Evaluation of a Trivalent Vaccine for *Aeromonas hydrophila, Flavobacterium columnare,* and *Edwardsiella ictaluri* Using In-pond Raceway Systems in West Alabama

by

Jesse Paul Bodam James

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 3, 2019

Keywords: Raceway, Vaccine, Channel Catfish, West Alabama

Approved by

Terrill R. Hanson, Chair, Professor and Extension Specialist, School of Fisheries, Aquaculture, and Aquatic Sciences (SFAAS)
Jesse Chappell, Co-chair, Associate Professor and Extension Specialist, SFAAS
Jeffery Terhune, Associate Professor, SFAAS
Luke A. Roy, Extension Specialist and Associate Research Professor, SFAAS
Benjamin Beck, Research Physiologist and Research Leader, USDA ARS Aquatic Animal Health Laboratory

Abstract

A trivalent vaccine, for virulent *Aeromonas hydrophila*, *Flavobacterium columnare*, and *Edwardsiella ictaluri*, was tested using in-pond raceway systems (IPRS) located in three active commercial catfish ponds. The goal of this study was to determine the effectiveness of this new vaccine against naturally occurring incidences of the target diseases. The study was conducted using three identical IPRS units in three ponds owned and operated by Williamson Cattle Company (WCC). Each IPRS was capable of housing four replicates per treatment.

The fish were vaccinated in May and stocked into the raceways in June of 2017. At the time of stocking mean fish weight was 36.79 g per individual. The fish were harvested in September of 2018 and average individual weight and total weight was collected for each treatment. The average individual weights at harvest ranged from 715.9 g to 933.6 g. Survival ranged from 46.7 % to 65.2%. There were no significant differences among treatments for any of the production endpoints measured.

A separate laboratory challenge trial was carried out with fish vaccinated via injection, as well as an equal amount of control fish. At the end of the field trial some of the vaccinated and control fish were transported to Auburn, AL for controlled laboratory disease challenges. Two challenge trials were conducted for each of the target diseases. A statistically significant difference in mortality rate was only found in one of the challenges using vAh bacteria. In that challenge, the vaccinated group had a 50 ± 16 percent mortality rate compared to the 95 ± 5.8 percent mortality rate of the control group ($P < 0.0364$).

Acknowledgments

I would like to thank my wife and family for their support during my academic career and for pushing me to achieve what at the time seemed out of my reach. I would like to thank Dr. Luke Roy for his guidance and expertise that has been extremely valuable throughout my time with Auburn. I would also like to thank the staff at the Alabama Fish Farming Center for all of their help and support they have given me during my time there. I am grateful for the experience that I gained working with Bill Hemstreet, and the wisdom that he has shared with me on all aspects of the catfish industry. I want to thank Dr. Benjamin Beck, Troy Bader and the rest of the USDA-ARS staff that provided tremendous support during the most difficult aspects of this project. I am thankful for the guidance I received from Dr. Jesse Chappell and Dr. Terry Hanson through the master's program. I would like to thank Mike Owens, Randy Hollingsworth, and Williamson Cattle Company for allowing me to conduct this project on their farms and for the assistance they provided. I would like to thank the USDA ARS Aquatic Animal Health Laboratory (Auburn AL), Alabama Agricultural Experiment Station; Alabama Department of Ag and Industries, Alabama Catfish Feed Mill, and the Alabama Catfish Producers association who financially supported this project. I would also like to thank the other people who had a hand in supporting this study: Sunni Dahl, Esau Arana, Grant Harless, Colton Granger, Alex Crawford, Daniel Creel, and Lee Collins.

Table of Contents

List of Terms and Abbreviations

Alabama Fish Farming Center (AFFC) – Auburn University and Alabama Cooperative Extension System's aquaculture diagnostics, research, and extension center located in Greensboro, Alabama.

In-pond Raceway System (IPRS) – A system of fish culture compartments within a pond that are supplied with continuous water flow.

Williamson Cattle Company (WCC) – The name of the company that owns and operates the catfish farm on which the study was conducted.

Cell – One individual compartment in an IPRS.

Dissolved Oxygen – The concentration of oxygen that is dissolved in the water.

Open Pond – Refers to the area of the ponds outside of the raceway systems.

Survival – refers to the amount of fish remaining from the original stocking number expressed as a percentage.

List of Tables

List of Figures

Chapter 1

Introduction

1.1 Current Status of the US and Alabama Catfish Industries

Catfish farming is an important industry in several areas of the southeastern United States (US). For many years, catfish farming has been a major driving force behind the economy of rural communities in Alabama, Arkansas, Mississippi, Louisiana, and Texas. Since its conception, farm based production of channel catfish *Ictalurus punctatus* and channel X blue catfish hybrids (*Ictalurus punctatus* ♀ x *Ictalurus furcatus* ♂) has grown to become the largest aquaculture industry in the US, reporting over $355 million in sales of food size catfish in 2017 and 341 million pounds in 2018 (NASS 2018; Hanson 2019). Although catfish farming has seen its share of difficulties and challenges it has held strong and continued to provide a valuable source of income for many people in the rural south.

Today there are currently 61,860 water acres actively involved in catfish production, a 1,070 acre increase from 2017, of which 17,450 are located in the state of Alabama (Hanson et al. 2018; NASS 2018). Since its peak of 662 million pounds in 2003, the U.S. catfish industry has experienced a dramatic reduction in production down to 307 million in 2014 (Hanson and Sites 2015). Since 2014 the industry has slowly started to regain its footing and production has risen to 330 million pounds in 2017 and 341 million pounds in 2018 (NASS 2018).

Catfish farmers, including farms in Alabama, have faced a number of obstacles over the years. Recession, rising feed and fuel costs, and cheap foreign imports have forced many farms out of business. An additional factor that has played a role in reducing profitability, especially on

Alabama farms, is fish mortality caused by infectious disease outbreaks. Enteric Septicemia of Catfish (ESC), *Flavobacterum columnare*, and Virulent *Aeromonas hydrophila* are the three most impactful diseases affecting commercial catfish farms in Alabama. According to an annual survey conducted by the Alabama Fish Farming Center, these diseases were responsible for the loss of over 5.5 million pounds of catfish and $9.7 million on Alabama farms in 2018 (Hemstreet 2019).

1.2 Traditional and Alternative Production Systems

Over the years catfish farmers have employed several different systems for food-fish production. One of the biggest limiting factors for catfish farmers is space. Due to increasing land prices in the US the ability to maximize production per acre has become increasingly important. In an attempt to increase production potential over traditional yields a number of different intensive pond-based systems have been developed and tested that focus on increasing stocking density and controlling water quality to improve overall yield. Although, some newer systems have been implemented, the traditional levee pond system remains the most widely used throughout the industry.

Open Pond Systems

Levee ponds are the most commonly used culture system for catfish production in the US. This system is popular among catfish farmers due to its simple construction and ease of management. These systems are constructed by forming raised earth levees around the outer edges of the desired pond area. In order to make harvest more efficient the inner banks of the levees typically have a slope of 3:1 or 4:1. The size of commercial ponds have declined over the

years as farmers have found that the smaller ponds enable them to boost their feeding and management efficiency. In the current industry, most newly constructed ponds will have a surface acreage between 3 and 5 hectares and an average depth of 1.7-1.8 m (Steeby and Avery 2002). An additional benefit that comes with the use of smaller ponds is the ability to limit disease outbreaks to smaller populations of fish.

Ease of management and efficiency are very important factors when choosing a production system for catfish. Levee ponds simplify management by requiring very little maintenance, providing access on all sides of the pond, and enabling a seine through harvest method. Other than aerators, feed trucks, oxygen monitoring equipment, and a reliable water source, there is very little equipment required for managing a levee pond. The relatively low operating cost of this type of system is what makes it such an appealing system to catfish farmers. Because of the high feed cost associated with catfish production, farmers need to maintain all additional production costs as low as possible in order to make a profit.

While levee pond systems have proven to be simple to operate they do have a few drawbacks that have influenced some farmers to pursue alternative systems. It is common practice for ponds to remain in continuous use for a number of years without being drained and this often leads to heavy sediment loading. Ponds that are kept in continuous operation for 15 years will contain an average sediment load of 40 cm (Steeby et al. 2003). This buildup of sediment leads to inefficient harvest, reduced pond volume, and can cause poor water quality. Another issue with levee ponds that has gained attention in the catfish industry recently is the abundance of oversized fish at harvest. These fish are often leftover fish that have been missed for several harvests, allowing them to reach a size that is undesirable for processors. It has been

found that the price dockage for oversized fish has a significant impact on the net returns received by catfish farmers (Gosh 2018).

Another form of pond that is commonly used in Alabama are hillside or watershed ponds. These ponds are built by constructing a single levee in a watershed depression to capture runoff rainfall from the surrounding area. These ponds are managed for catfish production in generally the same way as a levee pond system. Due to the rolling prairie topography of the Blackbelt region of west Alabama, these ponds are quite common. The main drawback for hillside pond systems is that they are more difficult to harvest efficiently, due to their irregular shape.

Intensive aeration is a method of intensifying production in traditional open ponds that has gained some popularity among farmers (Bott et al. 2015). These systems utilize an increased level of aeration to increase the carrying capacity and feeding rates of open pond systems. Although this generates much higher electrical expenses it has a significant impact on the amount of fish produced per hectare. When it comes to intensification, this system is likely the most easily adoptable for the catfish industry, as it does not require major pond reconfiguration.

Problems that are associated with all forms of open pond systems include predation and treatment expenses. Because open pond systems involve large volumes of water, chemical treatments require bulk amounts of chemical to reach effective concentrations. Due to the high cost of treatment chemicals this can become quite expensive. Fish eating predators, such as aquatic birds and mammals, are difficult to control in open pond systems due the ease of access that these animals have for feeding in the ponds. These dense populations of fish attract these animals away from their natural feeding grounds in large numbers. Depredation permits from the USDA APHIS wildlife services division are available for farmers that legally allow them to deter aquatic birds from feeding in their ponds (USDA 2019)

Split-pond systems

In an attempt to further increase production some U.S. catfish farmers have begun to adopt split-pond production systems. In 2016, the amount of split pond systems in production had reached 890 hectares in the U.S (Kumar 2016). To build a split-pond system, a levee is constructed to divide a traditional open pond into two unequal parts. One side that consists of about 20% of the original pond area is used for fish culture and the other 80% is used as a waste water treatment area. Water is exchanged between the culture area and the treatment area to remove waste and supply oxygenated water and supplemental aeration is provided to the culture area when necessary. (Kumar et al. 2016) Confining the fish to a smaller area of the system helps to increase feeding and harvesting efficiency, reduce disease treatment costs, and reduce predation (Tidwell 2012; Kumar et al. 2016).

Management of a split-pond systems is certainly more involved than that of traditional open ponds. However, with the experience and knowledge of water quality management that most traditional pond managers have developed, split-pond management is easily within their abilities. Split-ponds maintain sufficient dissolved oxygen levels in the culture area by utilizing the daytime activity of the algal bloom in the pond. The majority of the oxygen production takes place in the larger waste water treatment portion of the pond. This freshly oxygenated water is then pushed through the small fish culture area of the pond by either a slow-rotating paddlewheel or a screw pump where it can be utilized in the respiration process of the fish. During night time hours, all water exchange is stopped and supplemental oxygen for the culture area is provided by mechanical aeration (Tidwell 2012; Tucker et al. 2014). Managing this system requires close monitoring of dissolved oxygen and other water quality parameters, as well as fine-tuned control of water exchange and aeration to optimize efficiency.

