BIOECONOMICS OF *Flavobacterium columnare* VACCINE POND TRIALS FOR CHANNEL CATFISH *Ictalurus punctatus*

by

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ABSTRACT

The US farm-raised catfish industry has had a variety of problems in the last decade, both in production and marketing. Increased disease pressures have come primarily from the ubiquitous pathogens responsible for columnaris (COL, *Flavobacterium columnare*), enteric septicemia of catfish (ESC, *Edwardsiella ictaluri*), and virulent *Aeromonas hydrophila* (vAh). Pond trials were conducted with vaccinated fish using an experimental live-attenuated COL vaccine, and non-vaccinated channel catfish (*Ictalurus punctatus*). Data were collected to evaluate the COL vaccine for survival, growth, feed conversion, antibody presence, and economic benefit.

These trials were a follow-up to promising laboratory results, which confirmed that the 17-23 vaccine increased protection against *F. columnare* in channel catfish fry. Pond trials represent an environment more akin to commercial catfish operations which use earthen ponds. Channel catfish fingerlings were split into either a control or vaccinated treatment, with five ponds per treatment (n=5). Control fish were stocked at a rate of 741 fish per pond, while treatment fish were vaccinated through bath immersion before being stocked into their respective ponds (777 fish/pond). Fish were fed a 32% crude protein floating pellet from stocking in April 2019 until harvest in October 2019. Routine and periodic fish growth sampling and blood collection were performed throughout the trial. No COL disease challenge was conducted, although the production timeline overlapped with two known columnaris outbreak peaks which occur in the spring and fall as seasonal water temperatures change. Experimental units included ten 0.04-hectare watershed ponds, each with one 0.5 hp aerator running from 6pm to 8am daily.

Blood serum samples were processed and analyzed for antibody concentrations with an indirect ELISA (enzyme-linked immunosorbent assay).

Results showed no significant increase in survival of vaccinated fish compared to control fish ($P = 0.7127$), and there were no natural outbreaks of COL observed during the trials. Results of an indirect ELISA determined that the anti-COL antibody concentrations were significantly higher in vaccinated fish 4 weeks post-stocking ($P < 0.05$), but not at 8 weeks post stocking ($P=0.334$). Vaccinated fish (357.5 ± 30g) had a significantly higher average weight at harvest than control fish (289.3 ± 20g) ($P =0.0013$), and the FCR of vaccinated fish (1.35) was lower than control fish (2.13), representing a 37% FCR improvement ($P < 0.0001$). The total amount of feed fed decreased from the control to vaccinated treatment by 14% but was not significant due to high variability among vaccinated ponds ($P = 0.1195$). Without a large mortality event we were not able to assess the vaccine's impact on survival, but the vaccine impacts to production parameters (feeding amounts, FCR, biomass harvested) are encouraging. Our partial budgets present the minimum economic benefit to a producer on the research level, as well as potential net benefits on a more applicable commercial scale. Feed efficiency appears to be the catalyst for the benefits observed in this trial in which there was no observable or measurable incidence of columnaris. While the data is encouraging for commercial farmers, further production studies are warranted in which measured disease incidences are recorded to further corroborate the benefits of disease management through this columnaris 17-23 vaccine adoption.

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LIST OF TERMS AND ABBREVIATIONS

COL	Columnaris
DPH	Days Post Hatch
DO	Dissolved Oxygen
FCR	Feed Conversion Ratio
TAN	Total Ammonia Nitrogen
VFD	Veterinary Feed Directive

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INTRODUCTION

Status of US Catfish Industry

Catfish farming is an economic staple for many rural families in the southern United States (U.S.), producing both channel catfish (*Ictalurus punctatus)* and hybrid catfish (*I. punctatus, ♀ x* blue catfish*, I. furcatus, ♂).* Live food-size catfish earned $360 million in 2018 for producers in the top producing states of Mississippi, Alabama, Arkansas, and Texas (NASS 2018). Since the peak of U.S. catfish production in 2003 (300 million kg of catfish processed), both the market demand and farm production have decreased steadily. In 2014, processing had dropped over 50%, when only 139 million kg were processed in the U.S. (Hanson and Sites 2015). Production factors associated with this drop can be characterized by either their effect on the market or the farms themselves.

The current market demand for catfish is located within the southern states, states along the Mississippi River and into Central Canada, but struggles to gain national acceptance, especially within Eastern and Western coastal states. Local food movements encourage northern consumers to purchase lake-caught whitefish such as perch over catfish. Foreign products such as swai catfish (*Pangasius bocourti*) and tilapia (*Oreochromis niloticus*) are also competing in the supermarket. Pangasius is farmed intensively in South East Asia with a lower production cost. Even after import, these products are typically sold at prices lower than U.S. farm raised catfish products. In January 2020, frozen pangasius fillet imports from Vietnam were valued at

$2.82/kg ($1.28/lb), while U.S. farm raised catfish frozen fillets were valued at $9.50/kg ($4.31/lb) (Hanson 2020; NOAA 2020).

On the farm, production costs continue to increase. Feed is the most expensive variable cost to any aquaculture operation, and catfish farms are no exception. Multiple batch production makes it difficult for farmers to control exact populations or know their inventory, thus management is more subjective and difficult. Inconsistent seining at harvest leaves market-size fish in the ponds and has created a “big fish” problem (Creel 2020). Harvest-size fish (0.45-1.81 kg) missed over multiple years combined with continued feeding has led to oversized “big fish” (>1.81 kg). Even when harvested, these large fish create issues for processing equipment, and in return result in reduced or, in some cases, no compensation at all for the farmer (Creel 2020). Disease pressures have also increased in recent years, causing fish kills and immediate financial losses to the farmer. The newly emergent pathogen *Edwardsiella piscicida* has been increasingly reported in cases by the Aquatic Research and Diagnostic Laboratory (ARDL) at the Thad Cochran National Warmwater Aquaculture Center in Stoneville, MS since 2007 (Griffin et al. 2014; Griffin et al. 2019). The pathogens responsible for enteric septicemia of catfish (ESC, *Edwardsiella ictaluri*), virulent Aeromonas (vAh, *Aeromonas hydrophila*), and columnaris (COL, *Flavobacterium columnare*) have consistently topped mortality cases (Hemstreet 2018). These warm-water pathogens are considered ubiquitous throughout commercial catfish ponds and therefore complete eradication is unlikely (Schacte 1983; Tekedar et al. 2017; Kumar et al. 2019).

Disease Management Options

Current disease management strategies are dependent upon the pond environment and resource availability. Levee or watershed earthen ponds used in catfish production typically range from 3 to 10 ha per pond, with an average depth of 1.5 meter (Avery no date). The large pond volume limits the ability to treat fish effectively during outbreaks. At first sign of disease, farmers routinely restrict feeding for several days to reduce horizontal disease transmission due to contact during feeding activities. This action results in immediate loss to the producers in number of feeding days and subsequent fish growth. Shoemaker et al. (2003) also demonstrated in laboratory studies that catfish withheld from feed were highly susceptible to columnaris infections. Beck et al. (2012) reported the molecular basis for this result. However, if disease intensifies, medicated feed becomes the next logical option. U.S. regulations require that producers obtain a veterinary feed directive (VFD) for use of antibiotic feeds (FDA 2016). TERRAMYCIN®200 for fish (oxytetracycline dehydrate) is approved in catfish for motile Aeromonas septicemia (*Aeromonas hydrophila*) and pseudomonas disease (*Pseudomonas spp*.). ROMET®30 and ROMET®TC (sulfadimethoxine and ormetoprim) and Aquaflor® (florfenicol) are both approved for use in catfish for mortalities due to ESC, though Aquaflor ® requires a VFD (Kelly 2013). Once a prescription is obtained, the producer is then approved to purchase medicated feed from a licensed feed mill, if it is locally available. The VFD approval process stalls the critical time to treat fish, and again causes losses to the producer in reduced survival, lost growth, lost feed days, and the cost incurred to purchase medicated feed. Sick fish also often do not eat, so the medicated feed may be distributed but not consumed.

Detrimental effects of commercial antibiotic use are coming to light as antibiotic resistant bacteria are discovered across all sectors of agriculture. In aquaculture production, antibiotics have previously been prescribed prophylactically in order to reduce stressors of intensive rearing. Increased antibiotic use in the environment killed susceptible bacteria, and artificially selected for resistant individuals, leaving those resistant to repopulate the environment (Cabello 2006).

With the rise in antibiotic resistance, consumer preferences have also evolved. Perception reports state that consumers have taken notice of antibiotic use in farm-raised animals since 2008, with over 30 percent of surveyed consumers avoiding foods treated with antibiotics (Brewer and Rojas 2008; Gulab 2018). With this push to move away from antibiotics, the traditional mindset of merely treating diseases as they appear is also beginning to disappear. Instead, the catfish industry needs to find disease solutions through prevention of these top diseases, which makes vaccines a highly viable solution.

West Alabama Disease Impacts

Alabama is the second largest catfish producing state, estimated to produce 33% of all U.S. catfish, and over 2,600 jobs within the western rural regions of the state (Hanson *et al*. 2018). Columnaris disease has a considerable impact on this region and draws attention from Auburn University (Auburn, AL), the Alabama Fish Farming Center (Greensboro, AL), and the USDA-ARS Aquatic Animal Health Unit (Auburn, AL) in terms of research and diagnostic support to commercial producers. Yearly farmer surveys record production data and disease incidence for local farmers. In 2018, columnaris was responsible for 24% of all recorded mortalities, accounting for over 3 million fish deaths and a corresponding 1.5 million pounds lost that year (Hemstreet 2018). In neighboring east Mississippi, similar losses due to columnaris have been reported. In 2016, regional farmers reported 600,000 kg of fish lost to *F. columnare*, which translates to $1.4 million in losses (Peterman and Posadas 2019).

