Evaluation of the Live-Attenuated Vaccine AquaVac-COL® on Hybrid Channel x Blue Catfish Fingerlings in Earthen Ponds

by

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Abstract

The evaluation of the use of AquaVac- COL® was conducted with hybrid catfish, which are a cross between female channel catfish (*Ictalurus punctatus*) and a male blue catfish (*Ictalurus furcatus*), to determine production rates in field conditions. Fry were stocked at rates of 25,000 fry/ha in 0.1 ha earthern ponds and cultured for 172 d in one of two treatments. The treatments (n=4) were 10-day post-hatch fish sham-vaccinated and 10-day post-hatch fish vaccinated with AquaVac- COL® and then stocked in the ponds. Fingerling mean standing crop averaged 2,802 ± 268 kg/ha for sham- vaccinated and 2,676 ± 424 kg/ha for vaccinated treatments. No significant differences occurred between treatments. Mean individual fish weight at harvest averaged 16.8 ± 0.10 g for sham- vaccinated treatments and 21.1 ± 1.8 g for vaccinated treatments. No significant difference for mean individual fish weight was found between treatments. Feed conversion ratio (FCR) averaged 2.57 ± 0.18 for the sham- vaccinated treatment and 1.92 ± 0.38 for vaccinated treatments and were not significantly different between treatments. Mean percent survival for the sham- vaccinated treatment averaged 67.2% ± 7.2 while the vaccine treatment averaged 54.2% ± 12. Observed mortality for the sham- vaccinated treatments averaged 4.14% ± 1.2 while the vaccine treatment averaged 4.3% ± 4.0. The study determined no significant differences when considering the efficacy of AquaVac- COL® in respect to production parameters between ponds or between treatments.

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INTRODUCTION

Catfish production is the largest aquaculture industry in the United States. In 2009 processed farmed- raised catfish amounted to 166 million pounds (NASS 2010). The channel catfish, *Ictalurus punctatus,* is the primary species that the aquaculture industry relies upon in the United States (Goldburg et al. 2001). The most common method for the culture of channel catfish is earthen ponds at high density (Mingkang 2005).

Disease is one of the most reported reasons as to loss of standing crop during the culture period. Two primary diseases affect channel catfish, enteric septicemia of catfish (ESC) and columnaris (USDA 2007a). Columnaris is a bacterium that is found in waters all over the world and is attributed to causing loss in millions of dollars per year. As the channel catfish industry is a large economic entity, reducing the disease outbreak of columnaris is of primary concern. Management practices have been used to reduce stress as the bacterium infects the organism in this state. Other methods for reducing columnaris outbreaks include treatments such as salt, potassium permanganate, copper sulfate, and hydrogen peroxide (Wakabayashi 1991).

Hybrid catfish are a cross between female channel catfish and the male blue catfish, *Ictalurus furcatus.* Hybrids are commonly used in studies, as they tend to have consistently better growth, fillet yield, and harvestability (Giudice 1966; Dunham and Smitherman 1981; Smitherman et al. 1983; Dunham et al. 1987b; Dunham et al. 1990; Dunham and Brummett 1999, Bosworth et. al 2004, Tave et al. 1982; USDA 2007b; Dunham et al. 1982; Yant et. al 1976, Dunham and Argue 1998). Hybrid catfish have been observed to be less susceptible to

diseases, such as ESC and proliferative gill disease (Wolters et al. 1996). Although it is been observed that hybrid catfish are susceptible to columnaris, specific studies addressing susceptibility and control measures for columnaris disease in hybrid catfish have not been conducted. There are unknown differences in disease resistance to columnaris when comparing hybrids and channel catfish.

Vaccinations have proven to be effective when treating various diseases. The exploration of a vaccine for columnaris is still an on- going study. A live-attenuated vaccine called AquaVac- COL® produced by Schering Plough-Intervet, Inc. (Millsboro, Delaware) has been developed to reduce the disease effects of columnaris. Shoemaker et al. (2009) have shown AquaVac-COL® to be safe for eyed channel catfish fry and to aid in defense against columnaris in laboratory trials only. Bebak et al. (2009) have also seen that bass (*Micropterus salmoides floridanus*) species have lower risk of death when using AquaVac- COL® in a vat system.

The primary question to be answered from this study was: are the production characteristics (mean survival, feed conversion ratio, individual fish weight, and final standing crop) of hybrid catfish fingerlings grown in earthen ponds from the fry to fingerling improved when using AquaVac-COL®?

LITERATURE REVIEW

Channel catfish, *Ictalurus punctatus,* is a commonly cultured species in the United States. The development of the catfish industry has grown since the 1980s and is a fundamental contribution to the aquaculture industry in the United States. In 2004, channel catfish production grossed $480 million. The original purpose of culturing channel catfish was for stock enhancement of streams, lakes and ponds. The development of the catfish industry occurred when advances in technology occurred and a better understanding of the physiology of the catfish was accomplished. Spawning catfish was first achieved in 1914, which allowed catfish to be grown for stocking, and in the 1920s this was a common practice. People began to realize that catfish could be farmed for food fish, and, by the 1960s, Arkansas was producing 4000 ha of farmed catfish. The next development in the catfish industry was the feed mill in the 1970s. These developments are examples of what allowed the industry to grow, which in turn increased production significantly. By 2003, 64% of North American aquaculture production was attributed to the channel catfish, generating $348 billion dollars (Olin 2006). The industry has also seen growth because of an increase in demand, as capture fisheries are not able to meet this need.

Channel catfish farming has grown with the global trend, moving from 1,000 ha in the early 1960s to 77,000 ha in 1998 (Boyd 2000). Alabama, Arkansas, and Mississippi are the main producers of catfish providing 241 million foodsize fish on 4,856 ha in 2009. While the majority

of catfish provided were foodsize fish, the states also provided 877 million fry and fingerlings grown on 5,655 ha (USDA 2009). The catfish industry has grown to the largest aquaculture industry in the United States. The amount of farm-raised catfish processed in 2009 totaled 211 million kg (NASS 2010). Including feed mills, processors and supply companies, the total worth of the catfish industry is around seven billion dollars (USDA 2007b). In 2008, the most popular farm-raised fish or seafood consumed was catfish at number six and of the 7 kg of seafood consumed by Americans, 0.4 kg were catfish (NMFS 2008). Currently, 70% of the United States aquaculture industry relies on the channel catfish (Goldburg et al. 2001).