Split-pond systems are known for their ability to offer a significant increase in production over traditional open pond systems. During a seven-year study conducted using an experimental split-pond at Mississippi State University the systems net annual production ranged from 17,000 to 20,000 kg/ha (Tidwell 2012). Although a split-pond system requires a large population of fish to be crowded into a small area, water quality parameters such as ammonia and nitrite can be consistently held at or below acceptable levels in most situations. This can be attributed to the effectiveness of the natural waste removal processes carried out by the microbial communities in the waste treatment portion of the pond. Split-pond systems have gained some popularity in the commercial catfish production industry in recent years, however, they still only account for a small percentage of U.S. production. The small acceptance rate of this system could be attributed to several factors such as additional construction costs, increased risk, and fluctuating market prices. When fish prices are lower and feed costs are high, farmers tend to have less interest in adopting new systems, as those conditions would allow little room for failed ventures (Kumar et al. 2018). If the catfish industry continues to improve in the U.S. it could provide the appropriate conditions for more of these systems to be put into production.

In-pond Raceway Systems

Another alternative intensive-pond based system that has been used for catfish production in recent years is the In-pond Raceway system (IPRS). These systems are designed to increase the amount of fish that can be produced in a given area while also increasing the amount of control the farmer has over the fish population. Water quality management is the key to the IPRS's ability to increase its productivity over that of traditional open pond systems. By constantly adding clean oxygenated water and flushing out waste, the fish are held in a healthier

environment. This allows the fish to survive at densities that are significantly higher than what can be accomplished in open pond systems.

In-pond raceways are a system of fish culture compartments, typically called raceway cells, that confine the fish to a small area of the pond. These systems are equipped with some form of water moving device that forces water from the open pond through the cells. The cells themselves usually consist of solid bottoms and dividing walls with wire screens at the inflow and outflow ends. The system can either be fixed to the pond bottom or free-floating depending upon the design. For optimal water flow through the open pond area a baffle wall or levee is typically constructed. This wall forces the discharge water to circulate through the entire pond before it is cycled back into the raceways, allowing the maximum amount of time for natural waste removal and algal oxygen production to occur. (Brown et al. 2011; Tidwell 2012; Fullerton 2016)

In-pond raceway systems have been found to be capable of producing up to 7506 kg/ha of channel catfish and 13,034 kg/ha of hybrid catfish (Brown et al. 2011). IPRS have been found to reduce energy costs for aeration up to 50% compared to pond systems using conventional aeration. Through the combined effort of increased production and a reduced aeration expenses, these systems can offer a significant improvement in production efficiency (Tidwell 2012).

Although IPRS have shown potential for catfish production at the research level it has yet to be widely adopted by the commercial catfish industry, although it has gained widespread acceptance in Asia for culturing a number of different species (Roy et al. 2019). Some of the drawbacks that have been experienced by U.S. catfish farmers attempting to implement these systems for commercial use have been extremely high mortality rates, high startup expense, and increased management intensity. Due to the tight quarters that the fish are held at in these

systems the potential for disease outbreaks to quickly spread through the entire population is high. This reduces the amount of reaction time available to correct the problem and can lead to massive losses. Constructing an IPRS system requires that the farmer spend a large amount of money up front, which is a difficult and risky decision for most farmers to take when they already have a system in place that they know can cover its own cost. These systems often require a lot more attention than a traditional pond system and most smaller farms cannot afford to designate the manpower to run the system properly. However, some Alabama farms that already have these systems in place have been able to utilize them for production of other fish species, such as tilapia, and for industry research projects.

1.3 Major Diseases

One of the biggest setbacks catfish farmers face is losses due to disease outbreaks. When a disease occurs in a catfish pond it has the ability to spread very quickly through the entire fish population due to the high stocking density. It is not uncommon for a farmer to lose a substantial portion of the fish in a pond to a major disease occurrence. According to a farmer survey conducted by the Alabama Fish Farming Center in Greensboro, Alabama, Alabama farmers lost approximately 921 metric tons of catfish to disease in 2018 (Hemstreet 2019). Given that the average market value for catfish in that year was $2.53 /kg, these disease related losses cost the Alabama industry $6.4 million in fish losses alone (Hemstreet 2019). Of these losses 84% were caused by three primary diseases, Enteric Septicemia of Catfish (ESC), *Flavobacterum columnare*, and Virulent *Aeromonas hydrophila* (Hemstreet 2019). When other factors such as medicated feed cost, chemical treatments, and lost feeding days are factored in that total rises to $9.7 million (Hemstreet 2019).

Enteric Septicemia of Catfish

Enteric Septicemia of Catfish is an infectious disease that is caused by the bacteria *Edwardsiella ictaluri* (Hawke et al. 1981). The first instances of ESC are believed to have occurred in Arkansas as early as 1969, however, it was not officially recognized as an infectious disease until 1976 (Hawke et al. 1998). Not long after the initial outbreaks occurred this disease quickly spread throughout the U.S. catfish industry. In 2018 this disease was responsible for the loss of 195 metric tons of catfish on Alabama farms (Hemstreet 2019).

Fish that develop ESC usually express a number of physical and behavioral changes that can help in diagnosing this disease. One such behavioral change that can occur in a fish that has ESC is "whirling". When the *E. ictaluri* bacteria invade the brain of the fish it can cause it to perform an erratic rolling or tail chasing maneuver near the surface of the water. Another behavioral change that can be seen in an infected population is a reduction in feeding response or even a complete stop in feeding activity. These changes can be easily spotted through daily pond observations (Hawke et al. 1998). Some of the telltale external signs of an *E. ictaluri* infection include small raised bumps on the skin of the fish, exophthalmia (bulging of the eyes), ascites (swollen abdomen), and development of a subdermal hematoma or ulcer along the cranial foramen ("hole in the head") (Shotts et al. 1986). During the necropsy process, a diagnostician will look for fluid in the body cavity, a mottled coloration of the liver, and hemorrhaging of the intestines and muscle tissue (Hawke et al. 1998). These internal and external signs are a good indication that this disease is present. In order to confirm a positive identification, bacterial samples must be cultured and tested. Because *E. ictaluri* shares several clinical signs with its close relative *Edwardsiella tarda*, it is necessary to administer some tests to distinguish which

bacterial species is present. Unlike *E. tarda, E. ictaluri* is indole negative, Jordan Tartrate negative, and H_2S negative. The simplest method for distinguishing between the two bacterial species is to apply an isolated sample of the bacteria to a Triple Sugar Iron, TSI, slant media. When grown on this media *E. ictaluri* will produce a K/A result with no H_2S production (Hawke et al. 1981).

ESC is a seasonal disease that most commonly occurs during a distinct temperature range known as the "ESC Window". This seasonal window normally occurs during the spring and fall of each year as changing pond water temperatures pass through the 24-28°C range (Francis-Floyd et al. 1987). *E. ictaluri* invades the body of the fish through two pathways. One method of entry is via the intestinal walls. When the fish ingests the bacteria by eating infected carcasses or swallowing contaminated water, the bacteria pass through the intestinal membrane and quickly spread throughout the fish's body. It is common for fish that are infected through this pathway to die before any external signs are present, due to how fast the infection is able to reach a lethal level. The second pathway that *E. ictaluri* utilizes is through the nares (nasal openings). Bacteria present in the pond water are able to enter these openings and invade the delicate olfactory tissues. Infections that develop through the nares take longer to reach a lethal level than the intestinal pathway, which allows for the development of the associated external signs (Shotts et al. 1986).

In the event of an ESC outbreak there are a few treatment options that have proven to be effective in controlling the disease. One such treatment method that has been adopted by many farmers is withholding feed (Hawke et al. 1998). This is a cheap and simple way to address an outbreak, and it has proven to be effective in reducing the severity of disease events. A possible explanation for the success of this practice is that it reduces the amount of contaminated water

that fish are consuming while feeding. Due to the primary infectious pathway of an acute ESC outbreak being through the ingestion of infected carcasses and contaminated water, removal of dead fish will also help in limiting the further spread of bacteria to uninfected fish (Shotts et al. 1986; Klesius 1994).

When the occurrence of an ESC event is spotted and diagnosed quickly, the best option for treatment currently is via a medicated feed regimen. There are currently two antibiotics that are approved for use in commercial catfish production for the treatment of ESC, sulfadimethoxine and ormetoprim (trade name Romet) and florfenicol (trade name Aquaflor) (Bebak and Wagner 2012). While both these antibiotics work well against *E. ictaluri* it is recommended to have a sensitivity test performed by a diagnostics laboratory to determine which is most effective against the bacterial strain isolated from the fish. Another aspect farmers must take into consideration before applying a medicated feed is the withdrawal time before harvest associated with each drug. The withdrawal period for Aquaflor is 12 days and 3 days for Romet. If the fish in the infected pond are ready to be harvested it is best to use a medication with a shorter withdrawal period when possible, to get the fish to market sooner and limit the potential for any further loss of market ready fish.

A major drawback for using a medicated feed is its high cost, that has ranged from $970 - $1195/ ton (mean $1,064/ ton) for Romet, and $724 - $972/ ton (mean $773/ ton) for Aquaflor from January 2015 to May 2019, compared to 28% and 32% protein feed averaging $355 and $381 respectively (personal communication, Dr. Terrill Hanson). A farmer must take into account the value of the fish that could be lost without any treatment interference, and determine whether or not treating the fish with a medicated feed is a cost-effective solution. If a pond is experiencing a change in temperature and it is soon to be outside the optimal ESC temperature

range, the disease may resolve itself without treatment. All of the current approved medications for the treatment of ESC require a veterinary feed directive (VFD) from a veterinarian in order to be purchased (Hawke et al. 1998).

Vaccinations against *E. ictaluri* are currently at the forefront of the fight against ESC outbreaks. In 1990, serological research preformed on *E. ictaluri* determined that it was a suitable candidate for vaccine development (Bertolini et al. 1990). One vaccine that was commercially available to the catfish industry was Aquavac-ESC® (Shoemaker et al. 2002). This vaccine was a live-strain bacterin that was applied via an immersion treatment of catfish fry at an age of seven days post hatch or greater. The average cost of vaccinating fry at seven to ten days post hatch was approximately $0.004 per fish, which equates to $4,000 per million fry (Bebak and Wagner 2012). This ESC vaccine was commercially available starting in January 1999, but is not believed to be commercially available currently (personal communication, Dr. Benjamin Beck). A survey conducted by Bebak, and Wagner (2012) showed that the majority of the farmers using the vaccine felt that they were getting a better survival rate then they were without vaccination.

Columnaris Disease

Columnaris disease is one of the oldest and most impactful diseases in the catfish industry (Durborow et al. 1998). *Flavobacterum columnare,* the causative agent of this disease, was first described by Davis (1922). The cells of *F. columnare* are 0.3 to 0.7 μm wide by 3-10 μm long, making them large enough to be easily viewed with a light microscope (Farmer 2002). Its name is derived from the column-like formation of the cell masses that can be seen on

infected tissue samples (Wakabayashi 1991). Alabama farmers reported losses of 726 metric tons due to this disease in 2018 (Hemstreet 2019).

