

Science & Solutions



Nutrient sparing for sustainable profitability



Phytogenic feed additives
and nutrient sparing



Linking nutrition
and gut health

Editorial

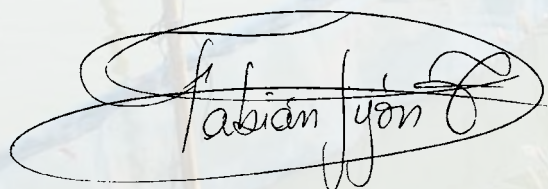
A Holistic Approach

Every industry faces new challenges. Aquaculture has been developing by upgrading its technological level and specializing in multiple areas in order to deal with a global growing marine protein demand. This industry develops among several technical disciplines, environmental, nutritional, sanitary and scientific, just to mention a few. Nowadays we must look at aquaculture as a complex industry that demands a wider, comprehensive view.

One of the greater challenges is gut health which directly relates to nutrition. Both have become cornerstones with a boom in the last decades, since the most important outbreaks hit the industry in shrimp aquaculture for example, WSSV, YHV, and now AHPND. BIOMIN supports these fights by developing **gut performance management** that includes preventive gut health and promoting gut performance, both areas in which we are global experts.

A strong R&D program allows us to help farmers face these challenges effectively and get focused on achieving sustainability; maximizing the return on investment in gut health and nutrition by understanding the nutritional, physiological and health processes. Combatting new diseases, nutritional challenges, and the constant efforts of nutritionists to provide nutrients in quantity, quality and availability for sustainable aquaculture go hand in hand with innovative feed additives.

We can only look at the present and future of the industry as a whole with elements connected to each other. We intend to address them with clear concepts that transform science into sustainable solutions; that is the BIOMIN gut performance management proposal.



Fabián JIJÓN
Technical Manager



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Photo: Ugurhan Betin

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By **Otavio Serino Castro, MSc**

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Nutrient sparing: a su long-term profitability



Photo: Kakaklor

By **Rui Gonçalves**, Technical Product Manager & **Carina Schieder**, Product Manager Phytogenics

Aquaculture production can address contradictory challenges such as costs and environmental footprint by using phytogenic feed additives that improve digestibility, nutrient retention, feed efficiency and reduce emissions.

sustainable tool for

Over the last decade, the aquaculture industry experienced consistent growth mainly in developing countries. Global aquaculture production will clearly continue to grow mainly as a result of improvements in production technology and increased demand for fish and shrimp products. However, aquaculture faces several important challenges in terms of efficient use of the raw materials that need to be addressed.

Contradictory challenges

Several trends put contradictory pressures on the aquaculture industry. Reliance upon scarce and costly raw materials, such as fishmeal, and the optimal use of alternative ingredients constitute a main challenge in aquaculture. Consumer awareness about environmental sustainability encourages producers to improve the production performance through sustainable aquaculture practices.

However, the use of less costly protein sources and low-nutrient dense diets most likely will lead to lower protein digestibility, higher amino acid imbalance, higher carbohydrate and fiber content in feeds. This can lead to inefficient nutrient use, resulting in increased

feed usage, greater susceptibility to disease and higher ammonia emissions—raising production costs and increasing the ecological footprint.

Gut performance holds the key

Optimum animal performance encompasses a number of factors, including genetic characteristics of the species, quality of the diets, environmental conditions and absence of disease outbreaks. Add to this competitive industry pressure and the need for efficient use and/or replacement of increasingly expensive raw materials—and the picture becomes even more complex. A focus on good gut performance and gut health can help to successfully navigate this large set of considerations and set the foundation for better growth. Phytogenic feed additives, consisting of herbs, spices, essential oils and extracts have gained considerable attention as an answer to these challenges.

The active ingredients, such as phenols and flavonoids, can exert multiple effects in animals, including improvement of feed conversion ratio (FCR), digestibility, growth rate, reduction of nitrogen excretion and improvement of the gut microbiota and health status. Examples of these ingredients with major active compounds are provided in *Table 1*.