The main form of catfish culture is earthen ponds and, in some cases, high-density systems, such as cages, pens, tanks, vats, and raceways (Mingkang 2005). In-pond culture has been practiced for around 50 years and is the most successful method for the aquaculture business in the United States (Engle 2003). Many improvements in methods have been developed in the catfish industry. Research has been focused in areas to aid in production and technology. Improvements in hatch survival rates and protection from disease are examples of improvements in production strategies (Small 2009; Carrias et al 2008). Nutritional studies have revealed better methods to obtain a more profitable feed conversion ratio from feed, temperature effect, diet compositions and many other related studies (Li 2008). Improvements of in-pond grading systems have allowed for less stress on the animal and more accurate sorting method (Greenland and Gil 1972; Trimpey et. al 2004).

The hybridization of a female channel catfish and a male blue catfish, *I. furcatus,* has proven to have more successful culture traits when compared to channel catfish production. In 2008, over 30 million hybrid fingerlings were produced (Li et al 2010). Hybrid catfish are bred for their superior genetics and vigor as compared to other commercially raised catfish, the hybrid

dominates in culture traits (Yant et al. 1976; Dunham and Smitherman 1987a; Argue 1996; Wolters et al. 1996; Dunham and Argue 1998; Dunham and Devlin 1998). The hybrid has faster growth to market size (Giudice 1966; Dunham and Smitherman 1981; Smitherman et al. 1983; Dunham et al. 1987b; Dunham et al. 1990; Dunham and Brummett 1999). It has been shown that hybrid growth rates during food fish grow-out are 12-31% faster than channel catfish (Yant et a. 1976, Li et al 2004; Dunham et al. 2008). Market size hybrids have also been seen to reach 680-794 g or larger when stocked at 12,355- 14,820 fish/ha (Li 2010).

Compared to channel catfish, the hybrid catfish has an increased tolerance to lower dissolved oxygen concentrations. A forced oxygen depletion by adding formalin to earthen ponds, cages, and concrete tanks with both channel catfish and hybrids resulted in hybrids having a 30-60% better survival rate (Dunham et. al 1983). The hybrid has a higher catchability by seine than the channel catfish, allowing for better harvestability (Tave et al. 1982; Dunham et al. 1982; Smitherman et al. 1983; Dunham et al. 1986, Yant et. al 1976, Dunham and Argue 1998).

Higher dress out percentages have been observed in hybrids when compared to channel catfish (Argue et al. 2003). A strain of NWAC 103 line channel catfish (*I. punctatus),* Norris line channel catfish, and channel catfish female x blue catfish male (*I. furcatus)* were used to compare meat yield and meat quality traits and it was seen that the hybrid had higher dress-out and fillet yields (Bosworth et. al 2004).

A greater feed efficiency is seen with the hybrid. When compared to channel catfish, the hybrid has been seen to consume more feed, gain more weight, convert feed more efficiently, and have a higher net production (Li et al. 2004). Higher yield has been seen in hybrids along with an increase in body weight of 18-100% when compared to channel catfish (Smitherman et al. 1983; Dunham et al. 1987b; Dunham et al. 1990; Dunham and Brummett 1999).

Hybrid fry and fingerlings require more capital than other food fish, but hybrid catfish fingerlings and food fish production generally is more profitable when compared to channel catfish at moderate prices (Li et al 2010). The most effective spawning process for producing hybrid catfish is artificial spawning, which involves stripping female channel catfish of eggs and sacrificing a male blue catfish. Producing fry becomes expensive, as the spawning process is more involved than channel catfish spawning. It is estimated that the increase in cost to produce a hybrid catfish fry is ½ cent per inch. Therefore, where a channel catfish costs around 1½ cent per inch, a hybrid catfish costs 2 cents per inch (Masser et al 1998). Due to the hybrids improved traits over the channel catfish, the additional cost should be recovered.

Survivability of hybrids is greater than channel catfish as the hybrids have greater resistance to some diseases (Dunham et al. 1990, Dunham et al 2008). Hybrids have been shown to have strong resistance to primary diseases of catfish such as, enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri,* motile aeromonas septicemia (*Aeromonas hydrophila), Ichthyophthirius multifilis,* and channel catfish virus (CCV) (Wolters et al. 1996, Masser et al 1998).

Columnaris disease is a wide spread bacterial disease in culture systems. *Flavobacterium columnare* is the pathogen responsible for this disease and is a gram-negative rod bacterium. This disease was first described by Davis (1922), who described it as *Bacillus columnaris* due to the cells appearing as column-like masses, which were isolated on tissues placed in wet mounts. The organism grows as a yellow-pigmented, rhizoid bacterium when grown on Shieh agar (Decostere 1998a). The disease-causing agent was first identified from hatchery-reared sockeye salmon, *Oncorhynchus nerka* (Ordal and Rucker 1944). The name of the organism then changed to *Chondrococcus columnaris*. The name columnaris was given to the disease because of its

appearance when examined with a microscope. The organism appears as columns in colonies that resemble haystacks. The bacteria are 0.3 to 0.7 μm wide x 3 to 10 μm long (Farmer 2002). After a succession of name changes, the current name is now *Flavobacterium columnare* as defined by Bernardet from molecularly identifying strain characteristics (Bernardet et al. 1996). *F. columnare* grows best at 25 °C and in a temperature range from 4 to 37°C (Amend 1982).

Catfish are susceptible to columnaris at any stage of life under varying water quality situations and during all seasons at appropriate growth temperatures for the bacterium. When *F. columnare* infections occur, there is a 24- hour period or less for incubation and mortalities will appear two to three days after exposure. In most cases, mortalities are dependent on temperature but can range from 10 to 100% (Holt et al 1975). While catfish are a primary species of interest from an economic stance, other fishes are susceptible to infection. Both warm water and cold water fishes are affected by columnaris. The disease is only found in fresh water and not in marine environments. The disease has been isolated from fishes in many countries around the world, including Japan, Korea, Canada, the United States, Taiwan and Europe. Many fish species can be infected, including eels (*Anguilla japonica* and *A. anguilla)*, oriental weatherfish (*Misgurnus anguillicaudatus*), goldfish (*Carassius auratus)*, common carp (*Cyprinus carpio)*, grass carp (*Ctenopharyngodon idellus*), pike (*Esox lucius*), tench (*Tincca tinca*), black bullhead (*Ictalurus melas Rafinesque*), rainbow trout (*Oncorhynchus mykiss*), and catfish (Wakabayashi 1991). The bacterium itself is naturally present in warm waters around the world and is opportunistic. Therefore, if an organism becomes stressed, the bacteria are able to primarily infect the organism, which can lead to secondary infections. The commonality of this pathogen around the world and in many water environments allows it to be a key pathogen of interest in order to prevent outbreaks from occurring in culture systems.

