Alabama Fish Farming Center ***Fish Farming News, 2024(1):1-16***

EXTENSION NEWSLETTER

White Spot Syndrome Virus:
An Emerging Disease in Crawfish

Anita Kelly, AFFC

Crawfish farming in Alabama is on the rise. Due to shortages of crawfish from Louisiana, it has become a profitable enterprise. Unfortunately, Alabama producers face an emerging disease confirmed in crawfish in eastern Alabama. White Spot Syndrome Virus (WSSV) is a devastating disease that has caused significant losses in shrimp farms globally. Initially found in Thailand, global trade has allowed WSSV to be translocated to several countries, including the U.S. WSSV has been found in crawfish in Louisiana since 2007. Cases have been reported in farmed crawfish every year since, usually in March or early April. Although white spot syndrome is technically a virus, it affects only crustaceans. It cannot infect people or other animals.

Figure 1. Jensen James, 6 year old son of AFFC Research Associate, Jesse James, helps his Dad trap crawfish on the family's crawfish farm.

Typical signs of a white spot syndrome outbreak are a drastic drop in catch over just a few days. Most medium-sized and large crawfish die, but small crawfish continue acting normally. Dead crawfish often float throughout the pond or windblown along the levees. Large crawfish that aren't dead are usually slow-moving and uncoordinated.

The producer faces severe economic loss when a pond "breaks" with white spot syndrome. Despite numerous documented cases, the factors that trigger these outbreaks have never been well understood.

Current research focuses on the genetic selection of crawfish unaffected by the virus. Currently, the Alabama Fish Farming Center, in coordination with Auburn University, is testing crawfish for WSSV. This testing is free and hopefully will prevent the disease from occurring in west Alabama.

Do big hybrid catfish cannibalize smaller catfish fingerlings on commercial farms?

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Since 2018, the Alabama Fish Farming Center has carried out research to evaluate the impact of big fish (fish that exceed the premium market size of 1.25 - 4 lbs) on the Alabama catfish industry. Using otoliths (small bones in the head of catfish that have growth rings) extracted from hybrid and channel catfish at a west Alabama processing plant, initial work confirmed that hybrid catfish reached this threshold at a much younger age than channel catfish due to a faster growth rate and could attain much larger sizes than channel catfish. More recently, field work in 2022 quantified the age structure of hybrid catfish remaining in commercial ponds after several harvests and immediately prior to restocking. This study determined that hybrid catfish exceeded 4 lbs at 2.7 years of age and 8 lbs at 3.2 years of age. The study also confirmed that large amounts of big fish remained in ponds even after time, effort, and expense had been expended by farmers to fully harvest these single-batch ponds prior to stocking a new crop.

There is little information in the literature on cannibalism in pond-raised catfish (Figure 1). The primary research study that is cited on this topic (Torrans and Ott 2016, *North American Journal of Aquaculture* 78(1):52-56), stocked fingerlings on top of market size hybrids (mean of 2.5 lbs at stocking). At harvest, these fish had attained an average size of 5.9 lbs by the end of the summer trial. The average weight (5.9 lbs) of the hybrid catfish at the end of the study was much smaller than the size potential of a hybrid catfish in commercial ponds. The largest hybrid catfish collected by the Alabama Fish Farming Center over the course of the two previously mentioned studies was 54 lbs (9-year-old fish), and hybrid catfish over 20 lbs were routinely collected in our age and growth studies. Farmers and processing plants have noted that the number of fish deemed to be big fish by the plant from a single pond can exceed 10,000 lbs (or more) in some cases. The effect of large numbers of carryover big hybrid catfish on fingerling survival is a scenario that is unstudied in the catfish industry.

Figure 1. Cannibalism by big hybrid catfish has been noted on multiple occasions by personnel at the Alabama Fish Farming Center in recent years.

Since initiating the age and growth work on catfish farms in west Alabama, we have heard quite a few anecdotal stories by catfish farmers of cannibalism on hybrid catfish fingerlings by larger carryover hybrids remaining in ponds. Many farmers suspect that cannibalism could be contributing to lower survival and production. This winter, one farmer informed us that while standing on top of a live haul truck as it delivered fingerlings to his farm, he observed several large hybrid catfish feeding on hybrid fingerlings as they were stocked into his pond. He noted that several large hybrids were directly under the ramp used to offload the fish with their mouths open while directly “inhaling” fingerlings as they were stocked. The Fish Center has documented catfish fingerlings in the stomach contents of large hybrid catfish on several occasions in big fish we have collected, including a 1.36 lb premium market size hybrid inside of a 27 lb hybrid catfish. Despite these anecdotal observations and several occasions where we have observed it firsthand, there is very little information available on this topic.

This summer, the Fish Center is coordinating with E.W. Shell Fisheries Station to carry out a preliminary pond experiment to further explore this issue. Big hybrid catfish have been sourced from west Alabama (6.25 – 29.6 lbs) and transported to Auburn for the study (Figure 2). These fish are much larger than the fish used by the Torrans and Ott (2016) study. The experimental treatments for this trial are as follows:

Treatment 1: Fingerlings with no big fish (fed a normal ration) to serve as a control
Treatment 2: Fingerlings + big fish (fed a normal ration)
Treatment 3: Fingerlings + big fish (unfed)

Figure 2. Big hybrid catfish were collected from a west Alabama farm for the cannibalism study.

This experiment is currently ongoing, and we will report the results at a future time. In the next few production seasons, we plan to examine the issue of cannibalism in further detail using controlled pond studies at the E.W. Shell Fisheries Research Station, as well as in commercial ponds in west Alabama.

Price Volatility in Catfish and Catfish Feeds

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Price fluctuations can be a major issue for fish farmers. In recent decades, we have observed increased price volatility for many agricultural commodities. Fluctuations in income and input costs create uncertainty for farmers, make financial planning and investment decisions more difficult, and can threaten their long-term profitability. In some agricultural industries, there are price stabilization programs and policy responses to price fluctuations. Currently, there are no such programs available to aquaculture producers.