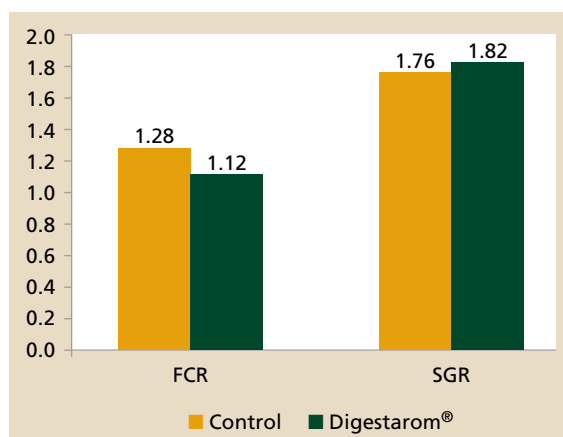
Table 1. Important constituents of selected essential oils.

Name (Botanical name)	Source	Important constituents
 Caraway (<i>Carum carvi</i>)	Seeds	carvone, limonene
 Oregano (<i>Origanum vulgare</i>)	Leaves	carvacrol, thymol, p-cymene
 Mint (<i>Mentha arvensis</i>)	Leaves	menthol, isomenthone, limonene
 Rosemary (<i>Rosmarinus officinalis</i>)	Leaves	1-8-cineol, α - and β -pinene, borneol
 Thyme (<i>Thymus vulgaris</i>)	Leaves	thymol, p-cymene, carvacrol

(adapted from Jänicke et al. 2013 and Tisserand and Young 2014)

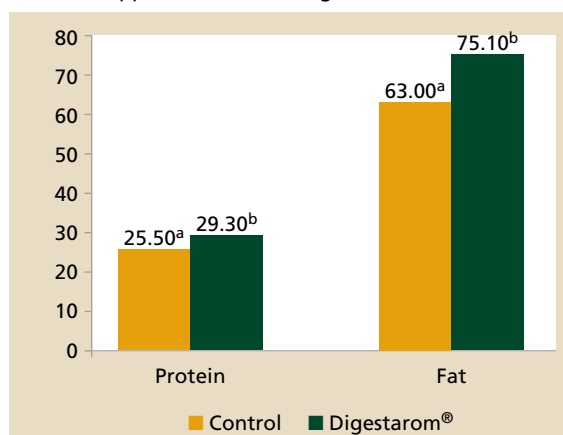
Photos: Vitalina Rybakova, David Clark, timsa, Branslavac, fotogal, Juha Huiskonen

Figure 1. Feed conversion ratio (FCR) and specific growth rate (%/day) of sea bream were improved with the supplementation of Digestarom®.



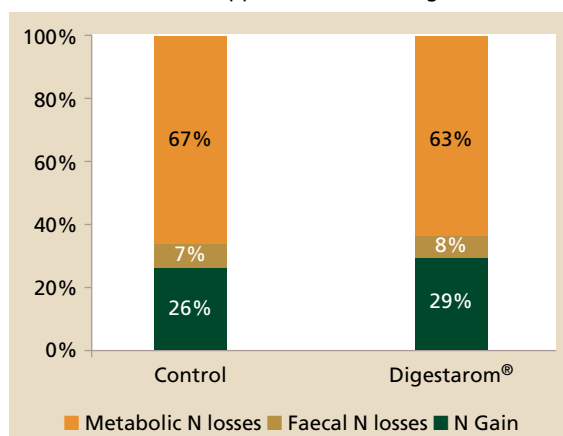
Source: BIOMIN trials, 2012

Figure 2. Nutrient retention of sea bream was enhanced with the supplementation of Digestarom®.



Source: BIOMIN trials, 2012

Figure 3. Nitrogen budget (gain, fecal losses and metabolic losses) in sea bream supplemented with Digestarom®.



Source: BIOMIN trials, 2012

How phytogenics work

Phytogenics may stimulate the digestive secretions, increase villi length and density and increase mucous production through an increase in the number of goblet cells. Through different strategies, such as matrix-encapsulation, volatile essential oils can be stabilized and may remain active throughout a greater section of the gastrointestinal tract (GIT), thus ensuring that positive effects are not only restricted to a smaller section of the GIT.

