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Impact of feed additives on surface mucosal health and columnaris susceptibility in channel catfish fingerlings, *Ictalurus punctatus*

Honggang Zhao [a], Chao Li [b], Benjamin H. Beck [c], Ran Zhang [a], Wilawan Thongda [a], D. Allen Davis [a], Eric Peatman [a, *]

[a] School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, Auburn, AL 36849, USA
[b] Marine Science and Engineering College, Qingdao Agricultural University, Qingdao 266109, China
[c] United States Department of Agriculture, Agricultural Research Service, Stuttgart National Aquaculture Research Center, Stuttgart, AR 72160, USA

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ABSTRACT

One of the highest priority areas for improvement in aquaculture is the development of dietary additives and formulations which provide for complete mucosal health and protection of fish raised in intensive systems. Far greater attention has been paid to dietary impact on gut health than to protective effects at other mucosal surfaces such as skin and gill. These exterior surfaces, however, are important primary targets for pathogen attachment and invasion. *Flavobacterium columnare*, the causative agent of columnaris disease, is among the most prevalent of all freshwater disease-causing bacteria, impacting global aquaculture of catfish, salmonids, baitfish and aquaria-trade species among others. This study evaluated whether the feeding of a standard catfish diet supplemented with Alltech dietary additives Actigen®, a concentrated source of yeast cell wall-derived material and/or Allzyme® SSF, a fermented strain of Aspergillus niger, could offer protection against *F. columnare* mortality.

A nine-week feeding trial of channel catfish fingerlings with basal diet (B), B + Allzyme® SSF, B + Actigen® and B + Actigen®+Allzyme® SSF revealed good growth in all conditions (FCR < 1.0), but no statistical differences in growth between the treatments were found. At nine weeks, based on pre-challenge trial results, basal, B + Actigen®, and B + Allzyme® SSF groups of fish were selected for further challenges with *F. columnare*. Replicated challenge with a virulent *F. columnare* strain, revealed significantly longer median days to death in B + Allzyme® SSF and B + Actigen® when compared with the basal diet ($P < 0.05$) and significantly higher survival following the eight day challenge period in B + Actigen® when compared with the other two diets ($P < 0.05$). Given the superior protection provided by the B + Actigen® diet, we carried out transcriptomic comparison of gene expression of fish fed that diet and the basal diet before and after columnaris challenge using high-throughput RNA-seq. Pathway and enrichment analyses revealed changes in mannose receptor DEC205 and IL4 signaling at 0 h (prior to challenge) which likely explain a dramatic divergence in expression profiles between the two diets soon after pathogen challenge (8 h). Dietary mannose priming resulted in reduced expression of inflammatory cytokines, shifting response patterns instead to favor resolution and repair. Our results indicate that prebiotic dietary additives may provide protection extending beyond the gut to surface mucosa.

1. Introduction

Fish currently provides three billion people with 20% of their animal protein requirements. However, environmental pollution and overexploitation threaten natural fish stock regeneration [1,2]. To meet the increased demand from the global market, aquaculture will play an increasingly important role in contributing to the volume and stability of global fish supplies. Commercial fish farming, in tanks or enclosures under monitored conditions, can increase production by controlling variables ranging from exclusion of predators and improved water quality to enhancement of diet and nutrition [3]. However, intensive aquaculture has been traditionally accompanied by increasing incidence and severity of disease outbreaks as environmental, genetic, or nutritional deficiencies are exploited by primary and opportunistic pathogens.

* Corresponding author.
E-mail address: peatmer@auburn.edu (E. Peatman).

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Developing dietary supplements and additives to provide complete mucosal health and protection of fish raised in intensive systems has emerged, therefore, as a high priority area with a great potential for significant improvements in aquaculture.

Teleost fish exhibit well-developed physical and immunological barriers at mucosal surfaces where a complex interplay of secreted mucus, commensal bacteria, and underlying mucosa-associated lymphoid tissue (MALT) elements serve to co-regulate immunity and maintain homeostasis in healthy fish [4,5]. While our understanding of host-pathogen-commensal-environment interactions are growing, our knowledge has been, until recently, focused in the gut mucosa, with relatively little study on skin and gill barriers. These exterior surfaces, however, are important primary targets for pathogen attachment and invasion. A key question is whether dietary additives, known to enhance gut immune health [6,7], may also stimulate beneficial, protective immunity at distal mucosal surfaces, either through transfer through the blood or by direct stimulation of immune receptors through the presence of whole or digested feed components in the water [8].

Flavobacterium columnare, the causative agent of columnaris disease, is among the most prevalent of all freshwater disease-causing bacteria. Often characterized as unpredictable and difficult to treat, columnaris impacts global aquaculture of catfish, salmonids, baitfish and aquaria-trade species among others [9,10]. Channel catfish, the predominant aquaculture species in the United States, are exceedingly susceptible to columnaris disease [11]. Catfish experiencing stress due to high rearing density [12], high organic loads [13], excessive handling [14], or high ammonia etc. [15] are more susceptible to *F. columnare* infection. Catfish gill and skin tissues constitute the primary route of entry for the pathogen, with infection often grossly evident soon after colonization in the form of pale discoloration, erosion or necrosis of these tissues [16]. Strategies to combat columnaris infections have long included lowering rearing density, salt baths, acid baths, and chemical therapeutants [17]. However, these approaches have failed to reduce columnaris disease incidence, as they are largely reactive measures after the onset of disease. Cost-effective, improved dietary formulations which improve immune readiness or decrease pathogen adhesion offer the potential of continuous, proactive mucosal protection [18,19].