Virulence factors of *F. columnare* are not well known, but are known to vary with the differing genetic groups of *F. columnare*. The ability of *F. columnare* to cause an infection is due to chondroitin AC lyase that causes tissue degradation after the pathogen has attached to the organism. This enzyme deteriorates polysaccharides found in the connective tissue allowing the infection to occur (Teska 1993). The effectiveness of degrading tissue varies with differing strains (Kunttu et al 2009). Virulence is also associated with capsule size, which is produced by the bacterium. Highly virulent strains range in sizes from 120-130 nm while low virulent strains range in size from 80-90 nm (Decostere et al. 1998b). Low virulent strains have a tendency to create necrotic lesions on the gills and body allowing systemic infections to occur. Highly virulent strains tend to have more affinity towards the gills. Mortalities caused by low virulent strains are seen after a period of days while high virulent strains have mortalities occur within a 24-48 hour time period (Rucker 1953; Pacha and Ordal 1970).

The common clinical signs that are associated with columnaris are associated with the tissue destruction that the infection causes. Signs of infection can be seen on the external portion of the fish. Usually, lesions are a dull white or yellow color located or around the mouth, on the skin or fins, or on the gills. A typical description of columnaris is known as 'cigar mouth', which stems from the infection causing the mouth of a diseased animal to appear as a brown color that is typical of cigars. The brown coloration will be seen along the lips and in the internal area of the mouth cavity. Presence of columnaris can be seen when erosion of the flesh occurs around the dorsal fin and down the lateral sides, which is known as a saddleback infection (Griffin 1987). The fins can also show signs of infection through the appearance of tissue rot as the fin erodes away. The disease causes a similar appearance to external tissue, which also appears as eroded and exhibits signs of necrosis. Along with the skin and fins, the gills are a primary area

that the disease infects and will also appear to be necrotic (Davis 1922). An initial diagnosis of columnaris can be determined when these signs are observed. These observations can also be confirmed for *F. columnare* through examination of a tissue scrap of the lesion and then examined on a wet mount under a microscope. While both low and high virulent strains are causing these clinical signs, the high virulent strain is also capable of producing high mortality without observable signs (Pacha and Ordal 1967). Once an area on an organism becomes infected, the bacteria spread quickly to tissue in the surrounding areas.

There are complications with isolating *F. columnare,* which has inhibited its pathogenicity from being determined. In many cases, when a fish is infected with *F. columnare* there typically is a secondary infection present. The secondary infection can occur from pathogens such as *Aeromonas spp., E. ictaluri,* or *E. tarda* (Hawke and Thune 1992). Due to the secondary infection, the agent causing the primary disease is difficult to identify. Therefore, the further understanding of the pathology of *F. columnare* has been hindered. The issue with multiple infections is the masking of the *F. columnare* by a number of situations. One situation is the presence of another *Flavobacterium*, which could veil the presence of *F. columnare* as the primary cause of the infection. The presence of a combative species, such as *Pseudomonas* or another gram-negative bacterium, could also inhibit the finding of *F. columnare* (Tiirola et al. 2002). The presence of these bacteria dominates *F. columnare*, which prevents the colonies from growing in media as a dominant species as the other species present grow more prominently. Thus, the identification of *F. columnare* can be overlooked and the fish misdiagnosed as to the cause of the infection.

The masking of *F. columnare* by other species is one issue with its identification while another issue with *F. columnare* is the inability for it to grow on common media, such as tryptic

soy agar, Mueller Hinton agar, or brain heart infusion agar. Therefore, isolation and culture of *F. columnare* has to be conducted on a specific agar. The necessary environment for growth of this bacterium includes a low nutrient high moisture content agar. Growing the sample on *Cytophaga* agar was originally conducted, but the more prominent bacteria grew more than *F. columnare*. The bacterium was found to resist the polymyxin B and neomycin in the agar. Adding these antibiotics to the agar allowed *F. columnare* to have more colonies present and prevent the growth of the combative bacterium. Shieh medium is also used to culture *F. columnare* and has been found to be more effective than *Cytophaga* agar. This type of media has a specific amount of salt that creates a more optimum growth environment specifically for *F. columnare.* Shieh medium supplemented with tobramycin has been found to also be an effective method for isolating *F. columnare* from other bacteria found in a sample (Decostere et al. 1997).

Once a diagnosis of columnaris has been confirmed, the next step is to treat the infected organism for the pathogen. There are different methods for treating columnaris in culture systems, but the most common are chemotheraputants. These are easily distributed in a culture system and have varying degrees of effectiveness for removing columnaris from the system. One method is to use salt as a controlling agent. An effective rate is to maintain salt levels around 3 ppt. Higher rates inhibit growth of *F. columnare* and clumping (Bernardet 1989). When salinity rates are at higher rates, mortalities were seen to decrease. This was attributed to the salinity preventing the pathogen from binding to the organism (Altinok and Grizzle 2001). Other preventative chemotheraputants are able to reduce the presence of this particular pathogen because the form of infection is topical. Therefore, treatments of these types must be able to remove pathogens from the surface of the organism. Common methods used are chemicals such as potassium permanganate, copper sulfate and hydrogen peroxide (Wakabayashi 1991). Each

treatment varies depending on the size of the culture system and the rate of the active ingredient within the chemical. Treatments of a specific culture system may also have to be done multiple times in order to ensure that the pathogen has been removed from the system. Another chemical that has shown to be beneficial as a candidate for treatment of *F. columnare* is the herbicide Diquat, otherwise known as 6,7-dihydrodipyrido (1.2a: 2, 1-c) pyrazidinium bromide. When compared with differing levels of potassium permanganate, chloarmine-T, hydrogen peroxide and copper sulfate, Diquat was seen to effective when conducted under bath conditions (Thomas-Jinu and Goodwin 2004). Other chemicals used in aquaculture systems have also been tested to determine their effectiveness for treating columnaris. In the same study by Thomas-Jinu and Goodwin (2004), a bacterial study was conducted which resulted in all groups having no mortalities when using the drugs Terramycin® and Romet®. Aquaflor® is an antibiotic that has proven effective in treating channel catfish for columnaris and has been approved by the Food and Drug Administration (FDA) (Gaunt 2010). These antibiotics can also be applied as feed. Treating infected fish with chemotheraputants, drugs or medicated feed should be avoided, if possible. The cost of treating with these methods can be an expensive option for removing a pathogen. Using certain chemicals as a management for pathogen control creates a withdrawal time in the treated organism if the organism is to be used as a food fish. The best treatment for preventing this pathogen from occurring is to manage the stress level of the culture system in order to prevent an infection from occurring.

The impact disease has on cultured species causes a significant decrease in return on investment. During the culture period, many factors contribute to costs, such as a transport, labor and feed. The effect of columnaris specifically has on cultured species can be seen worldwide. columnaris can infect many species of fish. Perhaps the most important economic repercussions

are seen in the catfish industry in the United States. Many farmed fish kills have been attributed to bacterial diseases. As of 2002, the diseases causing the most mortality were ESC and columnaris and 65% of fry and fingerlings deaths were attributed to these diseases by farmers. When columnaris was specifically identified as the pathogen causing the mortalities, some producers reported losing half of the infected population (USDA 2007a). Estimations of losses due to mortalities caused by columnaris alone reach into millions of dollars per year (Schrader 2008). Culture systems, such as tanks, have been reported to have half of the population killed when an infection from columnaris occurred (Plumb 1999).

