Enzyme booster
for improved performance

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Shrimp farming started to develop in the 1970s. In 2012, world shrimp production was larger than four million tons and more than 50 % of the shrimp eaten in the world today comes from aquaculture. South East Asia and China represent the largest and the most productive shrimp production area, accounting for 80% of the world production. Although, shrimp production has boomed during the last decades, farmers have to face a variety of issues to ensure their production. Shrimp are very sensitive animals and many disease outbreaks occurred in the past such as the white spot viral disease in 1994-1995 in south East Asia and some are still going on such as the Early Mortality in Shrimp syndrome since 2010 in South East Asia. Among them, one of the most important diseases is Vibriosis that kills some shrimp all along the production cycle. More than ten Vibrio species have been reported pathogenic for shrimp. Though Vibrio bacteria are part of the natural flora and culture environment of shrimps, Vibriosis can occur in a context of imbalanced environment and may cause total mortality of the reared shrimps. During the last two decades, mass mortality in growout ponds and hatcheries due to Vibrio were largely reported. Among the different Vibrio species, *Vibrio harveyi* is considered as one of the most important shrimp pathogens.

With very good management practices, it is possible to limit the Vibrio issue. In order to have a complete protection against the pathogen, it is important to find ways to avoid shrimp becoming contaminated by Vibrio. Antibiotics and chemotherapy are often used to manage disease outbreaks. However, these methods have limits, such as environmental hazards or the spread of antibiotic resistant bacteria.

Another way to prevent Vibrio disease is to improve the gut health. The gut is one of the most important entrances used by Vibrio to contaminate the shrimp. Favoring the natural defenses of the gut by preserving its natural balance to avoid Vibrio development and toxicity may be done. Olmix, with a unique expertise in clay and algae, has developed a new product aimed at the improvement of shrimp performance through boosted digestive enzyme activity and better digestive balance.

Clays and feed efficiency

While there is scientific evidence showing the benefits of clays in the prevention or treatment of digestive troubles and in the protection of the gut mucosa, much less is known about their capacity to improve feed efficiency. Yet, the improvement of the digestibility of feed is an integral property of clays. The mechanisms involved are thought to be multiple (Reichardt, 2008). The dominant hypothesis described in the literature is that clays slow down the transit of feed in the intestine, so the time for digestion is increased, hence a better digestibility of feeds and nutrients uptake. Nevertheless, it seems that the action of clays to enhance feed digestion in the intestine involves other mechanisms. Reichardt (2008) and Habold *et al* (2009) both report the ability of clays to favor the contact between enzymes and nutrients, and therefore to improve the rate of digestion of the feed. Indeed, digestive enzymes need to be in contact with their substrate in order for hydrolysis to occur. The physico-chemical interactions of the enzymes with clay particles seem to enhance the contact between the digestive enzymes and the feed, making clays a good supporting matrix for enzymes and acting as a meeting point for them to be in contact with their substrate. Indeed, Cabezas *et al* (1991) demonstrated that clay-enzymes complexes are formed at enteric pH values. These active stable complexes are resistant to proteolysis and increase the amount of active digestive enzymes in the intestine, thus improving nutrient digestibility. In the same way, Habold *et al* (2009) observed higher pancreatic lipase activity in rats supplemented with Kaolinite; Xia *et al* (2004) showed an increase in small intestinal digestive enzymes activities on broilers supplemented with Montmorillonite; and Paolo *et al* (1999) observed an increase in protein and energy retention coefficients for growing pigs supplemented with clay. Some studies also suggest that the increased activity of enzymes in contact with clay not only comes from their stabilization, but also from the presence of cofactors in the clay (Reichardt, 2008; Habold *et al*, 2009). Cofactors are defined as thermostable non-protein compounds that form the active portion of an enzyme system. In other words, cofactors are helper molecules required for enzymes to be active. They can be organic or inorganic, most commonly vitamins in the first case and metallic ions in the latter.

Clays are layered mineral materials, composed of a succession of aluminium and silicium based sheets, which order varies according to the type of clay. In Montmorillonite, several metallic ions replace some aluminium and silicium ions in the structure. Known as the substitution phenomenon, this event provides Montmorillonite part of its physico-chemical reactivity. Moreover, the presence of metallic ions may contribute to the activation of some enzymes, through their action of cofactors (Niederhoffer, 2000). Thereby, copper is known to activate lipase and phospholipase A (Jondreville *et al*, 2002) and zinc is a required cofactor of carboxypetidase (Williams, 1960), to mention only a few examples.

