Feed Additives Based On Quorum Sensing Disruption Could Aid Fight Against EMS/AHPN

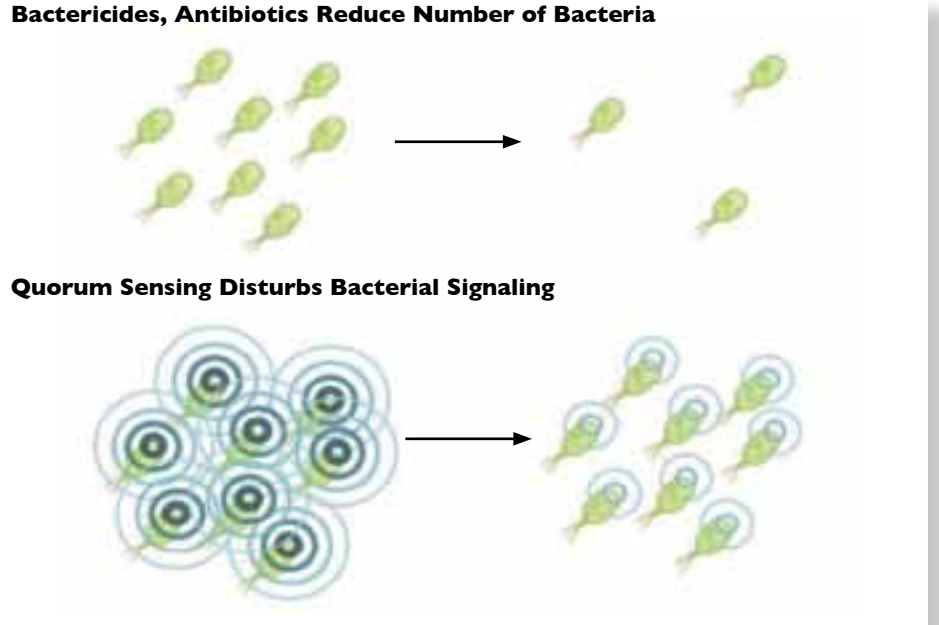

Figure 1. Quorum sensing inhibition.

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Summary:
The pathogenicity of early mortality syndrome in shrimp is likely regulated by quorum sensing, which allows the *Vibrio* bacteria that colonize shrimp guts to coordinate the release of the toxin that damages shrimp digestive systems. Research by the authors has shown that synergistic blends of natural antimicrobial compounds added to aquafeed can function as powerful interrupters of bacterial quorum sensing signaling in aquaculture pathogens such as *Vibrio harveyi* at concentrations well below minimal inhibitory concentrations.

Early mortality syndrome (EMS) or acute hepatopancreatic necrosis (AHPN) is a shrimp disease that has been disrupting production in major shrimp-producing countries since 2009. First reported in China, it has spread to Vietnam, Malaysia and Thailand, and just recently to Mexico and India.

EMS outbreaks typically occur within the first 30 days after stocking newly prepared shrimp ponds, and mortality can exceed 70%. EMS is caused by specific strains of a relatively common bacterium, *Vibrio parahaemolyticus,* which are transmitted orally, colonize the shrimp gastrointestinal tract and produce a toxin that causes tissue destruction and dysfunction of the shrimp digestive organ known as the hepatopancreas.

As reported during sessions on health management at GOAL 2013, the pathogenicity of EMS/AHPN is most likely regulated by a mechanism called quorum sensing, which allows the *Vibrio* colonies to coordinate the release of the potent toxin.

EMS Management

Because EMS is caused by a *Vibrio* that is difficult to eradicate from aquaculture production environments, its control requires a very different approach than the strategies used against white spot syndrome virus, which are based on specific biosecurity measures. Avoiding early contamination through the broodstock and larval stages, combined with continued control of microbial development – particularly during the initial month of the cycle – will be crucial to control EMS.

In this regard, intensive nursery systems are now being explored to produce juvenile shrimp throughout the critical stages affected by EMS. These systems allow superior control over nutrition and the microbial environment compared to direct stocking into growout ponds.

Microbial Control

The use of antibiotics to control microbial development throughout the production process is not desirable due to the risk of building up resistance and rejection by legislators and consumers. The shrimp industry requires alternative ways to control the microbial ecosystems in production systems.

Sustainable approaches to modulate the gut microflora in shrimp include the use of probiotics, selected bacteria that inoculate the gut; and specific natural compounds called botanicals or phytobiotics, which are capable of modulating the microflora toward a favorable composition.

Provided botanical formulations are heat stable, they can be easily incorporated into feed at the mill and therefore be present in every meal from the starter feed onwards without requiring major adaptations of the production protocols at nurseries or farms. Phytobiotics that promote healthy gut microflora also support the establishment of probiotic bacteria and therefore enhance the effects of probiotic inoculations in production systems. Various studies have demonstrated the ability of natural products to improve shrimp growth and survival

Functional Feeds

Functional feeds containing gut health promoters allow delivering with every meal an adequate concentration of natural antimicrobial activities into the shrimp gut. These feeds could be an

Table 1. Efficacy of a natural botanical product against aquaculture pathogens. (Nutriad Technology Center, in-house results, 2012.)

