Technical Bulletin

Information from Phibro Technical Services

# *The immune and metabolic mode of action of PAQ-Protex in shrimp*

## PAQ-Protex™ – Natural protection for superior growth performance

PAQ-Protex™ is a natural feed additive especially designed for shrimp. It contains a saponin blend from Yucca schidigera and Quillaja saponaria plants

# PAQ-Protex™
# Natural protection for superior growth performance

The shrimp farming industry is continually seeking to improve the health and biological function of shrimp to improve productivity. Feed supplements play a key role in shrimp farming, yet some products available in the market do not carry the benefits that an all-natural product can offer.

PAQ-Protex is a mixture of non-toxic quillaja saponins. It is obtained from two plants, *Quillaja saponaria* (quillaja) and *Yucca schidigera* (yucca), by a solvent-free process that does not contain any preservatives or carriers.

PAQ-Protex was developed to:

- Stimulate fish and shrimp growth and survival;
- Improve nutrient absorption and intestinal health;
- Enhance intestinal villi development;
- Improve feed efficiency (through reduced FCR levels);
- Reduce nitrogen loads in fish and shrimp ponds.

The beneficial effects of PAQ-Protex act on immunomodulatory, metabolic, anti-inflammatory and antioxidant processes. PAQ-Protex helps reduce disease incidence by initiating an immunological cascade and triggering an immune response, thereby contributing to the modulation of the shrimp immune system. Additionally, it improves production performance by promoting gut health and nutrient utilization. By providing a blend of phytochemical compounds such as polyphenolic antioxidants, glycosides, and quillaja saponins, PAQ-Protex promotes feed digestion and nutrient absorption. This also helps lead to a reduction of nitrogen input (in the form of ammonia and nitrite) in culture ponds (Cheeke, 1996).

## Immunological Mode of Action

Immune responses in shrimp are based on several defense mechanisms, such as lectins as recognition molecules, blood coagulation, melanization, the production of reactive forms of oxygen, phagocytosis, and encapsulation. In the presence of an injury, blood cells initiate the coagulation process to protect shrimp from excessive loss of liquids and to capture and immobilize invading microbes. Defensive enzymes are subsequently secreted to kill pathogens, which are then eliminated through phagocytosis and/or encapsulation. Finally, the process of melanization leaves the pathogens in an inert state and prepares them to be expelled by cuticular excretion or during the following molting cycle.

PAQ-Protex modulates the innate immune responses of shrimp through its quillaja and yucca components. The immune modulation properties of quillaja are driven by saponins, which can bind to proteins and receptors presented in the cell and block pathogens from infecting them.

Quillaja's cellular immune response results from the balance between its lipophilic (with an affinity for lipids) and hydrophilic (with an affinity for water) parts. Hydrophilic parts enable the immunological effect of quillaja saponins, as they can be easily absorbed through the cell wall and integrated into the pathogen's cell membrane where they form complexes with cholesterol. These processes are essential for controlling internal pathogens such as *Coccidiea*, gregarines, and bacteria, which have high concentrations of cholesterol in their cell membrane. When quillaja saponins penetrate the cell membrane they promote a pro-inflammatory response, thereby affecting the permeability of the pathogen, or creating pores in their cell membranes. This ultimately results in a disruption of the pathogen's biological function.

Another example of this immune process happens when intracellular bacteria *Vibrio parahaemolyticus* are able to escape into the cell cytoplasm and proliferate, by silencing the lysosome. Quillaja saponins can prevent this mechanism and trigger lysosomal activity by penetrating the epithelial cell using their hydrophobic side. This initiates phagocytosis by macrophages, representing the activation of the innate immune system in shrimp.

Similarly to quillaja, yucca contains beneficial saponins, oligosaccharides, and polyphenols. Furthermore, yucca also comprises resveratrol and yuccaols, two potent antioxidants, with the latter presenting excellent free radical scavenging activity.

This process is initiated by the recognition of bacteria and fungi. Generally, their detection activates extracellular cascades that amplify the signal and trigger downstream molecules to ultimately stimulate pathogen elimination (Figure 1). This results in the melanization of pathogens, leaving them inert for expulsion. However, melanin is highly reactive and can damage other cells. The mechanism to contain this relies on the yuccaols and resveratrol that are present in yucca, which are able to restrain melanin's activity exclusively to pathogenic agents due to their antioxidant properties, hence preventing further cellular damage (Figure 1). PAQ-Protex also contains phenols, a substrate for melanin production.

