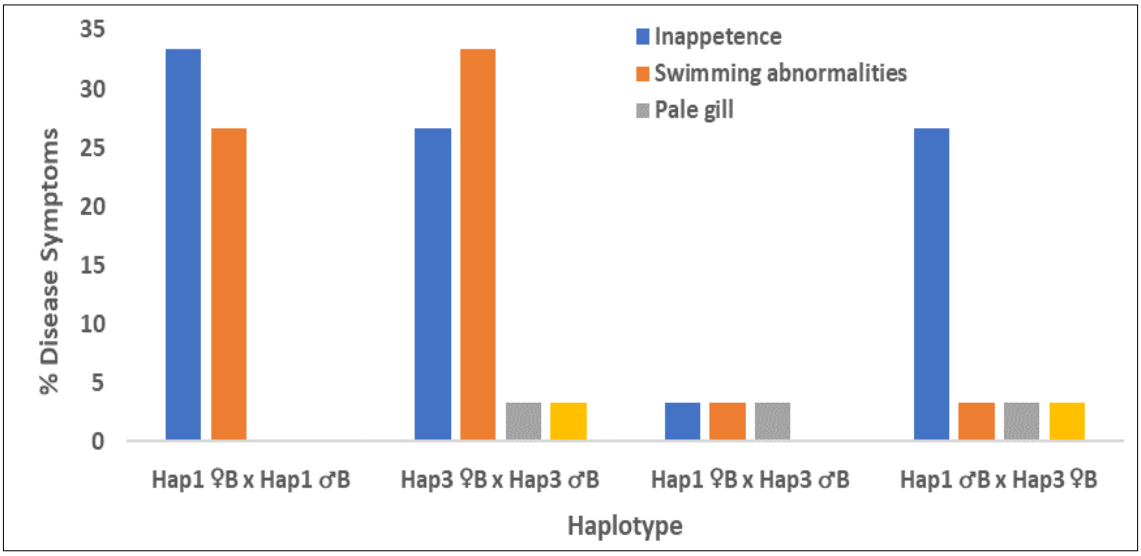

Fig 1: Percentage of fish with disease symptoms of Benue haplotype following experimental infection with *Aeromonas hydrophila*

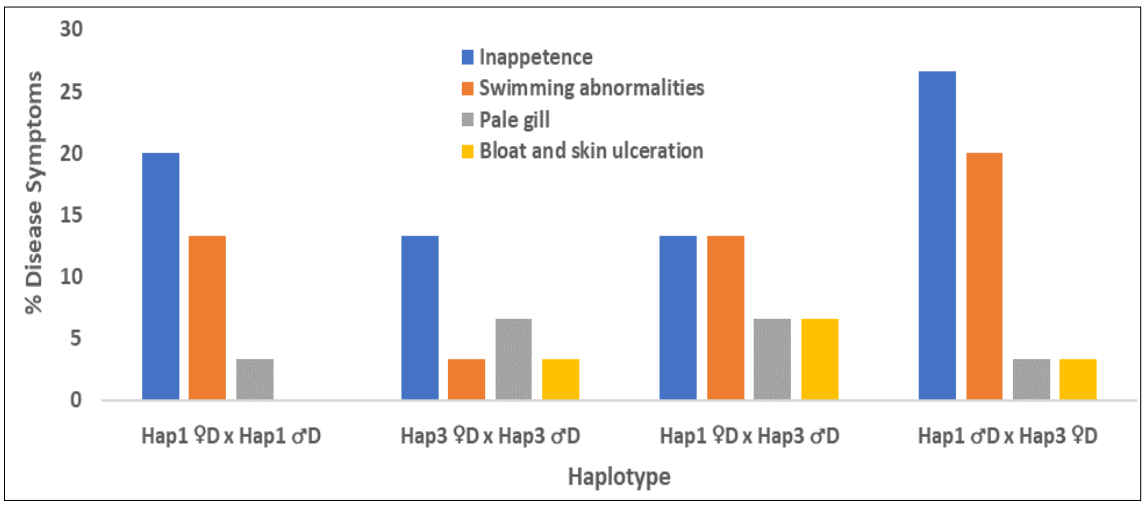

Fig 2: Number of fish with disease symptoms of Donga haplotype following experimental infection with *Aeromonas hydrophila*

Table 1: *Aeromonas hydrophila* characteristics and biochemical reaction

Test	*Aeromonas hydrophila*
MacConkey agar	Small, smooth and yellow colonies
Gram stain	-ve short rods
Motility	+
Gram staining	-
Gelatin liquefaction	+
Oxidase	+
Catalase	+
Arginine hydrolyses	+
NaCl free peptone water	+
Motility in distilled water	+
Arabinose fermentation	+
Aesculin hydrolysis	+
Growth on 5% NaCl	-
Nitrate reduction	+
Arginin hydrolysis	+
H_2S production	-

Growth rate of *C. gariepinus* Haplotype at 14 Days Post *A. hydrophila* infection Period

Table 2 revealed the results on growth rates of the infected and control specimens of the Benue *C. gariepinus* haplotype at the end of 14 days culturing period. The mean initial weights of the age group of the haplotype were: 18.03±0.21g (Hap1 ♀B x Hap1 ♂B), 18.13±0.55g (Hap3 ♀B x Hap3 ♂B), 18.1±0.10g (Hap1 ♀B x Hap3 ♂B) and 18.17±0.21g (Hap1 ♂B x Hap3 ♀B).