High intensity commercial rearing presents many opportunities for *F. columnare* to infect immunocompromised fish or present itself as a secondary infection. Environmental stressors such as high rearing densities, like those found on commercial farms, have been known to increase disease incidents (Suomalainen et al. 2005). However, as a warmwater disease, temperature remains an important factor in outbreaks. A 2006 study reported two distinct outbreak peaks impacted U.S. catfish farmers on an annual basis, with the largest amount of cases reported as the weather changes in April and September (Figure 1) (Mississippi State University 2006). However, a similar study carried out more recently determined that columnaris cases steadily rose from April to October (Mississippi State University 2017). This indicates that columnaris is now becoming a disease lasting the full production season. Losses in the fall represent a huge loss to farmers, as the cost of fingerlings in addition to feed purchased for an entire growing season can be lost in one fish kill. Columnaris is a well-known disease that has been studied for over 100 years but remains a poorly battled disease that desperately calls for management solutions for U.S. catfish farmers (Davis 1922).

Vaccine Adoption Obstacles

As previously mentioned, the farms of many producers are located in depressed economic regions, so market struggles have greatly impacted the profit margins of these farmers. In 2018, Mississippi State reported the average price paid to producers was $2.20/kg for premium-sized (0.45 – 1.81 kg) live fish (Breazeale 2018) and these are sold in grocery stores in the $13-$15/kg range. By comparison, salmon mariculture companies in the U.S. have integrated hatchery, grow-out and processing facilities that keep production costs down, and are sold in the $15-$26/kg range. Vaccines used in commercial catfish production have been shown to increase fingerling survival, however without a decent profit, farmers are understandably wary of increased production costs. With the addition of a vaccination, producers experience the expense before fish are stocked (Carrias et al. 2008; Johnson et al. 2014). For drug companies, the salmon industry is a much more attractive option, as the industry is much larger than the U.S. catfish industry and salmon producers can better afford to pay for vaccines as each fish is worth considerably more. Salmonid vaccines are often administered by injection, which provides significantly increased protection and production cost per fish (Thune et al. 1997). However, for larger fish like salmon (4.2 – 4.7 kg), the vaccine cost is spread out over a greater overall fish value (Davidson et al. 2016). Catfish farmers cannot afford this method for their smaller sized fish (0.5 – 1.8 kg), so other delivery methods are needed, such as immersion and oral delivery (Creel 2020).

The disparity in vaccine availability between salmonid and catfish aquaculture is blatant and highly visible. A total of 18 vaccines and bacterins are available for use in salmonids against a range of bacterial and viral pathogens, while only two vaccines available for catfish both protect against ESC (Shefat 2018). The AQUAVAC-ESC™ (Intervet) vaccine has been available to catfish producers since January 1999 but is used relatively infrequently in comparison to the losses caused by ESC (Bebak and Wagner 2012). Recent vaccine development with an avirulent *EiΔevpB* strain has been shown to provide significantly higher protection than AQUAVAC-ESC™ (Abdelhamed et al. 2018). In this study, *EiΔevpB* was shown to be safe and effective for both catfish fingerlings and fry against *F. columnaris* when administered through immersion.

Similarly, the AQUAVAC-COL™ (Intervet) vaccine was available in March of 2005 against *F. columnare* for catfish producers but is no longer available. Vaccine safety and increased survival were shown in the eggs and fry of channel catfish and largemouth bass (*Micropterus salmoides floridanus*) soon after appearing on the market (Shoemaker et al. 2007; Bebak et al. 2009; Shoemaker et al. 2011). Only one study was found that characterized the grow-out production impacts of a columnaris vaccine in catfish and found that there was no significant difference in survival between vaccinated and control fish (Kirkland 2010). This study was conducted using hybrid catfish, and to date there still has been no comparable study in channel catfish due to the market removal. Even when AQUAVAC-COL™ was still on the market in 2010, only 5% of producers used the vaccine. Most producers expect 100% of vaccinated fish to be protected, as seen with an increase in survival (Bebak and Wagner 2012). Eventually the vaccine was removed from the market due to low producer acceptance.

Developing the 17-23 Vaccine

Before the removal of AQUAVAC-COL™ from the market, efficacy was questioned by farmers and researchers alike. AQUAVAC-COL™ was developed using an avirulent rifampicin-resistant mutant of *F. columnare* from genomovar I that was proven to be less virulent (Zhang et al. 2006; Shoemaker et al. 2008). In response, a modified-live vaccine was developed with another rifampicin-resistant mutant from the virulent genomovar II, hypothesizing an increased level of protection (Olivares-Fuster and Arias 2011; Mohammed et al. 2013). Channel catfish mortality in an immersion challenge with genomovar II isolates reached 74%, which was significantly higher than the genomovar I challenge mortality (32%) (Arias et al. 2012). Lab trials by Mohammed confirmed that the genomovar II mutant 17-23 vaccine provided an increased level of protection to channel catfish fry than a vaccine from genomovar I (Mohammed et al. 2013). However, due to improved identification techniques, *F. columnare* is now preferably classified by genetic grouping. Recent research found that isolates from channel catfish of genomovar I (now genetic group 1) caused no mortalities, while genomovar II (now genetic group 2) were extremely virulent causing a 60% mortality rate (Shoemaker et al. 2008; LaFrentz et al. 2018).

The study presented in this thesis is a continuation of the genetic group 2 (formerly genomovar II) 17-23 vaccine trials. Previous laboratory studies were conducted with high levels of biosecurity in highly controlled environments that do not exist on commercial catfish farms in the U.S. Innumerable variables exist in earthen pond production that can directly impact vaccine success. By extending vaccine trials into ponds, we can better mimic commercial production. In accordance, stocked fish were vulnerable to natural *F. columnare* populations that are found ubiquitously in pond environments and subjected to seasonal water temperature fluctuations previously mentioned. The pond trial reported in this study was vital in assessing the future application of the 17-23 vaccine on a commercial scale.

Study Objectives

The hypothesis tested in this study was that vaccinated channel catfish grown in earthen ponds would have an increase in survival, improvement in feed conversion ratio (FCR), and an increase in total weight harvested in comparison to non-vaccinated fingerlings. An economic analysis of production variables was implemented to predict farm level impact and predict future adoption potential by the commercial catfish industry.

MATERIALS AND METHODS

Pond Production Trial

The pond trial was conducted in ten 0.04 hectare watershed ponds, located at the E.W. Shell Fisheries Station, Auburn University, Auburn, AL. Each of the ten ponds were dried for three weeks prior to filling. Liming of the ponds with agricultural lime was used to enhance fertilization and to minimize pH swings (Wurts and Masser 2013). The first treatment of 226.8 kg/pond of lime (5.6 MT/ha) occurred on April 1, and was broadcast across the dry pond bottom with buckets and shovels in order to raise alkalinity above 40mg/L (Boyd 1990; Boyd 2012). Ponds were filled with water from deep inlets in the pond bottom post-liming. A second liming occurred on May 8, in order to correct low alkalinity that was recorded at the time of (Tavares and Boyd 2003) stocking (April 29). Control ponds #1-5 and vaccinated pond #4 received an additional 453.6 kg of agricultural lime, while vaccinated ponds #1-3 and 5 received an additional 226.8 kg of agricultural lime to raise alkalinity to the prescribed level (> 40mg/L). A salt treatment occurred on April 8, with each pond receiving 136.1 kg (336.9 kg/ha) of salt (sodium chloride) spread across the water surface. This rate was used in order to obtain a chloride concentration of 100 ppm, which is recommended to prevent health issues related to nitrite toxicity. Pre-stocking treatment was supervised and performed by AU E.W. Shell Fisheries Station crew members.

Healthy channel catfish fingerlings (6” or 15.3 cm in length; approx. 8,000 individuals) were loaded onto a hauling truck and transported to Auburn University E.W. Shell Fisheries Station (April 29, 2019) from Georgia Select Fish farm, LLC (Bartow, Georgia). The hauling truck was equipped with four 1-m^3 tanks and aerated with fine-pore air stones using pure oxygen. Fifty fingerlings were removed randomly with a dip net from the four tanks, and sampled for weight (g) and total length (cm). The vaccine was then administered through a bath immersion into two of the four hauling tanks, which held half of the purchased fingerlings. The water levels in each of the vaccinated tanks were lowered to contain 100 gal of water per tank. One liter of the prepared *F. columnare* vaccine (17-23 against *F. columnare* genomovar II mutant) was then added to each tank, resulting in a dose rate of 2.25x10^6 CFU/mL. Fish were immersed in the vaccine for one hour before stocking an average of 777 fish per 0.04 hectare pond. Populations in the five vaccinated ponds ranged from 491 to 891 fingerlings. The non-vaccinated control fish were stocked at approximately 741 per 0.04 hectare pond into their respective five ponds while the vaccinated fish remained in the hauling tanks, resulting in a range of 773 to 788 fingerlings per pond.

Non-vaccinated control fingerlings were removed from the hauling tanks with dip nets and placed into baskets. The baskets were then transported by hand to the pond edge, lowered into the water, and fish were counted as they were released into the pond. The total number of fingerlings per pond was recorded as stocked, with a maximum rate of 800 fingerlings per pond. Stocking continued until all fingerlings had been removed from the two non-vaccinated hauling tanks. This procedure was repeated with the vaccinated fingerlings when stocking their respective ponds.

Due to the way channel catfish fingerlings were divided among tanks at the hatchery (by weight), the exact number of fingerlings was unknown, and control ponds were stocked unevenly. To correct this, at one week post-stocking (May 6, 2019) control ponds were seined in order to re-adjust populations. Once seined, a pre-calculated number of fingerlings were placed into a basket, and carried by hand from an overstocked control pond and released into an understocked control pond. Vaccinated pond populations were not adjusted, while the new control population counts were recorded following this event. As a result, the control pond average stocking rate of 741 fish per pond remained, but the range was adjusted from 741 to 742 fish per pond.

