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GENETIC DIFFERENCES IN RESISTANCE OF FARMED CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) CROSSBREEDS TO ENTERIC SEPTICEMIA AND COLUMNARIS DISEASES

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Abstract

Six different crossbreeds of channel catfish, *Ictalurus punctatus* derived from the South-east Fish Culture Laboratory (SFCL), Arkansas Foundation (AKFF), Hopper Steven (HS), BlackBelt (BB) and Goldkist Farms (GK1 and GK2), all in the USA, were evaluated at SouthFresh Farms, West Alabama, for resistance to enteric septicemia of catfish (ESC), and columnaris in May and June 2001. Fish were challenged in tanks with laboratory cultured isolates of *Edwardsiella ictaluri* and *Flavobacterium columnare*, the causative agents of ESC and columnaris disease, respectively. The AKFF × SFCL (female × male), GK1 × GK2 and BB × HS crossbreeds had higher ($P < 0.5$) resistance to ESC compared to the remainder of the crosses. Differences were also observed between reciprocal crosses in resistance to ESC. No differences ($P > 0.05$) were observed in resistance to columnaris among crossbreeds evaluated. Crossbreeding has potential for improving resistance of farmed channel catfish to *E. ictaluri* epizootics.

Introduction

One of the principal constraints to increased aquaculture production world wide is the incidence of infectious diseases that impact negatively on yield of commercially cultured species, including the channel catfish, *Ictalurus punctatus*. Farmed production of this species accounts for more than 50 per cent of all aquaculture production in the USA. (Stickney, 1993; Waldbiesser *et al.*, 1997).

Two major bacterial diseases that affect channel catfish are enteric septicemia of catfish (ESC) and columnaris. Enteric septicemia of catfish is caused by *Edwardsiella ictaluri* (Hawke, 1979), and is the most devastating infectious organism of cultured channel catfish (Plumb & Vinitnantharat, 1990). The causative agent of columnaris disease is *Flavobacterium columnare*, formerly *Flexibacter columnaris*, (Altinok & Grizzle, 2001).

Strains of catfish differ in resistance to bacterial and viral diseases (Dunham & Smitherman, 1984; Plumb *et al*., 1975). This phenomenon has also been reported for other fish species including common carp, *Cyprinus carpio* (Ilyassov, 1987) and Atlantic salmon, *Salmo salar* (Gjedrem & Aulstad, 1974). Gene frequencies may differ between strains of fish from genetic drift, natural selection, migration and differential mutation (Gardner & Snustad, 1981). This provides opportunities for improving disease resistance in fish through crossbreeding programmes. For example, crossbred channel catfish derived from experimental populations were more resistant to bacterial and viral diseases than pure strains (Dunham & Smitherman, 1984; Plumb *et al*., 1975).

Research examining crossbreeding to improve disease resistance in channel catfish has been limited to a few strains from experimental populations (Wolters & Johnson, 1995). There is need to evaluate farmed populations of channel catfish to identify strains that can be used to develop genotypes that are resistant to diseases

to improve aquaculture productivity. The objectives of the study were to assess resistance of six different farmed channel catfish crossbreeds to two bacterial diseases, ESC and columnaris, and to identify potential genotypes that can be used in selective breeding programs to enhance resistance to the two diseases.

Experimental

Six different channel catfish, *I. punctatus*, crossbreeds (F_1) derived from commercial populations were evaluated to assess their resistance to two bacterial diseases, enteric septicemia of catfish (ESC) and columnaris on-farm. The study was conducted from May to June 2001 on a commercial channel catfish farm, SouthFresh Farms, in West Alabama. The six crossbreeds were developed from crossing strains of channel catfish derived from the South-east Fish Culture Laboratory (SFCL), Arkansas Foundation (AKFF), Hopper Steven (HS), BlackBelt (BB) and Goldkist (GK1 and GK2) Farms, all in the USA. The crossbreeds were designated as AKFF × SFCL (female × male), GK1 × GK2, BB × HS, HS × SFCL, HS × BB and SFCL × AKFF

Production of channel catfish crosses

The six channel catfish crossbreds were produced by pairing female and male pure strains corresponding to the specific crosses in 1.9– 3.4 ha earthen ponds at a density of 1700 $kgha^{-1}$ from May to July 2000. The brood stock used were 4 years old. Initial average body weight of brood stock were 2.1 and 2.7 kg for females and males, respectively, for all the pairings, except for the GKI and GK2 strains. Average body weights of the GK1 and GK2 strains were 4.4 kg and 4.5 kg, respectively.