The Hap3 ♀B x Hap3 ♂B and Hap1 ♂B x Hap3 ♀B has significantly higher weight gain compared to Hap1 ♀B x Hap1 ♂B and Hap1 ♀B x Hap3 ♂B despite of their age bracket. Survivors of the *A. hydrophila* infected *C. gariepinus* had significantly higher growth rates than their age group controls among the haplotypes: 0.31±0.00% (control): 0.45±0.03% (challenged) in (Hap1 ♀B x Hap1 ♂B), 0.22±0.00% (control): 0.37±0.04% (challenged) in (Hap3 ♀B x Hap3 ♂B), 0.14±0.00% (control): 0.54±0.05% (challenged) in (Hap1 ♀B x Hap3 ♂B) and 0.19±0.00% (control): 0.35±0.03% (challenged) in (Hap1 ♂B x Hap3 ♀B).

Table 3 revealed the results on growth rates of the infected and control specimens of the Donga *C. gariepinus* haplotype at the end of 14 days culture period. The mean initial weights of the age group of the haplotype were: 18.07±0.21g (Hap1 ♀D x Hap1 ♂D), 18.00±0.23g (Hap3 ♀D x Hap3 ♂D), 18.03±0.05g (Hap1 ♀D x Hap3 ♂D) and 18.07±0.06g (Hap1 ♂D x Hap3 ♀D). The (Hap1 ♀D x Hap1 ♂D) and Hap1 ♂D x Hap3 ♀D had significantly higher weight gain compared to Hap3 ♀D x Hap3 ♂D and Hap1 ♀3 x Hap3 ♂D despite of their age bracket.

Survivors of the *A. hydrophila* infected *C. gariepinus* had a significant higher growth rates than the controls in the haplotypes: 0.30±0.00% (control): 0.46±0.03% (challenged) in (Hap1 ♀D x Hap1 ♂D), 0.21±0.00% (control): 0.49±0.03% (challenged) in (Hap3 ♀D x Hap3 ♂D), 0.25±0.00% (control): 0.59±0.03% (challenged) in (Hap1 ♀D x Hap3 ♂D) and 0.30±0.00% (control): 0.43±0.05% (challenged) in (Hap1 ♂D x Hap3 ♀D).

Similar trend was observed in both Benue and Donga haplotype of *C. gariepinus.*

Table 2: Growth Rate of *A. hydrophila* infected and control age group of Benue inbred and crossbred haplotype of *C. gariepinus* at 14 days Culture Period

	Haplotype			
Indices	**Hap1 ♀B x Hap1 ♂B**	**Hap3 ♀B x Hap3 ♂B**	**Hap1 ♀B x Hap3 ♂B**	**Hap1 ♂B x Hap3 ♀B**
Initial weight (g)	18.03±0.21[a]	18.13±0.55[b]	18.1±0.10[c]	18.17±0.21[d]
Final Weight (g)				
Infected	24.34±0.19[a]	23.31±0.34[a]	25.75±0.53[a]	23.10±0.44[a]
Control	22.35±0.00[b]	21.41±0.00[b]	20.10±0.00[b]	20.72±0.00[b]
Growth Rate (%)				
Infected	0.45±0.03[a]	0.37±0.04[a]	0.54±0.05[a]	0.35±0.03[a]
Control	0.31±0.00[b]	0.22±0.00[b]	0.14±0.00[b]	0.19±0.00[b]

Initial weight; mean with different superscript along the same row are substantially different at P = 0.05 while final weight and growth rate, mean with different superscript along the same column are significantly different at P = 0.05. Inbred = (Hap1 ♀B x Hap1 ♂B - Haplotype 1 female crossed with Haplotype 1 male and Hap3 ♀B x Hap3 ♂B - Haplotype 3 female crossed with Haplotype 3 male). Cross bred = (Hap1 ♀B x Hap3 ♂B - Haplotype 1 female crossed with Haplotype 3 male and Hap1 ♂B x Hap3 ♀B - Haplotype 1 male crossed with Haplotype 3 female).