The catfish industry is economically important in the United States for aquaculture, but other areas of the world have negative impacts on economy due to this disease. One rainbow trout farm in Turkey reported experiencing an outbreak of columnaris and attributed it to causing 30% of the mortalities in one day (Kubilay 2008). Perch populations in England were also significantly reduced due to an outbreak of columnaris (Morley 2009). These few examples can add up to major losses economically when considering a worldwide combination of all mortalities caused by columnaris. This disease causes a large loss in profit if it occurs in a culture system. Therefore, it is imperative that columnaris is identified and eliminated before it is able to cause mortalities in a culture system.

Vaccines have played a major role in improving animal health, animal productivity and safety in public health by reducing zoonotic disease, eradicating disease in countries, and have aided in preventing spread of disease by imported animals (Frey 2007). Interest for disease prevention began in aquaculture as increases in disease were prevalent due to the ability to have higher stocking densities in culture systems. The higher densities resulted in degradation of water quality conditions and easier spread of disease.

Increased efforts to investigate immunology and vaccination in fish began in 1935-1938 (Van Muiswinkel 2008). Protective immunity was shown against *Aeromonas punctatus* when injected with killed bacteria in the laboratory (Snieszko 1938). A further study showed that upon challenge, injection of killed or attenuated bacteria evoked protective immunity (Schaperclaus 1942). The first published study on oral vaccination was by Duff (1942), who showed that by feeding cutthroat trout a diet containing chloroform-killed bacterin (*Aeromonas salmonicida*) after injection or contact with clinically- ill fish protected against furunculosis (Duff 1942). The probable first report of vaccination on fish against a viral disease was done for spring viremia done by injection of formalin (Goncharov 1951). Immunization against bacterial diseases was also conducted on carp (Goncharov 1968, 1971). The prevention of vibriosis, enteric redmouth disease and furunculosis has been shown with the use of vaccines (Sakai 1999). Studies have shown promising results for *A. hydrophila* and *F. psychrophilum* (Rahman 2000, Crump et al. 2005, Dumetz et al. 2007, Högfors et al. 2008, LaFrentz et al 2008, LaPatra et al. 2010). Increased interest in live attenuated bacterial vaccines began in the 1990s (Norqvist et al. 1989; Vaughan et al. 1993; Thornton et al. 1994; Lawrence et al. 1997; Hernanz Moral et al. 1998; Marsden et al. 1998; Klesius and Shoemaker 1999; Thune et al. 1999). Vaccination can lower economic losses from mortalities, improve feed conversion and reduce length of time to marketability (Amend and Eshenour 1980; Tebbit et al. 1981; Horne and Robertson 1987, Lillehaug 1989, Klesius et al. 2006).

Disease management for fish at a young age proves to be the most difficult in the growth stage of cultured fish (Ellis 1988). During the early life stages, fish have non-specific and specific immunity. Non-specific defense mechanisms are innate factors, such as physical barriers and phagocytic cells, which help with diseases resistance. The non-specific defense system

prevents pathogens from attachment, invasion or replication on or in the tissue of the fish. This defense mechanism is temperature- dependent and takes more time to respond (Ellis 2001). The main physical barriers are the skin and mucous. Mucous is secreted from goblet cells in the skin, gills, and gastrointestinal tract preventing colonization of microbes. Phagocytic cells function to obtain, engulf, and destroy microbes.

Another form of defense is the specific defense mechanism, which develops later in life to aid long-term disease resistance. Fish naturally have humoral immunity that consists of B cells, which are triggered when antigenic stimulation occurs causing antibodies on the surface of lymphocytes to be secreted (Kaattari 1992). Antibody production time is 2 to 4 weeks in channel catfish, yet an infection from a pathogen could kill a fish within days. Antibody production is triggered in B cells after immunization in fish which appear in blood, bile and mucous (Wilson and Warr 1992).

Live- attenuated vaccines have been effectively used in livestock and poultry industries. Typically, attenuated vaccines are used when an endemic pathogen is present in the environment of cultured fish. Therefore, attenuated vaccines provide an advantage over killed vaccines in the aquatic environment. The use of modified live vaccine is able to repeatedly expose the host providing protective antigens to create immunity (Shoemaker 2009). Methods for vaccination of young fish are spray or bath vaccination but these methods tend to be less effective than injection, which is difficult to do with young fish. Vaccinating orally has been shown to be the least effective method while vaccination by injection has proven most effective (Ellis 1988). Young fish can be effectively immunized via immersion with modified live vaccines allowing for stimulus of the humoral immunity and long term duration of the vaccine (Shoemaker 2009). Attenuated vaccines, theoretically, can provide the capability for farmers to protect large

quantities of fish at a young age. Two vaccines have been approved for use on 7-10 day post-hatch catfish fry to prevent ESC and columnaris. These vaccines, AquaVac- ESC® and AquaVac-COL®, have proven to reduce mortalities for their respective diseases, and immunization from these vaccines has been shown to have a duration of 1 to 2 years (Panangala et al. 2006).

Vaccination against columnaris has had mixed results. Pond-reared channel catfish immunized with a formalin-killed bacterin had low-level protection from columnaris (Moore et al. 1990). Immersion immunization saw 60% protection in eels, while injection was not as effective (Mano et al. 1996). Heat and formalin- killed preparations provided some protection for carp and eel after immersion immunization (Liewes et al. 1982, Mano et. al 1996). Oral immunization of coho salmon at 3 months old had partial protection from a heat-killed vaccine (Fujihara et al. 1971).

As *F. columnare* causes infection by deteriorating the gills and infiltrating the body from there, a primary defense mechanism is mucus. Adhesion to gill tissue and the virulence of a strain of *F. columnare* are directly correlated (Decostere et al. 1999a). Adhesion on mucus-coated slides and the virulence of Finnish strains had no connection (Suomalainen et al. 2006). In another study, a less virulent strain of *F. columnare* mutant that forms smooth colonies had less adherence to both skin and gill tissues of channel catfish when compared to wild type strains that form hard colonies (Bader et al. 2005). Challenged catfish were seen to have higher mortalities due to the adhesion of the *F. columnare* to fry (Shoemaker et al. 2007). The ability of channel catfish to fight disease at a 10-day post hatch is of question. The immune system may not fully be formed by this age. A study done with *E. ictaluri* to determine significant antibody generation

showed that humoral immune response before four weeks of age in channel catfish might not be capable of making immunological tolerance (Petrie-Hanson et. al 1999).