Additional fish species were stocked after the initial fingerling stocking in April 2019. Common carp (*Cyprinus carpio*) from holding ponds at the E.W. Fisheries Station were stocked into the experimental ponds on June 17, in order to improve pond water quality by increasing turbidity as common carp are known to disrupt sediment while searching for food on the pond bottom (Milstein et al. 2002). Increasing turbidity of the pond water reduced light penetration and in turn reduced aquatic vegetation and algae populations which reduced oxygen demand in the pond over the course of the study. Common carp were stocked at a rate of 50 fish per acre, resulting in 5 fish stocked in each pond with an average weight of 0.17 kg. Grass carp (*Ctenopharyngodon idella*) were purchased from American Sport Fish Hatchery (Pike Road, Alabama) in order to reduce aquatic vegetation populations in the experimental ponds without applying herbicides (Fowler and Robson 1978). Grass carp were stocked at a rate of 20 fish per acre on August 1, resulting in two fish per pond (average weight of .65 kg each). Even with screens attached to water inlets, gizzard shad (*Dorosoma cepedianum*) were able to enter the ponds and were removed at harvest.

Throughout the trial, pond levels were maintained daily by standpipes and deep water inlets in order to maintain a consistent pond depth of four feet. Mortalities were removed with a dip net and recorded twice daily at the same time as dissolved oxygen readings. Mortalities were composted at the E.W. Shell Fisheries Station, and the collection net was sanitized using a Virkon® Aquatic Disinfectant (Syndel USA, Ferndale, WA) bath following each use.

Water quality parameters were measured in each of the ten ponds from stocking (April 29) until the last feeding event (October 21). Dissolved oxygen (DO) was recorded twice daily, once in the morning between 8am and 9am, and once in the evening between 3pm and 5pm. A MW600 Dissolved Oxygen Meter (Milwaukee Instruments, Rocky Mount, NC) was lowered to six-inches below the water surface to record the level of DO (mg/L) in each pond. Total ammonia nitrogen (TAN), nitrite (NO2-), chloride, hardness, and alkalinity were measured once a week in each pond using Tetra EasyStrips Ammonia Freshwater & Saltwater Aquarium Test Strip and Tetra EasyStrips 6-in-1 Freshwater & Saltwater Aquarium Test Strips (Tetra Werke, Melle, Germany). Channel catfish were fed a 32% crude protein floating pellet throughout the study (Alabama Catfish Feedmill LLC, Uniontown, AL). Feed was purchased in bulk by the E.W. Fisheries Station and stored in metal feed bins. As needed, two 80-gallon plastic tubs were filled with feed from the metal bins, and stored inside the research building's wet-lab. Five-gallon buckets were filled, covered with a lid, and moved via truck to the ponds at each feeding event. Buckets were carried to each pond edge by hand, and the lid was repeatedly hit against the bucket side in order to indicate time of feeding. A 0.95 L plastic container, which held 400 g of feed, was used to scoop feed from the bucket and broadcast across the center of the pond. Beginning on April 30, each pond was fed twice daily, at 8am and 5pm. Feed amount was limited to 50 g of feed per feeding event from April 30 (PM) to May 2 (AM). The limit was increased to 100 g of feed per event from May 2 (PM) to May 6 (PM). This limit was enforced in order to encourage feeding activity while not wasting feed. On May 5, we began feeding fish once a day in the evening between the hours of 3pm and 5pm. All ponds were fed to satiation from May 7 to May 10, and feeding amounts were calculated using the 90/7 method (see below) beginning on May 11.

The 90/7 feeding method is used to prevent over-eating, feed waste, and excess nutrient leaching into a pond and is a recommended best management practice used in research verification programs (Bott et al. 2015). This method allows feeding rates to be calculated according to individual pond activity, and is reassessed once weekly. Beginning on the first day, the individual pond was fed in small amounts up to satiation. The feed amount per day was then limited to 90% of the recorded satiation amount for the following six days. On the seventh day, each individual pond’s satiation amount was recalculated. This method was used from May 11 until the end of feeding on October 21.

Feed conversion ratio (FCR) was calculated for each pond by dividing the amount of feed fed to fish in a pond by the weight gain of the fish from that respective pond. The equation used is shown below:

$$FCR = [\text{Total feed fed (kg)}] / [\text{Harvest biomass (kg)} - \text{Stocking biomass (kg)}]$$

Sampling was carried out once monthly from May to October. Feeding was restricted one day prior to the scheduled sampling date. A one-inch mesh seine was pulled from one pond end to the other, fish were gathered in the seine sock, and baskets were used to scoop 30 random fish from the seine pull. Remaining fish in the seine sock were released back into the pond. The collected thirty fish in the sample were anesthetized before sampling by placing the fish into an 18 gallon plastic tub of water from the respective pond being sampled along with two bubble aerators. In a separate 14 L bucket equipped with a single bubble aerator, 1.3 g of MS-222 and 1.3 g of sodium bicarbonate were added to 13 liters of pond water for an anesthetic dose of 100 mg/L. Five sample catfish were added to the anesthetic bucket at a time. Once a fish was immobilized, weight (g) and length (cm) were measured. Afterward, fish were moved to a recovery tank basket within a cooler filled with the sampled pond's water and two bubble aerators. Fish remained in the recovery tank until all 30 sampled individuals had recovered from the anesthetic, and were then released back into their respective ponds. All equipment was cleaned following each sampling event using Liquinox® Detergent (Alconox, Inc, White Plains, NY).

Feed was restricted from all ponds two days before the scheduled final harvest. Pond levels were dropped one day prior to the intended harvest date by lowering the standpipes to half of their normal height. A mesh cover was placed inside each standpipe in order to prevent fish from escaping. Each pond was seined with a one-inch mesh seine with a maximum of five seine pulls per pond. Any grass carp, common carp, or shad removed during harvest were transferred to nearby ponds not involved in this trial. Catfish were scooped from the seine into baskets and each basket was weighed to record total weight (kg). The basket was then carried to the hauling truck, where individual catfish were counted and recorded as they were released into the hauling tanks. The hauling truck consisted of four individual hauling tanks, filled with 600 L of water, and one aerator supplied with pure oxygen in each tank. The final empty basket weight was recorded and was subtracted from the total weight to obtain the harvest fish weight. Harvest weights were summed by pond and divided by total fish counts to obtain the average weight per fish.

All non-vaccinated control fish were moved in the hauling tanks and restocked into station ponds for further grow-out. Vaccinated fish were moved by hauling tank to a counting shed, where they were transferred into coolers, and euthanized with MS-222 at a rate of 300 mg/L. Once euthanized, fish were layered with ice before being transported and incinerated by the Alabama State Diagnostic Laboratories (Auburn, AL). All used equipment was cleaned following harvest using Liquinox® Detergent (Alconox, Inc).

Enzyme Linked Immunosorbent Assay

The absorbance values (the relative level of anti-*F. columnare* IgM antibodies) for individual fish among the control and vaccinated populations were assayed at two time-points (4 and 12 weeks post stocking). The ELISA method was used to measure the catfish serum immunoglobulin M (IgM) antibodies as described with some modifications (Lange et al. 2016). Pierce 96-well polystyrene plates (ThermoFisher Scientific, Waltham, MA) were coated with 100 µL of 10 µg/mL of sonicated lysate derived from the vaccine isolate. Plates were then rinsed three times with 1x PBS with 0.05% Tween-20 (PBST) and then incubated for 1 h in blocking solution (PBST with 5% milk). One hundred µL of serum (1:100) was further serially diluted out to 1:1600 in 1x PBS on the horizontal axis of an antigen coated ELISA plate and incubated at room temperature for 1 h. Plates were rinsed as above and 100 µL of anti-channel catfish IgM mouse monoclonal 9E1 antibody (Miller et al. 1987) was added at 1:500 dilution in blocking solution. After 1 h of incubation at room temperature, plates were washed with PBST and 100 µl of sheep anti-mouse IgG HRP conjugated (GE Healthcare, Pittsburgh, PA) was diluted 1:5000 in blocking solution and incubated for 30 m at room temperature. Plates were rinsed three times with PBST, and 50 µL of 1-Step Ultra TMB-ELISA substrate solution (ThermoFisher, Waltham, MA) was added. The peroxidase reaction was stopped after 20 m with 50 µL of 3M H_2SO_4 and read spectrophotometrically at 450 nm with a BioTek plate reader operating under Gen5 software (Winooski, VT).

Economic Analysis

Using standard farm management techniques (Engle 2010; Kay et al. 2016; Kumar and Gaunt 2020), a partial enterprise budget comparing use of vaccinated fingerlings in place of non-vaccinated control fingerlings to produce channel catfish was developed. Partial budgeting accounts only for additional benefits and costs associated with a change, in this case changing from the use of non-vaccinated to vaccinated fingerlings in production. Budget analysis was performed at the research pond level (0.04 hectare) and extrapolated to a per hectare value and then to a 101- hectare commercial pond level. In partial budgets, the left column summarizes benefits associated with vaccinated fingerling production, including any increase in income, such as an increase in biomass harvested and any reductions in costs, such as the amount of feed fed. When added together the result is the total benefit from the change. The right-hand column displays additional costs associated with the change, such as reduced income or increased costs associated with using vaccinated fingerlings. The additional costs typically might include increased harvest and transportation costs of the additional biomass harvested, cost of the vaccine, and the additional loan interest required in the purchasing of these additional production inputs. The cost of this vaccine is not known, so a proxy cost of $5,000 per one million fry ($0.005/fingerling) was used based on hatchery ESC vaccine costs (T. R. Hanson, Auburn University, personal communication). By subtracting the additional costs from the additional benefits, a net benefit is found. A positive net benefit indicates that the change should be adopted, while a negative net benefit indicates that the change should be rejected.

Statistical Analysis

The mean antibody concentrations for each treatment at both time points were assessed for significance using a two-tailed unpaired *t*-test. Sample length and weight data were analyzed for differences with linear mixed models with pond as a random factor using the GLIMMIX procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC). There were 30 subsamples per pond per sampling event. Harvest data were analyzed with linear models for continuous response data (total harvest biomass, average harvest weight per fish, and FCR) or a generalized linear model for count data (survival expressed as a proportion) with a negative binomial distribution, and a log link function using the GLIMMIX procedure of SAS (SAS Institute 2016).