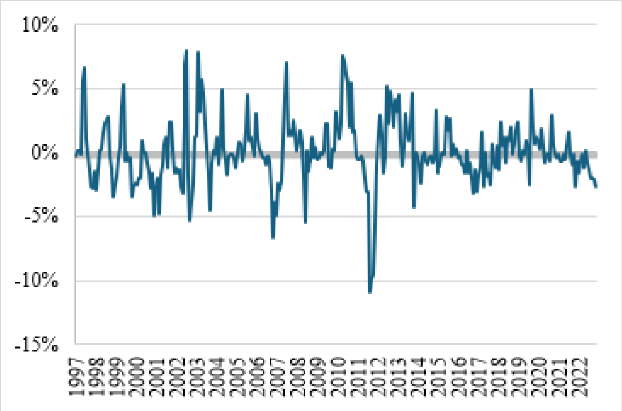

Figure 1. Percent change in monthly catfish price.

What causes price volatility?

Income volatility and feed price fluctuations are generally influenced by supply and demand dynamics but can be intensified by other macroeconomic variables such as the political environment, international trade, exchange rates, etc. As agricultural markets have become more open and international trade has increased, price and income volatility have also increased for many agricultural commodities.

Exogenous conditions, such as changing weather conditions and natural disasters, can also impact crop production and prices, which strongly influences the price of fish feeds. Existing studies have shown that volatility in many catfish feed ingredients translates to greater volatility in the price of catfish.

Price volatility in U.S. catfish

In the last decade, U.S. catfish production has stabilized at just over 300 million pounds. Since then, there has been considerably less volatility in the price producers receive compared to earlier time periods (Figure 1). High price volatility observed in 2007 – 2008 and 2011 – 2012 is consistent with other agricultural commodities affected by the global food price crisis, and higher volatility before 2007 was likely driven by fluctuations in supply (both domestic and imported).

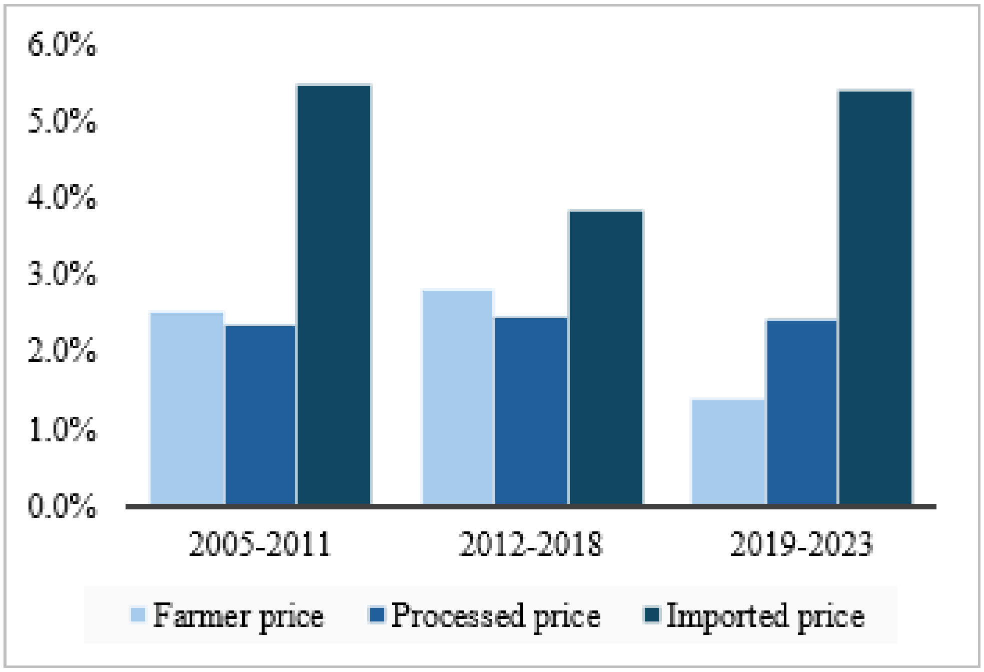

Figure 2. Volatility (standard deviation of percent change) in monthly farmer, processor and imported price.

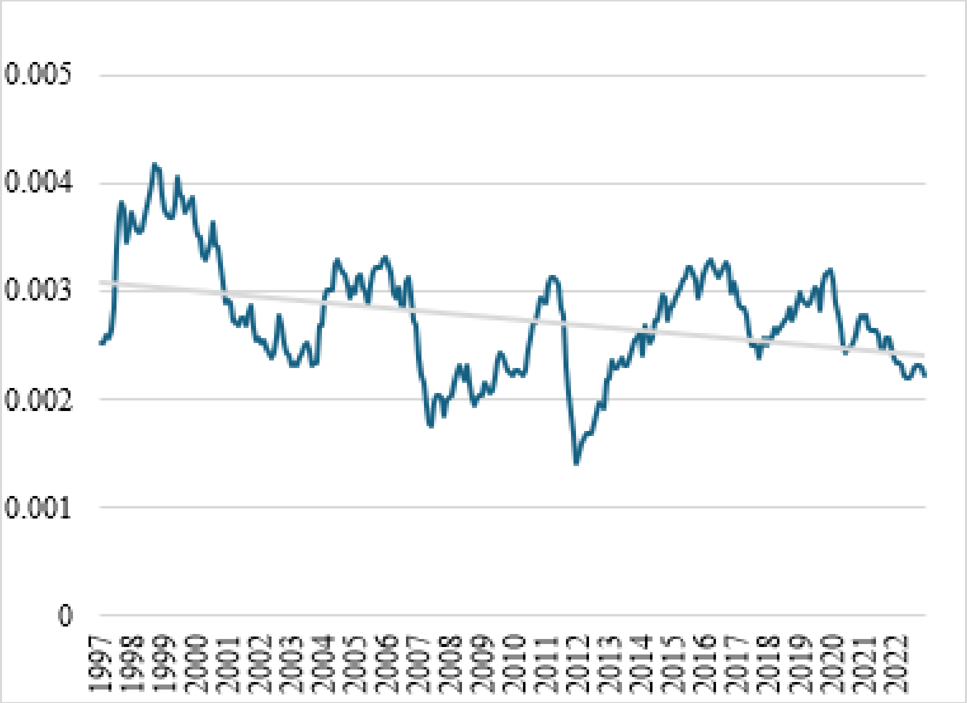

Figure 3. Ratio of catfish price to feed price.

Figure 2 shows volatility at the farmer and processor levels and for imported catfish fillets. The volatility in prices received by the farmers has declined in the last time period, however, price volatility has not declined at the processor level. Prices of imported catfish fillets are much more volatile across all time periods. This is not uncommon for imported products that are subject to exchange rates and transportation costs.

