EVALUATION OF THE GENETIC EFFECT OF STRAINS HYBRIDIZATION ON RESISTANCE TO *Aeromonas hydrophila* IN *Heterobranchus bidorsalis*

***[1]AKINWANDE, A.A., [2]OYEBAMIJI, F.E., [1]BOLU, S.A. & [3]A.A. YUSUF**

1. Department of Aquaculture and Fisheries, University of Ilorin, Ilorin, Kwara State.
2. Department of Fisheries Technology, Federal College of Freshwater Fisheries Technology, New Bussa, Niger State, Nigeria.
3. School of Life and Environmental Science, Deakin University, Australia.

*Correspondence: akinwande_fish@yahoo.com, +234 80546 97342

ABSTRACT

Estimates of heterosis, maternal effect and general combining ability for resistance to Aeromonas hydrophila in Heterobranchus bidorsalis were obtained from a complete diallel cross of nine (9) full siblings among three strains; Dadinkowa, Kainji and Owena using two male (♂) and four females (♀) from each ecological zones in Nigeria. Fishes (250 ± 16.8g) from all the genetic crosses were challenged using intraperitoneal injection and the survival was observed over a period of three weeks. Mean survival following challenge test was 67.18± 2.9% and ranged from 50.3 % in the Kainji (♂) X Owena (♀) to 82.6 % in the Dadinkowa (♂) X Kainji (♀). Specific heterosis was significant (P<0.05) in the Dadinkowa (♂) X Kainji (♀) cross which suggest that intraspecific hybridization in the two strains may increase disease resistance to A. hydrophila. Line effect was also significant (P <0.05) in the Dadinkowa strain. There were significant maternal effect and combining ability in the Dadinkowa and Kainji strains which implies there is prospect for improvement in diseases resistance against A. hydrophila in H. bidorsalis through continuous selection of the Dadinkowa line and crossing of this line of fish, with lines of the Kainji strain.

Keywords: *Heterobranchus bidorsalis,* strain hybridization, *Aeromonas septicaemia*, (♂) - male, (♀) - female

INTRODUCTION

Aeromonas hydrophila is a ubiquitous gram negative motile rod bacterium which sometimes causes disease among cultured and feral fishes (Cipriano, 2001). It is an opportunistic pathogen and has been considered to be of major economic problem in tropical aquaculture (Amin *et al*., 1985) and several species of freshwater and brackish water fishes have been reported to be susceptible to the aeromonas infection (Ogbulie and Okpokwasili, 1999; Obiajuru and Ogbulie, 2006). The development of effective strategies for decreasing disease mortality levels is one of the highest priority areas for improvement of aquaculture productivity ((Peatman *et al*., 2013) in order to meet the increasing demand for highly quality dietary protein source. The use of chemotherapeutic agents as preventive or curative measure against aeromoniasis has its attendant problem of bioaccumulation in both the fish body and environment and the threat of development of antibiotic resistant strains (Sahoo and Mukhereje, 1999). The development of fishes resistant to this bacterial infection through selection will bring about a longtime protection against the disease. In fish stock population (both within and between stocks), there has been documented report on the existence of genetically determined variations to infectious diseases (Fjalestad *et al*., 1993; Chevassus and Dorson, 1990). This genetic variation permits selective breeding effort either by producing specific diseases resistant strains of fish or by including disease resistance parameter into a composite breeding index (Midtlyng *et al*., 2002).

There have been comparatively few studies on production of strains of fish's resistance to specific diseases in aquaculture. Most studies on genetic (strain) selection in fishes have been geared towards improved growth rate, food conversion and fecundity. Difference in diseases resistance has been found in some strains of salmons (McGeer *et al.*, 1991; Withler and Evelyn, 1990). Variation in susceptibility to some diseases has been reported in several strains of the channel catfish (Lafrentz *et al.*, 2012; Bilodeau-Bourgeois, *et al*., 2008, Wolter and Johnson, 1995). Moreso strains with improved resistance to *Edwardsiella ictaluri* have also been developed in channel catfish (Peterson *et al.*, 2008). *Heterobranchus bidorsalis* belongs to the family Clariidae and it is one of the commonly cultured catfishes in Nigeria, because of its high commercial value. Cross breeding has been carried out to improve growth rate in *Heterobranchus* species in Nigeria but no selective breeding program to enhance diseases resistance in this species has been conducted. Since *A. hydrophila* is ubiquitous in the aquatic environment, this therefore necessitated the

need to compare the resistance of this pathogen in different strains of *H. bidorsalis*, in order to explore the possibility of selecting suitable strains with improved resistance.