RESULTS

Pond Production Trial

Water quality parameters remained within normal ranges reported for the commercial production of channel catfish (Table 1). Dissolved oxygen (DO) remained above 4.0 mg/L in all ponds, and with a control average of 9.2 ± 0.4 mg/L and vaccinated average of 9.3 ± 0.3 mg/L. An average pH of 7.2 was recorded for both treatments, while total ammonia nitrogen, nitrites, and chlorides all remained below 0.5 mg/L. Hardness and alkalinity levels remained within normal ranges following the liming treatments. Control ponds averaged 78.2 ± 16.0 mg/L in hardness and 15.5 ± 18.0 mg/L alkalinity. Vaccinated ponds averaged similarly with 77.7 ± 10.4 mg/L hardness and 10.2 ± 13.1 mg/L alkalinity. None of the measured water quality parameters were significantly different between control and vaccinated treatments.

Each month, the weight and length of 30 sampled individuals per pond treatment was measured and means were compared. There was no significant difference in either monthly weight or monthly length means until the October sampling. In October, the mean weight of the vaccinated sample (471.98 ± 85.32g) was statistically greater than the mean weight of the control non-vaccinated sample mean (358.66 ± 54.15g) (P = 0.0127) (Figure 2, 3). The vaccinated sample mean length (35.78 ± 1.67cm) was also significantly greater than the control sample mean length (33.74 ± 1.32cm) (P = 0.0352) (Figure 4, 5).

Survival in the control treatment averaged 83.76 ± 8.0 % while fish in the vaccinated treatment averaged 85.32 ± 6.0%. There was no significant difference in survival between the control and vaccinated treatments (P = 0.7127).

The total biomass of vaccinated fish harvested was 290.4 kg greater than harvested from the control fish (Table 2). There was a significant difference in total biomass harvested between the five control ponds and the five vaccinated ponds ($P = 0.0075$). The average weight of vaccinated individuals at harvest was significantly greater (357.5 ± 30g) than those of control fish (289.3 ± 20g) ($P = 0.0013$).

A total of 3,143 kg of feed was fed to all ponds (ten 0.04 ha ponds) throughout the study, with vaccinated fish consuming 1,456 kg (for five 0.04 ha ponds) and control fish consuming 1,687 kg (for five 0.04 ha ponds). This difference in feed fed when translated into reduced feed cost will be important to overall farm profitability. Figure 6 displays the average feed fed per day per treatment and shows that both treatments follow the same fluctuations, yet the vaccinated fish average was consistently lower than the control average beginning in the middle of June. While the difference in feed fed was not significant between the treatments ($P = 0.1195$), total feed fed to the vaccinated fish was reduced by 13.7% compared to control fish. The vaccinated ponds had a wide range and high level of variation, with a vaccinated treatment standard error of 52.0 compared to the control treatment standard error 18.7. The FCR of control fish was 2.13 ± 0.17, while the FCR of vaccinated fish was significantly lower at 1.35 ± 0.14 ($P < 0.002$) (Table 2).

Enzyme Linked Immunosorbent Assay

The absorbance values (the relative level of anti-*F. columnare* IgM antibodies) for individual fish among the control and vaccinated populations were assayed at two time-points (4 and 12 weeks post stocking) (Figure 7). At week 4, the mean ± standard error of absorbance for the control was (0.1578 ± 0.06) significantly less ($P < 0.05$) than the values noted in the vaccinated group was (0.1966 ± 0.11). At 12 weeks, the mean ± standard error of absorbance was 0.1245 ± 0.05 and 0.1364 ± 0.060 for the control and vaccinated groups, respectively, and no significant difference was observed (P = 0.334).

Economic Analysis

Partial budget additional benefits from using vaccinated fingerlings in channel catfish production totaled $1,590/ha. Additional benefits were result of increased biomass harvested increasing sales and reduced feed amounts relating to cost savings (Table 3). Additional costs come from the increased biomass harvest and their associated higher harvest and transport costs. Also included were vaccine and loan interest items for a total additional cost of $107/ha. The total additional benefits are greater than the total additional costs of using vaccinated channel catfish fingerlings over control fingerlings for producing channel catfish and result in a net benefit to the producer of $1,482/ha. A positive net benefit indicates that use of vaccinated fingerlings is beneficial to a producer, and due to the guidelines of partial budget analysis, we would recommend vaccine use within a farm management plan.

DISCUSSION

At the onset of the trial the biggest expectation was to observe a notable difference in treatment survival, however we did not experience a fish kill or columnaris outbreak at any time. In the original lab trials carried out with this vaccine in 2013, results concluded that the 17-23 vaccine provided an increased level of protection to channel catfish fry by significantly decreasing mortality. Mortality dropped from 63% (in genomovar I vaccinated fish) and 66% (in sham vaccinated control fish) to 41% in the 17-23 genomovar II vaccine used in this study (Mohammed et al. 2013). These observed survival values are a result of disease challenges including inoculation. Previous testing of AQUAVAC-COL™ was shown to significantly reduce mortalities in channel catfish fry from 30.7% in the sham vaccinated treatment to 13.3% in the vaccinated treatment (Shoemaker et al. 2007). The same result was also found in trials using largemouth bass where mortalities dropped from 43.8% to 11.1% in vaccinated fry (Bebak et al. 2009; Shoemaker et al. 2007). Even when the vaccine was administered to eyed-channel catfish eggs, significant differences in survival were noted, as the control treatment mean mortality (98.7%) was reduced through vaccination (49.3%) (Shoemaker et al. 2011). New research examining vaccination with a recombinant *F. columnare* DnaK protein vaccine has shown significant survival improvements (>30%) in vaccinated channel catfish compared to non-vaccinated controls (Lange et al. 2019). While the impact to survival is notable in these studies, it must be mentioned that all were carried out in a lab environment and most challenges used feed deprivation prior to disease challenge. Due to the market removal of AQUAVAC-COL™, we were unable to compare it’s efficacy during our trials. The only pond trial of AQUAVAC-COL™ was carried out in hybrid catfish fry 10 days post hatch (DPH), and no significant differences of any production parameters were noted (Kirkland 2010). The mean percent survival in the sham vaccinated treatment (67.2%) when compared to the vaccinated treatment (54.2%) showed a difference (Kirkland 2010).

Resistance to *F. columnare* in hybrid catfish has since been reported, supporting Kirkland's results when analyzing vaccine impact in hybrids (Arias et al. 2012). However, a similar report showed that even family lines in channel catfish have increased resistance to *F. columnare* (Beck et al. 2012). This report is a reminder to the catfish industry that we have many ways to reduce columnaris disease impacts, outside of medicated feed and vaccines.

Dietary interventions such as prebiotics and probiotics offer promise in combatting columnaris disease as well. Prebiotics are nutrients used to alter the microbiome environment in order to encourage the growth of preferential bacteria, and can target the gut, gills, and epithelial mucus layers. A prebiotic trial found a significant increase in the survival of *F. columnare* challenged channel catfish fingerlings following the addition of Actigen® to their diets (Zhao et al. 2015). Probiotics are live bacteria often added to feed in order to introduce preferential bacterial populations to the gut which compete to reduce attachment sites available for unwanted bacteria. In the case of columnaris disease, a *Saccharomyces cerevisiae* fermentation product (Diamond V Original XPC) can be added to commercial feed and is shown to increase total immunoglobulin counts and increase disease resistance in hybrid catfish (Mohammed et al. 2019). Copper sulfate used as a dietary supplement has been shown to significantly improve survival in channel catfish challenged with *F. columnare* (Farmer et al. 2017). External exposure of channel catfish fingerlings to copper sulfate in the water resulted in increased survival and extended protection from columnaris disease (Farmer et al. 2013). However, copper ions are able to bind to both organic and inorganic materials, which may limit the effectiveness of copper sulfate when used in a pond environment (Boyd 2012). Other mineral treatments such as kaolinitic clay can be used to treat the water. Kaolinitic clay has been found to improve survival by reducing the adhesion and colonization instance of *F. columnare* on the gills of channel catfish fingerlings (Beck et al. 2015). Though normally monitored in aquaculture for toxicity reasons, total ammonia nitrogen (TAN) levels can also be used to combat columnaris when there is a difference in host and bacterial pathogen TAN tolerance. In the lab, high total ammonia levels (15mg/L) reduced the concentration of *F. columnare* on the caudal fin of channel catfish and increased survival (Farmer et al. 2011). While there are many options for treatment, not all options will remain effective in the transition from lab to pond, and also from research scale to commercial scale.

F. columnare populations have been isolated from a variety of freshwater spp. including black mollies, catfish, eel, golden shiners, koi carp, rainbow trout, tilapia, and zebrafish (Declercq et al. 2013). However, related *F. columnare* vaccine trails have been almost exclusively performed on egg and fry stages within controlled laboratory settings. *Edwardsiella ictaluri* vaccines have been available to the commercial catfish industry for years, but high levels of associated mortalities are still reported (Klesius and Shoemaker 1999; Carrias et al. 2008; Hemstreet 2018). Unlike columnaris, continued ESC vaccine research has begun to include pond environments and commercial scale trials.

