The effects of dietary inclusion of a *Saccharomyces cerevisiae* fermentation product in a commercial catfish ration on growth, immune readiness, and columnaris disease susceptibility

by

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Abstract

Aquaculture is the fastest growing sector of animal agriculture. However, sustainable expansion and intensification of aquaculture worldwide has been severely hampered by disease. In the US catfish industry, the largest segment of US aquaculture, disease-based mortality levels can reach nearly 60% over the course of a production cycle. Columnaris disease, caused by *Flavobacterium columnare*, represents one of the largest sources of mortality in the industry. Despite its importance, there are currently few effective weapons available to combat this debilitating pathogen. Catfish producers are eager to gain protection against columnaris and other diseases in a more natural and cost-effective manner, i.e. through diets supplemented to provide for both mucosal health and performance. Here, we evaluated a *Saccharomyces cerevisiae* fermentation product (Diamond V Original XPC™). The trial featured four levels of inclusion which were added to a commercial 32% protein floating catfish ration. Following six weeks of feeding, we observed heightened resistance to columnaris disease and saw significant changes in the levels of immune effectors in the serum including lysozyme, complement, and immunoglobulin. Our results stress the importance of understanding and prioritizing the protective benefits of dietary ingredients for aquaculture species alongside more typical consideration of a species' minimal nutritional requirements.

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I. Literature Review

Background

Aquaculture

Aquaculture, or "aqua farming", is defined by the Food and Agriculture Organization (FAO) as the production of fish, crustaceans, mollusks, and marine plants, with these being in turn processed into products for human consumption, especially seafood. As the human population continues to increase, so does the need for food. Aquaculture has been responsible for impressive growth in the supply of fish for human consumption. Aquaculture provided only 7% of fish for human consumption in 1974. It then increased to 26% in 1994 and then again to 39% in 2004 (FAO, 2016). Growth in the global supply of fish for human consumption has outpaced population growth in the past five decades, increasing at an average annual rate of 3.2% in the period 1961-2013, double that of population growth, resulting in increasing average per capita availability (FAO, 2016). In 2013, world aquaculture production reached 97.2 million tonnes (live weight) with an estimated value of US $157 billion. The production of farmed food fish (finfish, crustaceans, molluscs, and other aquatic animals) was 70.2 million tonnes, up by 5.6 percent from 66.5 million tonnes in 2012. Among the 70.2 million tonnes of farmed food fish, 47 million tonnes were finfish, 6.7 million tonnes were crustaceans, 15.5 million tonnes were molluscs, and 893,000 tonnes were other aquatic animals. In addition, the production of 27 million tonnes of farmed aquatic plants had a 13.4 percent increase from 2012 (FAO, 2015).

When looking at top world producers of aquaculture products, China leads aquaculture production. China accounted for 45.5 million tonnes in 2014, or more than 60% of global fish production from aquaculture (FAO, 2016). Following China (62%), other major producers are India (6.6%), Indonesia (5.7%), Vietnam (4.6%), and Bangladesh (2.6%). Outside of China and the few additional 'major' producers, aquaculture is much smaller and often not growing rapidly. Many regions have declining or stagnating aquaculture production over the last years, e.g., North America (2010-2014: 1.2%-0.8%), Western Europe (2010-2014: 0.6%-0.4%), or the Caribbean (2010-2014: 0.06%-0.05%) (Pauly and Zeller, 2016). The United States was ranked number 17 of the top 25 aquaculture producers in 2014, producing only 425,900 metric tons of total aquaculture products; 178,300 tonnes which came from inland finfish (FAO, 2016), almost entirely catfish. Meanwhile, the overall trend for capture fisheries over the last few decades has been one of decline in global catch contributions (Pauly and Zeller, 2016). Increasing US consumer demands for "safe, sustainable, and local" seafood, set against the backdrop of declining and/or environmentally-destructive commercial fisheries, should support growth in domestic aquaculture in coming decades if other barriers to growth, including disease (below) are reduced.

US Catfish Industry

In the United States, channel catfish (*Ictalurus punctatus*) and their hybrid (*Ictalurus punctatus* x *Ictalurus furcatus*) represented approximately 57% of total U.S. aquaculture

production by weight and 26% by value in 2012 (Upton, 2012). The industry, in its current form, is centered in Mississippi, Alabama, and Arkansas. Despite the oversized contribution to US aquaculture from what has largely been a regional seafood item, the industry has been declining over the last decade. U.S. farm-raised catfish has declined among highly consumed seafood products in the face of rapid rises in imported seafood choices including tilapia and pangasid catfish, with Americans consuming only 0.56 pounds of catfish per person per year (Hanson and Sites, 2015). Since its peak in 2003, when 662 million pounds of round weight catfish were processed, only 301 million pounds were processed in 2014. This was down 32 million pounds (-10%) from 334 million processed in 2013. In total from 2003-2014, the industry faced a 360 million pound decrease (-54%) in production (Hanson and Sites, 2015). While production has shown stability in recent years (316 million pounds and 319 million pounds in 2015 and 2016, respectively; pers. comm. Dr. Terry Hanson), a significant portion of production acreage has likely been taken permanently out of production (declining to 69,910 acres from a high of 196,760 acres in 2002). Contributing heavily to this decline has been the concomitant rise of imports of frozen catfish and catfish-like fillets. In 2003, U.S. catfish imports were 2500 metric tons; by 2012, U.S. imports increased tremendously to 108,800 metric tons (Upton, 2012). Currently, imports account for 80% of all U.S. sales of frozen catfish fillet products (Hanson and Sites, 2015). Fluctuations in price paid to catfish producers and catfish feed prices have also impacted the industry. Following fish shortages associated with high fish prices in 2014-2016, the catfish market is currently saturated with product in 2017 and price to producers has declined to approximately $1.09/lb (Dr. Terry Hanson, *pers. comm*). However, feed prices in recent years

have been relatively stable ($368 average/ton in 2016;2017), and combined with reasonable fish prices, are supporting a potential bottom on the decline of the US catfish industry.

In order to remain competitive domestically, increase profitability, and contribute in the global marketplace, catfish producers need to optimize and intensify production practices. However, increasing biomass in the culture systems has contributed to rising incidence of disease and rising risks associated with production. Optimization of fish diets, alongside selection of genetically resistant fish lines and strains, is needed to operate profitably in this new landscape (Zhao, 2015).

Prominent Diseases of Ictalurid Catfish

Diseases represent a significant barrier to intensification of aquaculture, accounting for as much as 45% of losses (Murray and Peeler, 2005). This is particularly true in the US catfish aquaculture industry. Higher rearing densities in existing ponds or more recent innovations (split-ponds, raceways, etc.) are often accompanied with a significantly heightened risk of disease as lower dissolved oxygen levels, high ammonia and nitrites, and elevated fish stress depress catfish immune responses. Even at more traditional pond densities, catfish producers face a number of historical and emerging pathogens, and routinely place disease at the top of their list of problems. In 2015, the Aquatic Research and Diagnostic Laboratory (Stoneville, MS) reported that columnaris disease (*Flavobacterium columnare*) accounted for 54% of cases, enteric septicemia of catfish (*Edwardsiella ictaluri*) accounted for 27%, and proliferative gill

disease (PGD) accounted for 9.2%. Since 2009, disease case distributions have differed in Alabama, as a virulent *Aeromonas hydrophila* (vAh) strain has increased in incidence (Peatman et al. 2017). There also, columnaris disease predominates (36% of cases), followed by vAh (32%), and *Edwardsiella* (*ictaluri* and *tarda*; 23%; pers. comm. Bill Hemstreet, AL Fish Farming Center). As in other sectors of aquaculture, disease issues can be confronted by several means including changing environmental parameters (oxygen levels, nutrient loads, algal bloom management), eliminating pathogens within the culture system (filtration, sterilization, chemical treatments) (Plumb and Hanson, 2011), fish vaccination (Moore et al. 1990; Shoemaker et al. 2007), genetic selection for resistant host fish (Arias et al. 2012; Dunham et al. 2002), and by optimizing diets to maximize natural immune function (Iwashita et al. 2015b). In open pond culture of lower-valued species (e.g. catfish, tilapia), pathogen elimination and biosecurity are often difficult or impossible to achieve economically. In these environments, containment and handling of fish for treatment and/or vaccination purposes can also be economically and physically unfeasible. Daily feeding is often the only opportunity to intervene in pond culture. Maintaining higher levels of disease resistance through dietary additives, therefore, is a high priority for pond aquaculture. Columnaris disease, as described above, as the long-term leading cause of disease mortality for catfish, is particularly important in this regard.

Columnaris

Flavobacterium columnare (*F. columnare*), the causative agent of columnaris disease, is a Gram-negative bacterium and one of the most well-known and lethal diseases of freshwater fish (Beck et al. 2016). Columnaris disease was first described by Davis in 1922 when observed among warm water fishes from the Mississippi River (Davis, 1922). Davis (1922) reported large numbers of slender, motile bacteria present in the lesions and noticed column-like structures. The organism was hence named *Bacillus columnaris* due to its appearance and given the name of columnaris disease.

Epidemiology

F. columnare is distributed worldwide, infecting many different wild and cultured freshwater fish species such as carp, channel catfish, goldfish, eel, perch, salmonids and tilapia and many tropical freshwater aquarium fish (Bernardet and Bowman, 2006; Decostere et al. 1998; Figueiredo et al. 2005; Morley and Lewis, 2010; Rehulka and Minarik, 2007; Soto et al. 2008; Suomalainen et al. 2009). Most species of fish are susceptible to columnaris following some type of environmental stress and when water temperatures are high. Columnaris is seen in channel catfish when water temperatures are in the range of 25 to 32°C in the spring, summer and fall (Durborow et al. 1998). *F. columnare* can also be part of bacterial microbiota of freshwater fish, eggs, and the rearing waters they live in (Barker et al. 1990). Fujihara and Nakatani (1971) reported that fish may reside in a clinically healthy carrier status sheltering an *F. columnare* isolate remaining from a previous outbreak of columnaris disease and may act as an

infection source for other fish, releasing viable bacteria into the water source. Fijan (1968) indicated that *F. columnare* can survive for extended periods in water due to the influence by physical and chemical characteristics of the surrounding water. When outside the host, *F. columnare* can survive in static, sterile river water, sterile river mud, and fish feed (Ross and Smith, 1974; Bullock et al. 1986; Wakabayashi 1993). Also, *F. columnare* can change from a virulent to a less virulent form with an altered colony morphology to save energy (Kunttu et al. 2012).