Rectangular and cylindrical milk cans were placed in the ponds for fish spawning. The volume of the milk cans were 73. 8 litres and 62.4 litres, respectively. Egg masses were collected at 2–3 days intervals from the milk cans placed in the ponds. The eggs were sorted by cross and hatched separately in different paddle wheel troughs under commercial hatchery conditions. At hatch, fry were siphoned quickly into bowls filled with water and stocked immediately in 0.77 × 0.72 × 0.14 m^3 floating commercial wooden troughs placed in a 6.1 × 0.81 × 0.89 m^3 concrete tank at 180,000 fish m^{-2} (100,000/trough) in a flow-through system.

Swim-up fry were fed to satiation with a 52 per cent crude protein starter feed for 10 days in the hatchery, then, stocked separately according to cross in 0.8–2.4 ha earthen ponds at the rate of 50,000–1,000,000 fish ha^{-1} in nursery ponds. Fish were grown with 48 per cent crude protein diet in the ponds for 1 year (2000 – 2001).

Enteric septicemia of channel catfish (ESC) challenge

The study was conducted from June 8 to 21, 2001. A recirculating system was constructed on the farm for the ESC challenge. The recirculating system comprised two circular 1,600-litre fiber-glass tanks and an earthen pond. Commercial plastic fish holding baskets, 48 cm in diameter and 43 cm deep (Aquatic Ecosystem Inc., Apopka, Florida) were adapted for use in the experiment. A plastic mesh was used in lining the interior sides of the basket with part of the mesh projecting 40 cm above the top of the basket, to prevent fish from escaping.

Fish were conditioned for 6 days before start of the experiment. Two hundred fish per genotype were used for the six genotypes evaluated. Two replicates per genotype were used for the experiments. Fish were held in duplicate baskets at stocking rates of 100 fish per basket inside the two circular fibre-glass tanks. Stocking rate in each replicate tank was 19 kg (2 kg m^{-3}). The mean ($\pm$ SD) weight of the crosses ranged from 27.1 $\pm$ 4.5 to 38.2 $\pm$ 4.8 g. The baskets were labelled to identify cross. Each cross was represented in each circular tank. Fish were not fed during the study.

Water was supplied to the recirculating system from a 1.0 ha earthen pond using a 115 V pump

(The Campbell Group Harrison, Ohio). The earthen pond contained only grass carp, *Ctenopharyngodon idella*. Water was delivered to the tanks through a 5-cm diameter polyvinyl chloride (PVC) pipe from a height of 30 cm above the tanks. Water was discharged back into the same pond through a 7.4-cm diameter PVC central pipe. The tanks were not artificially aerated. Morning dissolved oxygen ranged from 3.5 to 7.0 mg l^{-1} and temperature ranged from 25.8 to 31.5 ^{o}C.

An *Edwarsiella ictaluri* isolate, originating from a natural disease outbreak, was obtained from the Alabama Fish Farming Center, Greensborro, Alabama, in 2001, and was grown in brain heart infusion broth at 25 ^{o}C with constant agitation using magnetic stir plates. A 1-litre stock solution of the bacteria was diluted into a 2-litre (final) solution and used to inoculate the fish. Bacteria were identified by methods described by Hawke (1979). Bacteria were inoculated for 24 h at 30 ^{o}C and plated in brain heart infusion (BHI) agar plates in duplicate. Cell counts were made by serial 10-fold dilution (Vinitnantharat & Plumb, 1992). Bacteria were counted after 48 h of incubation.