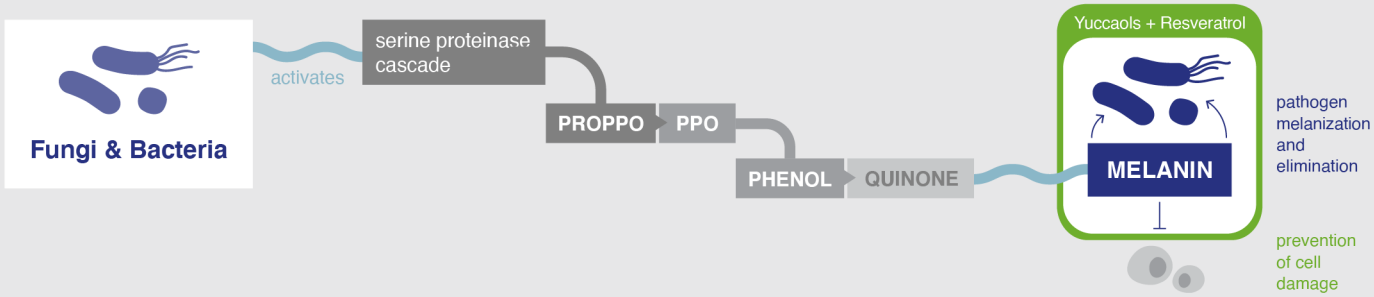


Figure 1. Yuccaols' free radical scavenging activity.

Lysozymes are proteins that catalyze reactions through hydrolysis, assuring immunity properties and acting against viruses, inflammation, and cancerous cells. As such, lysozymes display antimicrobial activity against bacteria, including species such as *Vibrio* that are pathogenic to shrimp. It has been shown that PAQ-Protex has the ability to increase lysozymal activity and haemocyte count in shrimp (Figure 2).

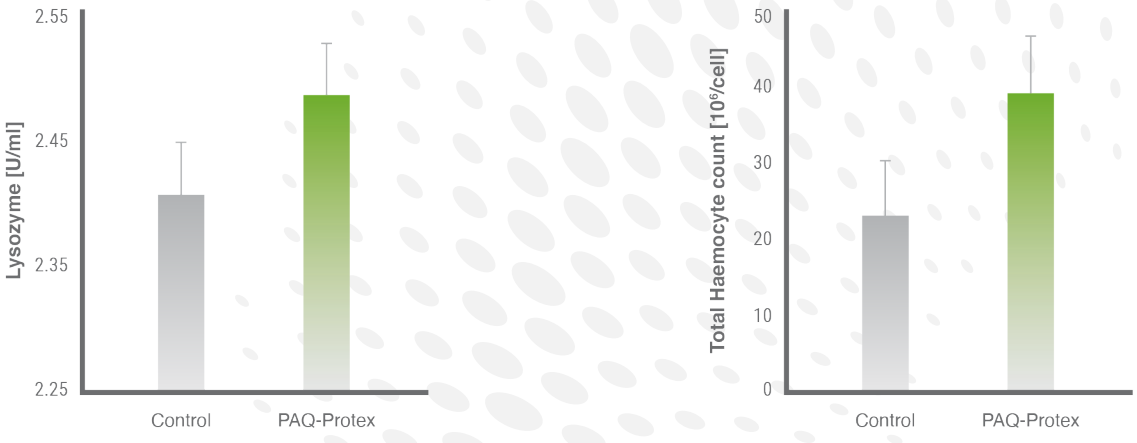


Figure 2. Immunological data on the effects of PAQ-Protex on lysozymes and haemocytes in shrimp.

In summary, PAQ-Protex has the ability to improve immunity through its quillaja and yucca components, which trigger immune mechanisms and pathways that work on several different levels of the shrimp immune system (Figure 3).

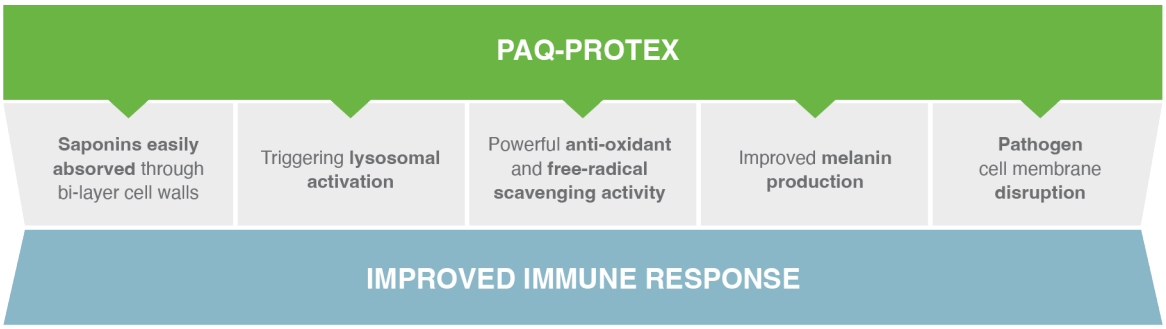


Figure 3. Summary of the immunological mode of action of PAQ-Protex.