Control of Columnaris

Control of columnaris disease is a combination of good management practices, appropriate use of the available chemotherapeutics or antibiotics, and vaccination when feasible. Good management practices are crucial in any aquaculture operation and are the foundation of all disease prevention programs (Mohammed, 2015). Fish culture conditions that may make fish susceptible to columnaris disease are high rearing densities (Shoemaker et al. 2003), high organic loads (Chowdhurry, 1988), excessive handing (Wakabayashi, 1991), and water quality issues, such as ammonia (Farmer et al. 2011; Cunningham et al. 2012). Management methods that can be used to combat columnaris disease include reducing the fish density (Suomalainen et al. 2005b), ozone treatment of water (Conrad et al. 1975), salt and acidic bath treatments (Suomalainen et al, 2005a), kaolinitic clay (Beck et al. 2015), and Diquat (Darwish and Mitchell, 2009). Besides optimizing and adjusting management practices, chemical agents can also be

adopted as a preventative measure (Declercq et al. 2013). Davis (1922) determined that treating fish for 20 min in a copper sulfate (CuSO4) bath at 37 mg/L or by adding copper sulfate to pond water at 0.5 mg/L could be preventive to episodes of columnaris. Rogers (1971) suggested the addition of potassium permanganate (KMnO4) to the water at 2 mg/L to reduce mortality due to *F. columnare* while Riley (2000) suggested a prophylactic treatment of channel catfish with 15 mg/L of chloramine-T.

Another method to prevent columnaris disease in fish is through vaccination. Although vaccination trials have not always been successful, success rates have increased with the increasing knowledge on fish immunity and its role in the defense against bacterial diseases (Declerq et al. 2013). Bath immunization with a bacterin was shown to protect carp (Liewes and Van Dam, 1982) and channel catfish (Moore et al. 1990) against columnaris disease compared to unvaccinated fish. Coho salmon obtained protection against columnaris disease by oral immunization with heat-killed cells of *F. columnare* incorporated into fish feed (Fujihara and Nakatani, 1971). Prolonged feeding (over three months) of formalin-killed bacteria provided high levels of protection (Ransom, 1975). Also, strains containing sialic acid were shown to serve as potential vaccine strains capable of protecting channel catfish from columnaris disease (Ourth and Bachinski, 1987). Currently, a modified live *F. columnare* vaccine, derived from a genomovar I isolate, is available for commercial use to prevent columnaris disease in channel catfish (AQUAVAC-COLTM (Merk)) (Shoemaker et al. 2011). However, as genomovar II strains have been found to be more virulent than genomovar I strains (Shoemaker et al. 2008) and causing mass mortality (Mohammed and Arias, 2014), a protective vaccine derived from

genomovar II strains is needed. Recently, a new attenuated vaccine (genomovar II mutant 17-23) has been developed and demonstrated to provide greater protection against genomovar II columnaris strains in relation to commercial vaccines (Mohammed et al. 2013; Zhang et al. 2017). However, this vaccine is not currently available on the commercial market (Zhao, 2015).

Another form of control of columnaris disease is the use of antimicrobial agents or antibiotics. External treatments are possible only in the early stages of the disease when the infection is still superficial (Bullock et al., 1986). Bath treatments using chloramphenicol (Snieszko, 1958), nifurpirinol (Amend and Ross, 1970; Ross, 1972), nifurprazine (Shiraki et al., 1970; Deufel, 1974) and oxolinic acid (Endo et al. 1973; Soltani et al. 1995) have been used effectively. However, in advanced stages of columnaris, administration of antimicrobials in the feed is necessary. Antibiotics used in catfish aquaculture are oxytetracycline (OTC), trimethoprim-sulphamethoxazole, florfenicol, nalidixic acid, and ampicillin (Zhao, 2015). Three antibiotics, Romet, oxytetracycline (Terramycin) and Aquaflor, are currently available for use as medicated feed in aquaculture. Romet 5:1 (sulfadimethoxine/ormetoprim) is typically mixed with floating feed and applied in catfish culture (O'Hara et al. 1997; Sarter et al. 2007). Aquaflor (florfenicol), an approved U.S. Food and Drug Administration (FDA) antibiotic, is used for treatment of columnaris disease in catfish (Zhao, 2015). Oxytetracycline (terramycin) is permitted for the treatment of several aquatic animal diseases such as furunculosis, columnaris, and Pseudomonas disease (Serrano, 2005). However, the excessive use of antibiotics in aquaculture has several drawbacks and consequences: (1) Antibiotics are expensive and infected fish usually lose appetite reducing the antibiotic intake, (2) Withdrawal periods after antibiotic

feed has been fed is needed before the fish can be marketed as a food item which adds to the production cost, (3) The extensive use of antibiotics can lead to the emergence of antibiotic resistant strains (Antibiotic resistance has already been detected in *F. columnare* strains (Mohammed and Arias, 2014; Declerq et al. 2013), (4) The discharge of antibiotic wastes into the environment can lead to emergence of drug-resistance in environmental bacterial communities due to the transfer of resistant traits between bacteria species (Schmidt et al. 2000; Serrano, 2005; Miranda and Rojas, 2007), and (5) possible allergic reactions elicited in the consumers after food contact (Serrano, 2005).

Dietary additives in aquaculture

A promising alternative to antibiotics is the use of feed additives in aquaculture. Feed additives, as defined by Barrows (2000), are non-nutritive ingredients or non-nutritive components of ingredients that are included in formulations to either influence physical or chemical properties of the diet or affect aquatic animals' performance or quality of resulting products. Numerous feed additives, containing direct and indirect modes of action, can replace the effects of in-feed antibiotics used for growth promotion and can be used in addition to chemotherapeutic agents and vaccines (Iwashita et al. 2015a; Dawood et al. 2017a). The most commonly researched feed additives include probiotics, prebiotics, synbiotics, acidifiers, plant extracts, nucleotides and immunostimulants such as β-glucan and lactoferrin (LF) (Misra et al. 2006; Yokoyama et al. 2006; Dawood et al. 2015a,b, 2016c, 2017b; Dawood and Koshio 2016;

Hossain et al. 2016). Previous studies on several of these additives have evaluated their effects on growth performance, immune response, disease resistance, and intestinal microbial communities for various aquatic animal species (Balcazar et al. 2006; Kesarcodi-Watson et al. 2008; Merrifield et al. 2010; Ringo et al. 2010a,b). These feed additives are also necessary for the immunological defenses of fish. The innate immune system is hypothesized to be activated in two ways, by directly stimulating the innate immune system or by enhancing the growth of commensal microbiota (Kuhlwein et al. 2014). Fish depend on their innate immunity to counteract pathogens due to limitations in their adaptive immune system (Esteban et al. 2005; Little et al. 2005). This is achieved by the production of different cells and soluble components involved in the immune response. Leucocytes are the main influence in the immune system and include lymphocytes, phagocytes, and auxiliary cells. The head kidney, the principal immune organ, is responsible for phagocytosis, antigen processing, the formation of IgM and immune memory through melano-macrophage centers (Tsujii and Seno, 1990; Meseguer et al. 1995). The spleen, a secondary lymphoid organ, is needed for immunological memory during longer-term antigen processing (Uribe et al. 2011; Secombes and Wang, 2012).

Probiotics

Probiotics are live microbial feed supplements or water additives in the form of monocultures, mixed cultures, or in combination with prebiotics or other immunostimulants that are administered through the gastrointestinal (GI) tract to improve the health and viability of the

host (Fuller, 1989; Havenaar et at. 1992; Skjermo and Vadstein, 1999; Gatesoupe, 1999). Probiotics in aquaculture include bacteria and non-bacteria that are applied via as a feed supplement or to the rearing water (Fuller, 1989). Probiotics are beneficial to the host by improving growth (Kumar et al. 2006; Boonthai et al. 2011; Silva et al. 2013), improving disease resistance (Irianto and Austin, 2002; Newaj-Fyzul et al. 2007; Silva et al. 2013), enhancing physiological and immune responses (Dawood et al. 2016), improving water quality (Lalloo et al. 2007; Taoka et al. 2006), and reducing the use of chemicals and antibiotics (Azad and Al-Mazouk, 2008; Hai and Fotedar, 2009).

Prebiotics

Prebiotics are non-digestible feed ingredients (typically fibers) that beneficially stimulate the growth or activity of one or a limited number of bacterial species in the gut of the host (Gibson and Roberfroid 1995; Roberfroid 2005). The immunomodulatory activity of prebiotics is facilitated through direct interactions with the innate immune system, or by enhancing the growth of commensal microbiota (Song et al. 2014; Dawood et al. 2015b, 2017b) which can play a major role in improving host health (Delgado et al. 2011). Common prebiotics used in aquaculture species are inulin, oligofructose, xylooligosaccharide, mannanoligosaccharide (MOS), fructooligosaccharide (FOS), galactooligosaccharide, and β-glucan (Dawood et al. 2017b) Prebiotics have shown promising results in improvement of disease resistance, growth performance, hormonal regulation as well as immune stimulation (Ringo et al. 2010b, 2014) in various aquatic animal species.

Synbiotics

Synbiotics are products that contain both probiotics and prebiotics (Akhter et al. 2015). Gibson & Roberfroid (1995) stated that the use of the synbiotics concept may give the benefit of both prebiotics and probiotics on growth of fish mainly due to the synergistic effect. This combination may improve the survival of the probiotic organism because fermentation can be implemented more effectively as its required specific substrate is readily available; hence, simultaneous presence of live micro-organisms (i.e. probiotic) and prebiotics results in advantages to the host (Collins & Gibson, 1999). Recent studies on the use of fructooligosaccharide, mannanoligosaccharide and inulin as a prebiotic in combination with probiotics (synbiotics) showed results that synbiotics could significantly enhance growth performance, digestive enzyme activity, and the immune-haematological response in fish (Hosseinifar and Mahious, 2007; Cerezuela et al. 2011; Hoseinifar et al. 2014; Dawood et al. 2015b; Dawood and Koshio, 2016; Ringo and Song, 2016).

Immunostimulants

Immunostimulants are naturally occurring compounds that enhance the immune system by interacting directly with cells of the system, activating them and by increasing the hosts' resistance against diseases caused by pathogens (Raa 1996; Sakai 1999; Bricknell and Dalmo,

2005). Immunostimulants used to stimulate the immune system are inactivated natural microbes or microbial products such as: β-glucans, lipopolysaccharides, lactoferrin (LF), chitin, fucoidan and peptidoglycans (NRC, 2011). Dietary supplementation of LF has been reported to enhance mucus secretion (Yokoyama et al. 2006), growth and nutritional status (Kakuta, 1996), phagocytosis (Sakai et al. 1995; Esteban et al. 2005), lysozyme activity (Kumari et al. 2003), iron absorption, and alter stress responses (Kakuta, 1996, 1998; Kakuta et al. 1998; Yokoyama et al. 2005; Welker et al. 2007a).