Two fish samples from each strain were taken to the Fish Disease Laboratory, Alabama Fish Farming Center, Greensboro, Alabama, a day prior to the disease challenge to determine if fish were infected with ESC before the start of the disease challenge. No *E. ictaluri* was isolated from the samples before the start of experiment.

Fish were inoculated with the *E. ictaluri* isolate in the recirculating tank by stopping the water flow and reducing the water level to a depth of 10 cm. The 1-litre bacterial stock solution was diluted to 2 litres using water from the recirculating tank and carefully poured into the tank. Fish were exposed to the bacteria for about 1 h under static conditions after which water flow was resumed in the recirculating tank. Concentration of *E.ictaluri* during the challenge was 1.0×10^{5} ml^{-1}.

Mortality was monitored daily in the tanks for 14 days. Dead fish were removed when observed and clinical signs of ESC were recorded following the method of Dunham, Brady & Vinitnantharat (1994). Two dead or moribund fish from each strain were sent to the Fish Disease Laboratory, Alabama Fish Farming Center for necropsy. Isolates from the brain, trunk kidney and liver were streaked unto a BHI agar plates for re-isolation of *E. ictaluri*, and subjected to biochemical evaluation (Hawke, 1979). In few instances, *E. ictaluri* could not be isolated in pure culture because an individual fish had become over-grown with other bacteria due to autolysis and post mortem invasion (Meade *et al.*, 1994). Mortality in these fish was attributed to ESC based upon clinical signs, and because *E. ictaluri* was isolated in concurrent sampled dead fish.

Columnaris challenge

The experiment was conducted from 3 to 16 May 2001. Six channel catfish crossbreeds, with the same genotypes as those used for the ESC challenge, were also evaluated for resistance to columnaris in the study. A 4,397 m^3 ($6.1 \times 0.89 \times 0.81$ m^3) concrete tank was used for the columnaris challenge in a flow-through system.

A *Flavobacterium columnare* isolate obtained from Auburn University Fish Disease Laboratory was used to establish an experimental infection. A 1- litre stock culture was inoculated into a 2 - litre (final volume) of HSU shotts (working culture) and agitated with a stirring bar for 36 h at 25 ^{o}C. A sample of working culture was diluted with sterile saline in 1:10 serial dilutions to 1×10^{-10}. Duplicate HSU shotts agar pour plates were made for bacterial enumeration. Fish were held in 12 baskets with identical size as used for the ESC study. The 2-litre bacteria stock solution was applied to each tank. Water level was reduced in the tank to 10 cm depth (0.5 m^3) to ensure bacterial contact with the fish. Exposure was for 1 h. Water flow was resumed after the exposure.

Mortalities were monitored and fish were sent to the laboratory for necropsy. Morphology of *F.*

columnare isolates was examined using the Gram's stain. Motility of bacteria isolates was determined by wet mounts, using phase contrast microscopy at 400 × magnification (Walters & Plumb, 1978). Standard plate count technique was used to enumerate the bacteria. Serial dilutions were made in duplicate petri dishes with Butterfields phosphate dilution water (FDA, 1998). Bacteria were enumerated using a Darkfield colony counter (Reichart Jung, Quebec). Number of columnaris colonies was estimated at 4.0×10^4 ml^{-1}. Analysis of fish was conducted on brain, trunk kidney and liver for detection of *F. columnare* isolates. The mean (± SD) weight of the crosses ranged from 27.2 ± 1.6 to 36.0 ± 6.0 g.

Water quality monitoring

All water quality parameters were monitored at 07.00 h. Dissolved oxygen in the tanks was measured daily using a YSI model oxygen meter (Yellow Spring Instrument Co. Yellow Springs, Ohio). Tank water temperatures and *p*H were measured with a mercury thermometer and a pin-point *p*H meter (American Marines), respectively. Ammonia-nitrogen and nitrite-nitrogen were measured once every 2 days using a Hach Kit (Model FF-2, Hach Company, 1993). Total hardness and alkalinity was measured using a Hach Kit at the commencement and at the end of the experiment.