Efficacy of AquaVac-Col® vaccine in pond- reared catfish has yet to be determined. The vaccine has been determined to be safe for 10-day post- hatch channel catfish fry and efficacy was seen in eyed channel catfish eggs and in10-day post hatch, 48-day post hatch, and 3 month old catfish in studies conducted in aquaria (Shoemaker et al 2009). The effectiveness of AquaVac-COL® vaccine was analyzed on 8-month old channel catfish fry 10 minutes post-vaccination in which the vaccine was seen to upregulate 28 expressed sequence tags that represent putative function in immune response (46%), signal transduction (21%), transcriptional regulation (11%), cell maintenance (11%), and unknown areas (11%) (Pridgeon et al. 2010). Largemouth bass fry were challenged with AquaVac-COL® vaccine where vaccinated fish were seen to have a 43% lower risk of death in one field trial utilizing large holding tanks (Bebak et al 2009). The vaccine exposes the protein to an underdeveloped immune system in young fish, which may prevent the immune system from responding to the antigen if the fish is exposed to it later in life (Kunttu 2010).

MATERIALS AND METHODS

On June 11, 2009, approximately 200,000 hybrid channel catfish fry were obtained from the Genetics unit of the E. W. Shell Fisheries Research Station, Auburn, Alabama. The hybrid strain of catfish used was Eagle strain channel females crossed with Rio Grand blue catfish. The fry were grown in the Genetics Unit and were obtained 10- d post hatch. Eight earthen ponds (0.1 ha each at a depth of 1 m) were used to grow the fry to fingerlings. The ponds were drained and dried for two weeks prior to refilling from a watershed located on the research station two days before fry were stocked. Before filling, screens were placed on inlet pipes to prevent wild fish from entering the study ponds. Fry in all treatments (approximately 25,000 fry/pond) were added to the ponds on June 11, 2009.

The fry were randomly separated into 2 treatments with 4 replicates per treatment. The two treatments were distinguished as a sham-vaccination and vaccination with AquaVac- Col®. The fry were treated with the vaccine and then placed into the earthen ponds on the same day. Briefly, fry were held in two rearing troughs until 10 days post hatch. An estimation of the number of fry stocked was based on an average weight of three samples of 25 fry each and numbers were extrapolated to estimate the total number of fry stocked. An estimated 25,000 fry were transported to each pond. Vaccination of fry was carried out on the pond bank according to the manufacture's recommendations for use on channel catfish fry. Based on 25,000 fish, 13.0 ml of vaccine was added to 4.8 L of water. The fry were submersed in the vaccine solution supplied with pure oxygen provided via air stones for 2 minutes. Afterwards, an additional 4.8 L of water

was added to the fish holding tank for an additional 30 minutes. The fry were then placed into the ponds. In sham- vaccinated control tanks, all fish underwent the same procedures minus the addition of the vaccine solution.

Each pond was fed twice a day by hand, once in the morning and afternoon with a commercially- available fry feed (Purina AquaMax™ Trout Diet, Gray Summit, MO). AquaMax™ Fry Powder containing 50% protein and 17% lipids was used to feed for weeks 1 to 4. Fish were fed with AquaMax™ ^{Fry} Starter 100 containing 50% protein and 17% lipid for week 5. AquaMax™ Fry Starter 200 containing 50% protein and 17% lipid was used to feed for weeks 6 to 8. AquaMax™ Fingerling Starter 300 containing 50% protein and 16% lipid was used to feed for weeks 9 to 13. AquaMax™ Grower 400 containing 45% protein and 16% lipid was used the remaining weeks of 14 to 22. Changing to a larger feed pellet was determined according to the behavior and size development of the fry in each pond. Fish were observed after every feeding and the activity of feeding was recorded. Fish were fed to approximate satiation each feeding based upon recommendations by Tucker and Robinson (1990), but never exceeding 114 kg/ha.

Dissolved oxygen (DO) and temperature were recorded twice daily in the morning and afternoon at dawn and dusk, using a dissolved oxygen meter (YSI model 55, YSI Inc., San Diego, California). In morning, supplemental aeration was provided to the pond. Aeration was provided nightly from evening to early morning and used emergency aeration as needed. A weekly monitoring of water quality parameters pH, temperature, chloride, total alkalinity, total ammonia nitrogen, and nitrite-nitrogen was conducted from June 11, 2009 to November 30, 2009. The measurement of pH and temperature was obtained with a portable Hach Sension1® pH meter (Hach Chemical Company, Loveland, Colorado, USA) on the pond bank.

Measurement of the remaining water quality parameters were obtained with a Hach® water quality kit (model FF-1A). Chloride levels were monitored weekly, and if observed to be below 50 ppm or 10 times the level of nitrites, salt was added to the pond.

Mortalities were monitored and recorded daily. Fish found suitable for necropsy were submitted to the Southeastern Cooperative Fish Disease Laboratory, Auburn University, Auburn, AL, for diagnosis. Liver, kidney, and spleen were taken from the samples and plated on brain heart infusion (BHI) agar media and Hsu-Shots agar media. To determine the cause of the disease and confirmation of the causative pathogen, biochemical testing of isolated bacterial colonies were run. Skin scrapes and gill samples were observed for parasite infestations. Confirmation of the causative disease gave direction to determining the treatment, if any, for the infected pond. Harvesting of the ponds began on November 30, 2009, and ended on December 4, 2009, in which fingerling fish were collected after 5 months of growing in the ponds. All fish harvested were weighed in bulk on a standard industrial bench scale (0.0001 lb readability) to determine the final standing crop of each pond. Subsamples from each pond, three 100 fish samples, were used to calculate average weight of the fish and then projected out to determine final numbers of fish harvested. Observed mortalities, total percent survival, and feed conversion ratio were calculated. Statistical analysis of all parameters was conducted using a paired sample *t*-test according to Zarr (1999), where significance was held at $p \leq 0.05$.