Nucleotides

Nucleotides are ubiquitous intracellular compounds of crucial importance to cellular function and metabolism that have essential physiological and biochemical functions: (1) encoding and deciphering genetic information, (2) mediating energy metabolism and cell signaling, and (3) serving as components of coenzymes, allosteric effectors and cellular agonists (Carver and Walker, 1995; Cosgrove, 1998). Dietary supplementation of nucleotides has shown to enhance immunity and disease resistance (Ringo et al. 2012), modulate innate and adaptive immune responses (Li and Gatlin, 2006), enhance growth (Ramadan et al. 1994; Burrells et al. 2001a; Sakai et al. 2001), increase stress tolerance (Li and Gatlin, 2006; Burrells et al. 2001b), and GI physiology and morphology (Ramadan et al. 1994; Burrells et al. 2001b; Cheng et al. 2011a,b). The need for nucleotide nutrition in fish remains unclear, but yeasts and yeast extracts serve as nucleic-acid rich substances.

Medicinal plants

Medicinal plants are commonly used as immunostimulants but have been investigated as chemotherapeutics in aquaculture due to their properties in growth-promotion, immune system stimulation, antimicrobial synthesis, and stimulation of appetite and anti-stress factors (Chang, 2000; Citarasu, 2010). Interest in the use of medicinal plants is growing because they are affordable, easy to prepare, nutrient rich, have fewer side effects during treatment of diseases, and are not harmful to the environment (Jian and Wu, 2004; Citarasu, 2010; Chang, 2000). Medicinal plants used include herbs, spices, seaweeds, herbal extracted compounds, traditional Chinese medicines and commercial plant-derived products (Dawood et al. 2017a). Several studies have shown increases in survival of fish fed herbal plants after challenge with pathogens (Gudmundsdottir and Majnadottir, 1997; Sakai, 1999; Brunt et al. 2007; Mohammed and Arias 2016).

Organic acids

Organic acids are short-chain fatty acids, volatile fatty acids or weak carboxylic acids, such as formic, citric, benzoic and lactic acid that have been used for decades in livestock feeds as a food preservative due to their antimicrobial properties. Along with their antimicrobial properties, organic acids have been used in aquatic animals due to their ability to enhance growth, nutrient utilization, and disease resistance (Ng and Koh, 2016). An advantage to using organic acids in aquatic animals may be to due to some evidence that organic acids inhibit the

growth of Gram-negative bacteria in the intestinal tract (Bai et al. 2015). Recently, many studies have determined the positive effects of organic acids and their salts on growth performance, feed utilization, nutrient digestibility, and disease resistance in several aquatic animal species (Ringo, 1991; Gislason et al. 1994; Vielma and Lall, 1997; Vielma et al. 1999; Lim et al. 2010).

Antioxidants

Antioxidants, molecules that inhibit the oxidation of other molecules, are divided into three main groups: functional amino acids, vitamins and trace minerals. Amino acids are crucial for fish because they are needed as energy substrates, for endogenous protein synthesis and to regulate metabolic pathways (Anderson et al. 2016; Yan et al. 2017). Recent studies have found increased disease resistance, enhanced innate immune response, and improved antioxidant capacity in fish fed feed supplemented with arginine (Buentello and Gatlin, 2001; Costas et al. 2011), tryptophan (Cuesta et al. 2008), glutamine (Cheng et al. 2011a,b; Hu et al. 2014; Pohlenz et al. 2012) and methionine (Elmada et al. 2016). Vitamins are necessary for normal cell function, growth, and development of aquatic animals (Dawood et al. 2017a). Vitamins C and E are major antioxidant additives and have been shown to enhance immune responses (Roosta et al. 2014; Kim and Kang 2015; Shahkar et al. 2015), reduce oxidative damage to tissues (Huang et al. 2017; Liang et al. 2017), facilitate in the absorption of iron (Hsu and Shiau, 1999), and reduce oxidative stress (Chien and Hwang, 2001; Tocher et al. 2003; Gao et al. 2013) in aquatic animals. Minerals are vital for the balance of osmotic pressure, structural constituents of tissues, transmission of nerve impulse and muscle contractions, and constituents of the exoskeleton

(NRC, 2011). Trace minerals that are required in small quantities are chromium, cobalt, copper, iodine, iron, manganese, molybdenum, selenium, and zinc (Antony Jesu Prabhu et al. 2017). Enhanced growth performance and immunological function has been seen in several fish species fed large amounts of copper (Lin et al. 2008; Sabatini et al. 2009; Mohseni et al. 2014) and selenium (Zhu et al. 2012, 2017; Ashouri et al. 2015).

Yeasts

Yeasts are ubiquitous microorganisms, which disseminate with animals, air and water currents, and can grow in various environments where organic substrates are available (Gatesoupe 2007). Industrial yeast is commonly used in aquaculture, either alive to feed live food organisms, or after processing, as a feed ingredient (Stones and Mills, 2004). Brewer's yeast (*Saccharomyces cerevisae*) has been used as a feed supplement for various animals. It contains various immunostimulating compounds such as β-glucans, nucleic acids, mannan oligosaccharides and other cell wall components (Li and Gatlin, 2003, 2005; Oliva-Teles and Goncalves, 2001) that are often used as immunostimulants in diets of terrestrial and aquatic monogastric animals (Sohn et al. 2000). β-glucans are polysaccharides composed of glucose molecules linked by β-1,3 and β-1,6 or β-1,3 and β-1,4 bonds. β-glucans with β-1,3 and β-1,6 bonding are most commonly found in the cell walls of yeast and mycelial fungi (Verlhac et al., 1998). It has been observed that *S. cerevisiae* can positively influence the non-specific immune responses (Ortuno et al. 2002; Siwicki et al. 1994) as well as growth performance (Abdel-Tawwab et al. 2008; Li and Gatlin, 2003, 2005; Noh et al. 1994; Oliva-Teles and Goncalves,

2001; Rumsey et al. 1991; Taoka et al. 2006) of some fish species. Several studies have indicated that dietary yeast and yeast extracts may influence hematological and serum biochemical parameters (Abdel-Tawwab et al. 2008; Andrews et al. 2009, 2011; Reyes-Becerril et al. 2008), which are valuable indicators of fish health and wellbeing (Hoseinifar et al. 2011). Li and Gatlin (2003, 2005) reported that enhanced weight gain and feed efficiency were generally observed in fish fed diets supplemented with yeast (at 1-4%) compared to fish fed basal diets. Similarly, Hoseinifar et al. (2011) reported that 2% *S. cerevisiae* var. *ellipsoideus* can be used as a growth promoter and intestinal microbial modulator for beluga juveniles without causing detrimental impacts on hematological and biochemical parameters. The growth promoting influences of baker's yeast have also been observed with Nile tilapia (*Oreochromis niloticus*) where optimum growth, feed utilization, and protein turnover were obtained with a level of 0.1-0.5% dietary yeast inclusion (Abdel-Tawwab et al. 2008). Lara-Flores et al. (2003) also reported that 0.1% inclusion of brewer's yeast elevated growth performance and feed utilization of Nile tilapia after 9 weeks feeding to satiation, and Ahilan et al. (2004) reported that dietary inclusion of 0.25% baker's yeast improved goldfish growth parameters. Additionally, Chiu et al. (2010) observed that grouper fed a diet containing live *S. cerevisiae* at the levels of 10^3, 10^5, and 10^7 CFU kg^{-1} displayed significantly increased weight gain compared to the control fed group. Research has been conducted intermittently on the effect of *S. cerevisiae* on channel catfish. Welker et al. (2007b) reported that 0.2% dietary whole cell brewer's yeast *S. cerevisiae* had no effect on the haematological parameters (WBC, RBC, Htc, Hb) of channel catfish (*Ictalurus punctatus*). Welker et al. (2012) performed another feed study with channel catfish fed diets supplemented

with MacroGard, Betagard A, or Levucell, yeast or yeast subcomponents, where no differences were seen among the diets after 1 week of pre-challenge feeding.

When looking at the catfish industry, a large body of research has been performed regarding the effects of feed additives included in commercial catfish diets (Table 1) over the last decade. However, the expense of these additives, difficulties in application, and uncertainty about efficacy in commercial settings have likely prevented commercial inclusion. Below I summarize literature on feed additives in catfish species.

Table 1. Summary of feed additives (excluding DVAqua/XPC products) used in catfish species.

Species	Feed additive	Dietary inclusion	Improvement	Reference
Prebiotics				
Channel catfish *Ictalurus punctatus*	MOS	2 g kg^{-1}	No effect on growth performance, lysozyme activity, SH50, antibody titre, and resistance against *E. ictaluri* Decrease in plasma cortisol	Welker et al. (2007b)
	MOS	2 g kg^{-1}	Increase resistance against *E. ictaluri* – by MOS-sink No effect on weight gain, SGR, and FCR	Peterson et al. (2010)
	Bio-MOS	4 g/kg	No effect on weight gain, SGR, and FCR Bio-MOS extruded at lower temperature:	Peterson et al. (2012)

			increase resistance against E. ictaluri	
	MOS	2 g kg^{-1}	No effect on lysozyme activity or SH50 Increased antibody titre	Welker et al. (2012)
	MOS	2 g kg^{-1}	Increase in survival No effect on SGR and growth	Hernandez et al. (2012)
	Yeast polysaccharide	0.1%, 0.2%, 0.3%	No effect on hematological parameters and gut microbiota	Zhu et al. (2012)
	Actigen (MOS)	0.10 g/100 g	No effect on growth, FCR, and survival Increased resistance to *F. columnare*	Zhao et al. (2015)
African catfish *Clarius gariepinus*	MOS	10 g kg^{-1}	Increase in lysozyme activity	Yoshida et al. (1995)
	AXOS	10 and 20 g kg^{-1}	Increase in acetate, propionate and total SCFA production No effect on butyrate production	Rurangwa et al. (2008)
Yellow catfish *Pelteobagrus fulvidraco*	KMOS, MOS	1 g kg^{-1}, 2 g kg^{-1}, 3 g kg^{-1} (KMOS) 3 g kg^{-1} (MOS)	>1 g kg^{-1} KMOS and 3 g kg^{-1} MOS: increase in RGR, SGR and lower FCR >2 g kg^{-1} KMOS: higher RGR, SGR, and lower FCR then MOS	Wu et al. (2014)
Probiotics				

