Functional feed additive helps during parasitic infestation of European seabass *Dicentrarchus labrax*

by Robles, R.[1], Isern-Subich, M.M.[2], Román, C.[1], Bermúdez, L.[1], Ceulemans, S.[2], Mazuelos, N.[3], Librero, M.[3], Medialdea, M.3, Albinyana, G.[4], Girons, A.[4], Tapia-Paniagua, S.T.[5], Moríñigo, M.A.[5] and Coutteau, P.[2]*

*[1]CTAQUA, Muelle comercial s/n. El Puerto de Santa Maria, 11500 Cádiz. Spain. *Present affiliation Robles and Co. Aquaculture. Temporal,8. Puerto Real, Cádiz-Spain.*

[2]Nutriad International, Hoogveld, 93. 9000. Dendermonde. Belgium

[3]Finca Veta La Palma, Puebla del Rio ,Sevilla. Spain

[4]Ictiovet, Provença, 392.PB. 08025, Barcelona. Spain

[5]Microbiology Dept. Facultad de Ciencias. University of Málaga. 29071 Málaga, Spain

Aquaculture production continues to increase following the demand for healthy and sustainable animal production. In 2015 world aquaculture production reached 106 million tons with a first sale value of US$160,200 million (FAO, 2016). As any other animal production industry, farmers are confronted with a variety of pathological issues that can endanger their production. Careful management practices, a holistic approach, functional nutrition and close surveillance of fish health are essential factors for a successful business.

In this study, a tank trial was carried out in the Atlantic area of South Spain, at the fish farm Finca Veta La Palma, located at the centre of the Guadalquivir River's estuary marshes. The farm produces European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) in earthen ponds. The objective of the study was to determine the effect of a functional, phytobiotic feed additive on the performance and health of seabream and seabass throughout the changing culture conditions during winter and spring. The use of unfiltered, untreated brackish water originating from the pond farm resulted in a natural disease challenge throughout the trial and imitated the fluctuating environmental conditions encountered in the pond farm.

Materials and methods

Sixteen out-door tanks of four cubic meter volume each have been used for the trial. The design applied for tank diet allocation was a randomised block design (Figure 1). Seabream and seabass juveniles were recruited from the grow-out ponds of the farm.

In total, 880 seabream juveniles and 800 seabass juveniles were stocked in 16 tanks (110 seabream per tank and 100 seabass per tank). Initial average body weight was 54,46±1,02g for seabream and 66,67±1,39g for seabass. Temperature and photoperiod were natural; experimental tanks are located at the exterior of the farm facilities.

Each tank had an individual water inlet and outlet; pure oxygen was supplied to each tank by a porous tube placed at the bottom of the tank. Tank water renewal was set at 66 percent per hour. The water was unfiltered and originating from the incoming water channels used for the pond farm. Flow rate per tank was controlled once per week. Dissolved oxygen and temperature were controlled three times per day per tank. Every two weeks salinity, total ammonia nitrogen (TAN) and nitrite of the culture water were checked.

Fish were fed six days per week with manual feeding and with belt feeders. Feed ration was calculated for all tanks based on feeding tables and as a percentage of biomass. Two diets have been tested per species, control feed, CF, (commercial diet,

Figure 1: Outdoor tanks used for the trial. In the image, harvesting of specimens during sampling

Table 1: Scoring system applied for the evaluation of the samples of gills, liver and intestine of the experimental individuals sampled at the beginning and the end of the trial. Numbers in parenthesis indicate the minimum and maximum value for each score using the number of features and the lowest or highest degree of affection.

Score	Histological feature	criteria
Gill injury (0 – 18)	Inflammatory infiltrate Lamellar hyperplasia Lamellar fusion Oedema Cell degeneration Vascular abnormalities	1 = lesion affecting up to 10% of gill surface 2 = lesion affecting 10-50% of gill surface 3 = lesion affecting more than 50% of gill surface
Gill parasites (0 – 9)	Monogeneans Trichodina sp Other ciliates	1 = single parasite detected 2 = less than 10 parasites detected 3 = more than 10 parasites detected
Liver injury (0 – 18)	Lipidosis	1 = hepatocytes presented with small intracytoplasmic vacuoles and central nucleus 2 = hepatocytes presented with marked intracytoplasmic vacuoles and central nucleus 3= hepatocytes presented with marked intracytoplasmic vacuoles and lateralised nucleus
	Hepatitis Cholangitis Haemorrhage Macrophage aggregates Exocrine pancreas atrophy	1 = focal lesion 2 = multifocal lesion affecting less than 10% of liver section 3 = multifocal lesion affecting ³10% liver section
Intestine injury (0 – 9)	Mucosa lymphocyte infiltrate Intraepithelial degenerative forms Submucosa mononuclear infiltrate	1 = very mild increased impact when compared with basal levels. 2 = multifocal marked increased impact when compared with basal levels. 3 = widespread marked increased impact when compared with basal levels.
Intestine parasites (0 – 3)	Coccidia	1 = single parasite detected 2 = less than 10 parasites detected 3 = more than 10 parasites detected