Channel catfish fry vaccinated with the commercially available AQUAVAC-ESC™ reported reduced mortalities even in pond trials, with 42.8% survival in sham-vaccinated fry increased to 61.5% in vaccinated fry (Nho et al. 2017). ESC vaccine research has also begun to include coated-feed technology as a delivery method, while maintaining high survival rates. Compared to non-vaccinated controls, vaccination significantly improved survival in coated-feed vaccinated fingerlings from 28.1% (control) to 54.3% (Wise et al. 2015). At harvest, the vaccinated fingerlings (90.7 kg) produced nearly double the biomass of the control (39.5 kg), with a 41% reduction in FCR from the control (2.2) to vaccinated treatment (1.3) (Wise et al. 2015). While our 17-23 vaccine trial results also found significant increases in biomass and feed conversion efficiency, vaccinated fish were fed less. Wise et al. found a significant increase in feed fed to the vaccinated treatment at 35 days post-stocking, however it was correlated directly with an increase in mortalities of the control group on that date (Wise et al. 2015). In a study that compiled data from 6 commercial catfish fingerling producers in Mississippi (from 2013 to 2016), production level effects of the avirulent *E. ictaluri* isolate (S97-773) vaccine used by Wise et al. have become apparent (Kumar et al. 2019). Again, channel catfish fry were stocked into ponds before being vaccinated using a coated-feed delivery method. Survival, total feed fed, and biomass harvested were all significantly higher for the vaccinated treatment, while average weight was not significant, and FCR was reduced. Kumar found significant survival improvements of ESC vaccinated channels (77%) over the control channels (58%), and FCR was reduced by 9% in the vaccinated fish (1.77) from control fish (1.94) (Kumar et al. 2019).

Trends from these two trials coincide with most of our production results, mainly by concluding that an FCR below 2.0 is possible with vaccine use. However, our trial vaccinated older fingerlings, which have a decreased feed conversion efficiency compared to fry due to body size and age (Robinson and Li 2015). Our study is novel in that our reduced vaccinated FCR (1.35) is a result of increased feed conversion efficiency rather than a difference in survival between treatments. Furthermore, without a large mortality event the production parameters are more comparable between treatments.

The economic analysis by Kumar et al. (2019) is a model example for our partial budget analysis of a commercial producer. His study found a much higher $6,072/ha in total benefits as a result of increased revenue (from fingerling yield) and no reduced costs compared to our total benefits ($1,590/ha from increased yield and reduced feed) (Kumar et al. 2019). Total costs ($2,124/ha) in their study were a result of $0/ha in lost revenue, plus the increased costs for additional feed fed to vaccinated fish. Our trial found only $107/ha in costs, though we vaccinated notably fewer individuals. Additionally in comparison, we must recognize that Kumar's study produced channel catfish fingerlings (average harvest weight = 33.5 g/fish) while we produced market-size channels (average harvest weight 1.73 kg/fish) from a total stocking rate of 18,983 fingerings/ha compared to their 300,602 fingerlings/ha.

The pricing of the 17-23 vaccine used in this trial was valued at $0.005 per vaccinated fish. However there is variation in prices, as AQUAVAC-COL™ was charged at $0.004 (C. Shoemaker, United States Department of Agriculture, personal communication). Using a price sensitivity analysis, a vaccinate unit price of $0.004 per fish would increase the trial net benefit to $1,491/ha (from $1,482/ha).

Utilizing the same method used to calculate net benefits for our research-level partial budget, we extrapolated those results to a 101-hectare farm, such as a typical farm size found in west Alabama (Creel 2020). In this scenario, there would be a potential total additional benefit of $160,560 due to an increase in harvest weight plus a decrease in feed cost. Total additional costs now total $10,831 as we would expect to vaccinate over 780,000 fingerlings and account for additional transport and loan interest costs. The result remains positive, predicting a $149,728 net benefit to the farm ($1,482/ha) from changing to columnaris vaccinated fingerling use. If a vaccination cost of $0.004 per fish were used, this would increase the net benefit to $150,547 ($1,491/ha).

A large mortality event would also have limited our understanding and application of the economic analysis. By detecting these changes in the production data, we were able to assume the minimum monetary benefits of the vaccine to producers without experiencing a columnaris disease outbreak. Survival is an instant measurement of success to a producer, as the image of a fish kill is immediately evident. Unfortunately, we cannot guarantee protection to producers with only this columnaris trial, but we are able to highlight results of the partial budget analysis. Even without an outbreak, a producer can theoretically save considerable amounts of money based on the improved feed efficiency observed in the vaccinated individuals. Costs associated with feed represents the highest variable cost for catfish farmers, so the ability to reduce feed fed is an instant benefit.

While our extrapolated data shows the potential benefits to a commercial producer when the vaccine is used on a whole-farm level, it also displays the benefits of vaccinating fingerlings over fry. The immune system in young fry are not fully developed in comparison to fingerling catfish, but are vaccinated at this stage for the convenience of producers during stocking from hatcheries into production ponds (Grace et al. 1981; Olivares-Fuster and Arias 2011; Nho et al. 2017). While immersion vaccine efficacy has been proven, the U.S. catfish industry demands low labor management techniques, resulting in limited efficacy due to the age of the fish at vaccination (Song 1986; Wise et al. 2015). The new coated-feed technology is a great bridge that can serve in both initial and booster vaccination events to any feed-trained fish. However, unlike the injection and immersion methods which guarantee equal vaccine exposure to all fish, coated-feed vaccine dosage is highly dependent on the individual's feeding amount (Nho et al. 2017).

Further research into the vaccine’s potential impact on survival is still needed and should consider including replicate ponds. The pond environment represents a difficult challenge due to variables associated with an outdoor and reduced-controlled environment. However, this variability is important for the adoption of vaccines within the catfish industry, and successful products must show benefits to the producer despite this variability. Our production results support advancement of the 17-23 columnaris vaccine towards commercial adoption, albeit further testing is needed in research and commercial ponds in which actual columnaris outbreaks occur to further validate the results of this study. Results from the partial budget analysis is an attractive benefit to catfish producers if the efficacy of the vaccine can be further substantiated under research and commercial settings. The vaccine success described in this study supports disease prevention methods as an effective farm management approach, rather than the reactionary method that is currently widely used through medicated feeds.

CONCLUSION

The results presented in this study provide a strong case for the continued research and direction of commercial application of the 17-23 columnaris vaccine. In summary, pond trials of the 17-23 columnaris vaccine improved production by using vaccinated channel catfish fingerlings in comparison to use of control non-vaccinated fingerlings, both from the biological and economic viewpoints. Initially, the columnaris immersion vaccination resulted in a significantly higher IgM mean antibody response in vaccinated fish over control fish, as detected through the ELISA. We suggest that this increased antibody response acted as a benefit to the vaccinated fingerlings, who were able to grow to harvest-sized catfish more efficiently. Efficiency was detectable through monthly sampling, which showed that vaccinated fingerlings did not vary in mean weight or length from their non-vaccinated counterparts until the final month before harvest (October). At harvest, we collected total biomass data from the pond and total feed amounts for both treatments. Overall, the vaccinated fish were fed less but produced a larger biomass at harvest, thus suggesting that vaccine 17-23 promoted an increased feed efficiency of the vaccinated channel catfish. This is further corroborated by the final FCR calculations, which showed a 36.6% reduction in the average feed conversion ratio from control to vaccinated fish, representing a 16% decrease in feed consumption. Without a large mortality event we were not able to assess the vaccine's impact on survival, but the vaccine impacts to production parameters (feeding amounts, FCR, biomass harvested) are encouraging. Our partial budgets present the minimum economic benefit to a producer on the research level, as well as potential net benefits on a more applicable commercial scale. Feed efficiency appears to be the catalyst for the benefits observed in this trial in which there was no observable or measurable incidence of columnaris. While the data is encouraging for commercial farmers, further production studies are warranted in which measured disease incidences are recorded to further corroborate the benefits of disease management through this columnaris 17-23 vaccine adoption.

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TABLES

Table 1. Water quality from a 25-week experimental pond trial in 0.04 ha ponds culturing vaccinated (n = 5 ponds) and control fingerlings (n = 5 ponds) at the E.W. Shell Fisheries Station, Auburn University, 2019. Data represents the mean ± standard error of values.

	Dissolved Oxygen (mg/L)	**pH**	**TAN (mg/L)**	**NO2- (mg/L)**	**Chloride (mg/L)**	**Hardness (mg/L)**	**Alkalinity (mg/L)**
Control	9.2 ± 3.5	7.2 ± 0.4	0.0 ± 0.2	0.0 ± 0.0	0.4 ± 0.4	78.2 ± 16.0	15.5 ± 18.0
Vaccinated	9.3 ± 5.2	7.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	77.7 ± 10.4	10.2 ± 13.1

Table 2. Production results for a 25-week pond trial conducted in ten ponds (0.04 hectare) for columnaris vaccinated (n = 5) and control non-vaccinated (n = 5) channel catfish fingerlings at the E.W. Shell Fisheries Station, Auburn University, 2019. Data represents the mean ± standard error of values.

Production Parameters	**Vaccinated**		**Control**		**p Value**
Avg. Stocking Rate (fish/ha)	19,430	± 156	18,535	± 22	< 0.001
Avg. Weight. at Stocking (kg/1,000 fish)	133	± 0	133	± 0	1.00
Total Weight Stocked (kg/ha)	535	± 4	510	± 0.6	< 0.001
Survival (%)	85	± 8	84	± 6	0.74
Total Feed Fed (kg/ha)	7279	± 1300	8437	± 466	0.12
Total Weight Harvested (kg/ha)	5,940	± 833	4,488	± 378	0.01
Avg. Weight at Harvest (kg/fish)	0.36	± 0.03	0.29	± 0.02	0.002
Feed Conversion Ratio (FCR)	1.35	± 0.14	2.13	± 0.17	< 0.001

Table 3. Partial enterprise budget showing the effect of changing from use of traditional non-vaccinated fingerlings to use of columnaris vaccinated fingerlings for channel catfish production on a per hectare basis, Auburn, Alabama 2019.