MATERIALS AND METHODS

Experimental fish and cross design

Wild strains of *Heterobranchus bidorsalis* used in this experiment were collected from three ecological zones in Nigeria and kept in separate broodstock tanks in the Hatchery of Federal College of Freshwater Fisheries Technology, New Bussa, and Nigeria. These were from; Sahel savannah (Dadinkowa dam, D); Rainforest (Owena River, O) and Guinea savannah (Kainji dam, K) and they were selected from these different ecological zones in order to obtain a very wide possible genetic difference between the strains (stocks). A diallel cross design (Gardner and Eberhert, 1966) between the three (3) *H.bidorsalis strains* was used to produce offspring of nine mating combinations.

Fry production and management

Hypophysation, dry fertilization and incubation of eggs was carried out using the method of Dada (1999). Two males (♂) and two females (♀) *H. bidorsalis* broodstocks of each strain were used and hatchlings (fry) from the nine genetic crosses were raised indoor in separate tanks in a continuous water flow- through system for five months when they have attained an average weight of 250 g.

Isolation and culture of *Aeromonas hydrophila*

Aeromonas hydrophilia was isolated from infected cultured *H. bidorsalis* showing gross pathological lesion and identified by biochemical characteristics as described by Surya *et al*. (2014). A preliminary LD50 experiment was carried out in order determine the challenge dose required to assure virulence of the pathogen. Bacteria isolated from the kidney of the dead fishes from the 1st- 4th day of injection were used to confirm that the cause of mortality was due to *A. hydrophila*. The bacteria were cultivated at 38 °C for 23 hours and maintained on a nutrient agar kept in a refrigerator at 2 °C until use. The viable cell count of the pathogenic bacteria on the plates was also performed manually. Isolation and culture of *Aeromonas hydrophila* was carried out at the Veterinary Research Institute of Nigeria (NVRI) Jos, Nigeria.

Experimental procedure and challenge test

A total of 150 fishes (250 ± 16.8 g) from each genetic cross were stocked in triplicates (50 fish/tank) in concrete tanks (2 m x 2 m x 1 m) which were continuously aerated. All fishes in each genetic cross were administered intraperitoneal injection containing 0.2 ml of *A. hydrophila* (4 $x10^5$ bacteria/ml). Dead fishes were removed daily and the challenge test was 21 days. Twenty percent of the dead fishes were necropised and *Aeromonas hydrophilia* isolated were identified from biochemical characteristics (Surya *et al.*, 2010).

Statistical analysis

Data collected at the end of 21 days challenge test were subjected to Analysis of Variance using the GLM procedure of Statistical Analysis System, (SAS Institute 2006, version 9.1 software) to know if the genetic cross as a main effect caused significant variation on survival. Differences among the genetic crosses were considered significantly different at ($P < 0.05$). Value for average heterosis, combining ability and maternal effect were computed from the mean survival rates of triplicates in each genetic cross that was compared with the series of estimate statements in the statistical procedure used. Survival rate was calculated as described by Fagbenro *et al*. (1992) as: $S(\%) = \frac{Ni-Nf}{Ni} \times 100$

Where $N_{f\,=}$ final number of fish at the end of the experiment and N_i is the initial number of fish at the beginning of the experiment. Heterosis here refers to the performance of the intra specific hybrids in terms of resistance to *Aeromonas hydrophila* infection relative to the parental strains expressed as percentage (Sash *et al*., 2011) , based on the equation: $H(\%) = \frac{Pi-Po}{Po} \times 100$

Where Pi = mean reciprocal of hybrid and Po = mean reciprocal of parent.