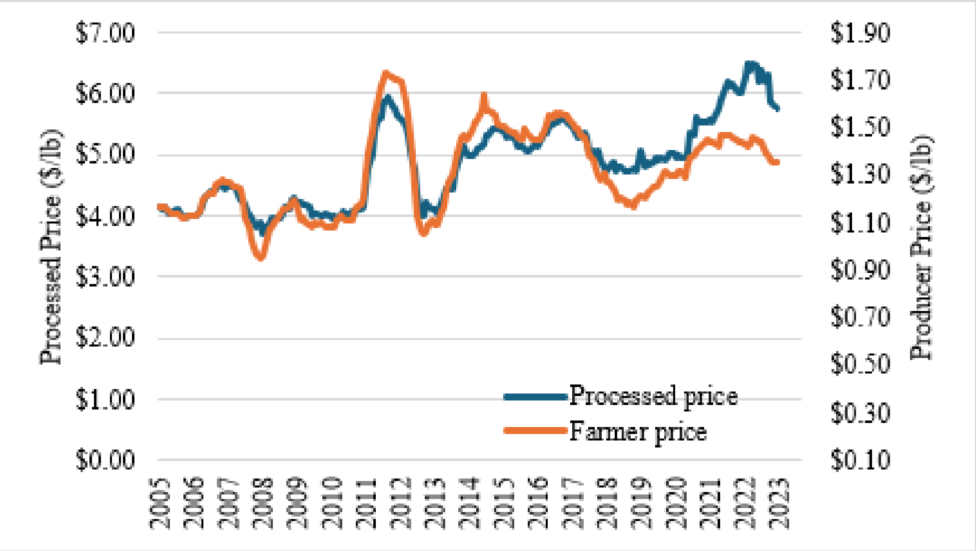

Figure 4. Producer price of premium sized catfish ($/lb) and processed price of frozen fillets ($/lb).

Dynamics in catfish feed prices

Feed is the largest cost in catfish production, accounting for about half of total variable costs. Feed prices have increased from the end of 2020 to early 2023, significantly impacting profit margins of catfish farmers. Volatility in feed price has shown a similar trend to catfish price – i.e., lower volatility in the last decade.

The ratio of catfish price to feed price is a broad indication of input costs relative to income (Figure 3). The ratio's overall decreasing trend indicates that the price of feed has risen more quickly relative to the price of catfish and suggests that already tight margins are becoming even tighter. Catfish farmers are price takers rather than price makers, and thus it is more difficult to pass on the increase in feed price. When catfish prices are low, farmers need to closely monitor feed costs to maintain a sustainable margin. We also find the ratio of the producer's price to the processed price of frozen fillets has declined in recent years. The producer price and processed price followed one another very closely up until 2017 (Figure 4). Up until this point, the ratio was relatively stable around 28%. Since 2017, the ratio has declined to about 23%, which may be the result of increased processing costs.

Managing price risks

There are some management practices to help mitigate challenges associated with price volatility. One option for coping with income volatility is to save earnings when margins are high and use reserves when margins are low. Another option is to diversify production across multiple commodities. Forward contracts and futures contracts can be useful to help reduce price risk when planning feed purchases. Many agricultural commodities have a futures market where standardized contracts are traded through organized exchanges. Forward contracts, on the other hand, are privately negotiated between the seller and buyer. Most seafood commodities, including catfish, are not traded in futures markets, but major feed ingredients like corn and soybeans are traded. Even if producers don't hedge using futures contracts, futures markets can still provide helpful information about what prices are expected to do in the future. Forward purchasing, or booking some feed inputs in advance, is a risk-management strategy and can be advantageous for financial planning and can partially protect the farmer from future increases in feed price. See Hanson and Sawadgo (2022) for more about futures markets and forward purchasing of feed inputs. Many other agricultural commodities have government programs to help producers mitigate their price risks. For example – the hog, beef cattle, and dairy sectors all have some type of margin insurance tools that can help offset losses when margins get very tight. While it has been considered in the past, catfish producers currently do not have any such tool.

RESEARCH ROUNDUP

Impact of Temperature on Antibiotic Treatments in Catfish

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Antibiotic resistance is a growing concern worldwide, affecting human and animal health. As the consumption of freshwater fish, like catfish, increases, so does the concern about antibiotic resistance in fish farming. In the U.S., catfish farming is a major industry but faces challenges from bacterial diseases that can cause significant losses. These diseases often depend on water temperature, with certain temperatures favoring the growth of different bacteria. When these bacteria cause disease outbreaks in fish, veterinarians can write a Veterinary Feed Directive for one of three FDA-approved antibiotics: Terramycin® (oxytetracycline hydrochloride), Romet® (a combination of sulfadimethoxine and ormetoprim), and Aquaflor® (florfenicol). These antibiotics work against a wide range of bacteria and are chosen based on their effectiveness at various water temperatures. However, how long these antibiotics last in the water and their impact on the bacteria and other microorganisms can vary with temperature. In addition, the temperature of the water plays a crucial role in shaping the bacterial community in production ponds. The makeup of these bacterial communities can significantly influence fish health, growth, and development.

To better understand how different temperatures and antibiotics affect antibiotic resistance in fish farms, a study was conducted at the Alabama Fish Farming Center in Greensboro, AL. We tested the three FDA approved antibiotics at different water temperatures (68°, 77°, and 86° F) in tanks. Specifically, Aquaflor® was given to fish in four tanks for 10 days, followed by a 15-day withdrawal period. Romet® was administered for five days, followed by a three-day withdrawal, and Terramycin® was used for 10 days with a 21-day withdrawal period. Fish in four other tanks received no antibiotics and served as the control group. We collected a 1-quart water sample from each tank before and after antibiotic treatment and at the end of the withdrawal period. These samples were processed to study how many and the number of different bacteria that were present. The results showed that the total bacteria present in the control water sample was highest at 86 ° F, and decreased with decreasing water temperatures. However, once the fish were provided antibiotics, no differences in bacteria counts were observed between the three water temperatures.