Reduced fishmeal content vs. feed efficiency

Replacement of fishmeal by plant protein, whether for economic or sustainable reasons, can decrease feed efficiency and suppress an animal's immune system due to less digestible raw materials or side effects in the gastrointestinal tract. Digestarom® (BIOMIN Holding GmbH, Austria), a matrix-encapsulated phytogenic additive, has proven to support animals to overcome these challenges and minimize the negative effects of fishmeal reductions and replacements, respectively.

Results in seabream

A first trial was performed with Gilthead seabream (*Sparus aurata*) at the University of Algarve, Portugal. In this trial, basal diet had 45 % crude protein and 18% lipid content. The majority of the dietary protein content was derived from plant ingredients; the diet contained only 14% fishmeal (FM). Additionally the treatment group received Digestarom®. The aim of the trial was to evaluate the effect of Digestarom® on feed efficiency, body composition and nutrient retention.

Dietary supplementation of Digestarom® showed a significant improvement of 16 FCR-points and an enhancement of the specific growth rate from 1.76% to 1.82% per day (Figure 1). Inclusion of the phytogenic mixture in the diet significantly enhanced ($p < 0.05$) protein and fat retention (Figure 2). The study also showed significant reductions of total nitrogenous losses, which were clearly associated with lower metabolic losses and increased utilization of protein for growth (Figure 3).

Replacement of fishmeal by plant protein can decrease feed efficiency and suppress an animal's immune system.

Photo: Alexander Hoffmann

Results in shrimp

A second feeding trial was conducted in collaboration with Ningbo University (China) to evaluate the efficacy of Digestarom® P.E.P. MGE as a tool to reduce the level of fishmeal in shrimp diets. The treatments consisted of 5 isoproteic diets (40% crude protein) with a positive control diet with 25% fishmeal inclusion, and four test diets with two lower levels of fishmeal (22% and 19%) with and without Digestarom® supplementation. Each diet was randomly assigned to 5 replicates of 30 juvenile white shrimp (approximately $0.33 \pm 0.00\text{g}$) and fed over 8 weeks.

The results indicated that the reduction in fishmeal reduced shrimp performance with the control diet (25% FM) having the best performance. Weight gain, feed conversion ratio (Figure 4), specific growth rate (Figure 5) and protein efficiency were improved for shrimp fed the phyto-genic additive supplemented diets compared to the lower fishmeal, non-supplemented diets.

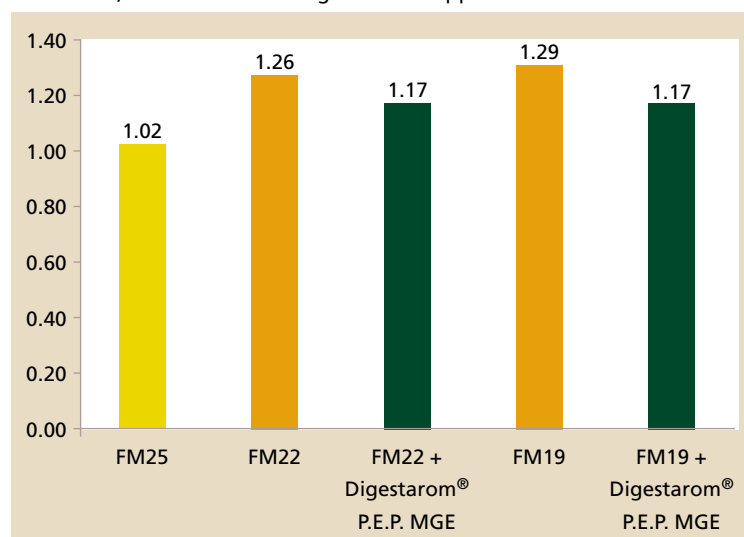
Analysis of mid-gut ultrastructure by transmission electron microscope indicated that shrimp fed the supplemented diets had an improved mid-gut microvilli structure compared to those fed the lower fishmeal diets only (data not shown). The performance improvement of the group given lower fishmeal diets supplemented with Digestarom® is an important result as part of a strategy to reduce costs.