The clinical signs of this disease are associated with the physical damage caused by the infection. Columnaris attacks the soft tissues such as the fins, gills, and skin where it slowly destroys and erodes away the tissue cells (Tomas-Jinu and Goodwin 2004; Declercq et al. 2013). Lesions on the skin of the fish usually appear as a dull discolored area with the outer edges covered with an opaque yellowish white colored film made up of swarms of *F. columnare* cells (Wakabayashi 1991). When the infection becomes more advanced the lesions can develop an open ulcer in the center exposing the muscle tissue (Durborow et al. 1998; Declercq et al. 2013). One easily distinguishable characteristic of columnaris is the development of one of these lesions around the base of the dorsal fin, known as saddleback condition (Durborow et al. 1998; Declercq et al. 2013). When the bacteria invade the mouth of the fish a yellowish-brown mucus will develop inside and around the edges of the mouth, which is commonly referred to as "cigar mouth" (Durborow et al. 1998). The oral ulcers that result cause the fish to stop feeding, most likely due to soreness, leading to death by starvation (Declercq et al. 2013). As *F. columnare* invades the gills of the fish, it will erode and destroy the delicate tissue that makes up the gill filaments leaving yellowish-brown patches of necrotic tissue (Durborow et al. 1998). This tissue destruction within the gills makes it difficult to exchange gasses properly which adds further stress on the fish.

A quick presumptive diagnosis of a *F. columnare* infection can be made by viewing a wet mount sample under a microscope (Noga 2010). The sample should be collected from the perimeter of a fresh lesion or from a gill clip. Under the microscope the long, thin rod-like bacterial cells (0.3-0.7 X 3-10 μm) can be easily viewed at 100 to 400 times magnification. The

bacteria are easily distinguished by their flexing and gliding motion and by the formation of column or haystack clusters (Durborow et al. 1998; Noga 2010).

In order to make a positive identification, sample swabs can be cultured on a growth media. *F. columnare* requires a low nutrient media with plenty of available moisture (Noga 2010). Selective growth media, such as Selective Cytophaga Agar and Hsu-Shotts, have been developed for the growth and isolation of this bacteria that take advantage of this bacteria's resistance to neomycin and polymyxin B (Durborow et al. 1998). These antibiotics will prevent the growth of most other aquatic bacteria while allowing *F. columnare* to grow freely. *F. columnare* usually forms a yellow colored rhizoid type colony on a solid growth media (Durborow et al. 1998; Declercq et al. 2013). The optimal growth temperature for this bacterium is between 25°C and 30°C and take between 24 and 48 hours of incubation time to form colonies (Decostere et al. 1998).

Columnaris is a seasonal disease with the majority of its outbreaks occurring between late spring and early fall (Noga 2010). Optimal water temperature range for an *F. columnare* outbreak is between 20°C (68°F) and 30°C (86°F), (Wakabayashi 1991). While this disease can occur under normal conditions, events such as exposure to poor water quality, crowding, and physical injury can weaken the immune system of the fish which can make it easier for a columnaris infection to develop (Durborow 1998). When fish develop a columnaris infection it is common to find an additional bacterial infection in the same fish. Other bacteria that are commonly found alongside *F. columnaris* are *Aeromonas* and *Edwardsiella* (Hawke and Thune 1992).

In order to prevent columnaris outbreaks, water quality, dissolved oxygen, and stocking density must be managed in a way that will prevent the fish from becoming overstressed.

Unfortunately, this is challenging in an intensive production system that focuses on maximizing the amount of fish that can be produced. Luckily there are a number of therapeutic treatment options for addressing a columnaris infection. Because most columnaris infections are external, chemical treatments can be added directly to the water to fight off the bacteria. Potassium permanganate ($KMnO_4$), copper sulfate ($CuSO_4$), and diquat (6,7-dihydrodipyrido [1,2-a: 2',1'-c] pyrazidinium dibromide) have been successfully used for treatment and prevention of columnaris infections (Davis 1922; Wakabayashi 1991; Durborow et al. 1998; Thomas-Jinu and Goodwin 2004). Another effective form of treatment for columnaris infections is through the use of antibiotics. The use of Terramycin and Romet have been found to be very effective, however, they are not currently labeled for use against *F. columnaris* (Thomas-Jinu and Goodwin 2004). The antibiotic Aquaflor® is currently the only available antibiotic drug approved by the Food and Drug Administration for use against columnaris disease for catfish (FDA 2019). Treatment with antibiotic feed requires a quick reaction time once the outbreak starts due to the tendency for fish that develop oral lesions to back off from feeding.

Since 2005 a live-strain vaccine, Aquavac-Col® had been commercially available to aid in the prevention of columnaris disease (Bebak 2012). This vaccine was applied as a bath treatment for eggs or young catfish. When administered to channel catfish fry at 10-48 days post hatch, this vaccine was found to provide a significant improvement to the percentage of mortalities due to *F. columnare* (Shoemaker et al. 2011). Currently, this vaccine is believed to not be commercially available (personal communication, Dr. Benjamin Beck). There are additional efforts to continue the development and commercialization of columnaris vaccines for the catfish industry.

Motile Aeromonas Septicemia

In recent years, severe outbreaks of motile *Aeromonas* septicemia (MAS) have been a major problem for west Alabama and east Mississippi catfish farms. In the 2018 survey conducted by the Alabama Fish Farming Center (AFFC), virulent *Aeromonas hydrophila* (vAh) was the leading cause of disease related mortalities on west Alabama Farms, causing 1588 metric tons of losses (Hemstreet 2019). Traditionally MAS is considered to be an opportunistic disease that takes advantage of the vulnerability of fish that are infected with other pathogens, such as *F. columnaris* (Xu et al. 2012). In 2009, an MAS outbreak caused severe losses on west Alabama catfish farms. Estimated losses due to this disease since 2009 have been approximately 1.36 million kg annually (Pridgeon and Klesius 2011). Unlike a traditional MAS outbreak the primary pathogen is *Aeromonas,* and the losses occur over a very short period of time. Bacteria isolated from the 2009 outbreak have since been identified as a new highly virulent form of *Aeromonas hydrophila* (vAh) (Pridgeon et al. 2013). Investigation into the origin of vAh suggests that the bacteria has an Asian origin that possibly made its way into the U.S. through the import of Asian carp, ornamental fish, or contaminated seafood products (Hossain et al. 2014).

Fish that become infected with vAh develop a number of external and internal symptoms that aid in its identification and diagnosis. Externally, fish will develop exophthalmia, petechial hemorrhaging, and preocular and ophthalmic necrosis (Baumgartner et al. 2017). At first glance vAh could easily be mistaken as ESC, due to the hemorrhagic characteristics that both of these infections often express. One distinct external sign that is often present with vAh that is absent in ESC infections is hemorrhaging in the eyes. Internally, vAh causes severe hemorrhaging to the

intestines and muscle tissues that can be easily recognizable during the necropsy process (Baumgartner et al. 2017).

The simplest method to identify this bacteria that is used by the AFFC diagnostics laboratory involves culturing samples collected from the brain, kidney, and liver of infected fish. The samples are streaked onto blood agar and myo-inositol plates and incubated for 24-48 hours at 30° C. The vAh bacteria has been found to have the ability to utilize myo-inositol as a sole carbon source (Hossain et al. 2013). This characteristic makes the use of myo-inositol growth media ideal for distinguishing this bacteria from other *Aeromonas* species. After the bacteria is successfully cultured it can then be identified using an API-20E test kit (BioMeriux, Durham, North Carolina).

Treatment of vAh infections is difficult due to the rapid rate at which this disease advances. Unfortunately, chemical treatments for this disease have thus far proven ineffective for vAh treatment. A study conducted by Bebak and Garcia (2012) found that the use of copper sulfate actually increased the mortality rate of fish infected with vAh. One treatment method that has been effective against these outbreaks is the use of medicated feeds. Currently Terramycin 200 is approved for use against bacterial hemorrhagic septicemia in catfish (FDA 2019). In order to utilize this medication to fight vAh, the farmer must get a veterinary feed directive (VFD) from his veterinarian. In addition to Terramycin, Aquaflor is also effective. However, Aquaflor can only be utilized if the diagnostician can also identify a columnaris infection on the fish since Aquaflor is not labeled for treatment of vAh.

Unlike the other diseases previously discussed, there are currently no commercially available vaccines for preventing vAh. However, there are a number of researchers that are

currently experimenting with vAh vaccination, including this study. Vaccination development is currently on the forefront of the fight to control vAh outbreaks in the catfish industry.

1.4 Use of IPRS to Answer Research Questions

The in-pond raceway system has not gained a large amount of attention in the catfish industry as a viable production system. However, it has been utilized quite effectively as a platform for conducting research. The biggest advantage to using an IPRS as a research platform is the ability to study a large number of sample groups in the same pond environment (replication). This is achieved through the construction of a desired number of cells that will each house its own population of fish. Traditionally, in order to conduct research with multiple treatment groups and a control group it would be necessary to have a large number of small ponds or conduct the study over a number of years with just a few ponds with experimental designs that were not robust. With this system, each cell can be monitored, fed, and harvested as if it were an independent pond and a large number of replicates can be used depending on how many cells the IPRS may possess. This allows the study to maximize its sample size and eliminate the need for a large pond based research facility.

An additional benefit that comes from having multiple replicates in one IPRS, is the removal of the environmental variable that would be present in a study using multiple ponds. In a multiple pond system, it would be next to impossible to ensure that all of the ponds have equal aquatic environments as the pond to pond variability would be enormous. With this particular system and experimental design, all of the cells in an IPRS are exposed to the same pond environment. This allows for an increased level of accuracy and confidence in the end results.

Using an IPRS enables researchers to carry out studies in active production environments without significantly impacting the efficiency of the pond. This allows researchers and farmers to work side by side to study and test many different aspects of catfish production in a real-world setting. This type of research could provide opportunities to study many aspects of catfish aquaculture that are difficult to replicate outside of a commercial production environment. The purpose of this study was to evaluate the effectiveness of a trivalent vaccine to combat the three most prevalent diseases in the U.S. catfish industry. To do this, controlled experiments were carried out in three in-pond raceways systems on a commercial farm in west Alabama in addition to controlled laboratory vaccine trials carried out at Auburn University.

1.5 Study Objectives

a) Determine the effectiveness of a trivalent vaccine, composed of killed *Edwardsiella ictaluri*, *Flavobacterum columnaris*, and virulent *Aeromonas hydrophila* cells, for reducing channel catfish mortalities due to ESC, Columnaris, and vAh disease outbreaks in an active commercial catfish production environment.

b) Test three different vaccine administration methods, intraperitoneal injection, immersion, and immersion with an adjuvant, in order to identify any possible differences in the amount of protection provided by each.