## Metabolic Mode of Action

Digestive enzymes (such as amylase and lipase) play a crucial role in shrimp development. Increased activity of these enzymes has been shown to improve growth performance, FCR, and feed consumption in different shrimp species (Akbary *et al*., 2017; Sankar *et al*., 2011). Saponins promote the activity of these enzymes and increase gut cell permeability, which improves nutrient uptake (Augustin *et al*., 2011).

The metabolic benefits of PAQ-Protex are also a result of quillaja and yucca saponin action, and lead to improved feed digestion through the:

- Enhancement of enzymatic activity;
- Increase of protein synthesis;
- Promotion of nutrient absorption in epithelial cells of the digestive tract;
- Increase in cell membrane permeability;
- Improvement of protein level and retention.

A suggested mechanism of action for quillaja and yucca saponins is the stimulation of intestinal absorption of dietary amino acids and fatty acids obtained after enzymatic digestion. These are likely to be associated with increasing protein synthesis, promotion of nutrient absorption in epithelial cells of the digestive tract, and cell membrane permeability to amino acid and other nutrients, which explains the higher nutrient retention levels (Francis *et al*., 2001).

Several studies have been conducted to evaluate the effects of PAQ-Protex in shrimp. A trial conducted in Vietnam measured the impact of PAQ-Protex on the enzymatic activity of Pacific white shrimp (*Litopenaeus vannamei*). The study compared a commercial feed supplemented with 2g/kg of PAQ-Protex with a regular commercial feed. Shrimp enzyme activity was measured in the hepatopancreas and gut. Results showed a significant increase in enzyme activity of amylase and lipase in the hepatopancreas and the gut after 28 days (Table 1).

Table 1. Results from a scientific study show that PAQ-Protex enhances the enzymatic activity in the gut and hepatopancreas of *Litopenaeus vannamei*.

**Enzyme activity in the hepatopancreas (IU/g)**

| Enzyme | Control | PAQ-Protex™ |
|---|---|---|
| Amylase | 311.79 ± 175 | **378.63 ± 129*** |
| Lipase | 58 ± 28 | 57.03 ± 42 |

**Enzyme activity in the gut (IU/g)**

| Enzyme | Control | PAQ-Protex™ |
|---|---|---|
| Amylase | 1242 ± 384 | **1811 ± 312*** |
| Lipase | 104 ± 25 | **123 ± 50*** |

**indicates statistically significant differences.*

Results demonstrate the important role that PAQ-Protex plays in enzyme modulation in shrimp, specifically on amylase and lipase activities. However, the enzymes and biochemical pathways involved in saponin biosynthesis are not yet fully understood. One hypothesis is that quillaja saponins trigger amylase activation. Amylase breaks starch down into sugars, producing a primary component of chitin, which is a key molecule for molting. By being involved in the molt cycle, amylase activity affects growth: the higher the levels of amylase activity, the higher chitin synthesis and induced molting, ultimately enhancing growth (Figure 4).

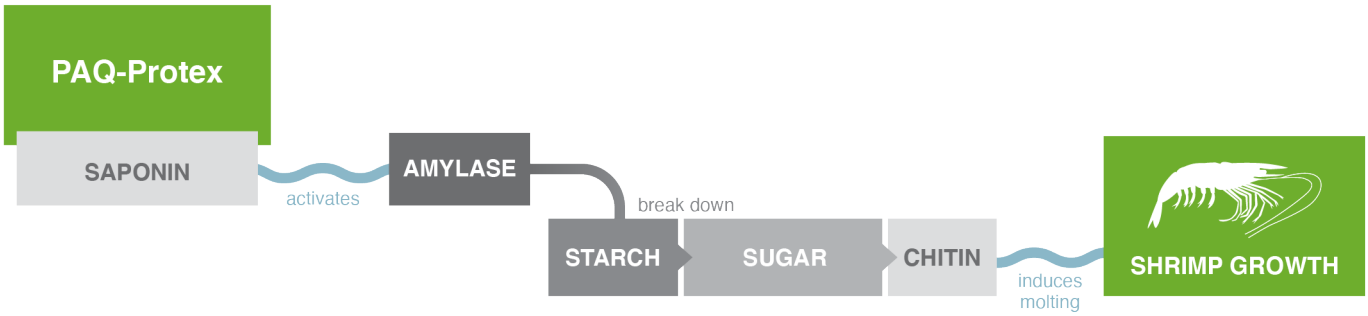


Figure 4. Quillaja saponins induce amylase activation, resulting in higher growth levels in shrimp.