Benefits			**Costs**		
A. Additional Income			**C. Reduced Income**		
I. Harvest Weight			*I. None*		
Difference in Weight Harvested, kg	Unit Price, $/kg	Value	None	Unit Price, n/a	Value
580	$ 2.404	$ 1,395			$ 0
B. Reduced Cost			**D. Increased Cost**		
I. Feed			*I. Vaccine*		
Additional Feed Fed to Control Fish, kg	Unit Price, $/kg	Value	Number of Vaccinated Fish	Unit Price, $/ea.	Value
463	$ 0.421	$ 195	7,772	$ 0.005	$ 39
			*II. Harvest and Transport**		
			Difference in Weight Harvested, kg	Unit Price, $/kg	Value
			580	$ 0.11	$ 64
			III. Interest on Variable Cost		
			Sum of I, II	Interest on Short-term Loan, %	Value
			$ 103	4.21	$ 4
Additional Benefits		**$ 1,590**	**Additional Costs**		**$ 107**
Add. Benefits – Add. Costs = Net Benefit		**$ 1,482**			
A positive net benefit indicates that we should accept the change to producing vaccinated fish.					

** The difference in mean total harvest weight of vaccinated treatments minus control treatments.*

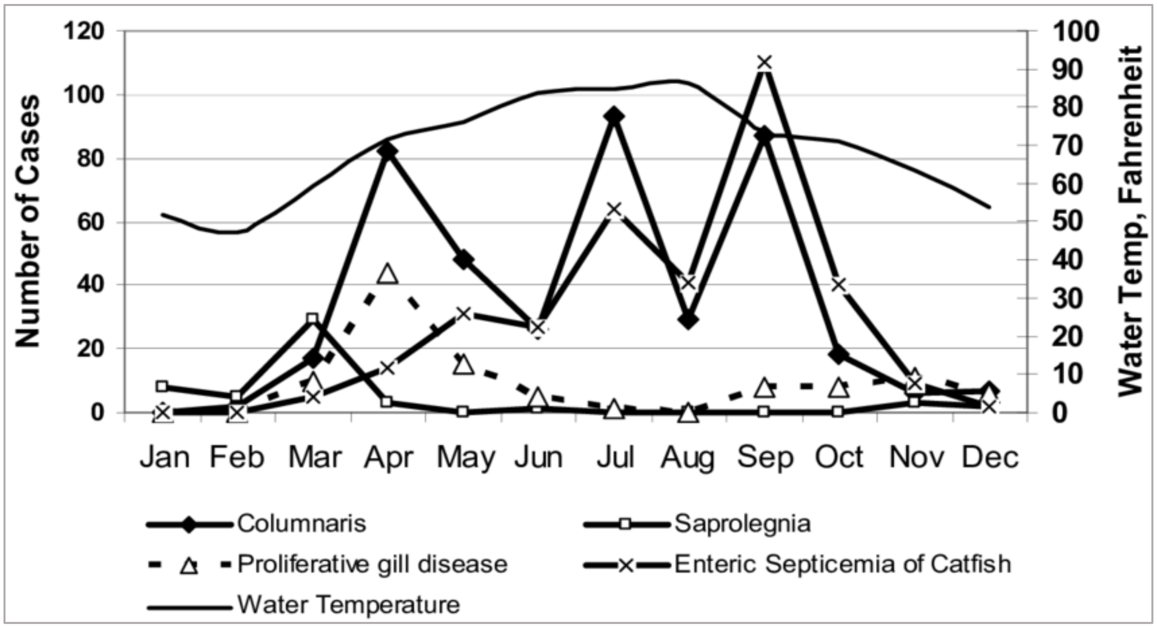
Table 4. Partial enterprise budget showing the effect of changing a 101-hectare farm from use of traditional non-vaccinated fingerlings to use of columnaris vaccinated fingerlings for channel catfish production, Auburn, Alabama 2019.

Benefits			**Costs**		
A. Additional Income			**C. Reduced Income**		
I. Harvest Weight			*I. None*		
Difference in Weight Harvested, kg	Unit Price, $/kg	Value	None	Unit Price, n/a	Value
58,610	$ 2.404	$ 140,896			$ 0
B. Reduced Cost			**D. Increased Cost**		
I. Feed			*I. Vaccine*		
Additional Feed Fed to Control Fish, kg	Unit Price, $/kg	Value	Number of Vaccinated Fish	Unit Price, $/ea.	Value
46,760	$ 0.421	$ 19,664	784,972	$ 0.005	$ 3,925
			II. Harvest and Transport*		
			Difference in Weight Harvested, kg	Unit Price, $/kg	Value
			58,610	$ 0.11	$ 6,469
			III. Interest on Variable Cost		
			Sum of I, II	Interest on Short-term Loan, %	Value
			$ 10,394	4.21	$ 438
Additional Benefits		**$ 160,560**	**Additional Costs**		**$ 10,831**
Add. Benefits – Add. Costs = Net Benefit		**$ 149,728**			
A positive net benefit indicates that we should accept the change to producing vaccinated fish.					

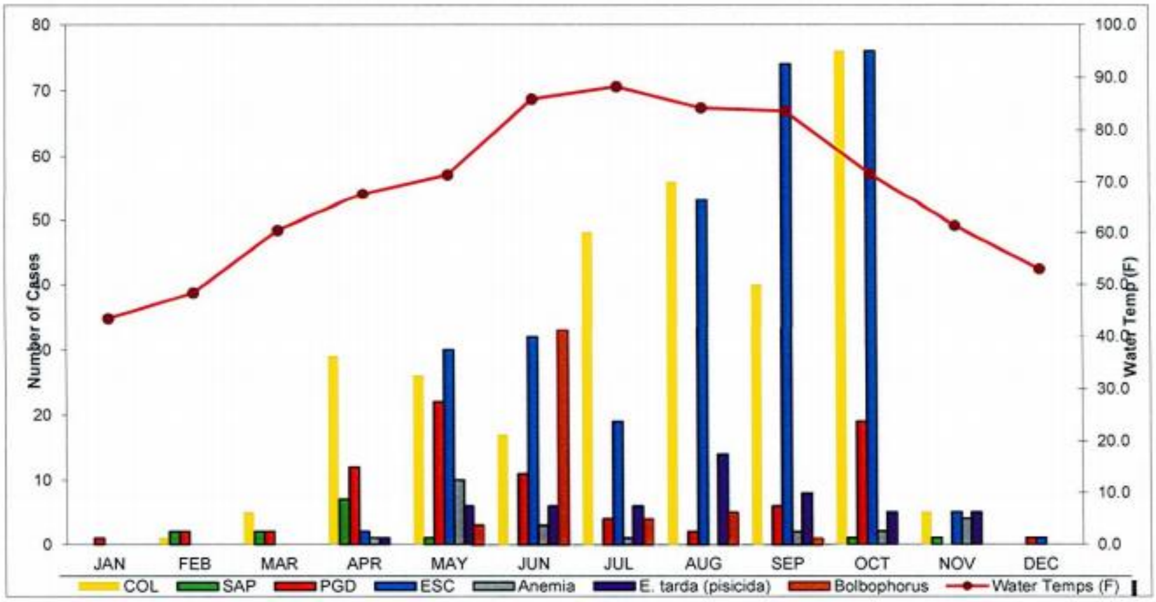
* The difference in mean total harvest weight of vaccinated treatments minus control treatments.

FIGURES

Figure 1. Comparison of seasonal occurrence of catfish diseases in the U.S. catfish industry in 2006[a] and 2016[b] as reported by the National Warmwater Aquaculture Center Diagnostic Lab in Stoneville, Mississippi[c].



[a]Source: Mississippi State University (2006).



[b]Source: Mississippi State University (2017).

[c]As measured by the number of fish submitted and diagnosed for a specific disease by the National Warmwater Aquaculture Center in Stoneville, Mississippi.

Figure 2. Comparison of control (2a) and vaccinated (2b) treatment average individual weights at monthly sampling events during pond trials using columnaris vaccinated fingerlings for channel catfish grow out at the E.W. Shell Fisheries Station, Auburn University, 2019.