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The combination of the matrix support provided by the clay and the cofactor effect coming from the metallic ions present in its structure can be referred to as **biocatalysis**: the improvement of performance of a biochemical reaction through the action of an external compound, a biocatalyst.

Not all clays are equal

Due to a large variety of clay minerals, one can imagine that all clays do not have the same potential for biocatalysis depending on their type, their purity, their source or their treatment. As such, clay structure can be modified and associated with other materials in order to potentiate its biocatalytic properties. Such technology has been developed by Olmix group (France) in the frame of its research program conducted on seaweeds and clays.

The micronized form allows a fine dispersion of the product in the intestine, providing many sites of reaction of enzymatic digestion with more easily accessible metal ions. Moreover, it benefits from a synergy between clay and seaweeds in the process of biocatalysis, as seaweeds bring in many diverse metallic ions, sometimes absent in the feed, which are required cofactors for the activation of several enzymes. This unique combination of seaweeds and clay makes it a unique tool to boost enzymes activities through the action of biocatalysis.

MFeed+, the only product benefiting from this new technology, has proven its efficacy in several studies. Among them, MFeed+ has been tested on shrimp by researchers in Kasetsart University (Thailand). The aim of the study was to evaluate the effect of MFeed+ feed supplementation on digestive and growth performance of *Penaeus vannamei*.

375 shrimp (6.3±0.2g weight) were distributed in fifteen 500 L glass tanks, containing 25 shrimp each. After a seven day period of acclimatization during when all shrimp were fed the basal diet, tanks were randomly allotted to one of three treatments (5 replicates per treatment): one control, fed the basal diet and two MFeed+ groups, for which the basal diet was supplemented with 0.1% or 0,2% of MFeed+. Growth performance parameters and mortality were recorded during the 60 days of supplementation. *Vibrio* bacteria were counted in the hepatopancreas and the intestine at 60 days as a marker of digestive health. Shrimp were fed three times a day to satisfaction. Feed amount was adjusted daily based on feeding ability of shrimps. Uneaten feed was siphoned out of the tank two hours after feeding. Water used in the experiment was seawater, with adjusted salinity to 12-15 ppt. Water in tanks was aerated with air stone and exchanged every two to three days at the rate 10-30% volume depending on its visible quality.

	Control	MFeed+ 0.1%	Variation over control	MFeed+ 0.2%	Variation over control
Average initial weight, g/ind	6.32 ± 0.13	6.32 ± 0.13	/	6.32 ± 0.13	/
Average final weight, g/ind	12.4 ± 0.8	13.09 ± 1.1	+5.6%	13.44 ± 0.1	+8.4%
Specific growth rate, %/d	1.22 ± 0.1	1.31 ± 0.1	+7.4%	1.34 ± 0.1	+9.8%
Total feed consumption, g/ind	8.66 ± 0.64	8.21 ± 0.92	/	8.52 ± 0.77	/
Feed Conversion Ratio	1.42 ± 0.1	1.22* ± 0.09	-14.1%	1.23* ± 0.1	-13.4%

** p-value < 0.05*

Table 1. Growth performance

	Control	MFeed+ 0.1%	Variation over control	MFeed+ 0.2%	Variation over control
Vibrio count at 60 days, x10^4 CFU/g					
· In hepatopancreas	3.07 ± 0.39	2.83 ± 0.31	-7.8%	1.17** ± 0.13	-61.9%
· In the intestine	1.67 ± 0.30	1.58 ± 0.25	-5.4%	1.13 ± 0.12	-32.3%
Survival rate, %	67.2 ± 4.38	78.4** ± 4.56	+16.7%	82.4** ± 3.58	+22.6%

*** p-value < 0.01*

Table 2. Health performance

Feed Conversion Ratio was greatly improved in groups receiving MFeed+. As a consequence, average final weight and specific growth rate of shrimp supplemented with MFeed+ tended to be higher. (See Tables 1 and 2). Moreover, the better digestion performance contributed to improve the digestive status of the shrimps, as shown by the lower Vibrio count in the hepatopancreas and the intestine and the improved survival rate of the juveniles. This study highlighted the interest of MFeed+ to improve digestive and zootechnical performance of shrimp.

More information

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