AQUACULTURE

DEVELOPMENT OF AN ORAL VACCINATION PLATFORM TO PROTECT CATFISH AGAINST ENTERIC SEPTICEMIA OF CATFISH

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Vaccines are an important component of disease management programs and have proven effective in preventing bacterial infections and reducing the use of medicated feeds in farmed fish. Initial attempts to vaccinate catfish against Enteric Septicemia (ESC) utilized killed vaccines and were for the most part unsuccessful. Vaccination failure has been attributed to poor antigen uptake during delivery and/or the inability of killed vaccines to elicit cellular immunity necessary to protect fish against an intracellular bacterial pathogen such as *E. ictaluri*. Alternatively, live attenuated vaccines are invasive and establish low-grade infections resulting in the stimulation of humoral and cellular immunity. Although safety can become a concern with live vaccines, especially if delivered to animals in suboptimal health, when delivered to healthy immunocompetent animals they typically result in long-lasting immunity.

Dr. David Wise discusses the latest technology from the vaccine delivery system with Steve Pomerleau of America's Catch catfish farm.

The main hurdle in developing effective catfish vaccines has not been the lack of an effective vaccine, but rather the inability to mass deliver live attenuated vaccines to fish beyond the fry stage of development in a commercial setting. At present, to conform to industry practices, vaccines are administered as an immersion bath to catfish fry between seven-12 days of age, when fish are transferred from the hatchery to nursery ponds. Developmental studies demonstrate catfish fry have an underdeveloped immune system and this is likely responsible for poor vaccine efficacies reported in previous commercial vaccination trials.

To circumvent this limitation, we investigated delivery of a live attenuated vaccine through the feed, allowing for the vaccination of an older more immunocompetent fish. This work began in 2008 with the attenuation of virulent strain of *E. ictaluri* isolated from a commercial catfish fingerling farm in Mississippi. The isolate was evaluated as a candidate for vaccine production in safety and efficacy trials conducted in the laboratory. All selection lines were shown attenuated but only one offered protection following exposure to the virulent parental field strain. A master

"THE VACCINE DELIVERY SYSTEM IS KEY TO THE VACCINATION PROCESS. SUCCESSFUL IMMUNIZATION IS DEPENDENT ON DELIVERING MEASURED VACCINE DOSES TO OLDER MORE IMMUNOCOMPETENT FISH IN THE POND."

David Wise

seed line designated S97-773-340X was developed and served as the seed stock for vaccine production. In 2009, procedures for preparing an oral vaccine were developed and tested in small experimental ponds with exceptional results. The immunization process proved highly effective but commercial scale application required the development of procedures for large quantity vaccine production and a mechanized delivery system allowing for accurate application of measured vaccine doses.

The USDA/ARS National Biological Control Laboratory (NBCL) in Stoneville MS, is a research facility designed for developing methods of mass propagation of beneficial microorganisms. In 2010, the Mississippi Agricultural and Forestry Experiment Station (MAFES) entered into research agreements with USDA-ARS, NBCL to develop commercial scale protocols for vaccine production. Vaccine was produced in 50 L floor model fermenters and processed according to procedures developed by MAFES scientists. Vaccine serials were shown safe and effective against *E. ictaluri* infection and stable for up to two years in cold storage without loss of potency.

In collaboration with MAFES scientists in the Department of Agricultural and Biological Engineering, a mechanized system for mixing and delivering the vaccine was developed using MAFES Strategic Research Initiative funds. The initial prototype was completed in 2013 and used in 1.0 acre experimental pond trials at National Warmwater Aquaculture Center. After minor modifications, the delivery system and processed vaccine was used in limited field trials in 2014. A second prototype with increased storage capacity was developed in 2015 and used in large scale commercial field trials in 2015 and 2106. A third generation delivery system has been constructed and validated for used in 2017. This final design has a modified conveyor screw and blower system to facilitate wet feed delivery, precision spray control technology to ensure accurate consistent application of vaccine to feed at continual variable rates, and programmable logic control system providing monitoring and documentation of performance parameters.

Commercial trials involving over 200 million

Table 1. *Production parameters of commercial vaccination trials conduction in 2013-2015.*
** denotes a significant difference within each production parameter between vaccinated and non-vaccinated fish (P≤0.05).*

	% Survival	Feed (Tons/ac)	Yield (lb/ac)	FCR	Size (lb/1000 f)
Channel Catfish					
Vaccinated (n=50)	**80.4***	**5.01***	**6,334***	**1.65***	**67.8**
Control (n=79)	**58.4**	**4.33**	**4,644**	**1.94**	**70.8**
Hybrid Catfish					
Vaccinated (n=43)	**78.5***	**7.62***	**8,204***	**1.84**	**99.4**
Control (n=67)	**69.5**	**5.85**	**6,258**	**1.95**	**90.4**

catfish fingerlings showed significant improvements in survival, feeding rates, and gross yield for both channel and hybrid catfish (Table 1). A partial budget analysis showed an increased net benefit of $1,877 and $2,589 per acre, respectively, for vaccinated channel and hybrid catfish, resulting primarily from improved survival and yield (Table 2). The improvement in survival from oral ESC vaccination was more pronounced in channels than in hybrids as ESC is less problematic among hybrid catfish. However, the economic benefit was slightly higher in hybrids (1.14 times greater) resulting from overall increased hybrid yield combined with a higher hybrid fingerling price.

These data clearly demonstrate the oral vaccination platform improves production efficiencies of both the commercial production of hybrid and channel catfish fingerlings. The vaccine delivery system is key to vaccination process. The application of precisely metered dosages at the point of feed delivery enables the oral distribution of live vaccines or any heat labile product to fish without destroying the organism or biological activity. The Mississippi Agricultural and Forestry Experiment Station at Mississippi State University is in the process of developing a commercialization strategy involving a public/private release of the technologies in efforts to maximize cost benefit ratios to producer in efforts to minimize application costs and maximize returns to producers.

Table 2. *Partial Budget analysis (per acre) comparing the economic effect of vaccinated channel and hybrid catfish on commercial fingerling operations.*

Category	Vaccinated Channels	Vaccinated Hybrids
Benefits		
Additional Revenue[a]	$, 2,702	$ 3,738
Reduced Costs	$ 0	$ 0
Total additional benefits	$ 2,702	$ 3,738
Costs		
Reduced Revenue	$ 0	$ 0
Additional feed cost[b]	$ 439	$ 1,149
Total Additional Costs	$ 439	$ 1,149
Net Benefit (Total benefits-Total Cost)	$ 2,263	$ 2,589

[a] Additional revenue is calculated as the product of additional yield in inches and price of fingerling/inch. Channel catfish fingerling prices = $0.0175/inch; hybrid fingerling price = $0.0275/inch.
[b] Additional costs are calculated as the product of additional feed and price of feed. Fingerling feed cost = $ 650/ton.