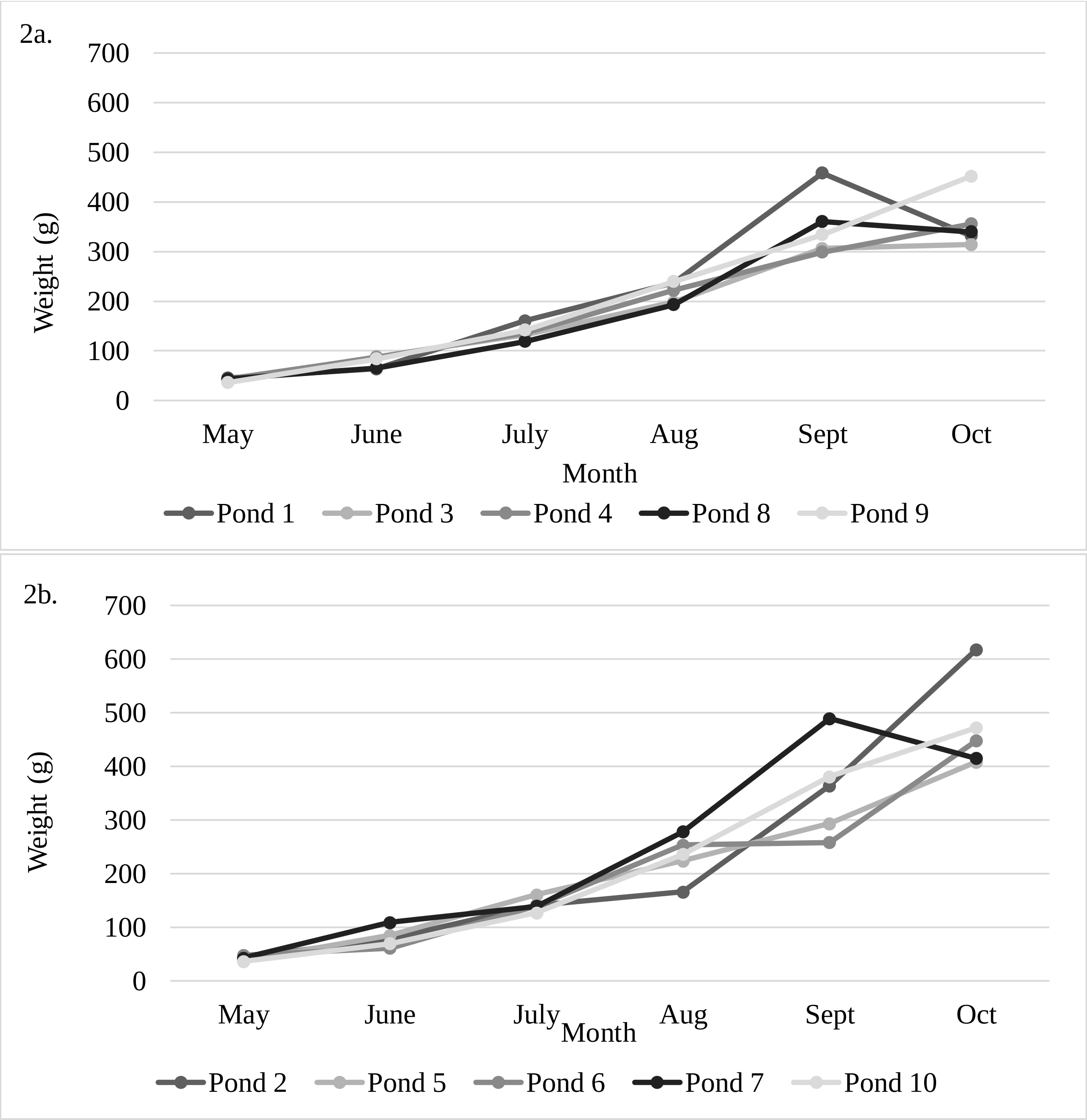


Figure 3. Comparison of monthly control and columnaris vaccinated channel catfish mean weights for channel catfish grow out at the E.W. Shell Fisheries Station, Auburn University, 2019.

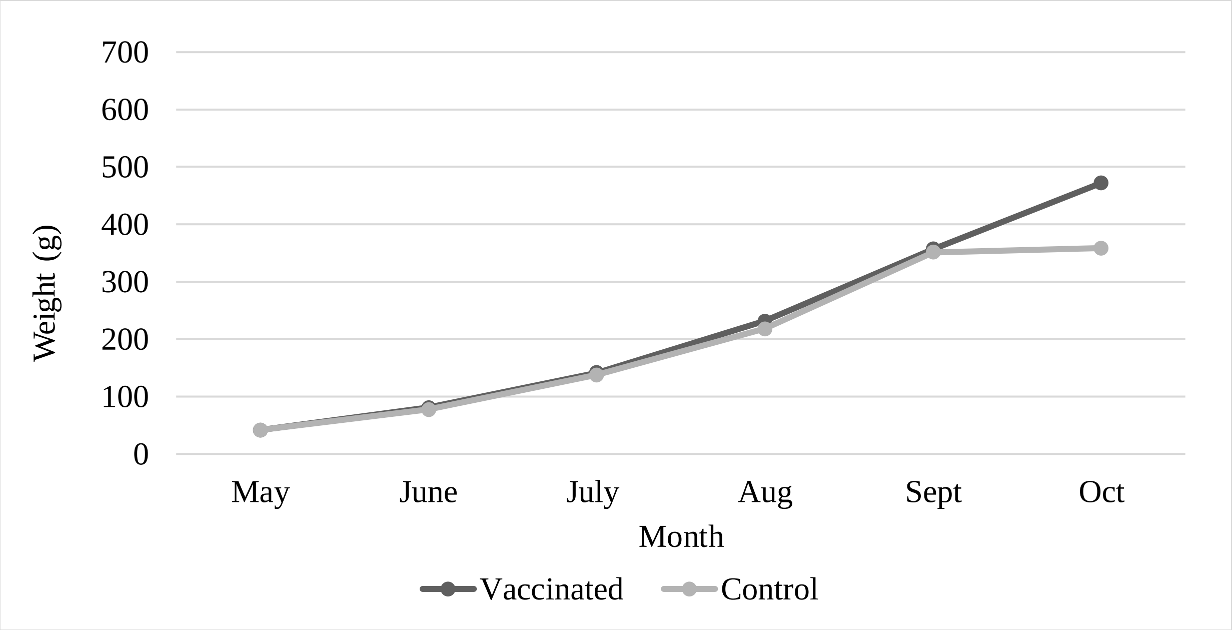
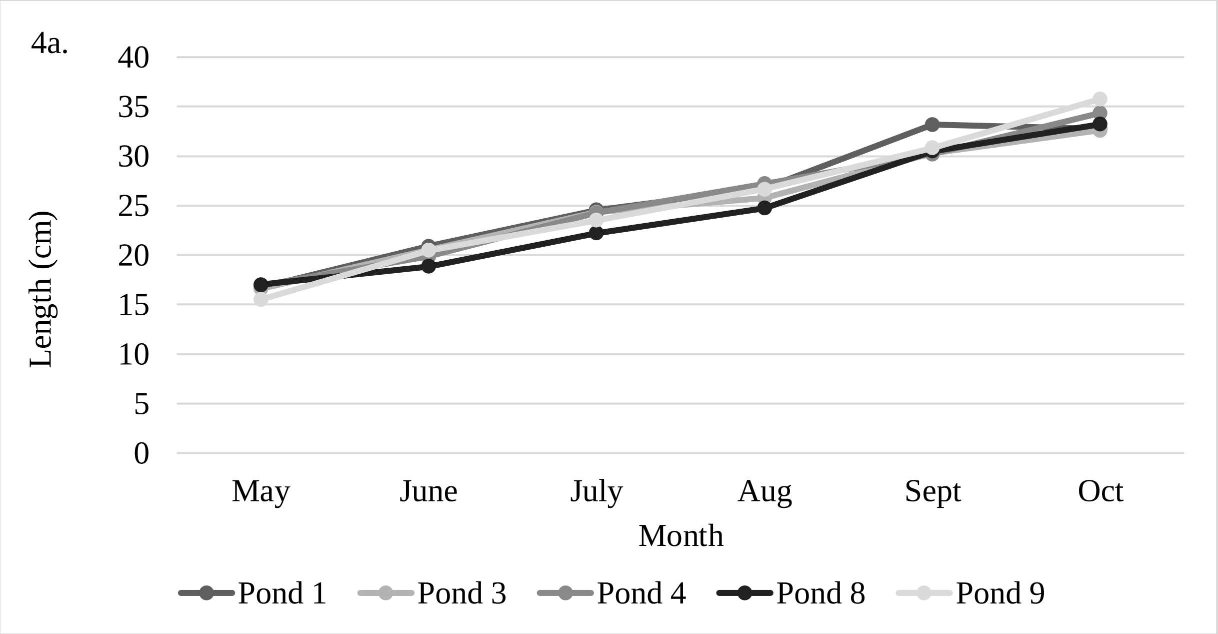


Figure 4. Comparison of control (4a) and vaccinated (4b) treatment average individual lengths at monthly sampling events during pond trials using columnaris vaccinated fingerlings for channel catfish grow out at the E.W. Shell Fisheries Station, Auburn University, 2019.



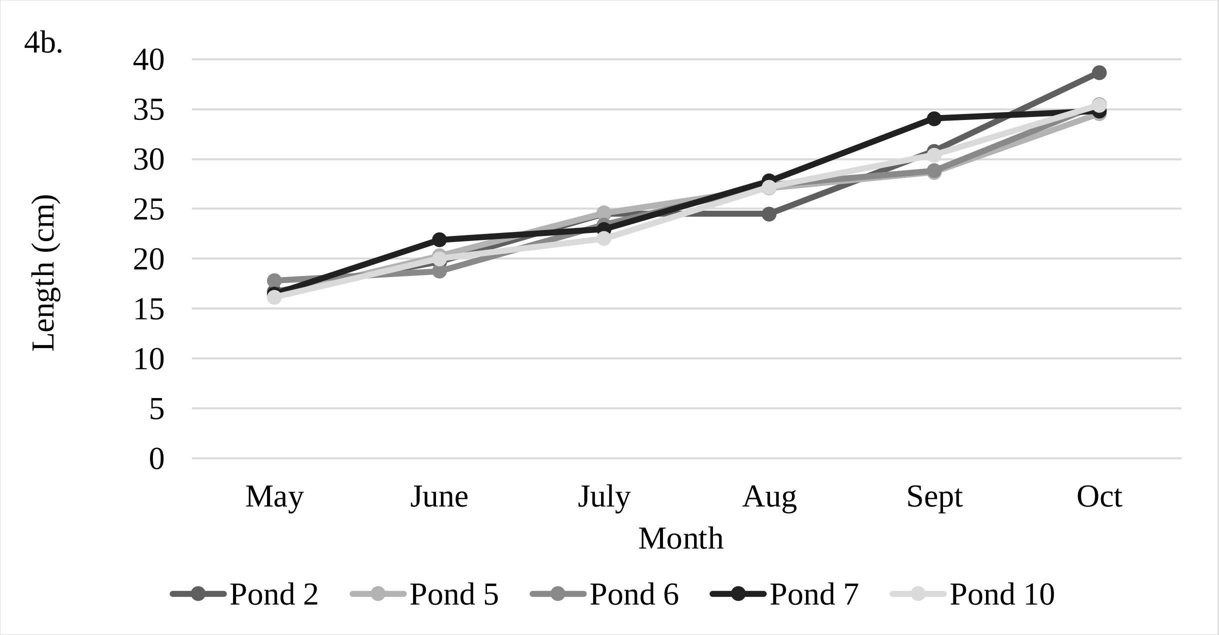


Figure 5. Comparison of control and columnaris vaccinated fish mean lengths from sampling events using columnaris vaccinated or control non-vaccinated fingerlings for channel catfish grow out at the E.W. Shell Fisheries Station, Auburn University, 2019.