Chapter 2

Materials & Methods

2.1 Study Site

The location for this commercial trial was a catfish farm owned and operated by Williamson Cattle Company (WCC) in Hale county just south of Greensboro, Alabama. This location was selected based on the past history of vAh outbreaks in the three selected ponds, and the close proximity of the farm to the AFFC. The commercial ponds containing the IPRS were stocked with multiple batches of channel catfish with a number of harvests and stockings taking place throughout the study period. All of the study ponds were managed according to WCC's historical management protocols in order to maintain a normal production environment. The three ponds used were labeled by WCC as R18 (IPRS Unit 2), R19 (IPRS Unit 1), and R22 (IPRS Unit 3), the area of these ponds was 4.45 ha, 4.86 ha, and 5.06 ha, respectively (Figure 2.1). Catfish in the study ponds were fed a 32% protein feed (Alabama Catfish Feed Mill, Uniontown, Alabama).

Figure 2.1. Satellite image of the study site showing the locations of the three ponds containing IPRS. (A) Pond R18, location for IPRS Unit 2. (B) Pond R19, location for IPRS Unit 1. (C) Pond R22, location for IPRS Unit 3.

2.2 In-pond raceway systems

Three separate in-pond raceway systems (IPRS) were constructed and subsequently installed in the aforementioned ponds to carry out a vaccine trial (Figure 2.2). Each IPRS consisted of 4 separate quads containing 4 individual 7.26 m^3 raceway cells. All three IPRS were constructed of the same materials and dimensions. The walls and dividers of each quad consisted of 2 mm black polyethylene liner with 2.54 cm x 2.54 cm angle aluminum framing for support. At the entrance and exit of each raceway there was a barrier in place comprised of 2.54 cm x 1.27 cm PVC coated steel wire mesh. The floor of each cell was lined with a single sheet of 4.87 m x 1.22 m thermoclear lexan. A dock was constructed around each IPRS to provide flotation and facilitate daily management of the system. The docks were constructed with a framework of 5.08 cm x 15.24 cm boards and a decking surface of pressure treated 2.54 cm x 15.24 cm decking boards. The flotation for the system was provided by 113.6 L plastic drums that were secured to the underside of the docks.

Aeration and flow was provided by an air-lift water moving system which utilized the force of rising and expanding air bubbles to generate a water current and facilitate oxygen diffusion (Fullerton 2016). A 1.2-kW regenerative blower (Sweetwater, Pentair Aquatic Ecosystems, Inc., Apopka, Florida) was utilized to supply air flow for four individual cells (4 blowers per IPRS unit). Each blower was connected via 50.8 cm flex hose to a distributor constructed of 76.2 mm PVC pipe that had four individual 50.8 mm outlets and control valves. Each air outlet was connected to a 0.91 m x 0.91 m diffuser rack using 5.08 cm flex hose. The four diffuser racks were mounted inside of a specialized housing which directed the vertical movement of the water horizontally into the front of the raceway cells. The diffuser housing, which is referred to as a hood, was constructed from angle aluminum and 2 mm black

polyethylene sheeting. These hoods were attached to the front of each raceway quad so that only the top edge was protruding above the surface. The four control valves of the distributor mounted on top of each quad allowed for adjustment of the flow rate in each raceway cell.

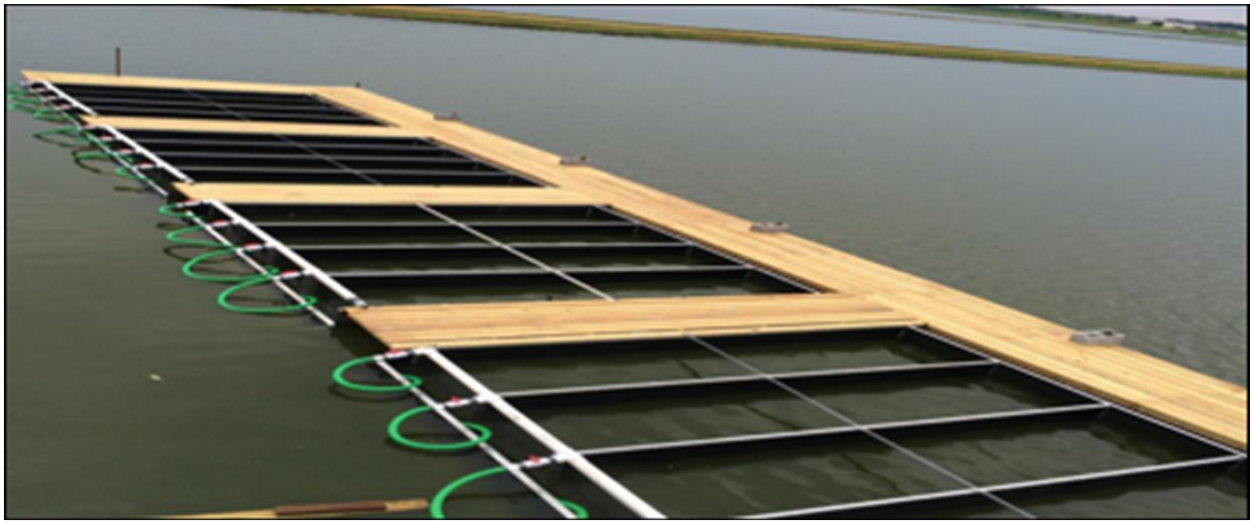

Figure 2.2. IPRS Unit 1 nearing the end of construction. Each section of four cells is referred to as a quad. There were four quads for a total of sixteen individual raceway cells in each of the IPRS units.

2.3 Vaccination

The trivalent vaccine used in this study was developed and produced by Kennebec River Biosciences in Richmond, Maine. This vaccine was designed to protect the fish against ESC, vAh, and columnaris. The diagnostics lab at the AFFC isolated all three of the bacteria strains used to create this vaccine from disease cases that occurred on the study farm. The trivalent vaccine utilized killed bacterins of each bacteria species to strengthen the immune response of the fish, hence allowing them to build immunity to the three diseases.

Vaccine administration was carried out at an outdoor holding facility on Auburn University's E. W. Shell Fisheries Research Station (Auburn, Alabama). The holding facility consisted of 12 flow-through raceway style holding vats (approximately 2600 L) with a flow rate of 20 L/min. The water for this system was pumped from the S1 pond which was located adjacent to the system. All of the catfish for each treatment were held in this system throughout the vaccination and recovery process.

Prior to vaccination the fish were separated into 4 equal groups, one for each treatment. The treatments for this study on channel catfish (*Ictalurus punctatus*) included a control channel catfish (CC), a manually injected trivalent vaccine into CC, a trivalent vaccine delivered through immersion using an adjuvant to increase adhesion in CC, and a trivalent vaccine delivered through immersion to CC. Treatments were each randomly assigned a letter of A, B, C, and D as well as a distinct identifying color (Table 2.1). Treatment A fish were anesthetized with MS-222 and then injected intraperitoneally with 100 µL of vaccine via a Kaycee injector with a .6x5 mm Unimed needle. The control group fish, treatment B, were not handled or mock vaccinated in any way. Vaccination in treatments C and D (the two immersion treatments) were administered

using the same procedures. Treatment C received the immersion vaccine without adjuvant and treatment D received the immersion vaccine with adjuvant. For immersion administration, fish were first crowded into an approximately 662 L portion of the raceway tank. After crowding, oxygen was supplied via a high-pressure tank in order to maintain a dissolved oxygen (DO) concentration above 5 mg/L throughout the vaccination process. Next, 3 L of vaccine was mixed in an 18 L bucket with some of the tank water and then the mix was added to the confined 662 L space occupied by the fish. Once the vaccine had been added, the fish were held in the confined space for an additional 15 min before releasing them back into the full space of the raceway tank. All of the vaccinated and control fish were held for an additional 14 days to allow for the fish to fully recover from the vaccination process and to develop an immune response. During this period, the fish were monitored daily for mortality and fed a daily ration of 36% protein feed (Cargill, Franklinton, Louisiana).

Table 2.1. Randomly selected letters and colors that identify each treatment.

Treatment	Letter	Color
Control	B	Red
Injection	A	Green
Immersion without adjuvant	C	Blue
Immersion with adjuvant	D	Black

2.4 Stocking

Stocking of the IPRS units occurred on June 5th, 6th, and 7th, 2017. All three IPRS were stocked with four replicates for each treatment, one replicate of each treatment per quad. The treatments within each quad were assigned to individual cells at random. Sample weights taken from all treatments prior to stocking indicated an average weight of 36.79 kg/1000. A hauling trailer with four separate tanks, one for each treatment, was utilized to transport the fish from the E. W. Shell Fisheries Research Station to the study site. Upon arrival, the fish were weighed out in baskets and stocked into their respective cells with a target stocking density of 1000 fish per cell or 36.8 kg per cell. Due to slight variations in total weight stocked per cell our actual stocking numbers ranged from 863 to 1127 fish per cell.

2.5 Growout

A commercial, 32% protein (6% lipid) feed (Alabama Catfish Feed Mill, Uniontown, Alabama) was offered to fish throughout the trial. Feeding was based on temperature and varied between once and twice per day depending on the time of year. A feed barrier screen (30.5 x 122 cm barrier of 6.35 mm mesh) was installed to prevent feed from escaping the raceway cells. Feed was administered using a 90% satiation feed approach where satiation feeding was determined every 7-10 days. All feeding was administered manually at the upstream end of each cell via 453.6 g scoops until active feeding stopped or the 90% satiation amount was reached.

Mortalities in the raceways were recorded and removed daily. Any moribund or fresh-dead catfish with little post-mortem change were collected, bagged, and labeled to be taken to the AFFC for examination and diagnostic evaluation. At the diagnostics lab, fish brought in from the IPRS were checked for external signs and parasites as well as a thorough internal

examination. Gill clips were collected from each fish for examination under a light microscope to identify any gill damage or parasites. Once the external examination was completed fish were necropsied in order to check the condition of vital organs and to collect bacterial samples. For identification of bacterial infections, swabs were collected from the brain, kidney, and liver of sick or dead fish and streaked onto blood agar and/or inositol media plates according to established techniques. Media plates were then incubated at 30°C for 24 hours to allow for bacterial growth. After incubation, any bacteria present would be streaked for isolation, if necessary, and subjected to either an API test or streaked on a TSA slant to identify the bacteria. The TSA slant media used allows for the identification of different strains of *Edwardsiella* bacteria based on the reactiveness the bacteria has with the media.

Dissolved oxygen and temperature were monitored daily in each raceway system using an YSI Pro20i (Yellow Springs, Ohio). Total ammonia nitrogen, nitrite nitrogen, and pH were measured two times a week. Total ammonia nitrogen and nitrite nitrogen were assessed according to Nessler's method (APHA et al. 1989) and Parsons et al. (1985), respectively. Measurement of pH was carried out using a pH meter (Pinpoint pH Monitor, Pentair Aquatic Ecosystems, Lake Apopka, Florida). Chloride, total alkalinity, and total hardness were monitored twice per month throughout the trial in each raceway system according to APHA et al. (1989). Pond water was sampled from the open pond directly in front of the intake of each IPRS system at a depth of approximately 0.3 m.