The significantly higher lipase enzymatic activity recorded in the gut with PAQ-Protex supplementation is also of great value for shrimp production. This is because the shrimp metabolism cannot tolerate high levels of dietary fat, and as such feed lipids should be restricted to levels between 5-8%.

Another study performed in Thailand investigated the effect of PAQ-Protex on proteolytic enzymes protease, trypsin, and chymotrypsin, and in protein digestibility of a soybean meal-based diet in whiteleg shrimp (*L. vannamei*). Three treatment groups were used in this study: a fish meal-based control group (FM), a soybean meal-based group, and a diet group based on soybean meal plus 0.1% PAQ-Protex. After assessing body composition, growth, and survival levels, the dietary inclusion of PAQ-Protex resulted in higher levels of total protein, chymotrypsin, protease activity, and the sum of all the essential amino acids (EAA) measured (Figure 5).

Table 2. PAQ-Protex increases protein digestability in *L. vannamei.*

| | **Control** (FM inclusion) | **Negative control** (Soybean based) | **Soybean based + PAQ-Protex 0.1%** |
|---|---|---|---|
| Protein | 75.8 | 75.0 | **75.3** |
| Arginine | 7.74 | 7.93 | **8.95** |
| Histidine | 0.71 | 0.73 | **0.74** |
| Isolucine | 1.2 | 1.3 | **1.59** |
| Leucine | 1.99 | 1.93 | **2.44** |
| Lysine | 3.69 | 3.76 | **4.44** |
| Methionine | 0.79 | 0.81 | **0.85** |
| Phenylalanine | 1.37 | 1.34 | **1.17** |
| Threonine | 1.21 | 1.36 | **1.07** |
| Tryptophan | 0.53 | 0.39 | **0.42** |
| Valine | 1.21 | 0.35 | **1.32** |
| Sum EAA | 20.4 | 19.9 | **22.9** |

**EAA – Essential Amino Acids*

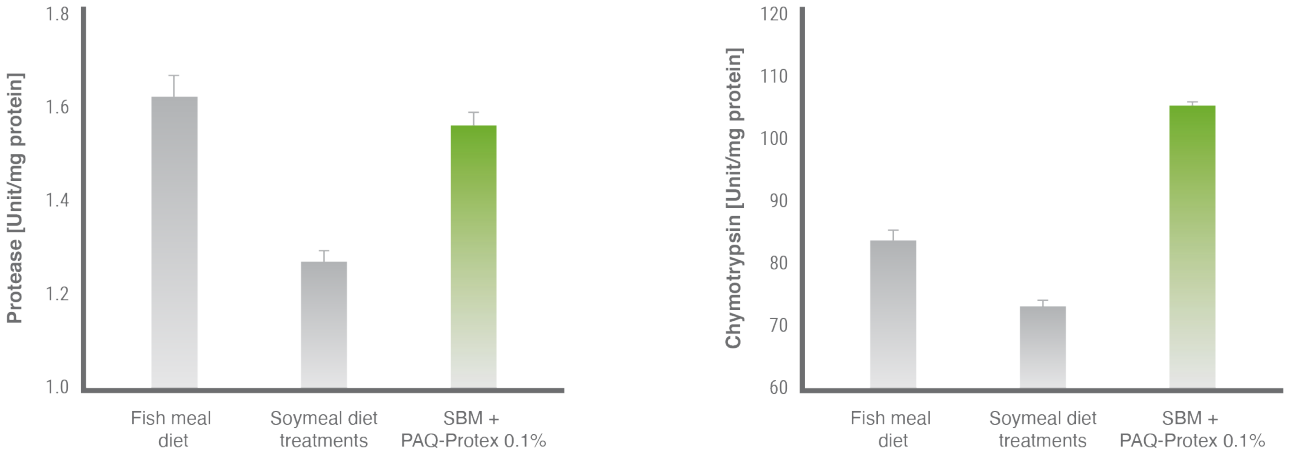


Figure 5. PAQ-Protex increases enzyme activity in *L. vannamei*.