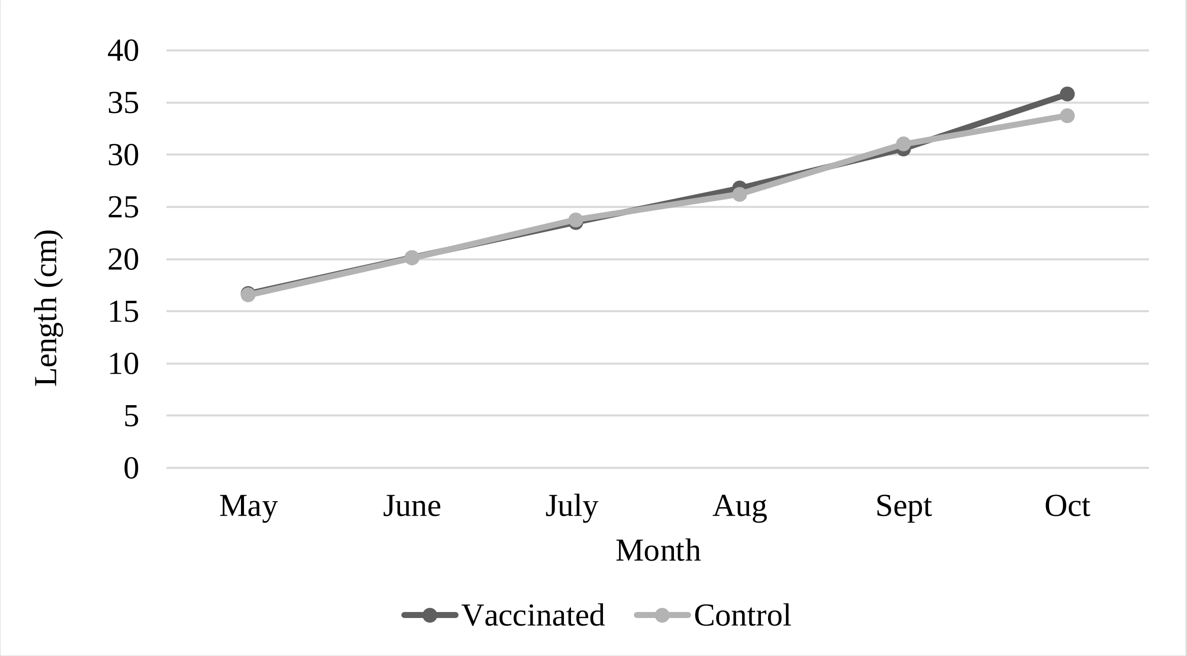


Figure 6. Average daily feed amounts fed to columnaris vaccinated and control non-vaccinated fish during the 25-week pond trials of channel catfish produced at the E.W. Shell Fisheries Station, Auburn University, 2019.

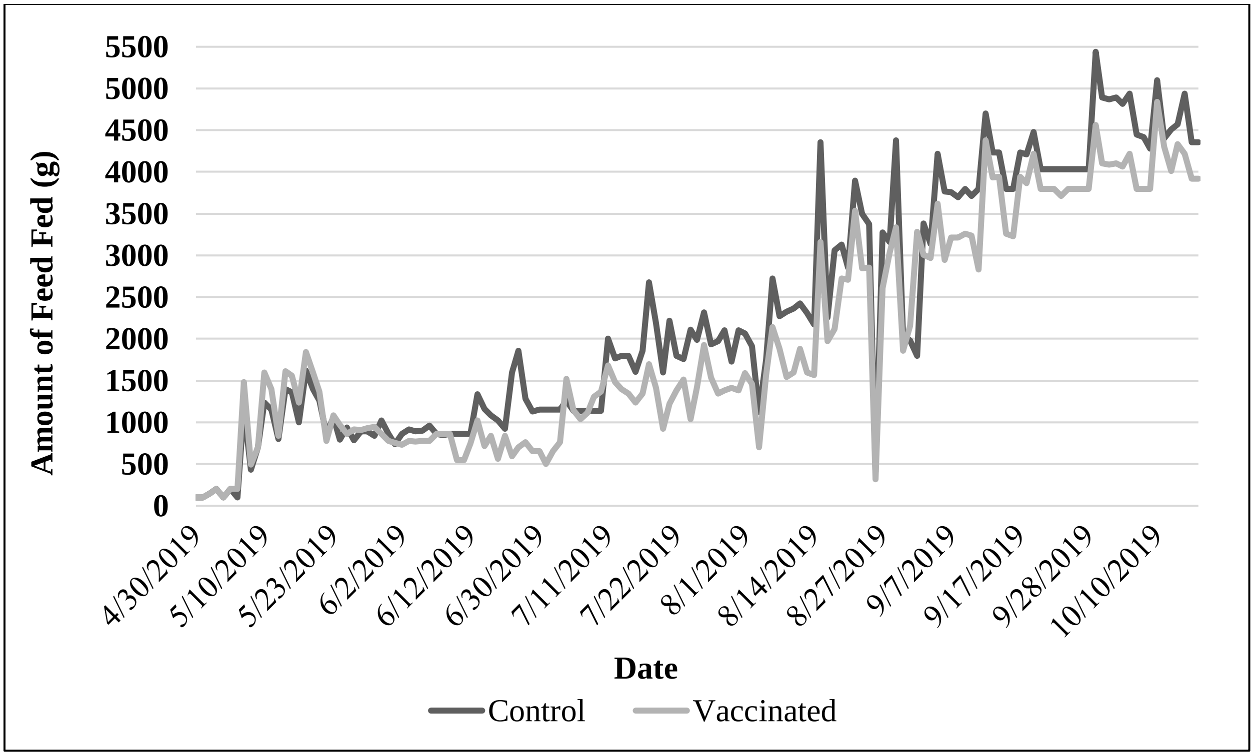
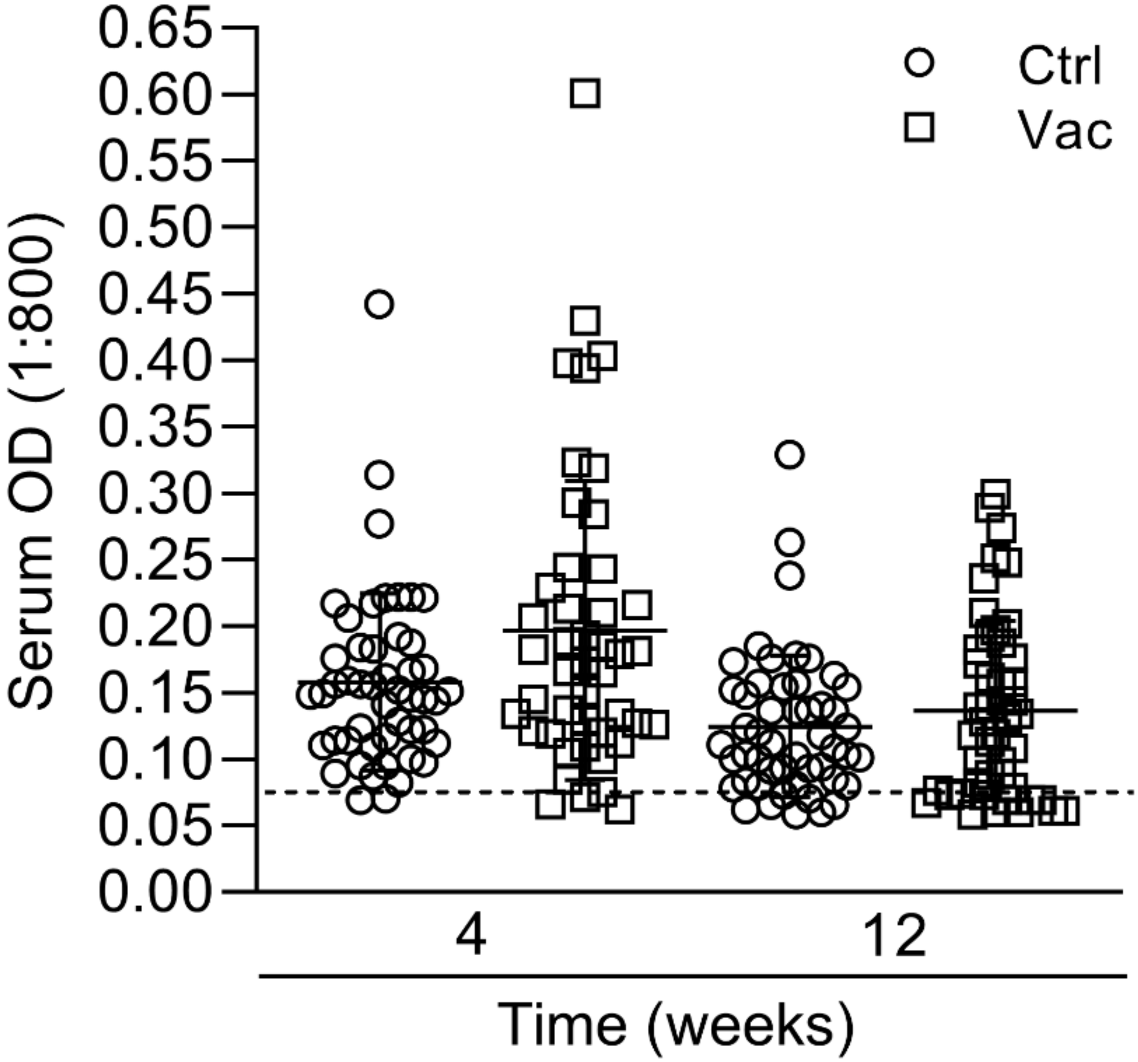


Figure 7. Mean immunoglobulin M (IgM) antibody concentrations of columnaris vaccinated and control fish from blood samples obtained at week-four and week-twelve post-stocking during experimental pond trials using vaccinated fingerlings for channel catfish produced at the E.W. Shell Fisheries Station, Auburn University, 2019.



APPENDIX

Appendix 1. Experimental pond layout of the 25-week trial conducted in ten 0.04 hectare ponds comparing columnaris vaccinated and control non-vaccinated channel catfish fingerlings produced at the E.W. Shell Fisheries Station, Auburn University, 2019.