RESULTS AND DISCUSSION

Water quality parameters were measured during the 172 days of the pond study including pH, total ammonia nitrogen, nitrite nitrogen, chloride, and total alkalinity. There was no difference between treatments considering the water quality parameters so for presentation purposes; overall means are presented in Table 1. Mean total ammonia nitrogen averaged 1.34 ± 0.15 mg/L for the sham- vaccinated treatments and 1.32 ± 0.27 mg/L for the vaccinated treatments. Unionized ammonia levels exceeded recommended values in the vaccine treatment in replicate 3 (0.9 mg/L) twice. Recommended levels are 0.5- 2 mg/L when held under constant temperature and pH for 96 -hour time frame (Hargreaves et al 2004). However, given the dynamic conditions of a pond situation, pH and temperature are on diurnal cycles and these types of constant exposure are not constant in this type of environment. Nitrite- nitrogen averaged 0.085 ± 0.016 mg/L for the sham- vaccinated treatments and 0.085 ± 0.01 mg/L for the vaccinated treatments. Chloride averaged 45 ± 1.87 mg/L for the sham- vaccinated treatments and 48 ± 2.01 mg/L for the vaccinated. Salt was added to vaccine replicate 3 and sham-vaccinated replicate 3 at a rate of 200 pounds to prevent nitrite from affecting fish, as the chloride levels fell below 50. Total alkalinity averaged 48 ± 1.23 mg/L for the sham- vaccinated treatments and 45 ± 1.63 mg/L for the vaccinated treatments. Weekly pond pH ranged from 6.75 to 10.2 over the study period (Figure 1). Recommended pH levels for catfish are 6.5 to 9 (Hargreaves et al 2004). Vaccine treatment replicate 1 exceeded (10.2) these recommendations once. Dissolved oxygen levels were within acceptable levels for culture of channel catfish accept

in vaccine treatment replicate one where dissolved oxygen fell below 3 mg/L twice, vaccine replicate 2 where dissolved oxygen fell below 3 mg/L once, and vaccine replicate 3 where dissolved oxygen fell below 3 mg/L once (Carter and Allen 1976). Vaccine replicate three there was an unexplained fish kill on 26 September where approximately 2500 mortalities were collected. No other mortalities were observed in this pond 14 days prior to the event or 4 days after the event. The dissolved oxygen level the night before the event was 3.2 mg/L and the morning level was 3.3 mg/L. Although fish were attempted to be necropsied, no know etiology could be concluded to the reason behind this group of fish dying on this say. Station personnel did find that no aerator was running at the time of the morning dissolved oxygen reading. Water quality parameters during the study were within known acceptable levels for culture of channel catfish (Tucker and Robinson 1990).

The production results for the trial conducted from June 11, 2009 to November 30, 2009 are seen in Table 2. Standing crop averaged 2802 ± 268 kg/ha for sham- vaccinated treatments and 2676 ± 424 kg/ha for vaccinated treatments. Individual fish mean weight averaged 16.8 ± 0.99 g for sham- vaccinated treatments and 21.1 ± 1.8 g for vaccinated treatments. Feed conversion ratio averaged 2.57 ± 0.18 for the sham- vaccinated treatments and 1.92 ± 0.38 for the vaccinated treatments. No significant differences are observed between treatments in considering standing crop, individual fish mean weight, and FCR. Survival ranged from 55% to 73% in the sham- vaccinated control and 29% to 92% in the vaccinated treatment (Table 3). The production parameters in this study are within what is expected for catfish fingerling production under commercial conditions stocked in similar densities (Tucker et al 2004).

The first mortalities of fingerlings expressing clinical signs of columnaris were observed on 26 August 2010 and was verified by the Southern Cooperative Fish Disease Laboratory at

Auburn University. Mortalities from columnaris were observed in all ponds from 26 August to 7 November 2010 when temperatures ranged from 27°C in August to 15°C in November. The last columnaris mortality was observed 7 November (Figure 2). The total number of mortalities collected during columnaris outbreaks determined the efficacy of the vaccine in terms of percent mortality observed. Vaccination benefits on production were also evaluated using mean standing crop, individual fish weight, and FCR.

Vaccine treatment replicate 4 had a partial dissolved oxygen kill, therefore, 29% represents the percent survival with the observed mortality excluded from the final standing crop of the pond. The observed mortality (4.3%) was included when calculating 37.3% for percent survival to indicate the percent survival if the dissolved oxygen event had not occurred as a dissolved oxygen event is not related to a disease outbreak. The final mean percent survival for vaccine treatment is 54 ± 13% and 67.2 ± 7.2% in the sham-vaccinated treatment excluding the observed morality. No significant difference was seen when comparing mean percent survival between treatments (Table 4) ($p= 0.32$). Observed mean mortality for the sham- vaccinated treatments averaged 4.14 ± 12% while that for the vaccine treatment averaged 4.3 ± 4.0 %. No significant difference was seen for observed mortality between treatments (Table 4).

In September 2009, *Ichthyophthirius multifiliis* was observed to be present on moribound fish that were also exhibiting signs of columnaris. Once *I. multifiliis* was determined to be present in the ponds, samples were taken from each pond every three weeks while the water was around 20°C. If *I. multifiliis* was found on sampled fish, formalin was used to eliminate potential disease episodes. One of the sham- vaccinated ponds and three of the vaccinated ponds were treated for *I. multifiliis* with formalin (Table 5). As formalin is not an approved FDA drug to treat columnaris, the use of formalin presumably did not affect the mortality rates (USFWS

INAD 9013). Columnaris mortalities continued in all ponds until 5 Nov 2009 and the mortalities stopped when pond temperatures were no longer conducive for columnaris growth.

Past studies evaluating the efficacy and benefits of using AquaVac-COL® vaccine in the laboratory have been mixed. It has been observed that the vaccine was able to upregulate sequence tags in different age fish (Pridgeon et al. 2010). Eyed channel catfish *in ovo* have also benefited from the vaccine as well as fry being vaccinated up to 10 days of age (Shoemaker et al. 2009). However, in comparison to the commercial vaccine with a second-generation vaccine under development, Olivares (2010) observed no increase in survival in aquaria challenges for the commercial vaccine compared to the control fish. These studies previously done with AquaVac-COL® have been conducted only in laboratory settings and not in pond environments where water quality and environmental conditions are changing and may place added stress on the fish that may go undetected through routine monitoring. In the only field trial known to date, Bebak et al. (2009) moved juvenile largemouth bass from ponds to fiberglass vats to evaluate AquaVac- COL®, therefore, reducing the effects of a true pond study.

The age of vaccinating catfish has been in question, as the immune system may not be developed enough to incorporate the vaccine into the immune system (Petrie-Hanson et al. 1999; Wise and Terhune 2001). AquaVac-COL® has been seen to be effective in channel catfish from 10-day post hatch to 3 month old (Shoemaker et. al 2009). However, with this columnaris vaccine being applied to fish at 10- d post hatch, it is unknown how long the vaccine organism resides in the fish to allow enough of an exposure to stimulate the immune system (Wise and Terhune 2001). Hybrid catfish have yet to be directly studied

with AquaVac-COL®, therefore the efficacy of the vaccination could be in question in relation to the ability for the vaccine to be effective to the immune system at a young age. Hybrid catfish are more disease resistant to ESC than channel catfish, but hybrid resistance to columnaris is still for the most part unknown.