General combining ability (GCA) estimate for each strain (line) was determined as the average value of the single crosses, each strain (line) entered into in the complete nine strain diallel set of data. This was estimated as described by Machikawa and Saetang, (2011) based on Griffing, method 1V (Griffing, 1956) as: $g_i = \frac{1}{n(n-2)}(nx_i - 2x \ldots)$ Where gi = GCA effect of the i^{th} parent; n = number of parent (strain) lines; X_i = mean (survival) value of the i^{th} parent; X..= grand mean.

RESULTS

The survival rate of all the genetic crosses at the end of the challenge test is shown in Table 1. The overall mean survival was 67.18 ± 2.9% with the highest value (82.6 ± 3.2%) in the Dadin kowa **(♂)** X Kainji **(♀)** and least value of 50.3 ±2.5% in the Kainji **(♂)** X Owena **(♀)**. Based on single trait analysis, the relative difference (for male and female) in the survival rates in all the genetic crosses were significantly different ($P<0.05$). Average heterosis in the genetic crosses was 5.16 ±2.66 while specific

heterosis ranges from 2.40 to 10.86 ±3.5 and this was significant (P < 0.05) in the cross involving the Dadinkowa x Kanji strain. Line effect in the parental varies from -7.93 to 6.77 ±3.1 and was significant (P < 0.05) in the Dadinkowa strain as shown in Table 2. Maternal effect also ranged from -12.55 to 19.9 ± 4.3 in the three parental strains. Significant negative and positive values based on this effect were obtained in the Dadinkowa and Kainji strains respectively. The general combining ability were -9.28, 13.38 and - 4.13 ± 3.7 in the Dadinkowa, Kainji and Owena strains respectively. These were however significant in both the Dadinkowa and Kainji strains.

Table 1: Survival of *Heterobranchus bidorsalis* from a diallel cross of Dadinkowa, Kainji and Owena strains following intraperitoneal challenge with *Aeromonas hydrophila (*Mean ± SE)

	Female Parent (♀)		
	Dadin Kowa (D)	Kanji (K)	Owena (O)
Male Parent (♂)			
Dadinkowa (D)	75.2 ± 4.1^{b}	82.6 ± 3.2^{a}	66.8 ± 2.4^{c}
Kainji (K)	64.4 ± 4.1^{c}	57.4 ± 2.2^{d}	50.3 ± 2.5^{e}
Owena (O)	74.1 ± 3.4^{b}	71.9 ± 4.3^{bc}	62.0 ± 3.5^{cd}

Table 2: Estimates (±SE) of specific heterosis, line effect, maternal effect and combining ability in *Heterobranchus bidorsalis* from a diallel cross among the Dadinkowa, Kainji and Owena strains

	Parameter					
Genetic cross	Specific heterosis	Line effect		Maternal effect		Combining ability	
Parental							
D (♂) X D (♀)		6.77*		-12.55*		-9.28*	
K (♂) X K (♀)		- 7.9	±3.1	18.42*	± 4.3	13.38*	(±3.7)
O (♂) X O (♀)		- 7.28		7.35		-4.1	
Intraspecific hybrids							
D (♂) X K (♀)	10.86*						
D (♂) X O (♀)	2.70	± 4.6					
K (♂) X O (♀)	2.40						

* Significant at P < 0.05

DISCUSSION

Mortality was very high at the beginning of the challenge test (injection of the pathogen) but reduced to a minimal level by the 10th day in the entire genetic crosses. *A. hydrophila* is known to be a secondary pathogen which requires a stressed fish or fish already infected by a primary pathogen before it can cause clinical condition (Omeje and Chukwu, 2014). In some strains of common carp (Jorgen *et al*. 2010) and two different base population of *Clarias macrocephalus* (Na-Nakorn *et al*. 1994) challenged with the same pathogen, high mortality were also recorded within the first few days of the post challenged test but the average survival rates differs from those obtained in this present study. This could be due to difference in the species involved and/or dosage of the pathogen used. Diallel cross is usually carried out to improve growth performance and disease resistance in which the data obtained based on the genetic effect are used in selecting the most suitable strain(s) for crossbreeding (Owolade *et al*., 2009; Wolters and Johnson, 1995).