In ponds, bacteria can develop antibiotic resistant genes that can be transferred to fish. The presence of antibiotic resistant genes was similar between all temperatures tested. However, the types of antibiotic resistant genes increased as water temperatures increased. Interestingly, the effect of the same antibiotic can change with the water temperature. For example, after treating fish with Aquaflor, certain antibiotic resistant genes considered very important by health experts, like glycopeptides, lipopeptides, and beta-lactams, increased significantly only when the water was at 86 °F.

This study reveals how the types of microbes and their resistance to antibiotics in catfish farming waters vary with water temperature and antibiotic use. We discovered that the response of the antibiotic resistant genes is affected by temperature, with higher prevalence observed at more extreme temperatures. Furthermore, warmer temperatures, especially at 86 °F, significantly enhanced the diversity of antimicrobial resistant genes. We also found a temperature effect on the overall mix of bacteria in the water, and this effect remains even after using antibiotics. Understanding these dynamics is essential for effectively managing catfish farms, ensuring the health of cultured fish populations, and preventing practices that may heighten the risk of developing antibiotic resistance, thereby safeguarding both aquaculture sustainability and food safety.

Understanding the Effect of Elevated Water Temperature on Growth and Health of Channel Catfish

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Figure 1. Catfish pond in Dallas County, Alabama.

Channel catfish is the most important and popular fish species commercially produced in the United States. Catfish production in the U.S. is dominated by three states: Mississippi, Alabama, and Arkansas. Among these three, Alabama accounts for about 30% of production. The optimal growth of channel catfish occurs between 82 - 85°F. Thus, the warmer southern states provide the best conditions for channel catfish growth. However, catfish farmers in the US face various challenges in operating an economically profitable farm.

Feed cost is one of the main challenges catfish farmers face. Initially, this was due to the high cost of fish meal, which is the primary protein source in catfish diets. To minimize this problem, farmers shifted many years ago to a diet with higher plant protein sources than animal protein sources. Currently, catfish diets are based on soybean meal as the primary protein source, and animal proteins such as fish meal, porcine meal, and poultry meal are used in lesser amounts.

Catfish farmers in west Alabama are facing another major problem. There have been many instances where farmers have reported decreased feeding responses in catfish, particularly during summer. During this period, the water temperature is the highest and rises above the optimal temperature for catfish growth. In some cases, when temperatures remain high for extended periods of time, fish become more lethargic and feeding efficiency is reduced. This leads to a reduction in production and profitability. Initial investigations of this problem by personnel at the Alabama Fish Farming Center in west Alabama concluded that affected fish were healthy, but had a large amount of undigested feed in the gut, especially when water temperatures approached or exceeded 90°F for prolonged periods of time. Based on the full guts of many fish, it was suspected that plant and alternative animal-based proteins other than fish meal might not be as digestible at high temperatures, causing problems.

To investigate this problem, a growth trial for channel catfish raised at two different temperatures with different protein sources was conducted at E W Shell Fisheries Station, Auburn University. The trial was conducted for 8 weeks in two recirculating systems (higher temperature and lower temperature), each with twelve polyethylene tanks with a volume of 210 gallons. The higher temperature system was maintained at 86±1°F while the lower temperature system was maintained at 79±1°F. Each tank was stocked with 15 channel catfish, weighing approximately 0.5 lbs each. Tanks were randomly assigned to one of four dietary treatments.

Diets were formulated to contain soybean meal as a primary protein source and supplemented with a 6% alternative protein source either with a) fishmeal, b) porcine meal, c) poultry meal, or d) beef bone and meat meal in the diet. The fishmeal diet was treated as a basal control in this study. Catfish were counted and bulk-weighed at eight weeks to determine survival and growth performance. Blood and liver were collected from 3 fish per tank for plasma lysozyme assay, cortisol assay and gene expression.

Under stable water quality (DO=6.8±0.8 mg/L, pH=7.0±0.1), at the end of the study, there were no differences in total biomass of the tank, mean weight, or weight gain percent; however, food conversion ratio (FCR) was increased in the lower temperature system compared to the higher temperature system. Consequently, there was an improved survival in the lower temperature system compared to the higher temperature system.

Plasma lysozyme, one of the first lines of defense in catfish, was also influenced by temperature, where fish in the lower temperature system had higher lysozyme concentrations than fish in the higher temperature system. Cortisol in blood plasma was also determined, as this is a measure of the stress level in fish. However, no differences were found among different temperature systems or different treatments. Some of the growth-related gene expressions were also analyzed. Again, no differences were found among dietary treatments or temperature for these genes.

The results from this study highlight important temperature effects on feed efficiency and survival of channel catfish during periods of high temperature, such as the hot summer months, which can inform effective management decisions for commercial catfish production. As this problem prevails also in hybrid catfish, more studies are underway to assess the effect of high-water temperatures on their growth and health. In the future, studies need to evaluate slightly higher water temperatures than were achieved in the higher temperature treatment in this study, such as water temperatures > 90°F.

Figure 2. Polyethylene tanks used for the trial.

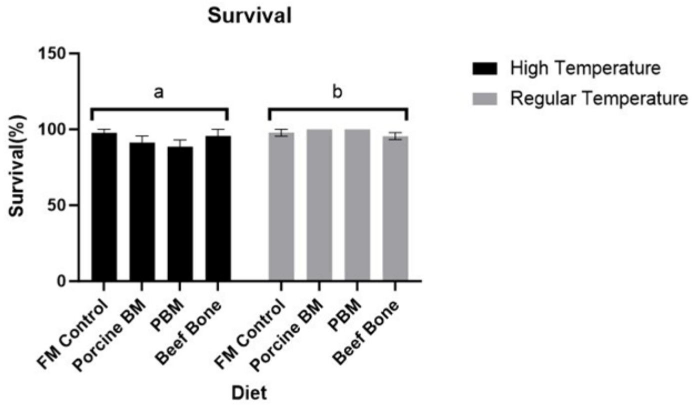

Figure 3. Survival of channel catfish after 8 weeks.