Channel catfish *Ictalurus punctatus*	*Bacillus* sp.	$2x10^9$ CFU/mL	Increase in survival and net production	Queiroz and Boyd (1998)
	Enterococcus faecium, *Bacillus subtilis* + *B. licheniformis*, *Pediococcus acidilactici*, *Lactobacillus* spp	$1x10^8$ CFU/g, $1x10^6$ CFU/g	No effect on protein, immunoglobulin, complement, or lysozyme	Shelby et al. (2007)
African catfish *Clarius gariepinus*	*Lactobacillus acidophilus*	$3.01x10^7$ CFU/g	Increase in pathogen inhibition and hematological parameters	Abdullah et al. (2011)
	Lactobacillus acidophilus	$3.01x10^7$ CFU/g	Increase in growth performance, nutrient utilization, survival, and hematological parameters	Al-Dohail et al. (2009)
	Biogen	0.5%, 1%, 1.5%, 2%	>0.5%: increased growth and nutrient utilization	El-Haroun (2007)
	Lactobacillus sp.	10^9 CFU/ml	Increase in growth, nutrient utilization, and hematological parameters	Aderolu et al. (2013)
Yeasts				
Channel catfish *Ictalurus punctatus*	MacroGard, Betagard A	1 g/kg, 0.1 g/kg	Increase in stress resistance No effect on growth, hematology parameters or immune function	Welker et al. (2007b)
		1 g/kg, 0.1 g/kg		

	MacroGard, Betagard A Brewer's yeast Baker's yeast *S. cerevisiae*, β-glucan (Sigma)	1%, 2% 2.7% (Baker's yeast), 0.2% (Sigma)	Immune parameter variation Improved weight gain and FER No effect on nonspecific immune responses	Welker et al. (2012) Li et al. (2001) Duncan and Klesius (1996)
Asian catfish *Clarias batrachus*	β-1,3 glucan (Sigma) β-1,3 glucan (Sigma)	0.1% 0.1%	Immune parameter variation Improved disease resistance and immune parameters	Kumari and Sahoo (2006b) Kumari and Sahoo (2006a)
African catfish *Clarius gariepinus*	Baker's yeast (*Saccharomyces cerevisae*)	0%, 2%, 4%, 6%, 8%	>2%: increased growth and feed utilization; no effect on hematological parameters 4%: best growth	Aderolu et al. (2011)

CFU: Colony forming units

DVAQUA®

DVAQUA®, a dietary *Saccharomyces cerevisiae* fermentation product, is a naturally fermented product consisting of yeast cell walls (β-glucans and mannan-oligosaccharides) and cell soluble materials (vitamins, proteins, peptides, amino acids, nucleotides, lipids, organic acids, oligosaccharides, esters, and alcohols), which seldom has living cells in the product (Burgent et al. 2004). Several studies suggest that DVAQUA® functions as a prebiotic-like feed additive that assists in growth, survival, and immune response in cultured aquatic species (Table 2) including rainbow trout (*Oncorhynchus mykiss*) (Barnes et al. 2006a,b; 2007; Barnes and

Burden, 2010), hybrid tilapia (*Oreochromis niloticus x O. aureus*) (He et al. 2009; 2010; Zhou et al. 2011), and Pacific white shrimp (*Litopenaeus vannamei*) (Burgents et al. 2004; Tipsemongkol et al. 2009).

Table 2. Summary of DVAQUA® studies performed in aquatic species and their effect on growth, survival, and immune parameters.

Species	Dietary inclusion of DVAQUA®	Improvement	Reference
Pacific white shrimp *Litopenaeus vannamei*	0.5%, 1.0%	1.0%: greater survival No effect on growth	Burgents et al. (2004)
	0.125%, 0.25%	0.25%: increase in weight gain, survival, and immune parameters	Tipsemongkol et al. (2009)
Rainbow trout *Oncorhynchus mykiss*	0.125 g kg^{-1}, 0.25 g kg^{-1}	0.25 g kg^{-1}: improved survival and growth compared to 0.125 g kg^{-1}	Barnes et al. (2006a)
	0.125%, 0.25%	0.25%: improved growth, FCR, and survival compared to 0.125%	Barnes et al. (2006b)
	0.25%	increased growth and survival No effect on FCR	Barnes et al. (2007)
	0.125 g kg^{-1}	improved growth, FCR, and survival	Barnes and Durben (2010)
Brown trout *Salmo trutta*	0.125%, 0.25%	0.125%: improved growth and FCR No effect on survival	Barnes et al. (2006b)

Lake trout *Salvelinus namaycush*	0.25%	0.25%: improved growth, FCR, and survival	Barnes et al. (2007)
Chinook salmon *Oncorhynchus tshawytscha*	0.125%, 0.25%	0.125%: improved growth, FCR, and survival	Barnes et al. (2006b)
Hybrid tilapia (*Oreochromis niloticus x O. aureus*)	0.125, 0.25, 0.5, 1.0, and 2.0 g kg^{-1}	No effect on growth and FCR >0.125 g kg^{-1}: stimulated colonization of some bacteria >0.25 g kg^{-1}: improved non-specific immune parameters	He et al. (2009)
	0.5 g/kg	Improved nonspecific immunity, intestinal bacterial count and bacterial diversity	He et al. (2010)
	unknown	No effect on growth Improved survival	Zhou et al. (2011)

Burgents et al. (2004) was the first to report on DVAQUA® application to assess impact on disease resistance and growth in Pacific white shrimp. For the growth experiment, shrimp were fed 0% and 1% DVAQUA® for 4 weeks while for the disease experiment, shrimp were fed three diets, 0%, 0.5%, and 1% DVAQUA®, for 4 weeks and were tested for disease resistance at the end of every week. Results showed that DVAQUA® had no effect on growth in shrimp fed the 1% supplement diet. Results on disease resistance showed that after 1 or 2 weeks on the test diets, there were no significant differences in mean survival but after 3 weeks, mean survival of shrimp fed 1.0% DVAQUA® was significantly higher than that of control shrimp. Another study

was performed on the same species (Tipsemongkol et al. 2009) that showed different results. Shrimp were fed three diets, 0%, 0.125%, and 0.25%, in tanks for 50 days and two diets, 0% and 0.25%, were fed to shrimp in ponds for 90 days to assess growth, survival, and immune response. For the tank experiments, shrimp fed with 0.25% DVAQUA® had a significantly higher ($p < 0.05$) average body weight than that of the control group while there was no significant difference in body weights between the control group and the group fed 0.125% DVAQUA®. The survival rates of shrimp fed DVAQUA® diets were significantly higher ($p < 0.05$) than the control group. The immune characteristics of shrimp fed 0.25% DVAQUA® diets included significantly higher ($p < 0.05$) total hemocyte counts, percentage phagocytosis and superoxide dismutase activity than the 0.125% DVAQUA® and control groups. For the pond experiments, shrimp fed the DVAQUA® diet had higher immune parameters and survival compared to the control groups.

Along with shrimp, salmonid species, such as rainbow trout, brown trout, Chinook salmon, and lake trout, have shown increased growth and survival when fed a diet supplemented with DVAQUA®. Barnes has performed many studies on these species (Barnes et al. 2006a,b; 2007; Barnes and Durben, 2010) feeding DVAQUA® levels of 0.125% or 0.25%. Rainbow trout fed 0.25% DVAQUA® had greater weight gain (682g ± g), FCR (1.09 ± 0.01), and lower mortality (3.4% ± 0.2) than control groups weight gain (623g ± 106g), FCR (1.23 ± 0.21), and mortality (4.9 ± 0.7) respectively. Brown trout fed 0.25% DVAQUA® had a 10% improvement in weight gain and FCR compared to control groups. Chinook salmon had increasing weight gain with increasing DVAQUA® supplementation (0.125% and 0.25%) (Barnes et al. 2006b). Barnes

et al. (2007) reported the same results in rainbow trout and lake trout fed diets supplemented with 0.25% DVAQUA®. Barnes and Durben (2010) reported that rainbow trout fed a 0.125 g kg^{-1} DVAQUA® diet had improved growth, FCR, and survival. In hybrid tilapia, He et al. (2009) reported that dietary DVAQUA® showed no significant effects on the growth performance, feed conversion efficiency and survival but enhanced non-specific immune parameters at dose levels between 0.226 and 0.5 g kg^{-1} diet. He et al. (2010) reported improved nonspecific immunity and increased intestinal bacterial count and bacterial diversity in the same species fed diets supplemented with 0.5 g/kg DVAQUA®.

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II. The effects of dietary inclusion of a Saccharomyces cerevisiae fermentation product in a commercial catfish ration on growth, immune readiness, and columnaris disease susceptibility

Introduction

As the global population grows, the demand for high-quality animal proteins is increasing. According to the UN Food and Agriculture Organization (FAO), aquaculture continues to be the world's fastest-growing animal food producing sector, accounting for nearly half of the world's yield of food fish (FAO 2017). However, the fast development of aquaculture and the intensification of fish culture systems to maximize production has led to magnification of fish stressors and consequently higher incidence of diseases (Iwama *et al.* 2011). Partial or total loss of aquaculture production due to increased disease outbreaks associated with culture intensification has been reported (Bondad-Reantaso *et al.* 2005) and with the constant expansion of intensive aquaculture, it is anticipated that global aquaculture production will be confronted with more disease outbreaks. Disease-related mortalities, such as those caused by columnaris disease, are considered a major threat to aquaculture production and account for tremendous annual economic losses (Balcázar *et al.* 2006, Dorsey and Robertson 2013, Verschuere *et al*. 2000).

Columnaris disease, caused by the Gram-negative bacterial pathogen *Flavobacterium columnare*, has been a significant problem in numerous wild and cultured freshwater finfishes

throughout the world, particularly in the intensively farmed aquaculture species (Beck *et al.* 2016, Pulkkinen *et al.* 2010). The economically important foodfish, channel catfish (*Ictalurus punctatus*), and other members of the family *Ictaluridae* are extremely susceptible to this disease (Arias *et al.* 2004, Peatman *et al.* 2013). Columnaris also assails many aquarium ornamental fishes (Decostere *et al.* 1998, Roberts *et al.* 2009). Currently, columnaris disease is the most frequently reported bacterial disease affecting farm-raised catfishes in the USA and costs the US catfish industry major financial losses estimated at 30 million dollars, annually (Declercq *et al.* 2013a, Shoemaker *et al.* 2008, Wagner *et al.* 2002).