Conclusion

Beyond the clear positive effects on improving feed efficiency, nutrient sparing can be a powerful solution to limit the nitrogen discharge to the environment. Phyto-genic feed additives can decrease ammonia emissions through improved protein usage, reducing the loss of nitrogen into the nature.

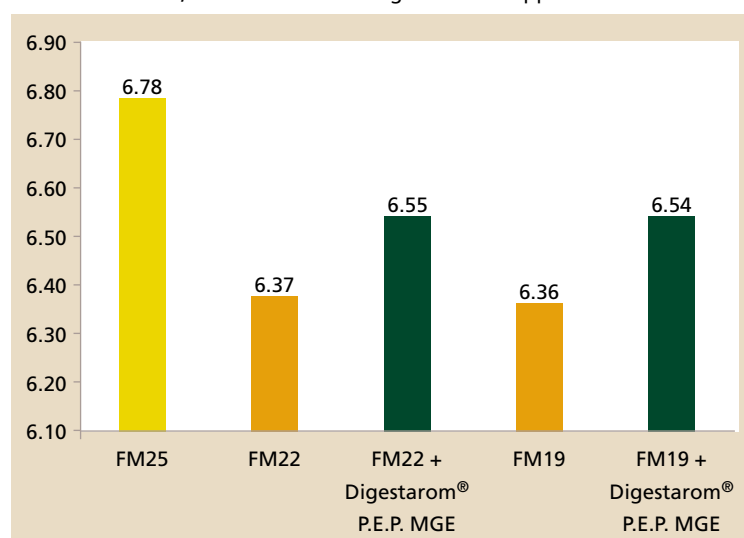
The presented result shows that the phyto-genic feed additive Digestarom® can be used as a nutrient-sparing tool for more efficient and cost-effective diets formulation.

Figure 4. Feed conversion ratio of shrimp fed diets with different levels of fishmeal, with and without Digestarom® supplementation.



Source: BIOMIN trials, 2012

Figure 5. Specific growth rate (SGR, %/day) of shrimp fed diets with different levels of fishmeal, with and without Digestarom® supplementation.



Source: BIOMIN trials, 2012

The Role of Nutrition

By **Otavio Serino Castro**, Technical Sales Manager

Nutrition along with environment and host physiology comprise the three main factors for strong gut performance. This second article in our gut health series reveals how nutrition can influence gut health.

Figure 1. Potential harm factors to gut health inherent to aquafeed production.



Due to recent losses caused by diseases outbreaks, several efforts are being adopted by the aquaculture industry to increase biosecurity and animal health. The use of functional feeds and cost minimization strategies—two common strategies in feed formulation—have several consequences for overall gut health.

From the formula to feed

Aquaculture nutritionists have the daily mission to optimize formulation costs and growth performance

while facing challenges such as raw material prices and availability, market trends, industrial process limitations, competitor's actions and higher quality standards. In this dynamic environment, professionals often need to take difficult decisions to stay on the narrow road towards profitability. The cost reduction orientation, especially in grow-out diets, can lead to “formulation myopia” where optimal profitability is not reached. A misstep can generate significant economic losses, leading to reductions in zootechnical performance or generate long-term health problems.

in Gut Health

How feed can affect gut health

The main objective of aquafeed is to assure the rational supply of essential nutrients that regulates animal lifecycle and production. A nutritional imbalance can directly impact growth and health status. In ideal conditions when animals have good gut health, nutrient digestion and absorption occurs efficiently while an intact gut structure and balanced gut microbiota helps protect animals against external threats. Yet, even a well-designed nutritional formula can contain hidden surprises that disrupt diet performance, affecting gut health directly or indirectly. Raw material quality, storage problems, feed processing deviations and formulation imbalances can all have negative unintended consequences (*Figure 1*).

The fish meal dilemma

Recent studies have demonstrated that premium fish meal can be successfully replaced with alternative protein sources at different levels for distinct species, such as shrimp, salmon, marine fish and etc. Replacements hinge on the fact that aquatic species do not require fish meal itself, but rather the nutrients it contains.

Efficient substitution of essential nutrients including amino acids and fatty acids should produce similar growth performance—something easily demonstrated in the lab trials using refined ingredients. However, full-scale adoption of alternative raw materials for fish meal replacement incurs several limitations such as antinutritional factors, mycotoxins and other restraints which can negatively impact nutrient availability, digestive processes and the gut environment.