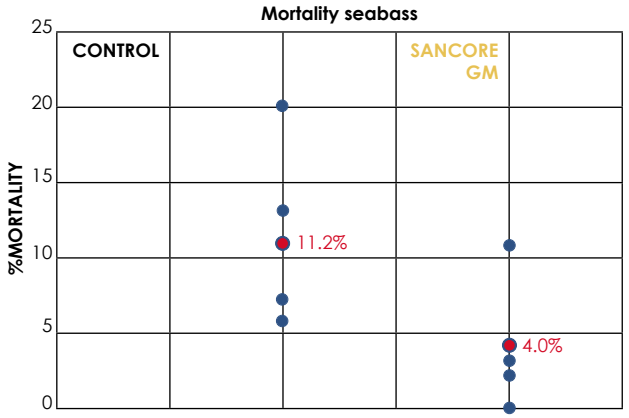
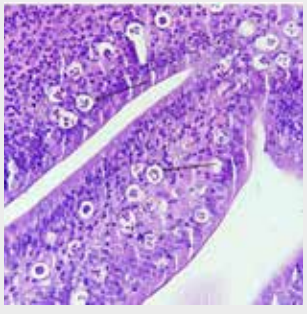


Figure 2. Mortality of European seabass during the parasitic episode. Average value per treatment in red (blue color dots correspond to the 4 replicates per treatment).

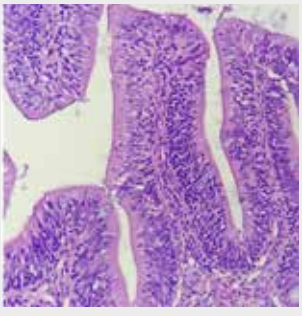
Figure 3: *Trichodina* sps in the gills of European seabass (arrows).

Figure 4: *Diplectanum sps* in the gills of experimental European sea bass.

Below; Figure 5: Coccidian parasites in the intestine of experimental European seabass



Below; Figure 6: Lymphocyte infiltrate in the intestinal mucosa of experimental Gilthead seabream



Portugal) and treatment feed, S (same commercial diet supplemented with 0.3 percent Sanacore GM®, Nutriad International, Belgium). Four replicate tanks were run per diet. Fish were fasted during 24 hours prior to sampling. Total duration of the trial was 150 days (from mid-December to mid-May).

Four samplings (initial, two intermediate and final) have been performed during the trial to control productive parameters: growth, feed conversion and survival as well as somatic indexes and the general health status of the fish. At each sampling moment, gill samples were observed on wet mounts to evaluate the presence of parasites. At the initial and end of trial sampling gill, liver and intestine samples fixed with 10 percent buffered formalin were processed following routine paraffin-inclusion protocol. Haematoxylin & eosin (H&E) 4m thick sections were used for histopathological analysis under light microscope.

A semi-quantitative score system was used in the histopathological analysis in order to evaluate impact of different histological lesions. Each tissue section was scored zero (absence of lesion), one (mild impact), two (moderate impact) and three (marked impact) for each histological lesion. Injury score to evaluate gill, liver and intestine damages were calculated adding individual scores. In addition, a parasite score for gill and intestines were also used as summarised in Table 1.

The intestinal microbiota was evaluated using the DGGE technique. Intestine samples of farmed sea bass and seabream specimens from both treatments were dissected at two sampling moments: initial (T0) and at the end of the experimental period after 150 days of culture (T150). All samples were kept at -20ºC until further processing at the microbiology lab (University of Málaga).

Intestinal samples from each treatment were homogenised in 1ml PBS (pH 7.2), and then the aliquot was centrifuged at 1000×g for 5min. Total DNA was extracted from samples according to Martínez et al. (1998), with some modifications as described by Tapia-Paniagua et al. (2010). Further processing and analyses of the samples were done according to the standard procedures of the lab. Species richness (R) and Shannon's diversity index (H') of microbial communities were determined (Tapia-Paniagua et al., 2010), considering that a relevant band must be present in the DGGE profiles of at least three of specimens analysed per diet.

Results and discussion

Average water temperature during the trial was 16,33±3,10 ºC with the minimum of 11ºC in January and maximum of 23ºC in May. According to the registry of the farm, temperature has been unusually high for that period of the year. Average salinity was 12 psu (fluctuating between 9 psu and 17 psu), TAN and nitrite values were lower than 1ppm during the whole trial and average dissolved oxygen in all the tanks was above 100 percent saturation.