Despite the global distribution and the significant losses attributed to columnaris disease, safe and effective therapies are not yet available. External treatments are possible only in early stages of the disease, when the infection is still superficial (Bullock *et al.* 1986). Common treatments widely used against the disease include administration of medicated feed, but the use of antimicrobial agents and chemical drugs has known different success rates and has multiple negative impacts on the environment and on human health; e.g. the emergence of resistant bacterial strains (Declercq *et al.* 2013b, Mohammed and Arias 2014) and residual accumulation in fish tissue (Cabello 2006, Romero *et al.* 2012). Another approach to cope with columnaris disease is through vaccination. Attenuated immersion vaccines represent a promising tool for controlling the disease (Mohammed *et al.* 2013, Shoemaker *et al.* 2011). However, vaccination trials have not always been successful and are associated with the risk of releasing live antibiotic resistant strains in the environment (LaFrentz et al. 2011). Hence, disease management in modern aquaculture should focus on environmentally friendly and lasting alternatives (Irianto

and Austin 2002, Reverter *et al.* 2014). Disease prevention plays a central role in sustainable aquaculture and, therefore, has been one of the most commonly adopted strategies in the US catfish industry. Modification of feeding regimens through dietary administration of pro- and/or prebiotics represents a simple intervention to control diseases in production settings, and therefore, gaining marked interest by fish producers worldwide.

In recent years, natural immunostimulants have shown profound potential for enhancing disease resistance in fish and shellfish. As a result, there has been a growing interest in the use of immunostimulants as they offer a safe and effective alternative strategy to antibiotics and other antimicrobial compounds. Whole-cell yeast, yeast-fermentation products, and yeast subcomponents comprise a rich source of nutrients, mannanoligosaccharides, and β-glucans that can possibly enhance gut health and immunity of terrestrial monogastric and aquatic animals, which consequently translate into better growth performance and reduced risk of diseases (Price *et al.* 2010, Sohn *et al.* 2000). Yeasts are considered part of the normal microbiota of both wild and farmed fish and their beneficial effects on fish health and nutrition are numerous (Navarrete and Tovar-Ramírez 2014). Recent studies have shown the stimulatory properties of *Saccharomyces cerevisiae* supplemented diets in fish. Supplementation of diets with yeast enhanced growth performance, feed efficiency rate, blood biochemistry, survivability, and non-specific immunological responses in *Uronema marinum* infected olive flounder, *Paralichthys olivaceus* (Harikrishnan *et al.* 2011). In other feeding trials, *S. cerevisiae* improved growth, feed efficiency, and non-specific immunity in Israeli carp, Nile tilapia, and rainbow trout (Lara-Flores *et al*., 2003; Noh *et al*., 1994; Pooramini *et al*., 2009). Hybrid striped bass (*Morone*

chrysops×*M. saxatilis*) fed *S. cerevisiae* showed a significantly higher survival (73.3–90%) following immersion challenge with *Streptococcus iniae* compared to control fish (53.3%) fed basal diet (Li and Gatlin Iii 2004). Feeding a *S. cerevisiae* fermentation product has been shown to enhance non-specific immunity markers lysozyme, complement, and respiratory burst activity in hybrid tilapia (He *et al*., 2009). Recently, in channel catfish, the feeding of a standard diet supplemented with a concentrated source of yeast cell wall-derived material including mannan oligosaccharides (MOS) improved survival of channel catfish following a columnaris challenge (Zhao *et al.* 2015).

To this end, the present study was conducted to evaluate the effect of dietary Diamond V Original XPC (Cedar Rapids, IA), a *S. cerevisiae* fermentation product rich in bioactive metabolites, as well as mannanoligosaccharides and β-glucans, on growth, immune readiness, and susceptibility of hybrid catfish to columnaris disease.

Materials and Methods

Experimental Fish and Rearing facilities

Hybrid catfish from Jubilee Fish Farms, Indianola Mississippi were reared at the Aquatic Animal Health Research Unit, Auburn, Alabama and fed a basal diet containing 92% dry matter, 7% ash, 3% lipid, and 34 % crude protein (Alabama Catfish Feed Mill, Uniontown, Alabama) for an acclimation period of 10 days prior to stocking. At the end of the acclimation period, fish

with an average weight of 54.13 ± 2.49 g were randomly stocked into sixteen 110-L aquaria at a density of 30 fish per aquarium. Aquaria were supplied with flow-through de-chlorinated heated city water in an initial rate of approximately 0.8 L/min and increased gradually to 1.0 L/min by week 6. Water was continuously aerated using air stones. Water temperature and dissolved oxygen of the aquaria were measured every day in the morning using an YSI model 58 Oxygen Meter (Yellow Spring Instrument Co., Inc., Yellow Spring Ohio). During the study, water temperature and dissolved oxygen averaged 27.48 ± 0.15 ºC and 5.63 ± 0.07 mg/L, respectively. Photoperiod was maintained at a12:12 h light/dark schedule.

Experimental diets and Feeding

The basal diet was extruded to prepare the Original XPC commercial catfish test diets. The basal diet was supplemented with three levels of XPC (2.5, 5.0 and 10 lb per ton) plus a control group (fed basal diet, 0 lb per ton XPC) with 4 treatments and 4 replications per treatment. The commercial basal diet was finely ground with a feed mill and supplemented with the different levels of additive. The dry ingredients were thoroughly mixed for 10 min in a Hobart mixer (Hobart Corporation, Troy, Ohio). 360 mL of deionized water/kg of diet was added and mixed for another 10 min. The moistened mixture was extruded through a 3-mm diameter die in a Hobart meat grinder. The resulting moist pellets were dried at room temperature (22-25 ºC) using an electric fan to a moisture content of 10%. Pellets were ground into small pieces, sieved to obtain appropriate sizes, and stored frozen in plastic bags at – 20 ºC until fed. Fish in four randomly assigned aquaria were fed one of the four experimental diets

twice a day to apparent satiation for six weeks. The amount of feed consumed was weighed and recorded daily for each aquarium. Once a week the aquaria were scrubbed and the waste was siphoned. On cleaning days, fish were fed only once in the afternoon. Fish in each aquarium were group-weighed and counted every two weeks. Fish were not fed on sampling days. Following each sampling period the growth, average weight gain, average feed intake, feed conversion rate, and feed efficiency rate data were calculated.

Hematological Assays

At the end of the feeding period, four fish were randomly chosen from each tank and anesthetized with tricaine methanesulfonate (MS-222) at 150 mg/L, and blood samples were collected from the caudal vein with heparinized (100 IU/ml) 1 ml syringes with 25 gage 1" needles for hematological assays. Total cell count, red blood and white blood count were performed in duplicate for each sample by diluting (1:10,000) whole blood and enumerating in a Spencer Bright Line Hemocytometer. Hemoglobin was determined using Total Hemoglobin kit by Pointe Scientific Cat # 2366306 for hemoglobin reagent and Cat # 2366304 for hemoglobin standard. Hemoglobin values were adjusted by Cyan methemoglobin correction factor for channel catfish described by Larsen (1964). Hematocrit for each fish was determined in duplicate using micro hematocrit method (Brown 1988). Other blood parameters (Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)) were calculated using red blood cells count, hemoglobin and hematocrit values for each fish.

Total Immunoglobulin

At the end of the 6-week feeding period, five fish per tank were bled using non-heparinized tuberculin syringes and allowed to clot at 4°C overnight. Serum samples were collected following centrifugation and stored at -80°C until used for subsequent assays of total immunoglobulin, lysozyme, and spontaneous hemolytic complement (SH50) activities. Serum total immunoglobulin (Ig) was determined following the method of Siwicki and Anderson (1993). The assay was based on the measurement of total protein content in serum before and after precipitating the immunoglobulin molecules employing a 12% solution of polyethylene glycol. The difference in protein content is considered the total immunoglobulin content. Serum total protein concentrations were calculated using bovine serum albumin as an external standard.

Lysozyme Assay

Serum lysozyme activity was determined by the method of Litwack (1955) as modified by Sankaran and Gurnani (1972). The assay is based on lysis of lysozyme-sensitive Gram positive bacterium *Micrococcus lysodeikticus* (Sigma Chemical Co., St. Louis, Missouri) by the lysozyme present in the serum. A suspension of 0.25 mg/mL freeze-dried *M. lysodeikticus* was prepared immediately before use by dissolving in sodium phosphate buffer (0.04 M Na_2HPO_4, pH 6.0). Serum (15 µL/well in duplicate) from each of the five fish per tank was placed in a microtiter plate and 250 µL of bacterial cell suspension was added to each well. Hen egg white lysozyme was used as an external standard. The initial and final (0- and 20-min incubation at

35°C) absorbance of the samples was measured at 450 nm. The rate of reduction in absorbance of samples was converted to lysozyme concentration (mg/mL) using the standard curve.

Natural Hemolytic Complement Activity

Serum natural hemolytic (alternative pathway) complement activity was adapted from Sunyer and Tort (1995) and modified for use in microtiter plates as described in Yildirim-Aksoy et al. (2007), except 0.85% phosphate buffered saline (PBS) containing $MgCl_2$, $CaCl_2$ and gelatin (PBS^{3+}) was used instead of GVB^{2+}as the assay solution. This assay is based on the hemolysis of sheep erythrocytes (Remel Inc., Lenexa, Kansas) by complement present in fish serum. Sheep erythrocytes were washed three times with potassium phosphate buffer containing $CaCl_2$, $MgCl_2$, and gelatin (PBS^{3+}) and standardized to 5×10^7 cell/mL in PBS^{3+} prior to use. A twofold serial dilution was made in 96-well round-bottom microtiter plates by adding 40µL of serially diluted serum from five fish per tank into the wells plated with 40µL of PBS^{3+}. The volume in each well was adjusted to 200µL by PBS^{3+}. Thereafter, 40µL of sheep erythrocyte cell suspension were added to each well. Positive controls (100% lysis) of distilled water plus sheep erythrocytes, negative controls (spontaneous lysis) of buffers and sheep erythrocytes were also processed in each plate. Samples were incubated in room temperature (22°C) for 1 hour with regular shaking. The reaction was stopped by placing plates on ice. The plates were centrifuged at 800 × *g* for 10 minutes at 4°C to avoid unlysed cells. Supernatants were pipetted into a new flat-bottom 96-well microtiter plate and the absorbance is measured at 570 nm using an Epoch-2 microplate reader (BioTek, Winooski, TX, USA). Complement hemolytic activity was expressed as ACH_{50} value

which represents the volume of serum necessary to produce lysis of 50% of the target cells under standard conditions and results presented as units/mL. The degree of hemolysis was estimated and the lysis curve for each sample was obtained by plotting Y/(100-Y) against the volume of serum added (mL) on a log-log scaled graph.