An additional study developed in Indonesia conducted a metabolic analysis of shrimp supplemented with PAQ-Protex, where a commercial feed was compared to the same feed supplemented with 2g/kg of PAQ-Protex. After 60 days, an improvement in the protein levels and protein retention was detected in the PAQ-Protex group (Figure 6).

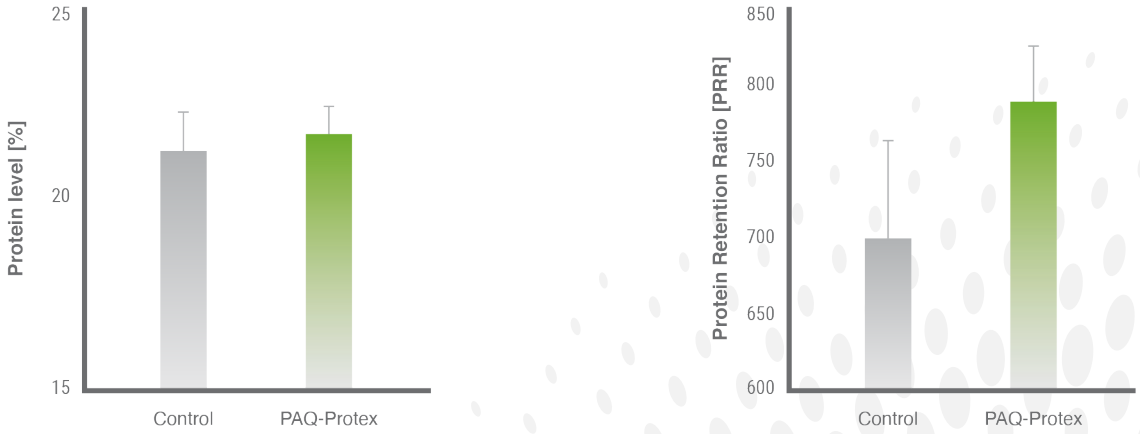


Figure 6. PAQ-Protex improves protein level and retention in shrimp.

These results suggest PAQ-Protex has a role in increasing digestive enzyme activity, which subsequently improves nutrient absorption, corroborating the results obtained in the aforementioned studies. Such changes in the enzymatic and protein parameters are usually associated with changing major raw ingredients in dietary groups. Since such significant changes were reported at low inclusion rates, the results of this trial highlight the ability of PAQ-Protex to improve shrimp health.

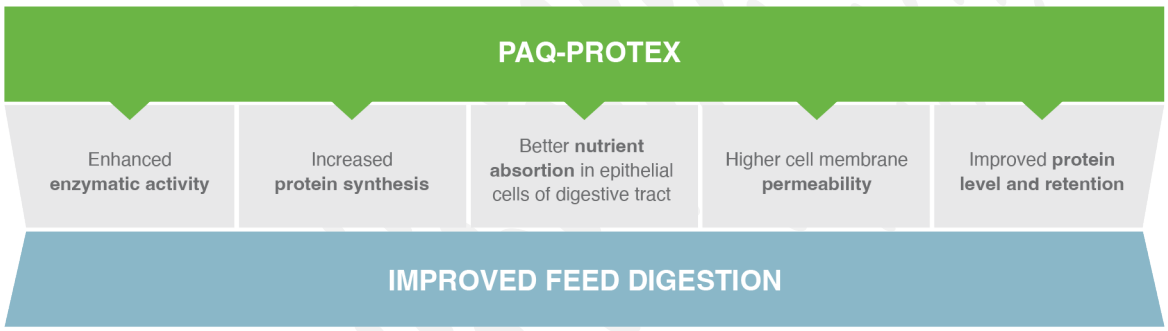


Figure 7. Summary of the metabolic mode of action of PAQ-Protex.

## Conclusions

PAQ-Protex stimulates feed efficiency, shrimp growth, and survival, thereby supporting an increase of total production yield and production profitability. By improving nutrient absorption, gut health, and feed use efficiency, PAQ-Protex also reduces the input of nitrogen compounds in aquaculture ponds, thus contributing to a healthier environment for shrimp to grow and thrive.

PAQ-Protex provides the necessary tools to enhance immune modulation through a saponin-driven mechanism. The metabolic mode of action of this product is based on the beneficial effects of quillaja and yucca saponins, which exert a positive effect on enzymatic activity and overall shrimp health.

Finally, in addition to presenting beneficial molecular properties and having an entirely natural origin, PAQ-Protex is highly palatable and presents very stable characteristics, which means that it can be added to feeds before the extrusion and pelletizing processes. This reinforces the advantages of adopting PAQ-Protex as a supplement for shrimp feed.

## References

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