2.6 Harvest

Fish were harvested using specialized nets custom built to tightly fit the contours of the grow-out cells of the IPRS. These nets were constructed from 2.5 cm diameter galvanized steel

conduit pipe welded into a 122 x 183 cm rectangle, and 1.26 cm netting was then attached to the steel frame using size 12 green nylon seine twine. The netting was attached in a manner that allowed for a center depth of 61 cm, allowing for a large quantity of fish to be captured in a single haul. To capture the fish, the net was lowered into a cell vertically at the upstream end, ensuring that no fish were behind the net. Once the net reached the bottom of the raceway cell it was slowly moved towards the downstream end. This process was facilitated with the aid of a rope tied to the bottom edge of the net which allowed gentle crowding of the fish to the back end of the raceway cell. When the bottom edge of the net reached the bottom rear corner of the cell the net was pulled up to the surface, using the attached rope, to capture the fish. When the harvest net was pulled up to the surface it was rested on the rim of the raceway cell while the fish were dipped out in baskets. Multiple passes were made in each raceway cell with the harvest net to capture any leftover fish that were able to evade the net on the previous pass.

Harvest of the IPRS took place at two different points throughout the trial. The first harvest was conducted on November 6th and 7th of 2017. This initial harvest was intended as a midpoint data collection to provide an estimation of how the fish were performing and to serve as an insurance policy in the event of a catastrophic failure of the system or a major mortality event due to a non-target disease. After the fish were captured and weighed during harvest they were returned to their respective raceway cells. The second and final harvest occurred on September 10th and 11th of 2018. After the fish were captured during this harvest they were released into the open pond to be seined out at a later date by the farm owner. During the final harvest 200 control group fish and 200 treatment A (injected vaccine) fish were held in two of the emptied cells of IPRS unit 3 to be subjected to a disease challenge in October. These fish were monitored and cared for as previously described.

In the spring of 2018, at the request of the Alabama Catfish Producers association (who partially funded the study), four cells from each IPRS unit were harvested and released into the open pond to make room for a feed evaluation study. Cells 5-8 of unit 1, 1-4 of unit 2, and 4-8 of unit 3 were selected for removal due to their proximity to the service dock that leads onto each raceway. Data collection for these cells was done in the same manner as the final harvest that took place in the fall of 2018. Unfortunately, this data could not be used in comparison with the final harvest data due to the difference in harvest dates. This change in the study reduced the number of replicates for each treatment from 4 per system to 3 but was requested by the funding source for the project and the decision was out of our control.

During the final harvest, total weight and average individual weight were determined for fish in each raceway cell. In order to accurately determine average individual weight, approximately 80 kg of fish were counted from each raceway cell. Collected stocking, harvest, and feed data was subsequently used to determine survival, feed conversion ratio (FCR), specific growth rate and weight gain.

2.7 Disease Challenge

Fish from the partial harvest in spring 2018 from treatments A and B were moved to the USDA ARS laboratory and the E.W. Shell Research Station for a laboratory bacterial challenge. This was in part due to the lack of verified disease outbreaks of the three pathogens the fish were vaccinated against in the IPRS field study. Briefly, 200 fish from each of the treatments A and B harvested in spring 2018 were placed in hauling tanks and transferred to the USDA ARS laboratory in Auburn Alabama. The fish were placed into two, 750 L tanks supplied by dechlorinated municipal water. The fish were treated with 2 mg/L of potassium permanganate for 1 hour in the hauling tanks before transfer to the laboratory holding tanks. Once in the tanks, the

fish were fed Romet for 5 days according to the manufacturer's recommendation to prevent bacterial disease onset.

In May 2018, a series of bacterial challenges were initiated. In each of the trials, a cohabitation design was utilized. In the first trial, 60 fish from each treatment were placed into 6, 1300 L tanks (10 fish from each treatment; 20 fish per tank) with 200 L of dechlorinated municipal water supplemented with 1 g/L salt (Instant Ocean, Blacksburg, Virginia). Water in the tanks were circulated through 10-gallon pool filters containing zeolite using 1/3 hp submersible pumps to remove ammonia. Either the left or right pectoral fin was removed to mark the animals as either control or vaccinated. The wild-type *E. ictaluri* strain used to prepare the vaccine was grown in brain-heart infusion broth for 48 hrs at 30 ºC. Enough bacteria were cultured to give an estimated dose of 1x10^7 colony forming units (CFU's) per ml. Actual dose was calculated based on standard plate count methods of the culture taken just prior to challenge. Fish were given a two-day acclimation period. Every third day, approximately 25% of the water was replaced with fresh dechlorinated water. The challenge was followed for 10 d with mortalities picked up twice daily and fish (either moribund or fresh-dead) were necropsied to confirm *E. ictaluri* infection. The second *E. ictaluri* challenge was conducted as above, however, the estimated challenge dose was 1x10^6 CFU/ml.

For the vAh challenge trials, fish were transferred to the challenge room area as described above with same fish densities and monitoring procedures. However, upon placement in the challenge tanks, the adipose fin was also removed from all fish as part of the challenge procedures described by Shoemaker et al. (2018) for this pathogen. The wild-type strain of vAh used was grown for 24 hours at 30 ºC in tryptic soy broth. Once the fish were stocked in the tank the bacterial culture was added. The estimated challenge dose was 1x10^6 CFU/ml. The

challenges were monitored for 10 d. The experiment was performed twice using identical methods.

Two columnaris challenges were performed with fish harvested in September 2018. Because of space logistics in the challenge room the fish were harvested for data collection from the IPRS and remained in separate groups within the units until October 16, 2018. The fish were removed from the IPRS units and transferred to the E.W. Shell Research Station challenge facility. The fish were marked as above for control or vaccinated treatment groups and immediately stocked into the challenge tanks. Two separate densities of fish were used and were analyzed as two separate challenge studies. In three of the tanks, 10 fish per treatment were placed in 200 L of water, while in three additional tanks 25 fish per treatment were added to 300 L of water. Fish were given a two-day acclimation period and then challenged via immersion with the wild-type *F. columnare* bacteria. The bacteria were grown in modified Sheih broth and followed procedures outlined by LaFrentz et al. (2012). The target dose of the bacteria in the tanks was 1x10^6 CFU/ml. Fish monitoring and water quality procedures were as outlined above.

Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, North Carolina). Data from the trivalent field trial was analyzed individually by IPRS unit using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple range test to determine differences among treatment means according to Steel and Torrie (1980) and Brown et al. (2011). Since one quad of each raceway system was harvested early to allow for an unrelated study at the request of the funding agency, statistical analyses were carried out on 12 raceway cells per IPRS (3 replicates per treatment). For the challenges, mortality rates were log transformed and an ANOVA with a blocking effect by tank was used to compare mortality

between treatments. Time-to-death of 50% of the animals was determined by using daily cumulative mortality to determine regression LT50 followed by in SAS using PROC GLM.

Chapter 3

Results

3.1 Water Quality

Water quality in all three ponds was closely monitored throughout the study by analyzing pond water samples that were collected from the ponds directly in front of the IPRS intakes. All water quality values are provided in Table 3.1.

Nitrite nitrogen concentrations were relatively low in all three ponds during the majority of the study, averaging approximately 0.1 mg/L. The highest nitrite nitrogen occurred in IPRS Unit 1 (1.92 mg/L). The recorded nitrite nitrogen never reached a point in any of the ponds high enough to surpass the recommended 10:1 chloride to nitrite ratio. During the winter of 2017 and 2018, heavy rainfall caused a severe drop in chlorides for all three ponds which housed the IPRS units. Fortunately, the chlorides in each pond started out at a relatively high concentration. This allowed for the chloride concentrations to remain at adequate levels even after the heavy dilution from rainfall.

The total alkalinity of all three systems was found to be well above the recommended 50 mg/L throughout the study (Boyd and Tucker 2014). Hardness for IPRS Units 1 and 2 did fall slightly below the recommended 50 mg/L for a short period, with the low points being 46 mg/L and 48 mg/L, respectively (Boyd and Tucker 2014). The mean pH for all systems was found to be within the 6.5 – 9.0 recommended by Boyd and Tucker (2014) for optimal growth and was typical of catfish production ponds in the region.

Dissolved oxygen (DO) was measured within the IPRS rather than the open pond in order to get a better representation of the conditions that the study fish were being exposed. Due to the

use of air diffusers in the water mover apparatus, it is likely that the DO concentrations inside the IPRS would be slightly higher than that of the open pond. The mean DO concentrations for all three IPRS ranged from 7.31 mg/L to 7.91 mg/L during the study period. The lowest recorded DO concentrations recorded were 1.9, 1.8, and 1.4 mg/L for IPRS units 1-3, respectively. At no point during the study were fish observed to be struggling due to low DO.

Throughout the study the mean water temperature in all three ponds was 23 degrees Celsius. The maximum water temperature for all three ponds of 33.33 degrees Celsius occurred on 7/21/17. The lowest recorded temperatures for each system all occurred on 1/5/18, and were 4.44°C for IPRS Unit 1 and 3.33°C for IPRS Units 2 and 3.

Table 3.1. Water quality variables measured in pond water samples collected at the intake of each IPRS system.

System	**Water quality variable**	Mean ± SD	Min	Max
IPRS Unit 1	Nitrite nitrogen (mg/L)	0.14 ± 0.27	0.00	1.92
	Chloride (mg/L)	48.00 ± 11.55	28.00	68.00
	TAN (mg/L)	1.01 ± 0.79	0.21	3.75
	Total alkalinity (mg/L)	109.81 ± 12.78	76.00	136.00
	pH	7.86 ± 0.90	5.42	9.66
	Total hardness (mg/L)	95.83 ± 41.74	46.00	170.00
	Dissolved oxygen (mg/L)	7.31 ± 3.18	1.90	48.00
	Temperature (ºC)	23.24 ± 7.83	4.44	33.33
IPRS Unit 2	Nitrite nitrogen (mg/L)	0.09 ± 0.10	0.00	0.36
	Chloride (mg/L)	39.68 ± 17.89	20.00	104.00
	TAN (mg/L)	0.76 ± 0.34	0.19	2.24
	Total alkalinity (mg/L)	114.88 ± 12.99	94.00	140.00
	pH	7.99 ± 0.92	5.38	9.65
	Total hardness (mg/L)	87.33 ± 39.76	48.00	178.00
	Dissolved oxygen (mg/L)	7.62 ± 3.54	1.80	57.00
	Temperature (ºC)	23.15 ± 7.85	3.33	33.33
IPRS Unit 3	Nitrite nitrogen (mg/L)	0.11 ± 0.19	0.00	1.21
	Chloride (mg/L)	53.68 ± 18.72	24.00	118.00
	TAN (mg/L)	0.66 ± 0.27	0.04	1.52
	Total alkalinity (mg/L)	116.62 ± 10.65	100.00	146.00
	pH	8.32 ± 1.00	5.36	9.99
	Total hardness (mg/L)	94.43 ± 36.34	54.00	180.00
	Dissolved oxygen (mg/L)	7.91 ± 2.83	1.40	15.50
	Temperature (ºC)	23.00 ± 7.80	3.33	33.33

3.2 Production Parameters

The feed conversion ratio (FCR) is the ratio of weight of feed fed to weight gained by the animal and is calculated by the equation (mass of feed fed / [final animal mass – initial animal mass]). The FCRs for the study ranged from 2.51 to 3.03 between all IPRS. SAS analysis of the FCRs for each treatment revealed no significant differences among any of the IPRSs. Additionally, no significant differences were found for average individual weight, percent weight gain, and specific growth rate between treatments in any of the IPRS units. Survival (%) among all treatments ranged from 46.68% to 65.23% with no significant differences among the treatments in any of the systems ($P > 0.05$). All production data can be found in Table 3.2.