Antinutritional factors and antinutrients

Antinutritional factors and antinutrients (ANFs) are endogenous substances present in feed and feedstuffs that can have negative effects on fish and shrimp metabolism. When ingested, depending on its nature, levels in feed and exposition period, these substances can disturb physiological processes. Antinutritional factors are found in feedstuffs of both plant and animal origin (*Table 1*).

Table 1. Main antinutritional factors present in feedstuffs used in aquaculture feed.

Feed Ingredient	Antinutritional factors
Soybean meal	Proteinase inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamin, phytosterols, allergens
Cottonseed meal	Phytic acid, phytoestrogens, gossypol, antivitamin, cyclopropenoid acid
Lupin seed meal	Proteinase inhibitors, saponins, phytoestrogens, alkaloids
Pea seed meal	Proteinase inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, antivitamin
Rapeseed meal	Proteinase inhibitors, glucosinates, phytic acid, tannins, erucic acid
Sorghum	Tannins
Raw/trash fish	Thiaminase
Crustaceans meals	Chitin, fluorine

Adapted, Francis et al. (2001).

They comprise a wide, diverse range of compounds regarding its chemical structure and effects in animal's metabolism. The aquaculture National Research Council (2011) lists 18 classes of antinutritional factors, each containing several compounds. For many of them, the mode of action, safety levels and impacts stills not well elucidated because of complex interactions with another dietary compounds and response variability among species. As illustrated in *Table 2*, the effects of antinutritional factors vary from an enzymatic activity inhibition on digestion to a direct effect on the intestinal epithelium.

Reducing antinutritional factors

The deactivation and elimination of antinutritional factors in feed and ingredients can be achieved by thermic and enzymatic process, chemical extraction and other processes like fermentation. Unluckily, complete inactivation of antinutritional factors is impractical in most cases, depending on the feed process and feedstuff. Therefore, antinutritional factor associated risks would



Table 2. Mode of action, effects on animals and nature of selected antinutritional factors present in aquafeed feedstuffs.

Antinutritional factor	Action	Effects on animals	Nature
Enzyme inhibitors	Proteinase (trypsin; chymotrypsin; elastase; enterokinase etc.), amylase and lipase inhibition	• Reduced protein, lipid and starch digestibility • Reduced growth performance	Simple or complex proteins. Denatured completely or partially by heating, alcohol extraction and fermentation.
Lectins	Binds reversibly specific mono - or oligosaccharide. Interacts with intestinal epithelial cells receptors.	• Disturbs intestinal functions and can cause systemic effects depending on the receptors affected • Can lead to histological alterations and inhibit glucose transport into intestinal epithelium	Soluble, heterogeneous (glyco) proteins. Partially resistant to heat denaturation and to digestive tract passage.
Saponins	Several biological effects including inhibitory action on protein digestion, vitamin absorption and glucocorticoid-like effects. Can alter epithelial membrane permeability.	• Negative effects on palatability and feed intake • Reduced cholesterol availability • Increased uptake of harm substances as allergens • Involved in soy-bean-induced enteritis	Diverse group of glycosides. Heat-stable and alcohol soluble compounds.
Glucosinolates/ Goitrogens	Generate toxic derivative compounds. Interferes on iodine uptake causing thyroid disruption and lesions mainly on liver and kidneys.	• Reduced palatability and decreased growth and production • Decrease on plasma T3 hormone levels and changes in thyroid histology • Can alter liver and kidney functions	Thioesters
Condensed tannins	Binds digestive enzymes and form complexes with proteins and minerals. Alters the feed organoleptic characteristic (bitter and astringent flavor).	• Decreased nutrient digestibility • Decreased palatability	Water-soluble phenolic compounds with molecular weight between 500 and 3000 (non-hydrolysable)

Adapted, NRC (2011).

vary regarding different suppliers, feed processing standards and feedstuffs source.