Other factors come into play, such as weather, predators and changing environmental pond dynamics. The variations in temperatures that occur throughout the growth season are conducive for varying pathogens that create for unpredictable disease outbreaks. Because all the mortalities could not be accounted for in this study (Table 4), as predators are present in the field that are not present in the lab inhibiting collecting 100% of the mortalities, it is difficult to determine real attributes of the vaccine in preventing Columnaris disease outbreaks. This bacteria has long been associated with stressors making the host susceptible to disease (Plumb 1999), however, this disease can be very unpredictable in regards to outbreaks because the bacterium is ubiquitous in all environments as well as being a primary and oftentimes a secondary pathogen.

Hybrid catfish have advantages over channel catfish when comparing production parameters. The ability to have better feed conversion ratio, growth to market size, and higher dress- out percentages (Li et al. 2004, Giudice 1966; Dunham and Smitherman 1981; Smitherman et al. 1983; Dunham et al. 1987b; Dunham et al. 1990; Dunham and Brummett 1999; Argue et al. 2003). Although hybrids have greater resistance to certain diseases, there is still more information to be discovered in respect to determining if hybrids are more resistant to columnaris compared to channel catfish (Wolters et al. 1996), as well as other physiological stress responses that may make the fish susceptible to disease.

Although the cost of hybrids may be more expensive at the onset of production, the most effective spawning process for producing hybrid catfish is artificial spawning, which involves stripping female channel catfish of eggs and sacrificing a male blue catfish. This action drastically increases labor cost for producing hybrid catfish. It is estimated that the increase in cost to produce a hybrid catfish fry is $0.025 per inch (Masser et al 1998). The cost of the AquaVac-COL® should be considered in farmers' economic outlooks. The possibility of discounts for fry producers to buy the vaccine is around $3000-5000 / million fry adding an additional $0.005 per fish to production cost (Kasha Cox, personnel communication, Schering Plough-Intervet, Inc.).

The study can be seen as a pilot study for the AquaVac-COL® vaccine on hybrid catfish in the field. No statistical differences were seen in this experiment in relation to comparing the benefits of vaccinated fish with AquaVac-COL® to sham- vaccinated fish. Future studies need to consider environmental changes and the possibility for alternate disease outbreaks that would also occur during columnaris outbreaks. As with other vaccination programs in fish and other livestock programs, the producers should observe consistent field results, overall reduced risk to disease, and overall improved production parameters. Given the unpredictability of this disease, additional pond studies should be performed to eliminate year to year variability that can affect results, as well as laboratory studies to better understand vaccination dynamics in hybrid catfish.

Table 1: Overall mean total ammonia- nitrogen, nitrite- nitrogen, chloride, and total alkalinity (mg/L) from 0.1 ha earthen ponds with either sham vaccinated or vaccinated fish after 172 d. Values are means (± standard deviation; n= 4). Significance difference occurred between treatments if $p < 0.05$.

Treatment	Total Ammonia Nitrogen	Nitrite Nitrogen	Chloride	Total Alkalinity
Sham-Vaccinated	1.34 ± 0.15	0.085 ± 0.02	45 ± 1.87	48 ± 1.23
Vaccinated	1.32 ± 0.27	0.085 ± 0.01	48 ± 2.01	45 ± 1.63
p-value	0.97	1	0.067	0.13

Table 2: Mean (± SD) standing crop, average weight, and FCR for hybrid catfish fry after 172 d in earthen ponds that were either vaccinated or sham vaccinated. Values are means (± standard deviation; n= 4). Significance difference occurred between treatments if $p < 0.05$.

Treatment	Standing Crop (kg/ha)	Ave. Weight (g)	FCR
Sham- Vaccinated	2802 ± 268	16.8 ± 0.99	2.57 ± 0.18
Vaccinated	2676 ± 424	21.1 ± 1.8	2.92 ± 0.38
P-value	0.77	0.99	0.37

Table 3: Percent survival between replicates ponds. No statistical comparisons were performed since replicate ponds are not paired with one another and are for presentation only.

	Replicate				
Treatment	1	2	3	4	Mean
Sham-Vaccinated	56%	55%	85%	73%	67 ± .07
Vaccinated	52%	44%	92%	29% (37.3%)	54 ± .12 (56.3)

Table 4: Mean percent ± SD, observed mortality, and total percent survival for hybrid catfish during the 172 day study period from ponds stocked with either sham vaccinated or vaccinated fry. Values are means (± standard deviation; n= 4). Significance difference occurred between treatments if $p < 0.05$.

Treatment	Observed Mortality (%)	Survival (%)
Sham- Vaccinated	4.14 ±1.2	67.2 ± 7.2
Vaccinated	4.3 ± 4	54.2 ± 13
P-value	0.96	0.32

Table 5: Study treatments and replicate ponds within study treatments that were treated with 20-ppm formalin for *Ichthyophthirius multifiliis* and date of first to last treatments.

Treatment	Date Beginning Treated	Date End Treated	Number of Treatments
Sham Vaccinated			
Replicate 3	24 Sept 2009	1 Oct 2009	3
Vaccinated			
Replicate 1	24 Sept 2009	29 Sept 2009	2
Replicate 4	29 Sept 2009		
Replicate 2	30 Oct 2009	3 Nov 2009	3

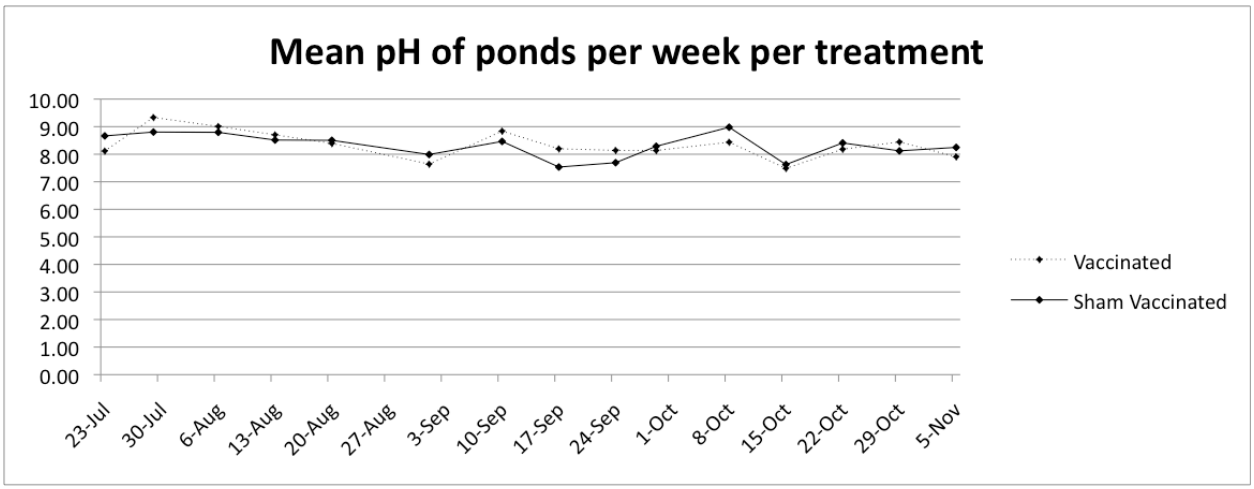

Figure 1: Weekly mean afternoon pH of ponds used to grow hybrid catfish from June 11, 2009 to November 5, 2009.