The high survival of the Dadin kowa strain and specific heterosis in the Dadinkowa x Kainji strain which were significant may suggest directional dominance for resistance to *Aeromonas hydrophilia*. This is at variance with the report of Jorgen *et al*. (2010) who found no significant difference in the survival rates of some strain of common carp injected with the same pathogen, thus showing no prospect for genetic improvement of resistance to the disease. A mortality rate of 29.3% and 66% has been obtained in the high and low resistant strains of the Atlantic salmon to infectious pancreatic necrosis (Storset *et al*., 2007). Similarly, Rodriquez- Ramilo *et al*. (2011) reported difference in resistance to *Aeromonas*

salmoncida in four turbot families based on quantitative trait loci (QTL) and suggest the possibility for selection, in turbots resistant to furunculosis. In selective breeding, line effect is influenced by both additive and genetic variation (Wolter and Johnson, 1995) and this was very significant in the Dadinkowa strain in this present study. This shows the possibility of improving resistance to *Aeromonas hydrophila* through selection in this strain.

Maternal effect which is the influence of the maternal genotype of the parental strains on the phenotype of the offspring (Hager *et al*., 2008) was significant and positive in the Kainji strain. This suggests that offspring's from the crosses having maternal Kainji parent may have higher resistance to the pathogen.

The general combining abilities of two parental individuals is a function of the expected performance value of a hybrid (Falconer and Mackay, 1996) and it is used as the measure of a male (female) average performance value of offspring from a male (female) when crossed to all other females (males) and expressed as a deviation from the means of all (female by male) crosses (Wang *et al*., 2006). These were significantly high in the Dadin kowa and Kainji strains which shows they are the best combiners for this trait under consideration. Variations in general combining ability has however been attributed to both additive interactions and additive x additive genetic effect (Falconer, 1989). Increased resistance to infectious pancreatic necrosis Virus (IPNV) through selection been achieved in the Atlantic salmon (*Salmon salar*) (Storset *et al*., 2007) and rainbow trout (*Salmon gairdneri*) in which the RF-201 commercial resistant strain of this fish had mortality rate as low as 4.3% (Okamoto *et al*., 1993). Difference in resistance to *Aeromonas salmoncida* in the brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) also exist among different geographical strains and some crosses between better performing strains, has produced progeny with greater resistance to this bacteria (Ehlinger, 1977). In two populations of *Clarias macrocephalus* with different genetic background, low heritability was obtained after one generation of mass selection for resistance to *Aeromonas hydrophila* (Na-Narkon *et al.*, 1994).

There has not been any documented report on disease resistance among strains/ genetic crosses of *H. bidorsalis*. The result of this present study shows significantly high survival and line effect in the Dadin kowa strain. Maternal effect were significant and positive in the Kainji strain and negative in the Dadinkowa strain, while combining ability was also significantly high in both the Dadin kowa and Kainji strains. This suggest that genetic difference may exist among *H. bidorsalis* strains and crosses for diseases resistance to *Aeromonas hydrophila*. Other crosses were not significantly different for this parameters examined. It is possible that probably with a larger sample and strain size, the difference in resistance to *A. hydrophila* may be more significant.

CONCLUSION

This study shows that the intraspecific hybrid of *H. bidorsalis* from Dadinkowa (male) x kanji (female) strains have higher resistance to *A. hydrophila* infection and the selections of this lines combination for breeding programme can improve its resistance to *A. hydrophila* infections which will increases the potential of *H. bidorsalis for* culture. Further research should also be carried out on resistance to *A. hydrophilia* in other strains of *H. bidorsalis* strains before the selection program can be fully utilized.

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