Practical implications

Animals exposed to antinutritional factors and other harmful compounds can exhibit slight behavioral alter-

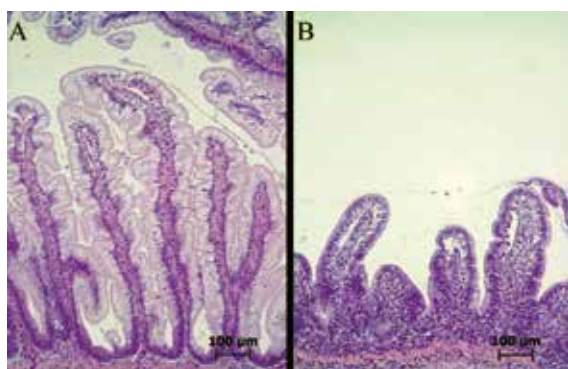
ations, performance and growth impairment or acute poisoning signals. The intensity of the response will be modulated not only by the doses and exposure time, but also by the interaction with others variables as environmental conditions, rearing phase and health status.

The main concern lies in the fact that negative impacts



For an overview of gut health in aquaculture, see Science & Solutions Issue #22

Figure 2. Diet impacts on salmon gut fold structure. A: fish meal fed animal distal intestinal mucosa; B: soybean fed animal distal intestinal mucosa, showing severe enteritis.



Adapted; Sahlmann (2013).

generally start silently, after few days of feeding, triggered by inflammatory process on the intestinal mucosa, microflora dysbiosis and reduced immunological responses. When the performance impairment becomes

Table 3. Gut health support mode of action of the several categories of feed additives.

Additive	Benefits
Probiotics	Intestinal epithelial barrier function enhancement, control of pathogenic bacteria, improved gut immune response, enzyme and VFA production
Phytogenics	Decrease on pro-inflammatory responses in gut mucosa, support to antioxidant system, endogenous secretion stimuli, immunostimulation, cell protection, reduced toxin challenges
Organic acids	Pathogenic bacteria control, increased nutrient and energy digestibility
Prebiotics	Immunostimulation, pathogenic bacteria adhesion
Mycotoxin deactivator	Counteract the negative effects caused by mycotoxins

Adapted, NRC (2011).

visible, critical physiological conditions may have been reached and secondary damages already occurred.

In Atlantic salmon smolts, for example, the soybean meal-induced enteritis illustrates very well the harmful consequences to the gut structure caused by exposure to antinutritional factors. After 7 days, inflammatory process becomes evident, and after 21 days of feeding, pronounced morphological effects in the mucosa can be observed, such as reduced fold height and complexity; shortened microvilli, decreased numbers and size of supranuclear vacuoles, submucosal leukocyte infiltration and a wider lamina propria (Figure 2).

Although salmon is a carnivorous species, the antinutritional factors effects are not limited to exigent species. Antinutritional factors from alternative raw material such as sunflower, cottonseed and rapeseed meal tend to cause similar impacts in omnivorous species including tilapia, catfish, pangasius and shrimp.

Counteracting negative impacts

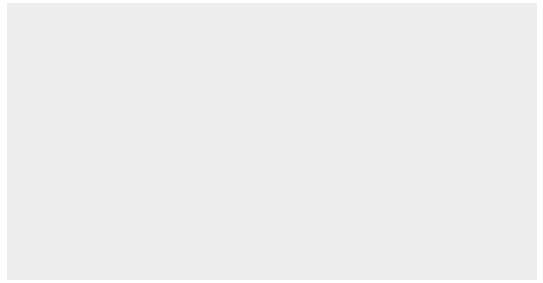
In commercial operations time and resources are limited, and even with a good surveillance program, some risky situations cannot be avoided.

In this scenario, innovative feed additives can act as nutritional tools that, in combination with functional nutrients, mitigate the risks of alternative feedstuffs economically, assuring gut health and increasing performance (Table 3).

A team effort

In order to reach high levels of excellence on gut health management, a strong multidisciplinary effort is required. Bringing together experts in nutrition, health, management, etc., to the table to develop and validate workable solutions is required in order to be successful.

The nutritional strategy success will be strongly linked to the sustainability of the aquaculture operations, which entails enhanced performance and profitability, lower nutrient release to the environment and improved animal welfare conditions.



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