Pathogenic species	Strain	Minimum Inhibitory Concentration (% extract)	Host Range	Quorum Sensing Documented
Flavobacterium columnare	LMG 10397	0.06	Tilapia, freshwater fish species	No
Listonella anguillarum	LMG 4411	0.23	Most marine fish species	Yes
Photobacterium damselae	LMG 7892	0.47	Sea bream, seabass, sole	No
Vibrio harveyi	BB120	0.47	Sea bream, common snook, penaeid shrimp	Yes
Vibrio alginolyticus	LMG 4409	0.94	Sea bream, grouper, most marine fish	Yes
Vibrio parahaemolyticus	LMG 4423	0.94	Marine fish, penaeid shrimp (EMS)	No
Edwardsiella ictaluri	LMG 7860	1.88	Catfish	No
Edwardsiella tarda	LMG 2793	1.88	Turbot, tilapia	Yes
Pseudomonas fluorescens	DVK1	3.75	Striped bass, white perch, yellow tail	No
Pseudomonas putida	DVK2	3.75	Ayu, freshwater fish species	No
Yersinia ruckeri	LMG 3279	3.75	Salmonids, mainly rainbow trout	No
Aeromonas hydrophila	LMG 2844	7.50	Salmonids, cyprinids, catfish, freshwater fish species	Yes
Aeromonas salmonicida	LMG 3780	7.50	Salmonids, cyprinids, freshwater fish	Yes
Streptococcus iniae	CCUG 27303	8.00%	Trout, tilapia and other freshwater fish	?

important component of any strategy to prevent EMS. However, the success of this approach will depend on the efficacy of the selected gut health promoter against the pathogenic bacteria involved in EMS.

Synergistic blends of natural compounds can be selected on their bacteriostatic and bactericidal properties against a specific range of pathogenic bacteria in vitro. In work at the Nutriad Technology Center, different *Vibrio* species, including *V. parahaemolyticus*, appeared to be highly sensitive to a natural feed additive composed of a synergistic blend of antimicrobial compounds (Table 1).

Quorum Sensing

Recent research has shown that, apart from direct bactericide/bacteriostatic effects, selected combinations of antimicrobial compounds are at the basis of more complex mechanisms to steer microbiota composition. In human medicine, compounds active in quorum sensing (Q.S.) disruption are being increasingly investigated as potential alternatives to antibiotics due to their efficacy at low concentrations and the low chance of bacteria developing resistance against these non-lethal molecules.

Quorum sensing is a form of bacterial communication based on the production and secretion of signaling molecules that can be detected by adjacent bacteria. When population density rises, these molecules accumulate in the extracellular environment, thereby providing a means for bacteria to quantitatively monitor the presence of other bacteria. When these signaling molecules reach a certain threshold concentration, they initiate intrabacterial signaling that culminates in the activation of specific genes.

Quorum Quenching

As determined in work by researcher Dr. Tom Defoirdt and co-workers in 2011, in most pathogenic bacteria for which quorum sensing has been studied, Q.S. was associated with pathogenicity, such as biofilm formation and the production of proteases, invasion factors or other virulence factors. In recent years, research focusing on ways to disturb Q.S. signaling – also called quorum quenching – has therefore gained interest.

Blocking communication is a novel way to prevent bacteria from triggering pathogenicity without exposing them to a selective pressure to survive (Figure 1). Research by the authors has shown that synergistic blends of natural antimicrobial compounds can function as powerful interrupters of bacterial Q.S. signaling in a typical aquaculture pathogen such as *Vibrio harveyi* at concentrations well below minimal inhibitory concentrations.

Recent scientific studies by Pande Gde Sasmita Julyantoro and Defoirdt have shown that quorum sensing-disrupting compounds are capable of increasing survival of crustaceans challenged with *Vibrio harveyi,* including larvae of giant freshwater prawns, *Macrobrachium* species, and the brine shrimp *Artemia*. Research by this article's authors similarly showed that strongly diluted extracts from a synergistic botanical product could protect *Artemia* during a challenge with *Vibrio harveyi* (Figure 2).

The determination of *Vibrio* concentrations in varied challenge treatments showed that the strong bactericide effect of the botanical product was responsible for this protection at the highest concentrations of the product. However, the negligible effect on *Vibrio* concentrations in *Artemia* as well as the culture water in the treatment exposed to the lowest dosage indicated the Q.S. disruption mechanism was responsible for the protective effect of the botanical extract at lower concentrations.

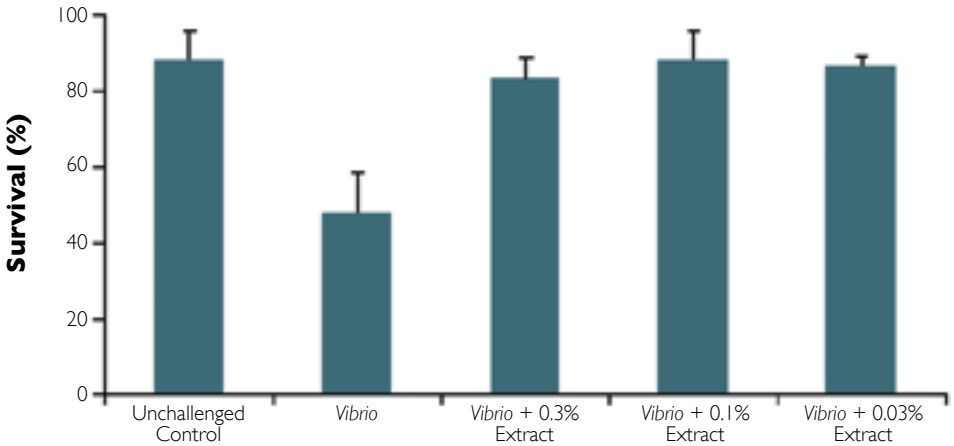

Figure 2. Survival of *Artemia* challenged with *Vibrio harveyi* and treated with different concentrations of an extract of natural compounds with antimicrobial activity.