Experimental Coinfection with Columnaris and Channel Catfish Virus Disease in Channel Catfish Fingerlings

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Bacterial pathogens are a significant concern for Mississippi and west Alabama catfish producers. The primary bacterial pathogens encountered are *Flavobacterium covae* (columnaris disease; previously under *F. columnare*), *Aeromonas hydrophila* (motile Aeromonad septicemia), and *Edwardsiella ictaluri* (hole-in-the-head disease). These diseases can move quickly through ponds, and it’s critical to understand the mechanics of these infections. In addition to bacterial pathogens, channel catfish virus (CCV) can also be an issue in production, and it primarily occurs in juveniles. This virus primarily impacts fingerlings during the summer when water temperatures are hot. For clinical signs, juvenile fish often present an extremely swollen abdomen, petechial hemorrhaging, and yellow mucus through the gastrointestinal tract. Although this virus has been researched for decades, several questions remain regarding pathogenesis, including the ability to be coinfective with other catfish pathogens. In aquaculture, bacterial coinfections exist when an animal is affected by one or more pathogens, and this may be any combination of bacterial, viral, or parasitic. A recent study was conducted to evaluate the dynamics of coinfection with *F. covae* and CCV in juvenile channel catfish. The primary objective was to evaluate the influence of a mixed infection on mortality. Additionally, samples were collected to evaluate changes to the host immune response and physiology via transcriptomic analysis of spleen and kidney tissues.

With this design, single infections of *F. covae* (isolate ALG-00-530) and CCV (isolate 2013-CCV-DRB) and a coinfection dose (both pathogens simultaneously) were used to experimentally infect fingerlings. Interestingly, the CCV isolate was recovered from frozen fish from a diagnostic case in 2013 (hybrid catfish), and it was shown to be exceptionally virulent. The catfish were challenged using an immersion model in a biosecure laboratory at the E.W. Shell Research Center (Auburn University). The catfish were exposed to each pathogen combination for a period of 1h. Following exposure, the tanks were provided with 28°C (82.4°F) flow-through water, and mortalities were collected and necropsied twice daily. After 13 days of observation, the single virulent *F. covae* infection group had a total cumulative percent mortality (CPM) of 21.3 ± 6.7 %. The single-infection CCV group was 77.0 ± 9.2 %. A coinfection half-dose combination of each pathogen demonstrated pronounced mortality (100.0 ± 0.0 %).

Trial results indicate changes in catfish mortality levels and survival trends from simultaneous exposure to this bacterial/viral infection. The project team is also finalizing transcriptomic analyses of catfish collected during the challenge period. This will allow us to understand how these pathogens influence changes to gene expression and the catfish immune response during this exposure.

For the pond culture of catfish, there are many different avenues of pathogen transmission, and the system's open nature allows diseases to spread quickly. By understanding the complex interactions of infections involving multiple pathogens, more customized and effective treatments, and mitigation strategies can be implemented at

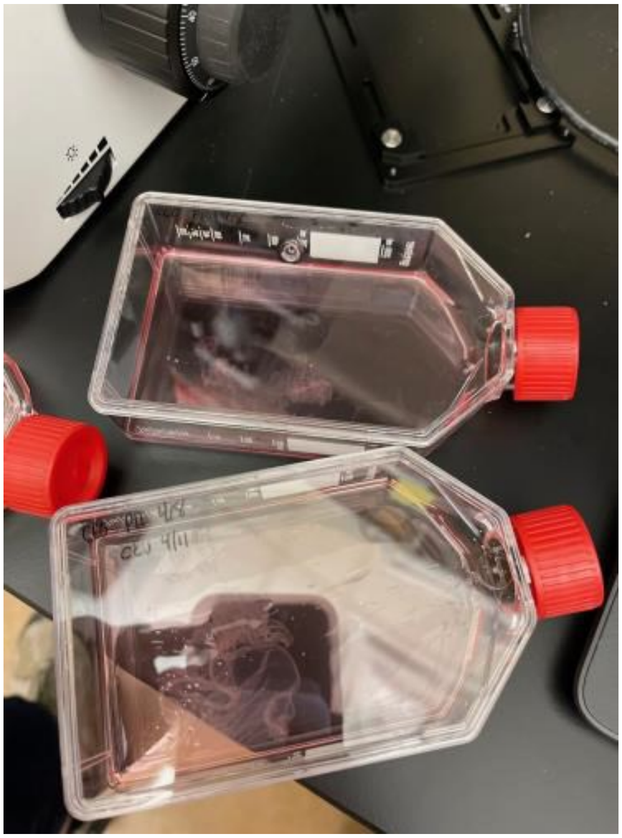

Figure 1. Channel catfish ovary (CCO) cells infected with 2013-CCV-DRB. These CCOs are used to propagate the virus for use in challenge studies.

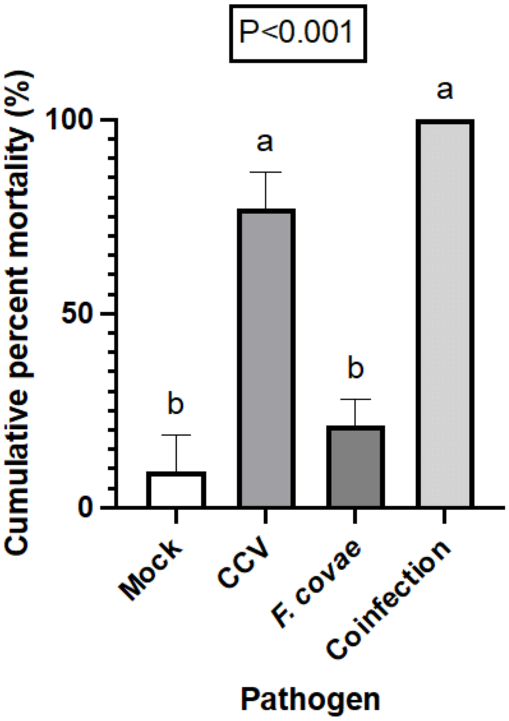

Figure 2. Endpoint cumulative percent mortality from the juvenile channel catfish challenge trial (13 days). Each bar represents the mean ± SEM (Standard Error of Mean) of three exposed tanks. Note, the coinfection group was simultaneously exposed to half-doses of the CCV and *F. covae*, whereas the single-infection groups received whole doses.

Are Heavy Metals a Concern in Catfish Aquaculture?

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Heavy metals like arsenic, cadmium, copper, lead, mercury, and selenium are naturally occurring elements that can have harmful effects on fish and people who consume them when present in high concentrations. Pond aquaculture could be a concern for heavy metal accumulation due to the large amounts of feed and chemicals (e.g., copper sulfate) that can be added to ponds year after year to control disease and algal blooms. This concern could be compounded by the fact that aquaculture ponds are rarely drained in some areas, leading farmers and consumers to ask, “Are heavy metals something I need to worry about?”