Gene Expression

A separate group of catfish reared and fed under identical conditions for 6 weeks were sacrificed at the end of the study to examine potential changes in intestinal gene expression in several genes important in catfish mucosal immunity. Samples for each dietary treatment were organized to create 4 pooled replicates each consisting of proximal intestine (~3 cm) pieces from 5 fish each. Gene specific primers were designed using Primer3 software (Table 3). Total RNA was extracted using the RNeasy Plus Universal Mini Kit (Qiagen) following manufacturer's instructions. First strand cDNA was synthesized by qScript™ cDNA Synthesis Kit (Quanta BioSciences) according to manufacturer's protocol. The qScript chemistry used a blend of oligo-dT and random primers. All the cDNA products were diluted to 250 ng/µl and utilized for the quantitative realtime PCR reaction using the PerfeCTa® SYBR® Green FastMix® (Quanta BioSciences, Gaithersburg, MD) on a CFX96 real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The thermal cycling profile consisted of an initial denaturation at 95 ºC (for 30 s), followed by 40 cycles of denaturation at 94 ºC (5 s), an appropriate annealing/extension temperature (58 ºC, 5 s). An additional temperature ramping step was utilized to produce melting curves of the reaction from 65 ºC to 95 ºC. Results were expressed

relative to the expression levels of 18S rRNA in each sample using the Relative Expression Software Tool (REST) version 2009. The biological replicate fluorescence intensities of the control and treatment products for each gene, as measured by crossing-point values, were compared and converted to fold change by the relative quantification method. The mathematical model was based on the correction for PCR efficiencies (assumed as 2) and the mean crossing point (Ct) deviation between sample groups and control groups. Expression differences between groups were assessed for statistical significance using a randomization test (≥ 2000 randomizations) and plotted using standard error (SE) estimation. Test amplifications were conducted to ensure that 18S and target genes were within an acceptable range. A no-template control was run on all plates. QPCR analysis was repeated in triplicate runs (technical replicates) to confirm expression patterns.

Table 3. Primers used for quantitative RT-PCR (5' to 3').

Gene	Forward (5'-3')	Reverse (5'-3')
18S	GAGAAACGGCTACCACATCC	GATACGCTCATTCCGATTACAG
IL1B	AAGGTTGTGCGGTGCACTAT	AGTGTAGGCCGAGAGGTTGA
IL8	CTTCACGATGAAGGCTGCAA	TTTGGCAGAAACAACGCTCT
IL8v2	CTTCACGATGAAGGCTGCAA	CCCCATCCTGCAGAAACTT
iNOS2B	CTGGCCCGTGTTAATGAGGT	TTGCGTGCATCAAACACCTG
MUC2	TGCAGAAGAACCAGAAAGAT	TTTTGGCAGTCTGTTAAGGT
LYC	GATGGATCAACGGACTATG	CTGTCTCACTATGGTCTTG

IL1B, Interleukin 1 beta
IL8, Interleukin 8
iNOS2b, inducible nitric oxide synthase 2b
MUC2, mucin 2
LYC, lysozyme C

Bacterial Challenge

The ALG-00-530 isolate of *F. columnare* is a genomovar II isolate that was recovered in 2000 from a diseased channel catfish in Alabama. A standard frozen glycerol stock of ALG-00-530 was used and the frozen material was inoculated into 25 mL modified Shieh (MS) broth and grown at 28°C for 24 h with shaking at 175 rpm. Following 24 h culture, 200 µL of culture was inoculated into 200 mL MS broth and cultured at 28°C (150 rpm) for ~ 20 h. The OD540 of the culture was adjusted to 0.8 using sterile MS broth and then used for the challenge. The challenge doses were determined by diluting and plating the culture, enumerating colonies, and calculating the CFU mL^{-1}, following standard practices. In the low dose challenge study, fish were exposed to 2.2×10^4 CFU/ml, while in the high dose challenge fish were exposed to 2.9×10^5 CFU/ml. Fish were held in 30 L of aerated water containing the challenge inoculum for 30 minutes before water flow was restored at 0.5 L/min. Fish were monitored twice per day at which moribund and dead fish were removed.

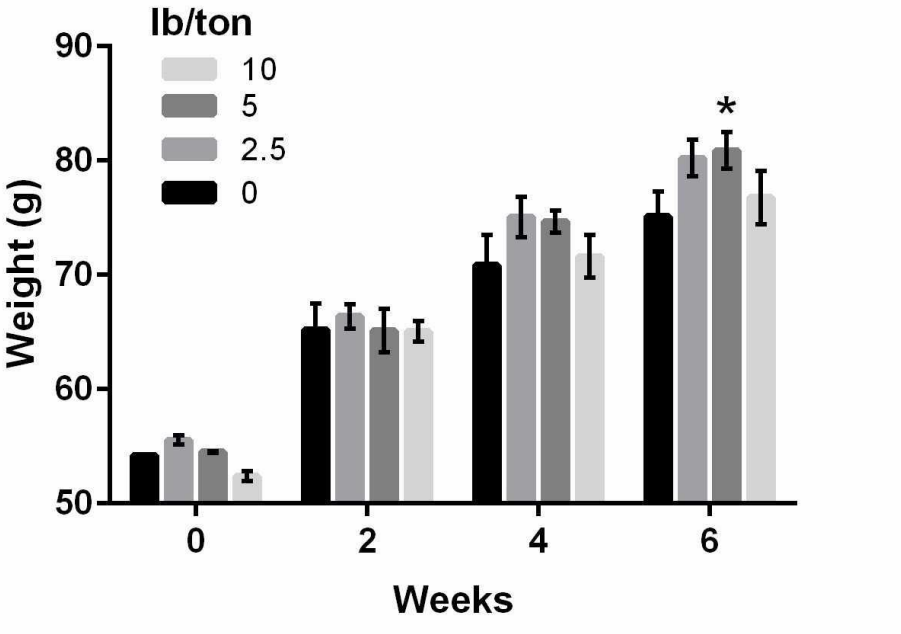
Statistics

Growth, FCR, hematology, and serum protein data were analyzed by one-way ANOVA. Duncan's multiple range tests were used to determine differences between treatment means. Differences were considered significant at the 0.05 probability level. All analyses were performed using the SAS program (Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, USA, 2001). Survival data were analyzed using Graphpad Prism (version 6.0) by Kaplan-Meier Log Rank Survival Analysis using the Mantel-Cox test.

Results

Growth rates were monitored at 0, 2, 4, and 6 weeks following the initiation of the feeding trial (Figure 1). There were no significant differences in growth except for the 5 lb/ton dose, which were significantly larger (80.7 $\pm$ 1.6 g) than control fish (75.2 $\pm$ 2.2 g) at the end of the 6 week trial (*P<0.05*). There were no significant differences between the feed conversion ratios (FCR) of any treatment. FCRs averaged 2.59, 2.23, 2.31, and 2.31 for the 0, 2.5, 5, and 10 lb/ton treatments, respectively.

Figure 1.- Growth of hybrid catfish fed a commercial catfish ration supplemented with graded levels of Diamond V XPC for 6 weeks. The asterisk denotes statistical significance between fish fed the control diet versus the indicated dietary inclusion level of XPC (P<0.05)



Hematological analyses (Table 4) revealed numerous differences in the cellular composition of the blood in XPC fed animals as compared to the control group. White blood cell numbers were substantially higher ($P<0.001$) in the 5 and 10 lb/ton treatment. White blood cell numbers were also greater in the lowest dose tested, but the difference was marginally outside the level of statistical significance ($P = 0.06$). Differences were also evident in the red cell compartment; however, the data do not reflect a clear dose-dependence. The absolute number of red blood cells was significantly greater in the 2.5 and 5 lb/ton treatments, while hematocrit (packed cell volume) values were significantly greater in the 2.5 and 10 lb/ton treatments. There were no differences detected in red cell size (mean corpuscular volume, MCV) or the total amount of hemoglobin in whole blood (Hb). The mean corpuscular hemoglobin (MCH), which is the average mass of hemoglobin per red blood cell was significantly lower in the 5 lb/ton treatment; however this difference was no longer significant when normalized to the hemoglobin content per unit volume of red cells (mean corpuscular hemoglobin concentration; MCHC).

Table 4: Mean total cell count, red blood cells, white blood cells, hematocrit value, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration of hybrid catfish (N = 16) fed diets containing various levels of Diamond V for 6 weeks. Means followed by the same letter within a column are not significantly different using Duncan's multiple range tests.

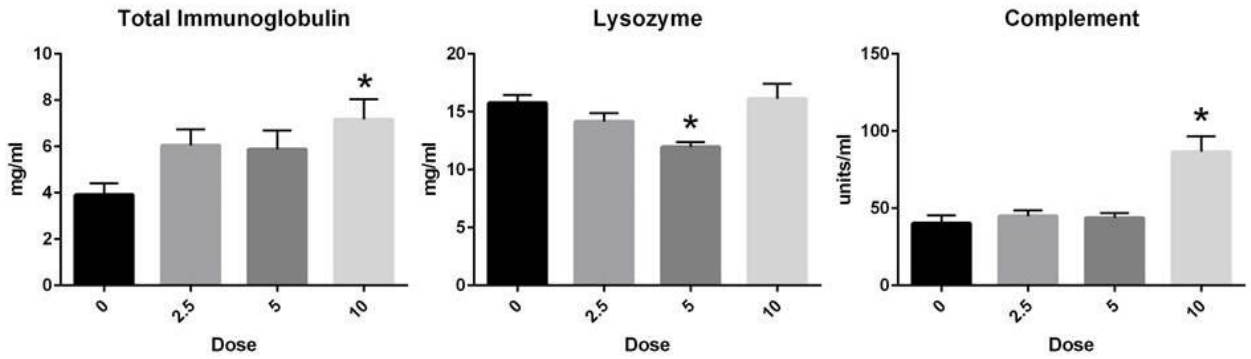
Treatments (lbs/Ton)	TCC/ul ($x10^6$)	RBC ($x10^6$)	WBC ($x10^5$)	HCT (%)	Hb (g/dl)	MCV (fl)	MCH pg	MCHC (%)
0	2.90^b	2.73^b	1.45^b	35.47^b	9.23	132.12	34.07^a	25.74
2.5	3.27^a	3.05^a	1.78^b	37.38^{ab}	9.32	123.91	30.79^{ab}	24.96
5.0	3.32^a	3.07^a	2.25^a	36.03^b	9.07	121.04	29.07^b	24.87
10.0	3.22^a	2.89^{ab}	2.32^a	39.13^a	9.74	133.03	34.36^a	25.16

TTC, total cell count
RBC, red blood cells
WBC, white blood cells
HCT, hematocrit value
Hb, hemoglobin
MCV, mean corpuscular volume
MCH, mean corpuscular hemoglobin
MCHC, mean corpuscular hemoglobin concentration