Table 3.2. Averaged individual weight, weight gain, specific growth rate, FCR (kg feed fed/ [final kg – initial kg]), and survival for each treatment within their respective IPRS systems.

System	Treatment	Average Weight (g)	Weight Gain (%)	Specific Growth Rate	Feed Conversion Ratio	Survival (%)
IPRS Unit 1	Injected Vaccine	858.75	2048.94	0.66	2.79	49.52
	Control	873.07	2084.46	0.67	2.81	51.71
	Immersion	933.59	2235.55	0.68	3.03	46.68
	Immersion + Adjuvant	893.65	2136.40	0.67	2.91	47.10
	P-value	0.414	0.418	0.441	0.734	0.745
IPRS Unit 2	Injected Vaccine	788.35	1872.78	0.65	2.45	64.10
	Control	751.05	1779.44	0.63	2.52	63.43
	Immersion	800.87	1904.10	0.63	2.51	62.35
	Immersion + Adjuvant	815.99	1941.94	0.65	2.52	61.76
	P-value	0.656	0.656	0.555	0.955	0.918
IPRS Unit 3	Injected Vaccine	793.85	1886.55	0.65	2.60	65.23
	Control	760.46	1802.99	0.64	2.74	59.33
	Immersion	715.90	1691.46	0.62	2.74	57.33
	Immersion + Adjuvant	746.74	1768.66	0.63	2.80	58.11
	P-value	0.448	0.449	0.456	0.647	0.306

3.3 Pond Data

The pond data for the three ponds housing IPRSs was provided by WCC from their farm records on a monthly basis from one month prior to the start of the study until the final harvest month. This data included average weight of feed fed per day, amount of feed fed since last harvest, total feed per hectare, number of fish harvested, and weight of pond inventory for each month. The pond data for the 2017 study months are shown in Table 3.3 and 2018 months can be found in Table 3.4.

Table 3.3. Monthly pond management data for each pond containing an IPRS in 2017. Provided by Williamson Cattle Company.

Pond Area	**System**	**Month**	**May**	**June**	**July**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**
R19 4.86 ha	**IPRS unit 1**	Average feed (kg/day)	418	254	471	295	306	454	215	57
		Feed since harvest (kg)	14095	4445	15536	4638	8289	15547	17849	18268
		Total feed/hectare (kg)	2903	916	3199	955	1707	3201	3675	3761
		Harvest (no. of fish)	15538	4900	17125	5113	9138	17138	19675	20138
		Inventory (kg)	20888	12717	23570	14742	15309	22680	10773	2835
R18 4.45 ha	**IPRS unit 2**	Average feed (kg/day)	293	343	549	643	249	227	176	23
		Feed since harvest (kg)	4457	16284	29620	50473	2393	7087	8720	8958
		Total feed/hectare (kg)	918	3354	6100	10393	493	1459	1796	1845
		Harvest (no. of fish)	4913	17950	32650	55638	2638	7813	9613	9875
		Inventory (kg)	14651	17172	27459	32129	12474	11340	8800	1134
R22 5.06 ha	**IPRS unit 3**	Average feed (kg/day)	162	112	209	282	221	448	204	11
		Feed since harvest (kg)	680	4037	8766	17202	23893	30788	33101	33418
		Total feed/hectare (kg)	129	768	1667	3269	4542	5852	6291	6352
		Harvest (no. of fish)	750	4450	9663	18963	26338	33938	36488	36838
		Inventory (kg)	8119	5589	10449	14080	11056	22396	10206	567

Table 3.4. Monthly pond management data for each pond containing an IPRS in 2018. Provided by Williamson Cattle Company.

Pond	System	Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.
R19 4.86 ha	**IPRS unit 1**	Average feed (kg/day)	238	91	131	195	324	405	510	567	308
		Feed since harvest (kg)	18813	91	1168	3674	10365	17531	14152	25832	2155
		Total feed/hectare (kg)	3874	19	241	757	2134	3610	2914	5319	444
		Harvest (no. of fish)	20738	100	1288	4050	11425	19325	15600	28475	2375
		Inventory (kg)	11907	4536	6532	9775	16193	20253	25605	28350	15399
R18 4.45	**IPRS unit 2**	Average feed (kg/day)	34	386	91	337	208	165	421	538	551
		Feed since harvest (kg)	9061	10830	11975	15059	1247	4581	17259	27805	46380
		Total feed/hectare (kg)	1866	2230	2466	3101	257	944	3554	5725	9551
		Harvest (no. of fish)	9988	11938	13200	16600	1375	5050	19025	30650	51125
		Inventory (kg)	1701	19278	4536	16874	10387	8255	21069	26898	27533
R22 5.06 ha	**IPRS unit 3**	Average feed (kg/day)	102	590	408	156	241	347	528	518	440
		Feed since harvest (kg)	33657	36446	39689	941	5931	11351	29608	41356	10727
		Total feed/hectare (kg)	6398	6928	7544	179	1128	2158	5628	7860	2039
		Harvest (no. of fish)	37100	40175	43750	1038	6538	12513	32638	45588	11825
		Inventory (kg)	5103	29484	20412	7802	12066	17327	26399	25923	22022

3.4 Disease

The diagnosed disease cases from the three IPRS units can be found in Table 3.5. During the study ESC was diagnosed in IPRS 1 and 2. This diagnosis was made a total of 11 different times, however, none of these cases produced caused a large number of observable mortalities. *F. columnare* was diagnosed in every treatment group for all three IPRSs during the study, with the exception of the injected vaccine treatment group for IPRS unit 3, for a total of 31 cases. Many of the *F. columnare* cases were also accompanied by secondary *Aeromonas* infections of non-target species such as *A. veronii* and the non-virulent strain of *A. hydrophila.*

Table 3.5. Total number of diagnosed disease cases for treatment cells in each IPRS system.

System	Cells	Treatment	ESC	F. col	vAh
IPRS Unit 1	1,11,13	Injected Vaccine	2	7	0
	3,9,14	Control	2	5	0
	4,12,16	Immersion	3	5	0
	2,10,15	Immersion + Adjuvant	2	4	0
IPRS Unit 2	6,9,13	Injected Vaccine	1	2	0
	8,10,16	Control	0	2	0
	4,11,14	Immersion	1	1	0
	7,12,25	Immersion + Adjuvant	0	1	0
IPRS Unit 3	2,12,16	Injected Vaccine	0	0	0
	4,11,15	Control	0	1	0
	1,10,13	Immersion	0	2	0
	3,9,14	Immersion + Adjuvant	0	1	0

3.5 Laboratory Disease Challenge

Following the partial and final harvests, fish from the injected vaccine and control groups were transported to the USDA ARS laboratory and the E.W. Shell Research Station in Auburn, Alabama to undergo disease challenges. No statistically significant differences in mortality rate between treatments was observed in the challenge trials for ESC and columnaris. In Trial 1 (vAh challenge), a statistically significant difference between the vaccinated and control groups, which had mortality rates of 50 ± 16 and 95 ± 5.8, respectively was observed ($P < 0.0364$). Trial 1 (ESC challenge) suffered a near 100% mortality in both treatments yielding an insignificant result in both mortality rate and time-to-death (TTD). ESC trial 2 also resulted in nearly 100% mortality for both treatments. Although, the mortality rates in ESC trial 2 were insignificant ($P < 0.2555$), there was a significance difference between TTD ($P < 0.0438$). The complete results of all challenge trials are provided in Table 3.6.

Table 3.6. Bacterial dose of culture, fish mortality percentage, and time-to-death for 50% of fish (TTD50) in days for fish challenged with virulent *Aeromonas hydrophila* (Aeromonas), *Edwardsville ictaluri* (ESC), and *Flavobacterium columnare* (columnaris) under laboratory conditions. Tank blocking effect p-value is also given for each trial. P-Value < 0.05 were considered significant and designated by an *.

Treatment	Aeromonas		ESC		Columnaris	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Challenge Dose	1.6 x 10^6	4.1 x 10^6	9.2 x 10^6	1.3 x 10^6	9.2 x 10^5	9.2 x 10^5
Tank p-value	0.8095	0.7516	0.2532	0.3735	0.0223*	0.9800
Control	95 ± 5.8	78 ± 20	97 ± 5.0	98 ± 5.0	60 ± 57	93 ± 12
Vaccinated	50 ± 16	88 ± 9.6	100	88 ± 15	66 ± 8.6	90 ± 8.6
Mortality p-value	0.0364*	0.8614	---	0.2555	0.5286	0.8020
Tank p-value	0.4934	0.588	0.0029*	0.0111*	0.1217	0.1058
Control TTD50	1.8 ± 0.31	4.0 ± 1.9	2.7 ± 2.0	3.5 ± 1.3	2.3 ± 1.1	6.8 ± 1.5
Vaccinated TTD50	9.1 ± 6.6	2.4 ± 0.79	3.0 ± 2.3	4.5 ± 1.9	2.6 ± 1.3	6.9 ± 1.6
TTD50 p-value	0.1168	0.2484	0.2834	0.0438*	0.3179	0.8492

Chapter 4

Discussion

The primary objective of this study was to assess the efficacy of an autogenous trivalent vaccine using field trial in in-pond raceway systems housed in commercial catfish production ponds. Due to the unpredictable nature of disease events in production ponds, the use of laboratory-based challenges at the end of the trial provided a valuable backup test. This enabled the confirmation of the IPRS results as well as the ability to test the vaccine against target diseases that were not present during the field trial.

Given the past prolific history of disease outbreaks in the ponds used in the field trial, the lack of sizeable incidence of disease, particularly vAh is quite surprising. The only disease that infected both the ponds and the raceway systems was columnaris. The columnaris present in the IPRS cells persisted as minor chronic infections, causing low numbers of mortalities over long periods. These infections occurred intermittently throughout the study in all three of the IPRS and over time, the slow accumulation of small numbers of mortalities were enough to have a substantial impact on the survival percentage of each treatment.

During the study, columnaris accounted for 31 of the 42 diagnosed cases. This disease was diagnosed in all treatments within each IPRS, with the exception of the injected vaccine treatment in IPRS unit 3. Roy et al. (2013, 2019), in an earlier study evaluating the incidence of various disease cases involving IPRS in west Alabama 2008-2013, also stated that columnaris was the disease that was most reported by commercial producers using this production system.

Additional studies with IPRS in research settings have also reported high incidences of columnaris (Brune et al. 2004; Fullerton 2016; Roy et al. 2019). Due to the high reported incidences of columnaris in this particular production system it was, in fact, a logical system in which to test a vaccine for columnaris. One potential explanation for the columnaris incidences in IPRS systems is the stressful nature of these systems. Stress factors such as crowding, mechanical injury, and rough handling can often lead to columnaris infections (Durborow et al. 1998). Since the occurrence of columnaris was high throughout the study, albeit expressed as smaller incidents of mortality over a long period of time, the vaccine did not prove effective for protection against this disease.