The fate of heavy metals is either to remain in the water column and enter fish tissue through the gills or be trapped and buried in the sediments (Figure 1). In other words, just because a metal is present in the water does not necessarily mean it can make its way into the fish and cause harm. Whether or not a metal can enter a fish through the gills or other avenues (in other words, toxic to the fish) depends on several factors, referred to as the “bioavailability” of a metal.

When discussing heavy metal bioavailability (toxicity), it is important to understand the environmental factors that can increase or decrease the toxicity of these elements. The two main factors of interest are the pH and the amount of dissolved organic matter (DOM) in the water. Water pH in most

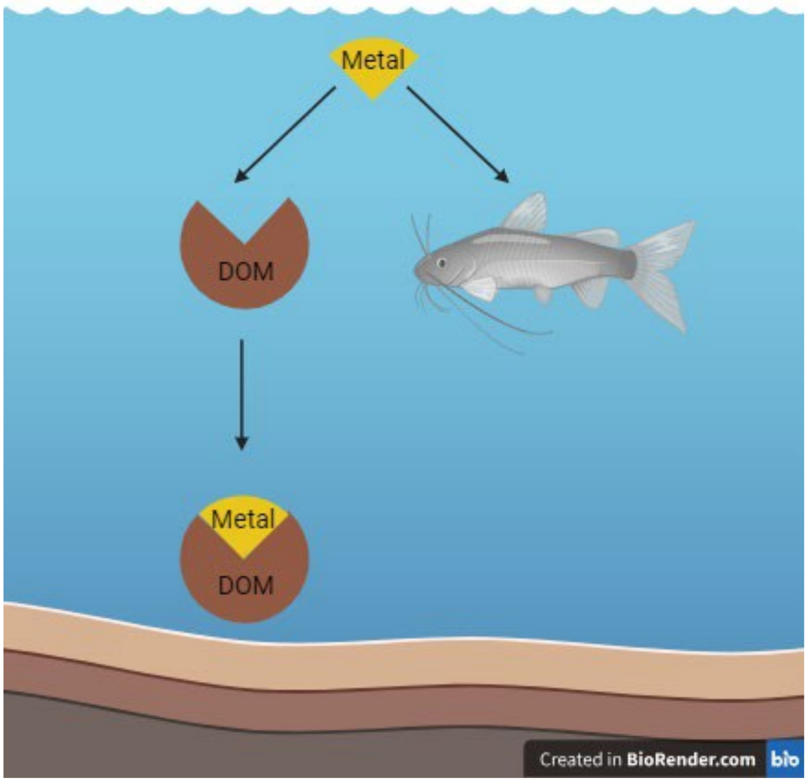

Figure 1. Metal ions in the water column can either be taken into fish through the gills or incidental ingestion or be bound to dissolved organ-

catfish ponds will fluctuate throughout the day, lowest just before sunrise and increasing until sunset. This is due to photosynthetic algae removing carbon dioxide from the water, raising the pH throughout the day. Dissolved organic matter refers to water material ranging from fish waste products to undigested feed to decaying algae and other organisms. Generally speaking, higher pH values make heavy metals less available and, therefore, less toxic to fish and other organisms. Similarly, high amounts of dissolved organic matter can reduce the toxicity of metals to fish. Dissolved organic molecules act as metal scavengers that can quickly take up metals, binding them and making them unavailable for uptake through fish gills.

The highly productive nature of catfish aquaculture ponds creates a unique environment that has the potential to minimize any toxic effects of heavy metals that enter them. The high amounts of nutrients allow for intense algal blooms that raise the water pH to levels above those typically seen in more natural systems and increase the amount of organic matter in the water as the cells naturally decay (Table 1). This creates an interesting scenario in which catfish farms may be uniquely situated to alleviate any harmful effects of heavy metals without any intervention from the farmer.

The USDA Food Safety and Inspection Service tested 737 catfish tissue samples from domestic and foreign sources from 2008-2009 and released the findings in a 2010 report. Of the 737 samples, the USDA found that only 17 tissue samples contained detectable levels of heavy metals (arsenic, cadmium, lead, and mercury), and none of the 737 samples exceeded regulatory

Aquatic System (Source)	Chlorophyll (µg/L)	pH	Dissolved Organic Carbon (mg/L)
Aquaculture Ponds (Wilson, unpublished)	257	7.73	31.8
Canadian Shield Lakes (Welsh et al., 1996)	n/a	6.20	5.68
Laurentian Great Lakes Region (Mahdiyan et al., 2021)	4.68	n/a	5.04
Fawn Lake (West et al., 2003)	n/a	5.9	9.1
Lake of Bays (West et al., 2003)	n/a	6.8	1.8

Table 1. Average chlorophyll (estimate for algal abundance), pH, and dissolved organic carbon (a form of dissolved organic matter) from aquaculture ponds and natural systems, according to published literature, or personal observations.

guidelines (Table 2). A more recent study carried out at Auburn University (2024) measured the heavy metal concentrations in water samples and fish tissue from West Alabama farms, and found no cause for concern in these systems.

Heavy Metal	Number of Samples Tested	Samples with Detectable Amounts of Heavy Metals
Arsenic	735	2
Cadmium	736	2
Lead	736	14
Mercury	737	0

Table 2. Results of catfish fillets from foreign (151) and domestic (586) sources tested for heavy metal residue. The 18 tissue samples with detectable amounts of heavy metals were below regulatory guidelines. Further detail can be found in the USDA Food Safety and Inspection Service's 2008-2009 report.

Conclusions

Heavy metals, in most cases, they appear to be less of a problem in catfish aquaculture. High water pH due to algal productivity coupled with high amounts of organic matter from fish waste, undigested feed, and decaying algae come together to create an environment that counteracts the toxicity of any present heavy metals.

Understanding How Phosphorus Could be Removed in Aquaculture Ponds by Gypsum

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Phosphorus (P) is a required element for all living organisms. However, P overloading in catfish aquaculture ponds can negatively impact fish production and water quality. For example, high concentrations of P, especially soluble reactive phosphorus (SRP), in aquaculture ponds are strongly linked to harmful blue-green algal blooms since SRP can be directly used by algae. Blue-green algal blooms and their release of toxins are seen as some of the most critical stressors facing catfish producers, especially in the warmer summer months and early Fall. Therefore, controlling P, especially SRP, in catfish aquaculture ponds is needed to mitigate algal blooms and enhance catfish production in aquaculture ponds.