Analysis of serum samples showed significantly higher levels of total immunoglobulin in the 10 lb/ton treatment. While 2.5 lb/ton and 5 lb/ton were slightly higher than the control, these differences were not significant. Curiously, when compared to fish fed the control ration, lysozyme levels were significantly lower in the 5 lb/ton treatment. No differences were detected between alternative complement levels in the 0, 2.5, and 5 lb/ton treatments; however fish fed the

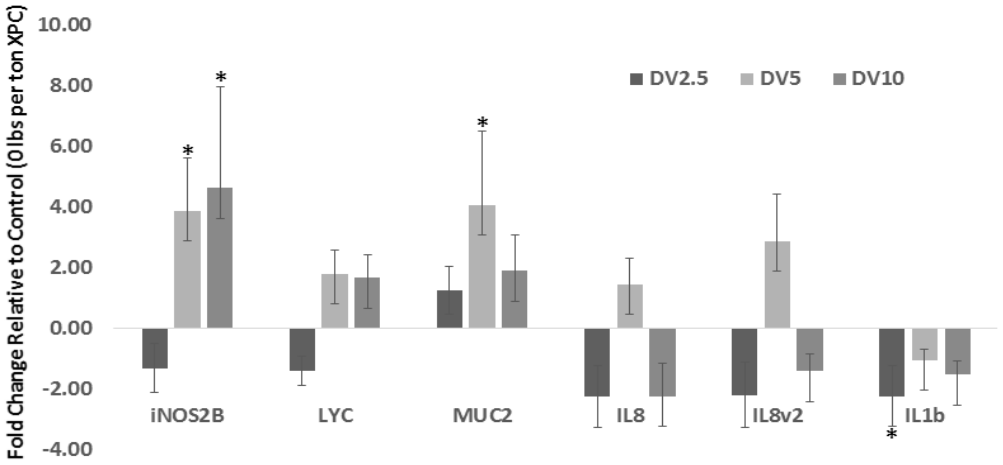
10 lb/ton level had significantly higher complement, nearly a two-fold difference, in their sera (Figure 2).

Figure 2.- Levels of important immune effectors in sera following the feeding trial. Asterisks denote statistical significance between fish fed the control diet versus the indicated dietary inclusion level of XPC (P<0.05).



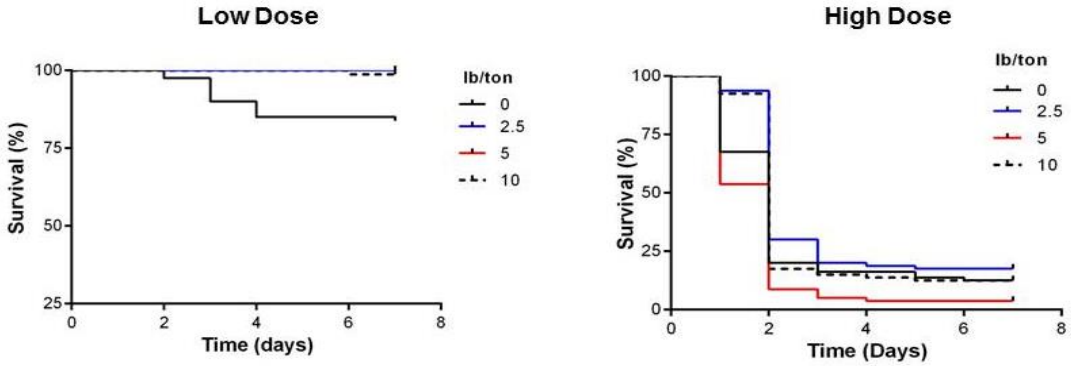
We also examined gene expression in a number of key genes linked in previous studies to broad-based resistance or susceptibility to disease in catfish (Peatman *et al*. 2013; Zhao *et al*. 2015). iNOS2b, correlated previously with columnaris resistance (Peatman *et al*. 2013), was significantly upregulated at the 5 lb/ton and 10 lb/ton doses (relative to control). Lysozyme C, however, somewhat consistent with serum sampling, showed no significant upregulation with XPC dose. Mucin 2 was significantly induced only in the 5 lb/ton treatment. Among pro-inflammatory cytokines, often associated in catfish with over-exuberant and damaging responses, we observed no significant upregulation in either of two forms of IL8 or IL1b. In fact, IL1b showed a pattern of repressed expression, albeit only significantly altered expression in the 2.5 lb/ton treatment (Figure 3; $p<0.05$).

Figure 3. Quantitative RT-PCR analysis of immune gene expression in proximal intestine following 6 week feeding. Fold changes are relative to the control (0 lbs/ton XPC treatment) normalized to the 18S housekeeping gene. Gene abbreviations are IL1B, Interleukin 1 beta; IL8, Interleukin 8; iNOS2b, inducible nitric oxide synthase 2b; MUC2, mucin 2; LYC, lysozyme C. Results are presented as mean ± log standard error (SE) of fold changes and the asterisk indicates statistical significance at $P < 0.0$



Two independent challenge studies, featuring a low and high dose, were conducted to examine the influence of Diamond V XPC on columnaris disease susceptibility (Figure 4). In the low dose challenge study, all animals fed XPC showed significantly better survival (Figure 4; left).

Figure 4.- Survival of hybrid catfish fed a commercial catfish ration supplemented with graded levels of Diamond V XPC for 6 weeks and challenged with *Flavobacterium columnare*.



However, the overall mortality was low with 16.3%, 0%, 0%, and 1.25% mortality observed in the 0, 2.5, 5, and 10 lb/ton treatments, respectively. In the high dose challenge, mortality was dramatically higher (Figure 4; right). There were no significant differences in cumulative survival across any treatment. However, while overall survival was not different, the survival curves were significantly different between both the 2.5 and 10 lb/ton versus the controls, as the chance of surviving at least one day were 93.5% for the 2.5 lb/ton group, and 92.5% for the 10 lb/ton treatment, versus 67.5% for the control, suggesting some degree of initial protection.

Discussion

Despite the continuous efforts that have been undertaken to develop new disease management tools for sustainable aquaculture; current practices remain unsustainable. Therefore, modern aquaculture is in desperate need of natural non-antibiotic alternatives (Reverter *et al.* 2014). Over the past few years, environmentally friendly aquaculture has been evolving globally to overcome the shortage of efficacious fish vaccines and the numerous drawbacks of using traditional chemotherapeutic drugs. Probiotics represent a significant advancement in technology that is currently gaining marked interest by fish producers worldwide to mitigate disease problems in aquaculture. The use of probiotics in aquaculture has been accompanied by a concomitant decline in the levels of antibiotics and a boost in growth performance as well as immune response (Irianto and Austin 2002). Anaerobically fermented *S. cerevisiae* products have been shown to positively influence growth and non-specific immune responses of several fish species; however, its effect has not been examined in hybrid catfish yet.

Therefore, in the present work, we examined the beneficial effects of dietary supplementation of commercial catfish diet with a *S. cerevisiae* fermentation product (Original XPC) on growth, immune readiness, and susceptibility to columnaris disease in hybrid catfish.

Growth performance results from studies that have fed products derived from *S. cerevisiae* have been variable. In the present study, no significant differences in FCR or in growth were detected between treatment groups except for the fish fed dose 5 lb/ton, which showed significant growth improvement (80.7 + 1.6 g) at the end of the 6 weeks feeding period compared to the control fish (75.2 + 2.2 g) fed basal diet (Figure 1). Likewise, dietary supplementation with commercially available yeast or yeast subcomponents for 4 or 6 weeks did not significantly affect total weight gain or FCR of channel catfish (Welker *et al.* 2007). In growth trials, Peterson et al. fed a yeast cell wall product, an immunostimulant composed of the outer cell wall of *S. cerevisiae* which is rich in mannan-oligosaccharides, supplemented diets to channel catfish and found no differences in weight gain, specific growth rate, or FCR among treatments after 6 weeks at 2 g/kg (Peterson *et al.* 2010) or after 9 weeks at 4 g/kg (Peterson *et al.* 2012). Similarly, no significant difference in growth rates was observed in Nile tilapia, *Oreochromis niloticus* fed probiotics (*Bacillus subtilis*, *S. cerevisiae* and *Aspergillus oryzae*) at 5 and 10 g/kg for 4 weeks, although the fish fed diets supplemented with probiotics had better FCR (Iwashita *et al.* 2015). Moreover, growth rate and feed efficiency were also unaffected in juvenile red drum, *Sciaenops ocellatus* and sea bass, *Dicentrarchus labrax* fed brewer's yeast for 6 and 12 weeks (Li *et al.* 2005, Oliva-Teles and Gonçalves 2001), respectively. Conversely, enhanced growth has been reported for rainbow trout, *Oncorhynchus*

mykiss (Staykov *et al.* 2007), European sea bass (Torrecillas *et al.* 2007), hybrid striped bass, *Morone chrysops*×*M. saxatilis* (Li and Gatlin Iii, 2003), koi carp, *Cyprinus carpio* (Dhanaraj *et al.* 2010), beluga sturgeon, *Huso huso* (Hoseinifar *et al.* 2011), and broiler chicks (Santin *et al.* 2001, Zhang *et al.* 2005) fed *S. cerevisiae* supplement diets. It is also noteworthy that the inclusion of the same product we used, Original XPC, in nursery diets of weaned pigs had no effect on body weight or average daily gain but resulted in greater weight gain following exposure to *Salmonella* than in pigs fed conventional nursery feed (Price *et al.* 2010).

The enhanced weight gain and feed efficiency in the aforementioned studies were attributed to increased beneficial bacteria within the gastrointestinal tract, stimulation of gastric development, secretion of digestive enzymes, enhanced energy gained by fermentation within the lower gastrointestinal tract (GIT), supply of certain essential nutrients, enhanced amino acid and mineral absorption, or other health benefits associated with host/prebiotic synergy, such as better intestinal motility and inhibition of toxin binding (Hoseinifar *et al.* 2011, Iji *et al.* 2001, Macfarlane *et al.* 2008, Price *et al.* 2010, Tewary and Patra 2011). Catfish are capable of digesting and utilizing around 60-70% of the complex carbohydrates, but the remaining portion is undigestible (Duncan and Klesius 1996, Wilson and Poe 1987). Therefore, enzymes released by *S. cerevisiae* could have improved the feed digestibility and subsequent absorption throughout the GIT. *S. cerevisiae* produce enzymes that are not produced by the host and the inclusion of yeast in the diet was reported to improve feed efficiency, organic phosphorus (phytic acid) utilization and fiber (complex polysaccharides including cellulose) digestion (Swain *et al.* 1996, Tewary and Patra 2011). Moreover, improved digestive and absorptive intestinal capacity was

reported in pigs due to increased width of jejunal villi in the jejunum of pigs fed XPC compared with controls resulting in better growth performance (Price *et al.* 2010). Enhanced intestinal morphology has been associated with greater weight gain in pigs (Pluske *et al.* 1996, Zijlstra *et al.* 1997).