High-throughput transcriptome sequencing (RNA-seq) offers several advantages over traditional microarray approaches for nutri-genomics [20]. It allows for capture of novel transcripts and splicing variants which may not be present on static arrays, it has a larger dynamic range, and it avoids the potential of cross-hybridization of similar probes resulting in inaccurate gene expression values [21]. Using RNA-seq approaches, previous work by our group has demonstrated that differing cytokine and lectin profiles in surface mucosa differentiate fish from families identified as resistant or susceptible to columnaris disease [4]. We have particularly focused on the role of a rhamnose-binding lectin (RBL1a) in facilitating pathogen attachment and invasion in the gill [4,22–24]. Our group found that pathogen attachment could be reduced in a dose-dependent manner through addition of a sugar ligand (rhamnose or D-galactose) and that levels of RBL1a were dramatically impacted by feeding status [22]. Given our improved understanding of mechanisms potentially governing host mucosal immunity in the context of columnaris, we were interested in examining whether commercially-available enhanced diets could increase catfish survival by modulating these same pathways. It was investigated whether the feeding of a standard catfish diet supplemented with Alltech dietary additives Actigen®, a concentrated source of yeast cell wall-derived material including mannan oligosaccharides (MOS) and/or Allzyme® SSF, a fermented strain of *Aspergillus niger* producing a complex of enzymes [25], could offer protection against *F. columnare* mortality.

2. Material and methods

2.1. Fish and diet composition

All procedures involving the handling and treatment of fish used during this study were approved by the Auburn University Institutional Animal Care and Use Committee (AU-IACUC) prior to initiation.

In order to evaluate the biological response of two dietary supplements (Actigen® and Allzyme® SSF meals, Alltech, Inc., Nicholasville, KY, USA), four practical diets (Basal diet (B), B + Allzyme® SSF, B + Actigen® and B + Actigen®+Allzyme® SSF) were formulated to contain 36% protein and 8% lipids and offered to juvenile (average size 4.1 ± 0.11 g) channel catfish over a nine week growth trial (Table 1). Fish were stocked in 36 aquaria (75L) at a density of 20 fish per aquaria with nine replicate tanks per dietary treatment. The fish in the replicate aquaria were randomly assigned to each dietary treatment and offered feed twice daily (8:00 am, 4:00 pm) based on a set percentage of body weight. Water temperature (27.82 ± 1.16 °C) and dissolved oxygen (5.63 ± 0.73 mg/L) were measured twice daily by YSI Model 58 Oxygen Meter (Yellow Springs Instrument Model 58, Yellow Springs, OH, USA) and pH (7.23 ± 0.40) weekly with a pH meter. A diel light:dark cycle was set at 14:10 h. Fish were weighed upon initiation of the trial and every two weeks thereafter. Feed inputs were adjusted based on observed feed consumption and biweekly feed conversion ratio. At the conclusion of the growth trial, final weight, weight gain and feed utilization were determined. All data was analyzed using one-way analysis of variance to determine significant differences ($P < 0.05$) among treatments. The statistical analyses were performed using the SAS® software package (SAS Institute Inc., Cary, NC, USA).

The basal diet was formulated to meet the known nutrient requirements of the fish and represents a typical fingerling diet. Diets were manufactured at Auburn Fisheries North Station, Auburn, AL under laboratory conditions. Each diet was prepared by mixing pre-ground dry and wet ingredients in a food mixer (Hobart, Troy, OH, USA) for 15 min. Boiling water was then blended into the mixture to attain a consistency appropriate for pelleting. The moist mash from each diet was passed through a die (2.4 or 3.17 mm) in a meat grinder, and the pellets were dried in a forced air drying oven (<50 °C) to a moisture content of less than 10%. Diets were stored at −20 °C and prior to use each diet was ground and sieved to an appropriate size.

2.2. Bacterial challenge and tissue collection

Fish were maintained in four 50 gallon tanks and acclimatized for 2 weeks at a temperature of 28 °C after transfer to the challenge lab in the CASIC building, Auburn University. Before challenge, the *F. columnare* bacteria (BGFS-27; genomovar II) [26] were cultured from a single colony and re-isolated from a symptomatic fish. The bacteria were inoculated in modified Shieh broth and grown in the shaker incubator (100 rpm) for 24 h at 28 °C. Challenge experiments were conducted by immersion exposure for 2 h at a final concentration 1×10^5 CFU/mL. After eight weeks of the above feeding regimen, fish from 3 replicate per treatment were challenged with *F. columnare* through standard bath challenge [10,22]. Daily and accumulative mortality was tracked to preliminarily estimate the effect of four diets.

At nine weeks, based on pre-challenge trial results, basal, B + Actigen®, and B + Allzyme® SSF groups were selected for further challenges with *F. columnare*. Four tanks were used for each group, three of which were challenged with *F. columnare* and one