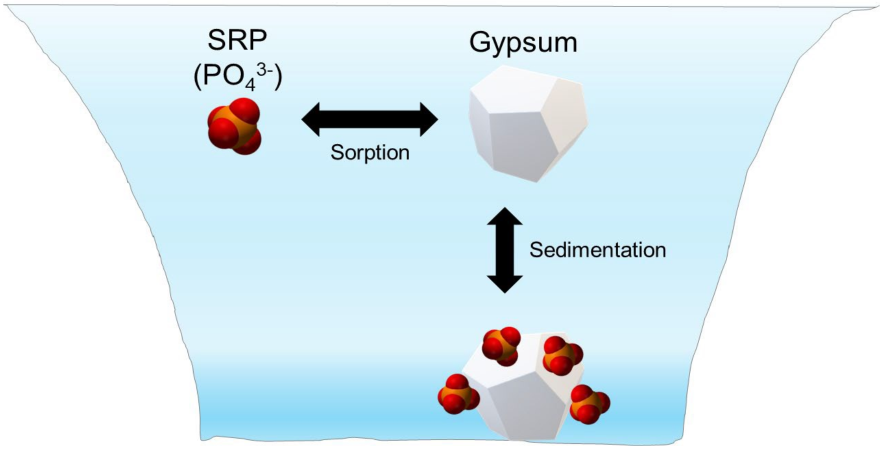

Figure 1. A schematic showing how soluble reactive phosphorus (SRP; solute) can be removed by gypsum via sorption, followed by sedimentation in the water column that eventually settles down at the pond bottom. Sorption refers to removing a compound (SRP) from water by a solid constitute (gypsum).

A particular type of gypsum called flue gas desulfurization (FGD) gypsum, an energy plant waste by-product resulting from sulfur removal, has recently raised attention as a cost-effective sorbent for removing SRP from water (Figure 1). Therefore, we performed experiments in laboratory-simulated systems to investigate the removal efficiency and mechanisms of SRP by FGD gypsum in water. The P concentrations used in the laboratory experiment encompass concentrations commonly found in ponds (13 oz per acre-foot) up to quantities in industry processing systems, such as in wastewater treatment plants (WWTPs).

We found two types of removal mechanisms, depending on the contact time between SRP and gypsum (Figure 1). Specifically, SRP removal increased quickly during the first 1 hour of contact and then increased slowly until an equilibrium was reached at approximately 24 hours. The initial rapid phase during 0–1 hour refers to the quick sorption of SRP onto gypsum surfaces until all active sites on gypsum surfaces are completely occupied. The second phase, during 1–24 hours, is the slower sorption step within the “interior” of the gypsum structure. The maximum sorption removal capacity of SRP by the FGD gypsum was calculated at ~1.0 lb SRP per 1,000 lb of gypsum (0.1%) in a simple water matrix.

The results from our laboratory-controlled systems suggest that the FGD gypsum can potentially remove SRP in water, but the removal efficiency is relatively low (0.1%). The removal of SRP by gypsum is most efficient during the first 1 hour, followed by a much slower removal efficiency after 1 hour (until 24 hours). However, caution is needed for farmers to translate our laboratory findings to their pond studies since water chemistry is significantly different from actual ponds. Ponds contain many biotic factors (algae, microorganisms, etc.), which are expected to significantly impact SRP removal by gypsum. In addition to the sorption potential (Figure 1), the FGD gypsum can release calcium cations (Ca2+) in aquaculture ponds, which will increase the water hardness of ponds and bring additional benefits to fish and water quality. The released Ca2+ from gypsum can form calcium phosphate (Ca3 (PO4)2) or hydroxyapatite (Ca5(PO4)3OH) minerals at alkaline pH conditions, which will further decrease SRP concentration in aquaculture ponds. However, future whole pond tests are needed to carefully test the benefits or adverse effects of the FGD gypsum on SRP removal, water quality, and algal blooms.

Evaluation of Orally Delivered *Aeromonas hydrophila* Vaccines in Channel Catfish

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In 2023, Alabama raised 96 million pounds of catfish and generated $112 million in revenue, making it the second-largest catfish producer in the United States. Eighty-three percent of losses were attributed to bacterial diseases. The most prevalent bacterial disease last year in Alabama (2023) was caused by virulent *Aeromonas hydrophila* (vAh). Farmers can lose over 50% of a harvest yield in less than a week due to vAh infection, thus increasing the urgency for an effective preventative measure. Vaccination is a promising avenue to control/prevent fish disease. One vaccine approach that has proven successful in aquaculture is bacterin vaccines. Bacterins are formulated using killed bacterial cells. Bacterins promote a strong immune response and produce specific antibodies, especially following a second (booster) dose. Frequently, bacterin vaccines are formulated by mixing with certain adjuvants. An adjuvant's role is to enhance the immune response to a vaccine, thus increasing overall immune protection. In the present study, we evaluated the oral delivery of two bacterin vaccines developed by our laboratory and whether an adjuvant could improve protection. Oral vaccination allows farmers to administer vaccines easily to fish in the ponds, ultimately reducing stress for the catfish. The two bacterins were formulated using killed cells of vAh (ALG-15-097), originally isolated from an outbreak in West Alabama; S14-452, originally isolated from an outbreak in Mississippi). Each bacterin was fed directly or after mixing with an adjuvant (water-in-oil emulsion; 70/30 ratio of adjuvant to antigen). Both bacterins were top coated onto feed at a cell concentration of about 1 - 2 X 10^8 CFU/gram of feed to evaluate the potential for oral vaccination of catfish against vAh. The study was also designed to simultaneously assess booster vaccination at nine weeks after the initial vaccination.

Figure 1. Fish feed top-coated with adjuvanted vAh (ALG-15-097), as shown, being fed to channel catfish.

The bacterins were top coated onto feed, and catfish (average body weight = 0.5 oz.) were fed at 3% body weight for seven days (Figure 1). For booster vaccination, the fish (average body weight = 2.0 oz.) were fed with the same bacterin at 3 % body weight for another seven days at nine weeks after initial feeding. Fish were challenged with vAh using the fin clip model at 3- and 12-weeks post-vaccination and booster-vaccination, respectively.