Development of columnaris vaccines has been an ongoing process in recent years and is slowly being adopted into some segments of the catfish industry. A survey conducted by Bebak and Wagner (2012), found that 16% of the area used for food fish production utilized fish vaccinated against columnaris. Research conducted by Shoemaker et al. (2011), when evaluating a commercially available live-attenuated columnaris vaccine using channel catfish fry, reported that the vaccine provided significant protection in laboratory challenges. Further adoption of vaccinated fish in the food fish industry could aid in reducing losses and increasing productivity in the future.

Recent columnaris research has revealed one potential shortfall of the currently available vaccine. Columnaris can be divided into three distinct genetic groups, genomovars. Of these groups, genomovar II is the most virulent to channel catfish. The Aquavac-Col® vaccine was created from rifampicin-resistant mutants from the genomovar I group. A study conducted by Olivares-Fuster and Arias (2011) identified and described rifampicin-resistant mutants of the more virulent genomovar II strain. A recent study by Mohammed et al. (2013) determined that

the use of these genomovar II mutants for vaccination provided better protection against genomovar II strains while also providing protection against genomovar I strains. The development of more effective columnaris vaccines using these more virulent genetic groups could provide a better method for reducing industry losses due to this disease.

The presence of columnaris throughout all treatments, coupled with the lack of significant difference in survival between treatments, indicates that the trivalent vaccine used in this study was not effective in protecting the fish against columnaris infections. In addition to the findings presented by the IPRS component of the study the results of the laboratory disease challenge also found no significant reduction in mortality rate between vaccinated and control treatments. These results differ from those of Shoemaker et al. (2011); however, that study utilized a live-attenuated vaccine as opposed to the killed bacterins used in the trivalent vaccine trials reported in this study.

ESC was the only other bacterial disease that was found within the study systems. This disease was diagnosed 11 times throughout the study, nine of which occurred in IPRS unit 1. The cases in unit 1 were distributed across all four treatment groups. The infection in IPRS unit 2 was only found in the Injected and Immersion without adjuvant vaccine treatments. None of the ESC cases found resulted in any sizeable numbers of mortalities. In the study carried out by Roy et al. (2013, 2019), ESC was also second behind columnaris in the number of diagnostic cases reported by commercial farmers utilizing IPRS in west Alabama.

IPRS unit 1 is the only system in the study to have documented ESC cases diagnosed in the control group. However, it was also diagnosed in all of the vaccinated treatments in that system. These ESC infections did not appear to contribute to any serious mortalities within any of the systems during the trial period. The lack of statistically significant differences ($P > 0.05$)

between the treatments of this system suggests that there was no observed beneficial effect of the vaccine on survival when exposed to ESC. This result contrasts with the findings of Thune et al. (1994), which reported that administration of a killed *E. ictaluri* vaccine orally and via immersion provided a significant improvement in relative percent survival of channel catfish fingerlings during laboratory challenges. Like the result of the IPRS portion of the study, laboratory challenge results also indicated that the vaccine was not effective in providing fish protection against ESC. However, due to the excessive dosages used in the challenge, this result may be inaccurate. The longer TTD of the vaccinated treatment in the lower dosage challenge suggests that the vaccine had some effect against ESC. Additional testing using proper challenge dosages are needed in order to more accurately determine the true effectiveness of this vaccine against ESC.

Contrary to our findings, many studies have found vaccinations to have a positive effect in terms of increasing survival during exposure to ESC. Research conducted by Wise et al. (2015) and Shoemaker et al. (1999), found that vaccinating channel catfish with live-attenuated ESC vaccines provided significant improvements in survival. Bebak and Wagner (2012) revealed that 19% of the food-size production within the US catfish industry was utilizing ESC vaccinated fingerlings at the time of the survey; and of that 19%, 42% of those farmers felt that the vaccine was effective. Unlike the aforementioned studies, the trivalent vaccine used in this study was composed of killed bacterins. Similar to the findings of this study, Thune et al. (1997), found that no protection was provided by vaccinations using formalin killed *E. ictaluri*. These results indicate that future work with ESC vaccinations would likely be more successful using live strain vaccines.

As vAh never occurred in any of the IPRS study systems in the west Alabama field trial, no indication of the vaccine's efficacy (either positive or negative) can be determined from that portion of the study. While this is unfortunate, carrying out field trials with vaccines in commercial settings is a valid step towards commercial acceptance of vaccines and for the other two pathogens examined, the approach proved viable.

Despite the lack of field exposure to vAh, the results of the laboratory challenge do provide some insight into the viability of the vaccine for this particular pathogen. The two laboratory challenge trials conducted with vAh yielded conflicting results. Trial 1 found a significantly lower mortality rate for the vaccinated group ($P = 0.0364$), while trial 2 did not ($P > 0.05$). One key difference between these two trials was the condition of the vaccinated fish. The vaccinated treatment group used in trial 2 had become aggressive, possibly due to low density, prior to the challenge leading to territorial fighting. This resulted in the fish having scrapes and sores present on their skin from biting and "finning" each other. These surface wounds likely aided in the induction of vAh in these fish but further confound interpretation of the results of the challenge. The study conducted by Zhang et al. (2016) on experimental induction of vAh determined that the best method for causing a controlled infection was to clip the adipose fin of the fish to create a portal of entry for the bacteria. The positive results observed in the first laboratory challenge (trial 1) are nevertheless promising and merit further investigation.

Although this study did not provide a definitive answer in regard to the vaccine's efficacy of reducing vAh losses, other studies have had better success. Shoemaker et al. (2018), experimented with a similar formalin-killed vAh vaccine and were successful in increasing survival of 4-7 g hybrid catfish fingerlings via immersion. Another vaccination method that has proven successful in laboratory settings is the use of recombinant proteins associated with

virulence factors of vAh, rather than the bacteria itself. In a study conducted by Zhang et al. (2015), they vaccinated channel catfish fingerlings with aerolysin and haemolysin proteins via intraperitoneal injection, and found a significant improvement in survival when challenged with vAh. One major difference between this vaccine study and the work done in other studies is that the vaccinated fish were not challenged until the fish reached harvest size. In most other studies the fish are challenged within a few weeks or months of the vaccination. Further work in the development of a suitable vaccine for vAh is very important for the future of the catfish industry. This disease has repeatedly been responsible for the largest portion of disease related losses on Alabama farms since it first appeared in 2009 (Hemstreet 2019).

Development of a vaccine is a difficult trial and error process. In this study, we observed that an experimental trivalent vaccine was not effective against columnaris and ESC. Our attempt at testing this vaccine against natural disease occurrences was only partly successful, as vAh never manifested itself and only a minor instance of ESC occurred. A suitable vaccine administration method could not be determined due to the lack of efficacy of the vaccine.

Chapter 5

Conclusion

The goal of this study was to evaluate the efficacy of a trivalent vaccine in channel catfish against virulent *Aeromonas hydrophila*, *Flavobacterium columnare*, and *Edwardsiella ictaluri*, in active commercial catfish ponds. As previously discussed, there is a great need for a viable method of reducing the losses that the U.S. industry suffers due to these three diseases. Unfortunately, the results found during this study did not reveal the trivalent vaccine to be successful in combating columnaris losses. Additionally, the combined results of the IPRS study and the laboratory challenges found that the trivalent vaccine provided no significant improvement in mortality related to ESC.

Because of the absence of vAh in the raceway systems and the inconclusive results presented by the laboratory challenge, no determination of efficacy against this disease can be made based upon the results of this study. In order to make an accurate determination of this vaccine's ability to fight vAh infections further testing is required. The outcome of this study indicates that the best method to efficiently test this vaccine in the future would be to eliminate the natural occurrence requirement. Testing should be conducted via periodic laboratory challenge of healthy vaccinated and control groups. While the use of multiple IPRS in three different ponds with history of disease outbreaks was an innovative research approach and environment in which to field validate a vaccine, there were no guarantees that an actual infection would occur.

The lack of observable differences among any of the treatments in the IPRS study indicates that the problem lies either with the vaccine itself or the lack of diseases to test the vaccine treatments adequately in the commercial pond settings or with the vaccine delivery methods. Because administration method could still play an important role in the efficacy of this vaccine it would be wise to incorporate comparisons of various methods into future studies. The practicality of the administration method needed to effectively apply a vaccine to catfish could have an important influence on the adoption of a successful vaccine into the catfish industry. Because of the large volume of fish that would need to be vaccinated in a commercial setting, and the added expense of doing so, it is highly important that future vaccine development also include the development of cost efficient administration methods.

Literature Cited

APHA (American Public Health Association), American Water Works Association and Water Pollution Control Association. 1989. Standard Methods for the Examination of Water and Waste Water, 17th edition, APHA, Washington, D.C.

Baumgartner, W. A., L. Ford, and L. Hanson. 2017. Lesions caused by virulent *Aeromonas hydrophila* in farmed catfish (*Ictalurus punctatus* and *I. punctatus* x *I. furcatus*) in Mississippi. Journal of Veterinary Diagnostic Investigation, 29: 747-751.

Bebak, J., and J. C. Garcia. 2012. Effect of Copper Sulfate on *Aeromonas hydrophila* Infection in Channel Catfish Fingerlings. North American Journal of Aquaculture, 74: 494-498.

Bebak, J., and B. Wagner. 2012. Use of Vaccination Against Enteric Septicemia of Catfish and Columnaris Disease by the U.S. Catfish Industry. Journal of Aquatic Animal Health, 24 (1), 30-36.

Bertolini, J. M., R. C. Cipriano, S. W. Pyle, and J. J. A. McLaughlin. 1990. Serological Investigation of the Fish Pathogen *Edwardsiella ictaluri*, Cause of Enteric Septicemia of Catfish. Journal of Wildlife Diseases, 26 (2): 256-252.

Bott, L. B., L. A. Roy, T. R. Hanson, J. A. Chappell, and G. N. Whitis. 2015. Research verification of production practices at an intensively aerated hybrid catfish operation in west Alabama. North American Journal of Aquaculture, 77(4): 460-470.

Boyd, C. E., and C. S. Tucker. 2014. Handbook for Aquaculture Water Quality. Craftmaster Printers, Auburn, Alabama.

Brown, T. B., J. A. Chappell, and C. E. Boyd. 2011. A Commercial-scale, In-pond Raceway System for Ictalurid Catfish Production. Aquaculture Engineering, 44: 72-79.

Brune, D. E., G. Schwartz, A. G. Eversole, J. A. Collier, and T. E. Schwedler. 2004. Partitioned Aquaculture Systems. Southern Regional Aquaculture Center, Publication No. 4500.

Davis, H. S. 1922. A new bacterial disease of fresh-water fishes. United States Bureau of Fisheries Bulletin, 38: 261-280.

Declercq, A. M., F. Haesebrouck, W. Van den Brorck, P. Bossier, A. Decostere. 2013. Columnaris disease in fish: a review with emphasis on bacterium-host interactions. Veterinary Research, 44:27, https:// doi.org/10.1186/1297-9716-44-27, accessed March 6, 2019.

Decostere, A., F. Haesebrouck, and L. A. Devriese. 1998. Characterization of four *Flavobacterium columnare* (*Flexibacter columnaris*) strains isolated from tropical fish. Veterinary Microbiology, 62: 35-45.