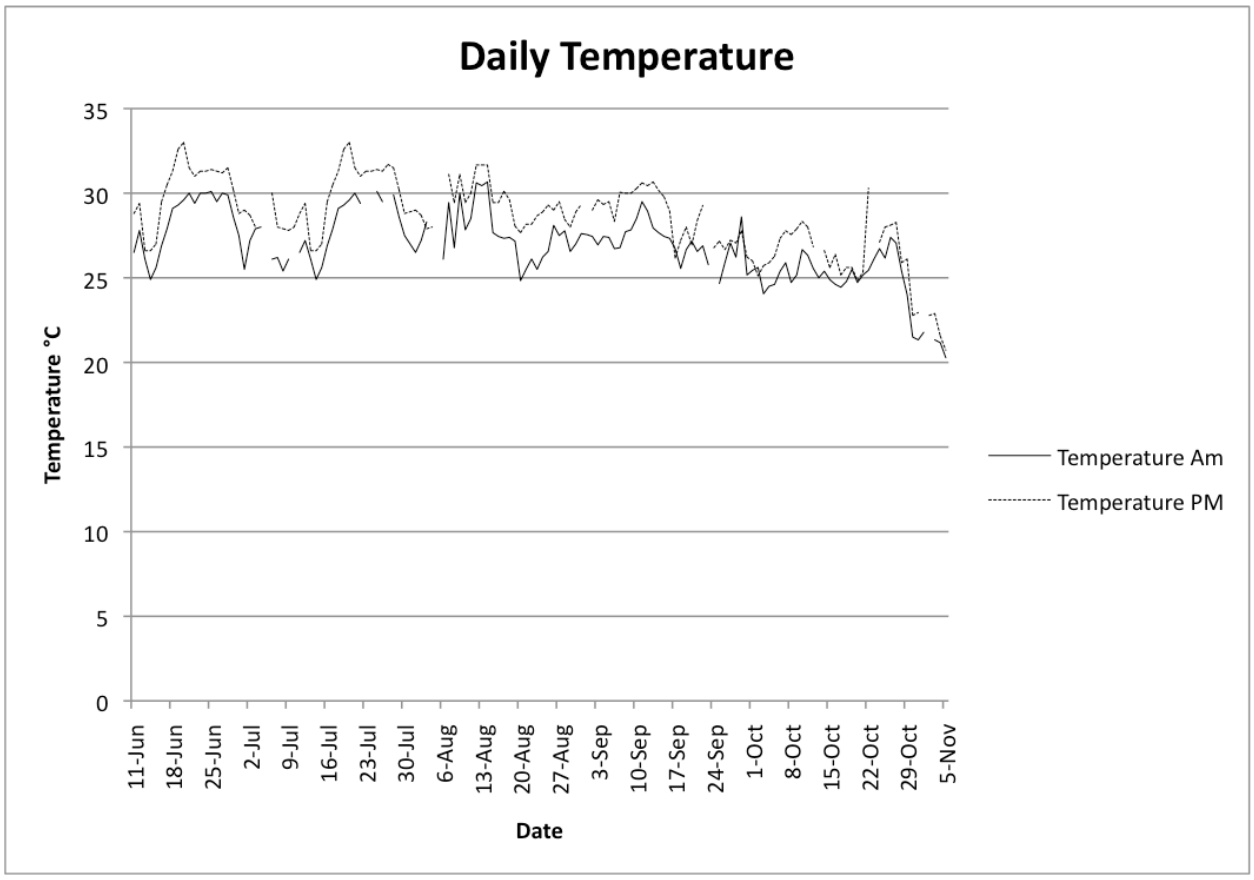

Figure 2: Daily mean temperature of ponds used to grow hybrid catfish from June 11, 2009 to November 7, 2009

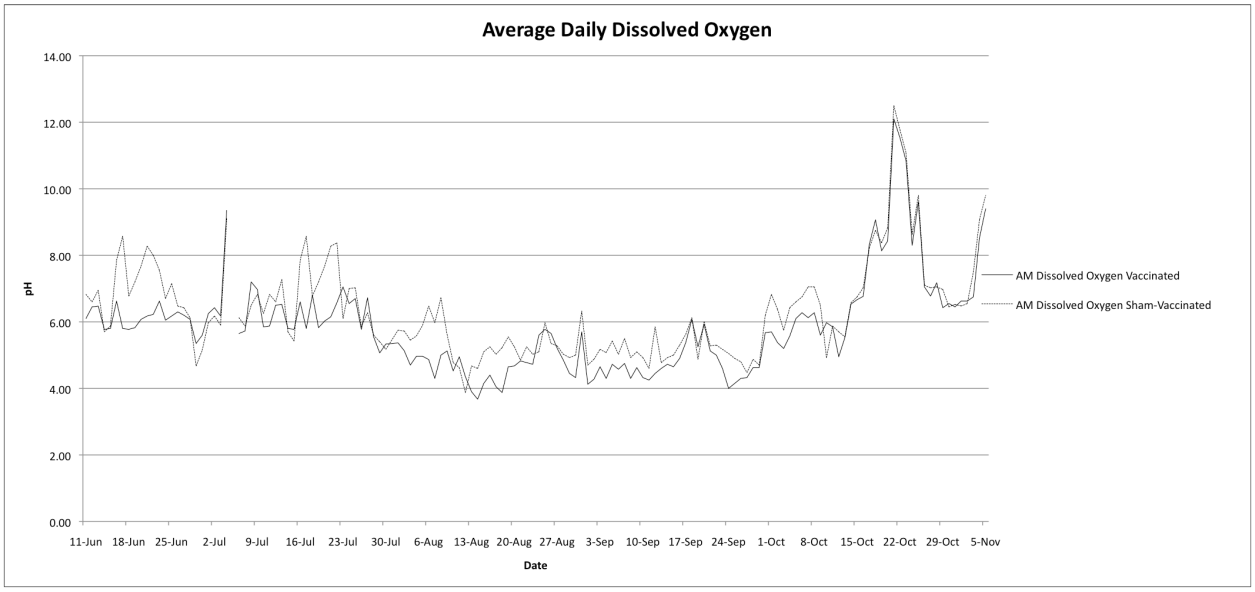

Figure 3: Average daily morning dissolved oxygen levels for the vaccinated and sham-vaccinated treatments from 11 July – 5 Nov 2009.

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