At 3 weeks post-vaccination, vaccinated fish exposed to vAh (ALG-15-097) without adjuvant exhibited significant protection compared to both adjuvant only and placebo controls (Figure 2) in the face of a severe challenge (> 90 % mortality in the controls). Marginal protection was seen for fish groups fed ALG-15-097 plus adjuvant and with the S14-452 bacterin alone (Figure 2). These results were consistent with an earlier trial (unpublished) that demonstrated marginal protection following oral vaccination at a similar bacterin vaccine dose with improved efficacy following booster vaccination.

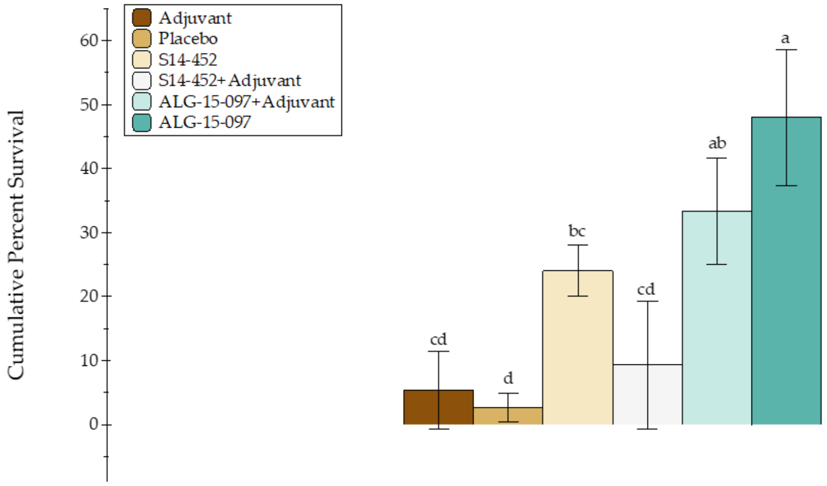

Figure 2. Cumulative percent survival of fish following exposure to vAh (ALG-15-097) at 3 weeks post-vaccination (n=3 tanks of 25 fish for each treatment).

At 12 weeks (2-weeks post booster vaccination), fish survival rates were significantly increased following challenge with either ALG-15-097 (Figure 3) or S14-452 (Figure 4) suggesting vaccination with the oral bacterin provided cross-protection to geographically different vAh isolates. The results also indicated that an adjuvant did not improve the bacterin(s) efficacy. Feeding the bacterin/adjuvant together did not result in less protection; it simply did not enhance protection.

When we began the research, we hypothesized that an adjuvanted formulation would enhance protection and potentially lead to a single 7-day oral vaccination regimen. The vAh whole-cell bacterin appears to be a potent vaccine regardless of delivery method (good protection is also observed when administered as an immersion vaccine). These results indicate that an orally delivered bacterin vaccine can protect channel catfish against vAh infection, especially after booster vaccination. Serum samples were collected and will be processed to examine the antibody response post-vaccination, which will lead to a better understanding of the catfish immune response against vAh. The deployment of these safe and effective vaccines could prevent mass mortality due to vAh, reduce antibiotic use, and ultimately improve farmer profits.

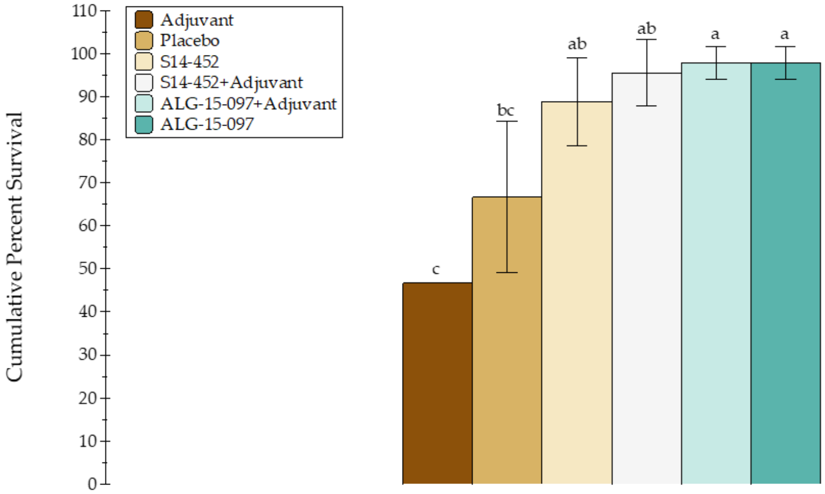

Figure 3. Cumulative percent survival of fish following exposure to vAh (ALG-15-097) at 12 weeks post-vaccination (n=3 tanks of 15 fish for each treatment).

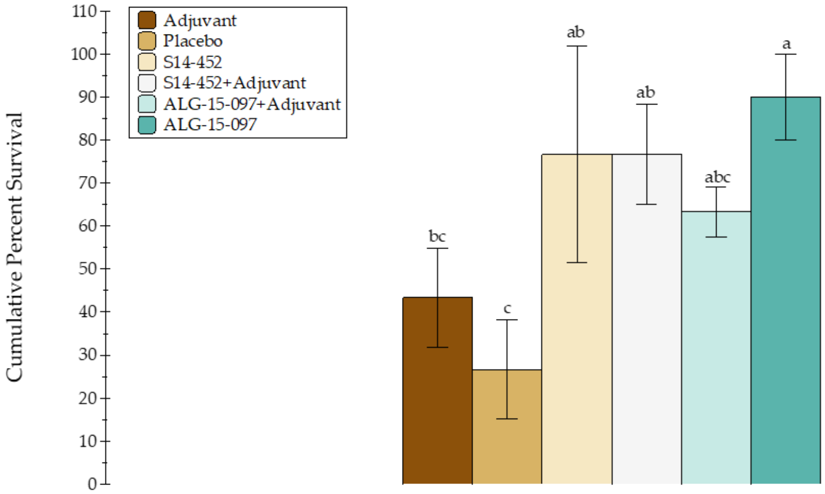

Figure 4. Cumulative percent survival of fish following exposure to vAh (S14-452) at 12 weeks post-vaccination (n=3 tanks of 10 fish for each treatment).

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