Haemogram and serum biochemical parameters are valuable indicators and pathophysiological reflectors of the general health status and often provide crucial information for assessment of fish physiological responses (Abdel-Tawwab *et al.* 2008, Decie and Lewis 1991, Hoseinifar *et al.* 2011). In our study, XPC fed fish showed a marked increase in red and white blood cell counts and hematocrit values as compared to the control group, while no differences were detected in MCV, total Hb, and MCH/MCHC (Table 2). Similar to these results, administration of *S. cerevisiae* biotic forms (whole yeast cell, its mannan-oligosaccharide extract, and a mixture of both) to Nile tilapia for two months resulted in a significant increase in erythrocytic count, packed cell volume, hemoglobin concentration, and total leukocyte counts in treated fish versus the control group (Abu-Elala *et al.* 2013). Moreover, the haematological parameters (red and white blood cells numbers) of channel catfish fed for a short-term (1 week) diets containing commercial whole-cell yeast or yeast subcomponents were somewhat lower for the control group in relation to the supplemented groups (Welker *et al.* 2012). Welker *et al.* (2007) reported significant elevation in hematocrit and hemoglobin values but no differences in red and white blood cells counts in juvenile channel catfish after 6 weeks of feeding yeast or yeast subcomponents from various commercial sources (Welker *et al.* 2007). Brewer yeast is a source of vitamin B complex as well as other haemotonic ingredients utilized in the process of

haematopoeisis (Abu-Elala *et al.* 2013). Moreover, they may stimulate the secretion of signaling molecules by the immune system (cytokines) which can induce the formation of new white blood cells (Raa 2000). This may explain the substantially higher white blood numbers observed in this study. However, blood parameters of *Labeo rohita* fingerlings fed diets supplemented with 0%, 5%, 7.5%, and 10% *S. cerevisiae* for 60 days were all within standard levels except for the 5% treatment, which were superior as compared to others (Tewary and Patra 2011). Replacement of fishmeal with brewer's yeast did not affect the haematological parameters (RBC, Hb, Htc, MCV, MCH, lymphocyte and platelet counts) of the hybrid catfish, Thai Panga (*Pangasianodon hypophthalmus*× *Pangasius bocourti*) (Pongpet *et al.* 2016). Dietary supplementation with probiotics (*Bacillus subtilis, S. cerevisiae* and *Aspergillus oryzae*) in juvenile tilapia for four weeks had no effect on Hb, MCV and MCHC and total leukocyte count, however, fish supplemented with 10 g/kg probiotic exhibited higher lymphocytes values on the fourth week, and on the sixth week, Ht level was higher in fish fed probiotics (Iwashita *et al.* 2015). Differences in fish species, age/size, strain of probiotic, dosage, feeding duration, mode of supplementation, environmental and/or rearing conditions may have accounted for some of the varying results present in the literature regarding the response of these same parameters in fish fed yeast-supplemented diets.

Immunoglobulins, the complement system and lysozyme are important defense molecules against microbial infections and their assessment often provide important information on the innate immunity of the host. Serum analysis in the present study revealed higher levels of total immunoglobulin in all XPC treatments than in the control group (Figure 2). While fish fed

the 10 lb/ton ration had higher (two-fold difference) alternative complement level, the 2.5 and 5 lb/ton treatments presented no differences when compared to fish fed control ration. Moreover, lysozyme levels in sera were similar among all treatments except for the group fed 5 lb/ton, which were significantly lower. Considerable variation exists in the available literature regarding the immunomodulatory properties of dietary *S. cerevisiae* in teleosts. Siwicki et al. reported that dietary intake of immunostimulant preparations containing *S. cerevisiae* by rainbow trout for 1 week elevated total plasma protein and total immunoglobulin levels (Siwicki *et al.* 1994). The concentration of lysozyme is directly proportional to the leukocytic count. In fishes, lysozyme is found mainly in tissues rich in leucocytes, where the risk of microbial invasion is high, such as skin, gills, head kidney, and the alimentary tract (Yano 1996). The addition of *S. cerevisiae* to *O. niloticus* diet remarkably increased alternative complement level and the leukocytic count, which in turn elevated lysozyme concentration and activity (Abu-Elala *et al.* 2013). In European sea bass and rainbow trout, there was a positive correlation between lysozyme and alternative complement pathway activities in blood and inclusion of dietary yeast cell wall, mannan-oligosaccharides (Staykov *et al.* 2007, Torrecillas *et al.* 2007). Inconsistent with these studies, Welker et al. concluded that dietary administration of *S. cerevisiae* commercial preparations following the manufacturers' supplementation recommendation was ineffective in positively affecting immunity or lysozyme levels in channel catfish (Welker *et al.* 2012, Welker *et al.* 2007). Similarly, hybrid striped bass fed diets with incremental levels (1%, 2% and 4% of diet) of dried brewer's yeast were found to have serum lysozyme levels within normal ranges (Li and Gatlin Iii 2003). Intake of high levels of yeast, either through prolonged

feeding, high dosage, or both was reported to cause immunosuppression in channel catfish (Welker *et al.* 2012), seabream (Couso *et al.* 2003, Ortuño *et al.* 2002), African catfish (Yoshida *et al.* 1995), Atlantic salmon (Robertsen *et al.* 1990), and rainbow trout (Jeney *et al.* 1997), which is likely due to an overload of glucan receptors on phagocytic cells reducing their ability to phagocytose bacteria in fish (Couso *et al.* 2003).

Immunostimulants derived from yeast have been shown to boost fish's innate defense mechanisms against infectious diseases (Engstad and Robertsen 1993, Ortuño *et al.* 2002, Tewary and Patra 2011). However, improved antibody response or innate immune function does not often predict resistance to disease. Channel catfish have previously shown some increase in nonspecific immune function but without a corresponding increase in resistance to *E. ictaluri* when fed diets supplemented with whole-cell or subcomponents of *S. cerevisiae* (Welker *et al.* 2007). Fish fed XPC in our study showed initial protection and heightened resistance (increased chance of survival) against columnaris disease over control fish (Figure 4). Equally, Nile tilapia fed a commercial *S. cerevisiae*-supplemented diets had better resistance to bacterial challenges with *F. columnare* and *Aeromonas hydrophila* (Abdel-Tawwab *et al.* 2008, Abu-Elala *et al.* 2013). In addition, dairy-yeast effectively reduced mortality rates in stressed golden shiners exposed to *F. columnare* (Sink and Lochmann 2008, Sink *et al.* 2007). In the grouper, *Epinephelus coioides*, dietary administration of *S. cerevisiae* at an optimal dose (10^7 cfu/kg) induced upregulation of innate cellular and humoral immune responses as well as increased resistance to disease challenges by *Streptococcus* sp. and a grouper iridovirus (Chiu *et al.* 2010). Furthermore, Li and Gatlin (2003) demonstrated that brewer's yeast administered to hybrid

striped bass for relatively long periods positively influence resistance to *Streptococcus iniae* infection without causing immunosuppression. In catfish studies, channel catfish injected with yeast β-glucans or fed diets supplemented with mannan-oligosaccharide showed reduced mortality relative to controls following challenge with *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish (ESC) (Chen and Ainsworth 1992, Peterson *et al.* 2010). Controversially, the results obtained by several other authors were not in agreement with the abovementioned results. Dietary supplementation of *S. cerevisiae* or its subcomponents while enhancing non-specific immune response, does not appear to improve resistance of channel catfish to ESC as fish fed yeast had mortality rates comparable (the increase in survival was not significant) to fish fed control diet following exposure to *E. ictaluri* infection (Duncan and Klesius 1996, Welker *et al.* 2007).

Although there was no significant difference in cumulative mortality among treatments in the high dose challenge (Figure 4, right), we observed better survival rates in the XPC supplemented groups. No mechanism has thus far been proposed to explain columnaris disease-subsiding assets of this product and the actual mechanism of action still needs to be determined. In addition to modulating the immune response, XPC may possess some antimicrobial-like activities. The soluble metabolites produced during the anaerobic fermentation of *S. cerevisiae* were associated with growth inhibition of *Candida tropicalis* and *Escherichia coli* in vitro (Jensen *et al.* 2008). Chen and Ainsworth (1992) hypothesized that the increased survival of channel catfish intraperitoneally-injected with β-glucan and challenged with *E. ictaluri* was related to increased phagocytic activity and bactericidal ability of phagocytes and neutrophils. It

is also possible that some simultaneous modifications in the associated microbiota, as suggested by Sink et al. (2007) and Li and Gatlin (2004), enhanced the survival of hybrid catfish, however, we did not characterize the microbiome in this study. Another protection mechanism against pathogenic bacterial infections is through stimulation of GIT bacteria to produce short chain fatty acids that are inhibitory to several pathogens and increase in number, therefore competing for attachment sites with pathogens on the GIT mucosa (Niba *et al.* 2009). Price *et al*. (2010) interpreted the increased *Salmonella* shedding from pigs consuming XPC, as an indication of rapid elimination of the pathogen from the GIT, which may result in reduced infection rates. Few studies have characterized microbial communities colonizing skin and gills of aquaculture species. *S. cerevisiae* supplementation could have played a critical role in shaping the fish's microbial community and/or increasing competition (competitive exclusion) with *F. columnare* on the fish surface. Inclusion of XPC in the diet of weanling pigs was shown to increase numbers of beneficial bacteria (*Bacteroides, Bacteroidetes,* and *Lactobacillus*) in the feces of pigs consuming XPC compared with animals eating control diet (Price *et al.* 2010). Moreover, dysbiosis of the fish's external microbiome was previously shown to increase catfish susceptibility to columnaris disease (Mohammed and Arias 2015) and when *F. columnare* is present in low numbers, it may not be able to compete with other naturally occurring organisms on the host (Suomalainen *et al.* 2005). Therefore, manipulation of the fish's microbial communities/microbiome composition was suggested as a more ecological strategy to combat columnaris disease (Suomalainen 2005).

In conclusion, results of the current study suggest that *S. cerevisiae* fermentation products have the potential to increase profitability of hybrid catfish industry by enhancing nonspecific immunity, limiting disease-associated mortalities, replacing antibiotics and chemotherapeutants in aquaculture. Further experiments are necessity to clarify mechanisms of action, as well as the optimum feeding duration and concentration required for each fish species to achieve maximum immunity (disease resistance) and to minimize the risk of disease outbreaks under different production scenarios.

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