Table 3. Growth Rate of *A. hydrophila* infected and Control Age group of Donga Inbred and Crossbred Haplotype of *C. gariepinus* at 14 days Culture Period

	Haplotype			
Indices	**Hap1 ♀D x Hap1 ♂D**	**Hap3 ♀D x Hap3 ♂D**	**Hap1 ♀D x Hap3 ♂D**	**Hap1 ♂D x Hap3 ♀D**
Initial weight (g)	18.07±0.15[a]	18.00±0.46[b]	18.03±0.06[c]	18.07±0.06[d]
Final Weight (g)				
Infected	24.47±0.21[a]	24.82±0.59[a]	26.25±0.25[a]	24.04±0.64[a]
Control	22.13±0.00[b]	21.04±0.00[b]	21.50±0.00[b]	22.14±0.00[b]
Growth Rate (%)				
Infected	0.46±0.03[a]	0.49±0.03[a]	0.59±0.03[a]	0.43±0.05[a]
Control	0.30±0.00[b]	0.21±0.00[b]	0.25±0.00[b]	0.30±0.00[b]

Initial weight; mean with different superscript at the same row are different at P = 0.05, while final weight and growth rate, mean with different superscript along the same column are significantly different at P = 0.05. Inbred = (Hap1 ♀B x Hap1 ♂B - Haplotype 1 female crossed with Haplotype 1 male and Hap3 ♀B x Hap3 ♂B - Haplotype 3 female crossed with

Haplotype 3 male). Cross bred = (Hap1 ♀B x Hap3 ♂B - Haplotype 1 female crossed with Haplotype 3 male and Hap1 ♂B x Hap3 ♀B - Haplotype 1 male crossed with Haplotype 3 female).

Discussions

Clinical Signs of *A. hydrophila* Disease in *C. gariepinus* Haplotype

Aeromonas hydrophila is a fermentative, oxidase-positive, facultative anaerobe commonly found in fresh water and sewage, and has been isolated from the skin, gills and visceral organ of raised *C. gariepinus* fingerlings [12]. The fact that all the infected specimens displayed inappetence, swimming abnormalities, bloat and skin ulceration behavior within 96 hours of *A. hydrophila* infection, whereas all control treatments did not, suggest that the *A. hydrophila* infected the challenged fingerlings.

There is a significant variation among individuals per treatment that showed inappetence, swimming abnormalities, pale gills, bloat and ulceration symptom (All the haplotype recorded higher inappetence and swimming abnormalities while pale gill and bloat skin ulceration were absent in inbred of Benue (Hap1 ♀B x Hap1 ♂B) and Donga (Hap1 ♀D x Hap1 ♂D) haplotype) despite the similarity in the number per treatment who displayed other symptoms, these could point to a distinct amount of reaction in the gene as conditioned by the magnitude of the pathogen reaction on the haplotype. It is so clear that *A. hydrophila* infection could have harmed this essential organ in the infected *C. gariepinus* specimen. In the diseased population, this could explain reported inappetence, pale gills, swimming abnormalities, bloat and ulceration problem and mortality.

Growth Rates of *C. gariepinus* Haplotype at 14 Days Post *A. hydrophila* infection Period

Results of the growth rates of the challenged and controlled *C. gariepinus* haplotypes revealed that the initial mean weight of the samples of the haplotypes differed despite their age group and having been reared under the identical conditions. The highest and the lowest weights were found in crossbred of Benue and Donga haplotypes. The fact that one crossbred had the lowest mean weight while the other had the highest mean weight among the haplotypes demonstrate that not all crossbreeding attempts will result in positive heterosis. Because the size disparity between haplotypes cannot be eliminated, it is widely assumed that the percentage growth rate of the haplotypes would be better measured based on differences between the initial and the final weights of each haplotypes in the reared challenged and control treatments [10].

The challenged specimen demonstrated a substantialy greater growth rate in all the haplotypes of Benue and Donga, indicating that the challenged specimens had responded to the infection by growing faster than their respective controls. This pattern may represent the coping mechanism of *C. gariepinus* genotypes in response to *A. hydrophila* infection. However, it has been determined that members of the secretory proteins, antigen 5, and pathogenesis-related proteins superfamily are found in a remarkable range of organisms spanning each of the animal kingdoms, with the majority of these having notable expression bias to the reproductive tract and immune tissues [13]. In the meantime, pathogenesis-related proteins can accumulate to levels ranging from 0.3 up to 1% of total protein content [14]. The superior growth rates in the infected treatments over the control may be due to increased mobilization for this immune response, which may have resulted in their relatively higher growth rates. The *A. hydrophila* challenge may have activated a signal for this immunological response, to which all of the challenged specimens reacted differently, resulting in faster growth rates than their controls. As a result, the disparities between challenged haplotypes could be due to variances in their intrinsic endowment for immunological response.

Individuals level of resistance to a pathogen can vary, with some being completely resistant, as reflected by a gene-for-gene connection [15].

Because of their genetic endowment to mobilize for higher cell growth and immune associated protein production in response to *A. hydrophila* disease challenge, the infected fingerlings of the examined haplotypes may have showed superior growth and survival rates relative to their controls.

Meanwhile, the high susceptibility of the crossbred compared to the inbred of the Benue and Donga haplotype agreed with the fact that hybridization may produce relatively less fit hybrid genotypes with decreased performance and reduced ability to cope with pathogens and hybrid response to disease may vary depending on the degree of genetic admixture [16].

Conclusion

In response to *A. hydrophila* infection, *C. gariepinus* haplotype from Benue and Donga River have the potential for high survival and growth rates. The information is useful for broodstock selection, increased production, and lucrative *C. gariepinus* culture in the face of *A. hydrophila* infection.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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