Durborow, R. M., R. L. Thune, J. P. Hawke, and A. C. Camus. 1998. Columnaris Disease: A Bacterial Infection Caused by *Flavobacterium columnare.* Southern Regional Aquaculture Center, Publication No. 479.

Farmer, B. 2002. Improved Methods for the Isolation and Characterization of *Flavobacterium columnare.* Master's Thesis. Department of Fisheries and Allied Aquaculture, Auburn University, Auburn, Alabama, USA.

FDA, United States Food and Drug Administration, 2018. Approved Aquaculture Drugs, https://www.fda.gov/animal-veterinary/aquaculture/approved-aquaculture-drugs, accessed May 9, 2019.

Francis-Floyd, R., M. H. Beleau, P. R. Waterstrat, and P. R. Browser. 1987. Effect of water temperature on the clinical outcome of infection with *Edwardsiella ictaluri* in channel catfish. Journal of the American Veterinary Medical Association, 191: 1413-1416.

Fullerton, G. C. 2016. Economic Viability of Floating In-pond Raceway Systems for Commercial Hybrid Catfish Production. Master's Thesis, Department of Fisheries and Allied Aquaculture, Auburn University, Auburn, Alabama, USA.

Gosh, K. 2018. Factors Affecting the Frequency of Oversized and Undersized Channel Catfish, *Ictalurus punctatus*, x Blue Catfish, *I. furcatus*, Hybrid Catfish at Food Fish Harvest and Their Economic Impact. Dissertation, Department of Fisheries and Allied Aquaculture, Auburn University, Auburn, Alabama, USA.

Hanson, T. R., and D. Sites. 2015. 2014 U.S. Catfish Database. Department of Agriculture Economics, Mississippi State University. https://www.agecon.msstate.edu/whatwedo/budgets/docs/catfish2014.pdf.

Hanson, T., L. Roy, and B. Hemstreet. 2018. 2016 Alabama Farm-Raised Catfish Industry Highlights. Alabama Cooperative Extension System. https://www.aces.edu/blog/category/fish-water/aquaculture/

Hanson, T.R. 2019. U.S. Catfish Processing Report. The Catfish Journal, Jackson, MS, Jan/Feb Vol. 33 Number 1.

Hawke, J. P., and R. L. Thune. 1992. Systemic isolation and antimicrobial susceptibility of *Cytofhaga columnaris* from commercially reared channel catfish. Journal of Aquatic Animal Health, 4: 109-113.

Hawke, J. P., A. C. McWhorter, A. G. Steigerwalt, and D. J. Brenner. 1981. *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicemia of catfish. International Journal of Systematic Bacteriology, 31(4): 396-400.

Hawke, J. P., R. M. Durborow, R. L. Thune, and A. C. Camus. 1998. ESC – Enteric Septicemia of Catfish. Southern Regional Aquaculture Center, Publication No. 477.

Hemstreet, B. 2019. Disease Survey Report: 2018. Fish Farming News, 2019(1): 5-7.

Hossain, M. J., G. C. Waldbieser, D. Sun, N. K. Capps, W. B. Hemstreet, K. Carlisle, M. J. Griffin, L. Khoo, A. E. Goodwin, T. S. Sonstegard, S. Schroder, K. Hayden, J. C. Newton, J. S. Terhune, and M. R. Liles. 2013. Implication of Lateral Genetic Transfer in the Emergence of Aeromonas hydrophila Isolates of Epidemic Outbreaks in Channel Catfish. PLoS ONE [online serial] 8:e80943.

Hossain, M. J., D. Sun, D. J. McGarey, S. Wrenn, L. M. Alexander, M. E. Martino, Y. Xing, J. S. Terhune, and M. R. Liles. 2014. An Asian Origin of Virulent *Aeromonas Hydrophila* Responsible for Disease Epidemics in United States Farm-Raised Catfish. mBio5(3):e00848-14.doi:10.1128/mBio.00848-14.

Klesius, P. 1994. Transmission of *Edwardsiella ictaluri* from Infected, Dead to Noninfected Channel Catfish. Journal of Aquatic Animal Health, 6: 180-182.

Kumar, G. 2016. Adoption of alternate catfish technologies. 2016 Annual report by Delta Regional Extension Center, 70-71.

Kumar, G., C. Engle, and C. Tucker. 2018. Factors Driving Aquaculture Technology Adoption. Journal of the World Aquaculture Society, 49(3): 447-476.

Kumar, G., C. Engle, and C. Tucker. 2016. Cost and risk of catfish split-pond systems. Journal of the World Aquaculture Society, 47(3): 327-340.

LaFrentz, B. R., S. E. Lapatra, C. A. Shoemaker, and P. H. Klesius. 2012. Reproducible challenge model to investigate the virulence of *Flavobacterium columnare* gemnovars in rainbow trout *Oncorhynchus mykiss.* Diseases of Aquatic Organisms, 101: 115-122

Mohammed, H., O. Olivares-Fuster, S. LaFrentz, C. R. Arias. 2013. New Attenuated vaccine against columnaris disease in fish: Choosing the right parental strain is critical for vaccine efficacy. Vaccine, 31: 5276-5280.

NASS (National Agricultural Statistics Service). 2018. Catfish Production. United States Department of Agriculture, Washington, D.C.

Noga, E. J. 2010. Fish Disease: Diagnosis and Treatment. second edition, Wiley-Blackwell, Ames, Iowa, USA.

Olivares-Fuster, O., and C. R. Arias. 2011. Development and characterization of rifampicin-resistant mutants from high virulent strains of *Flavobacterium columnare.* Journal of Fish Diseases, 34: 385-394.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1985. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, New York, USA.

Pridgeon, J. W., and P. H. Klesius. 2011. Molecular identification and virulence of the three *Aeromonas hydrophila* isolates cultured from infected channel catfish during a disease outbreak in west Alabama (USA) in 2009. Diseases of Aquatic Organisms, 94: 249-253.

Pridgeon, J. W., P. H. Klesius, L. Song, D. Zhang, K. Kojima, and J. A. Mobley. 2013. Identification, Virulence, and Mass Spectrometry of Toxic ECP Fractions of West Alabama Isolates of *Aeromonas hydrophila* obtained from a 2010 Disease Outbreak. Veterinary Microbiology, 164: 336-343.

Roy, L., W. Hemstreet, and T. Brown. 2013. Catfish disease cases in in-pond raceway systems in Alabama: 2008-2013. The Catfish Journal. 27(4): 17,21.

Roy, L. A., T. R. Hanson, L. B. Bott, J. A. Chappell. 2019. Production and economic comparison of single versus multiple harvests of hybrid catfish in a commercial in-pond raceway

system in west Alabama targeting two market outlets. Journal of the Southern Association of Fish and Wildlife Agencies. 6: 58-66.

Shoemaker, C. A., P. H. Klesius, and J. M. Bricker. 1999. Efficacy of a modified live *Edwardsiella ictaluri* vaccine in channel catfish as young as seven days post hatch. Aquaculture, 176: 189-193.

Shoemaker, C. A., P. H. Klesius, and J. J. Evans. 2002. In ovo methods for utilizing the modified live Edwardsiella ictaluri vaccine against enteric septicemia in channel catfish. Aquaculture, 203:221-227.

Shoemaker, C. A., P. H. Klesius, J. D. Drennan, and J. J. Evans. 2011. Efficacy of a modified live *Flavobacterium columnare* vaccine in fish. Fish and Shellfish Immunology, 30: 304-308.

Shoemaker, C. A., H. H. Mohammed. T. J. Bader, E, Peatman, and B. H. Beck. 2018. Immersion vaccination with an inactivated virulent *Aeromonas hydrophila* bacterin protects Hybrid Catfish (*Ictalurus punctatus* x *Ictalurus furcatus*) from motile *Aeromonas* septicemia. Fish and Shellfish Immunology, 82: 239-242.

Shotts, E. B., V. S. Blazer, and W. D. Waltman. 1986. Pathogenesis of Experimental *Edwardsiella ictaluri* Infections in Channel Catfish (*Ictalurus punctatus*). Canadian Journal of Fisheries and Aquatic Sciences, 43: 36-42.

Steeby, J., and J. Avery. 2002. Construction of Levee Ponds for Commercial Catfish Production. Southern Regional Aquaculture Center, Publication No. 350.

Steeby, J. A., J. A. Hargreaves, C. S. Tucker, and S. Kingsbury. 2003. Accumulation of sediment in commercial channel catfish ponds. Aquacultural Engineering, 30:115-126.

Steel, R. G. D., and J. H. Torrie. 1980. Principals and Procedures of Statistics: a biometrical approach. McGraw-Hill, New York, NY.

Thomas –Jinu, S., and A. E. Goodwin. 2004. Acute columnaris infection in channel catfish, *Ictalurus punctatus* (Rafinesque): efficacy of practical treatments for warmwater aquaculture ponds. Journal of Fish Diseases, 27: 23-28.

Thune, R. L., J. P. Hawke, and M. C. Johnson. 1994. Studies on Vaccination of Channel Catfish, *Ictalurus punctatus,* Against *Edwardsiella ictaluri.* Journal of Applied Aquaculture, 3:1-2: 11-24.

Thune, R. L., L. A. Collins, and M. A. Pena. 1997. A comparison of immersion, immersion/ oral combination, and injection vaccination of channel catfish *Ictalurus punctatus* against *Edwardsiella ictaluri.* Journal of the World Aquaculture Society, 28: 193-201.

Tidwell, J. H. 2012. Aquaculture Production Systems. Retrieved from https://ebookeentrial-proquest-com.spot.lib.auburn.edu.

Tucker, C. S., D. E. Brune, and E. L. Torrans. 2014. Partitioned pond aquaculture systems. World Aquaculture Magazine, 45(2): 9-17.

USDA (United States Department of Agriculture Animal and Plant Health Inspection Service). 2019. Aphis.usda.gov, accessed June 7, 2019. activities/ct_federal_permit_process.

Wakabayashi, H. 1991. Effect of Environmental conditions on the infectivity of *Flexibacter columnaris* to fish. Journal of Fish Diseases, 14: 279-290.

Wise, D. J., T. E. Greenway, T. S. Byars, M. J. Griffin, and L. H. Khoo. 2015. Oral Vaccination of Channel Catfish against Enteric Septicemia of Catfish Using a Live Attenuated *Edwardsiella ictaluri* Isolate. Journal of Aquatic Animal Health, 27: 135-143.

Xu, D., J. W. Pridgeon, P. H. Klesius, and C. A. Shoemaker. 2012. Parasitism by protozoan *Ichthyophthirius multifiliis* enhanced invasion of *Aeromonas hydrophila* in tissues of channel catfish. Veterinary Parasitology, 184: 101-107.

Zhang, D., D. Xu, and D. Shoemaker. 2015. Immunization with recombinant aerolysin and haemolysin protected channel catfish against virulent *Aeromonas hydrophila*. Aquaculture Research, 8(3): 875-882.

Zhang, D., D. Xu, and D. Shoemaker. 2016. Experimental induction of motile *Aeromonas* septicemia in channel catfish (*Ictalurus punctatus)* by waterborne challenge with virulent *Aeromonas hydrophila.* Aquaculture Reports, 3: 18-23.