Data analysis

Mortality data for the catfish genotypes exposed to ESC and columnaris were analysed using contingency tables and chi-square analysis. When significant differences were observed, the contribution of the deviations of observed mortality from expectations for each genotype were compared to the calculated chi-square, and was used to rank the genotypes (subclasses) as described by Snedecor & Cochran (1967). The Statistical System, SAS, release 6.12 (SAS Institute, 1989) was used for the analysis.

Results and discussion

Mean water quality parameters in the tanks used for the enteric septicemia and columnaris disease challenges for the commercial channel catfish, *I. punctatus*, crossbreeds are shown in Table 1. Mean mortality of the commercial channel catfish crossbreeds exposed to *E. ictaluri* and *F. columnare* is summarised in Tables 2 and 3, respectively.

Differences ($P < 0.001$, $\div^2 = 36.67$, df = 5) were observed in mean mortality percentage among the six commercial channel catfish crossbreeds when challenged with *E. ictaluri*, the causative agent of enteric septicemia of catfish (ESC) (Table 2). The SFCL × AKFF and HS × BB crossbreeds had a higher mean mortality than the remainder of the crosses (Table 2). Differences ($P < 0.001$, $\div^2 = 17.90$, df = 1) in mean mortality were also observed between the AKFF × SFCL and BB × HS crossbreeds and their reciprocal crosses when challenged with ESC (Table 2).

Two and one of the crosses involving maternal parents of the HS and SFCL genotypes, respectively, had significantly higher mortality when exposed to *E. ictaluri* compared to crossbreeds that were sired by these same genotypes: AKFF × SFCL and BB × HS (Table 2). No differences ($P > 0.05$, $\div^2 = 3.35$, df =5) in mean mortality were observed among the six different commercial channel catfish crossbreeds when challenged with *F. columnare*, the causative agent of columnaris although observed mean mortality of the HS × SFCL was lowest compared to the other crossbreeds (Table 3).

There was no relationship between body weight and mortality of channel catfish during the bacterial disease challenge. The correlation between body weight and resistance to ESC and columnaris was – 0.23 and – 0.03, respectively, and was not significantly different from zero.

Research has shown significant phenotypic variation among strains and families of channel catfish in resistance to enteric septicemia of catfish (ESC), indicating potential for

TABLE 1

Mean (± S.D.) water quality parameters in tanks used for the enteric septicemia (ESC) and columnaris diseases challenge of commercial channel catfish, Ictalurus punctatus, *on-farm in 2001*

Parameter	*Disease challenge*			
	ESC		*Columnaris*	
Temperature (^{o}C)	28.8 ± 1.8	(25.8 - 31.5)	24.3 ± 1.1	(22.5 - 26.0)
Oxygen (mg l^{-1})	5.1 ± 1.1	(3.5 - 6.6)	7.0 ± 0.5	(6.2 - 7.5)
*p*H	8.0 ± 0.3	(7.4 - 8.5)	7.7 ± 0.2	(7.2 - 7.9)
Ammonia-nitrogen (mg l^{-1})	0.3 1± 0.4	(0.0 - 1.4)	0.3 1± 0.5	(0.0 -1.4)
Nitrite (mg l^{-1})	≤ 0.06	(0.00 - 0.08)	≤ 0.08	(0.00 - 0.06)
Alkalinity (mg l^{-1} as $CaCO_3$)	141.0 ± 0.7	(140.0 -141.0)	141.0 ± 0.7	(140.0-141.0)
Hardness (mg l^{-1} as $CaCO_3$)	49.5 ± 1.0	(49.0 -50.0)	49.5 ± 1.0	(49.0 - 50.0)

[1]Ranges of water quality parameters are indicated in parenthesis.

TABLE 2

Mean (±SD) body weight and mortality of commercial crossbreeds of channel catfish, Ictalurus punctatus, *exposed to* Edwardsiella ictaluri *on-farm in 2001*

Genotype	Body weight (g)	Mortality (%)
SFCLXAKFF	30.4 ± 6.3	30.5[a]
HSXBB	28.4 ± 1.6	27.0[a]
HSXSFCL	38.2 ± 4.5	19.5[b]
AKFFXSFCL	27.1 ± 4.5	8.0[c]
GK1XGK2	33.3 ± 4.2	7.5[c]
BBXHS	35.2 ± 2.0	3.0[c]

[1]SFCL = South-east Culture Laboratory strain. AKFF = Arkansas Foundation Farms strain. HS = Hopper Stevens Farms strain. BB = BlackBelt Farms strain. GK1 and GK2 = GoldKist strains. Mean values with different superscripts within column are significantly different, chi-square test ($P < 0.001$). Mean body weights are not different ($P > 0.05$, ANOVA).

improvement of resistance to this bacterial disease through selection (Wolters & Johnson, 1995). However, selection in the Kansas strain of channel catfish did not improve resistance to the disease (Dunham, Brady & Vinitnanthorot, 1994). The observed superior performance of the AKFF×SFCL, GK1×GK2 and BB×HS channel catfish crossbreeds in resistance to ESC, which

TABLE 3
Mean (±SD) body weight and mortality of commercial strains of channel catfish, Ictalurus punctatus, *exposed to* Flavobacterium columnare *on-farm in 2001*

Genotype	*Body weight (g)*	*Mortality (%)*
AKFFXSFCL	29.2 ±1.4	21.5
GK1XGK2	32.5 ± 1.5	21.5
HSXBB	27.2 ± 1.6	20.0
BBXHS	36.0 ± 6.0	20.0
SFCLXAKFF	30.3 ± 0.5	19.5
HSXSFCL	31.3 ± 2.0	12.5

[1]AKFF = Arkansas Foundation Farms strain. SFCL = South-east Culture Laboratory strain. HS = Hopper Stevens Farms strain. BB = BlackBelt Farms strain. GK1 and GK2 = GoldKist strains. Mean mortalities and times are not different, chi-square test ($P > 0.001$). Mean body weights are not different ($P > 0.05$, ANOVA).

reflected in very low mortalities (< 10%) in the study, suggests the utilization of these crossbreeds for improving survival during *E. ictaluri* epizootics on-farm.

Wolters & Johnson (1995) who evaluated diallel crosses involving the Marion, Kansas and Norris strains of channel catfish found significant differences in ESC tolerance between some of the reciprocal crosses. These same authors reported that maternal effects were significant and positive for the Norris but negative for the Marion × Kansas parental lines in resistance to *E. ictaluri*. The significant differences in resistance between the SFCL × AKFF and BB × HS crossbreeds and their reciprocal crosses, in the study, collaborates the findings of these authors that paternal and maternal factors influence resistance to *E. ictaluri*. In the study, there appears to be maternal strain effect of the BB and AKFF strains in resistance to this bacteria. In contrast, paternal strain effects were apparent for the SFCL and HS genotypes in resistance to the same disease. Differential methylation of DNA between the maternal and paternal genomes due to genomic imprinting in germ cells (Lewin, 1999) and its corresponding effect on gene expression is a potential explanation for the observed differences in ESC in resistance between the reciprocal crosses in the study and that reported by Wolters & Johnson (1995).

The SFCL × SFCL cross is known to have poor resistance to columnaris (Dunham & Smitherman, 1984). However, this cross had equal or better columnaris resistance than three different channel catfish crossbreeds.The lack of significant differences in resistance among the crossbreeds evaluated, in the study indicates crossbreeding may be less efficient in improving columnaris resistance in channel catfish.

Conclusion

Crossbreeding has potential for improving ESC resistance in farmed channel catfish. Mickett (2002) observed greater genetic variation among farmed strains of channel catfish compared to research populations, using amplified fragment

length polymorphism (AFLP) DNA markers. Thus, crossing the divergent strains of channel catfish may result in positive heterosis for enhanced resistance to ESC on-farm. In contrast, selection of columnaris-resistant strains of channel catfish is crucial to developing genotypes that have superior performance when exposed to this bacterial disease.

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