An unexpected gill fluke infection occurred during the month of February affecting only the seabass tanks. The diagnosis confirmed an ectoparasite infestation by *Diplectanum sps* as predominant species. A treatment with 100ppm formaline bath was applied twice during the episode. Average mortality in this period was 11,15±6,74 percent for control and 4,05±4,66 percent for the treatment (Figure 2). No mortality was recorded in the seabream tanks and the evaluation of the gill-wet mounts did not show the presence of any parasite.

Due to the lack of challenging temperatures or disease events, seabream exhibited good production parameters as well in the control feed tanks as in the Sanacore GM fed tanks (survival > 92%; SGR 0,55 %/d; FCR 1,7) and no significant differences were detected between treatments. In the case of seabass, the parasite incident did not allow to obtain clear data for evaluation of production results.

The histopathological analyses of European seabass gill samples indicate that concerning the gills, the degree of gill injury between the CF and S groups were similar, however the impact of *Trichodina*

infection was higher in the CF group (Figure 3). Similarly, impact of *Epitheliocystis* intracellular colonies was slightly higher in CF group. Prevalence of monogenean parasites (*Diplectanum sps*.) was low but monogenean related pathology was significant in this species (Figure 4).

The gill damage score provides information on tissue response to insult (e.g. inflammation, lamellar hyperplasia, lamellar fusion and edema). Gill damage score was slightly higher in group CF (8,92) than in group S (7,75). With regard to liver injury, the score was markedly higher in group CF fish (4,57) in comparison to group S fish (2,40) (Table 2). Higher score was a consequence of a higher impact of lipidosis signs at moderate levels and focal degenerative/inflammatory lesions on group CF fish.

Intestine scores were slightly higher in group CF fish. This was a consequence of CF fish presenting moderate to severe lymphocyte infiltrate in intestine mucosa that could be related to mild enteritis signs. Accordingly, coccidian parasites (Figure 5) were detected in both groups but impact was slightly higher in CF fish.

Histopathological findings in Gilthead seabream, indicate that the gill injury scores in fish from group CF (8,67) and group S (8,33) were very similar. Small differences were detected on the impact of certain parasites. In the case of *Trichodina*, seabream from CF group presented a slightly higher impact. However, group S presented a slightly higher impact of *Epitheliocystis* (Table 3). In the case of the liver, seabream liver scores for group CF presented a slightly higher liver score (2,15) than the group S livers (1,61). Intestine scores were similar between group CF (4,17) and group S (3,83). Intestinal mucosa showed moderate to severe lymphocyte infiltrate and seabream from group CF presented slightly higher counts of lymphocytes (Figure 6).

Table 2. Summary of the histopathological analyses results from European seabass experimental individuals.

DIET	Gill injury score	Liver injury score	Intestine injury score	Intestine coccidian score
Control	8,92	4,57	4,33	1,17
Sanacore GM	7,75	2,40	3,92	0,67

Table 3. Summary of the histopathological analyses results from Gilthead seabream experimental individuals.

DIET	Gill injury score	Liver injury score	Intestine injury score	Intestine coccidian score
Control	8,67	2,15	4,17	0,17
Sanacore GM	8,33	1,61	3,83	0,00

Table 4. Microbial indexes at T0 and T150 days of the trial for experimental Gilthead seabream and European seabass.

		Richness R	Shannon's Index H'
Gilthead Seabream *Sparus aurata*	T0 initial	32±3.0	2.5±0.1
	T150 Control feed group	30±1.3	2.39±0.1
	T150 Sanacore GM group	33±0.7	2.72±0.1
European Seabass	T0 initial	35±2.0	2.98±0.1
	T150 Control feed group	29±1.0	2.80±0.1
	T150 Sanacore GM group	36±1.0	3.17±0.1

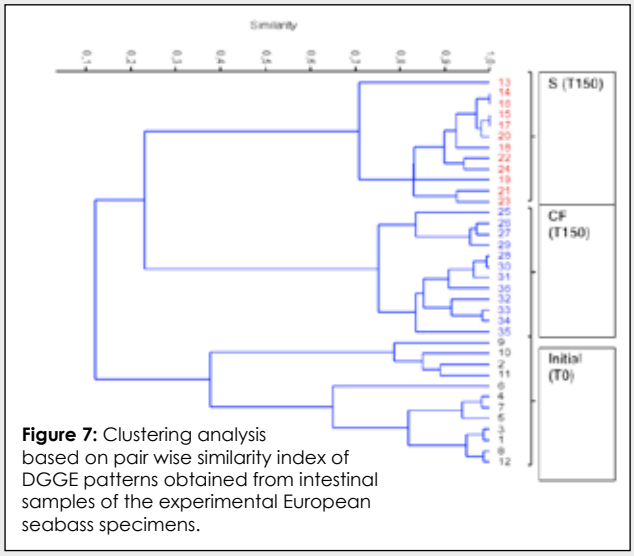


Figure 7: Clustering analysis based on pair wise similarity index of DGGE patterns obtained from intestinal samples of the experimental European seabass specimens.

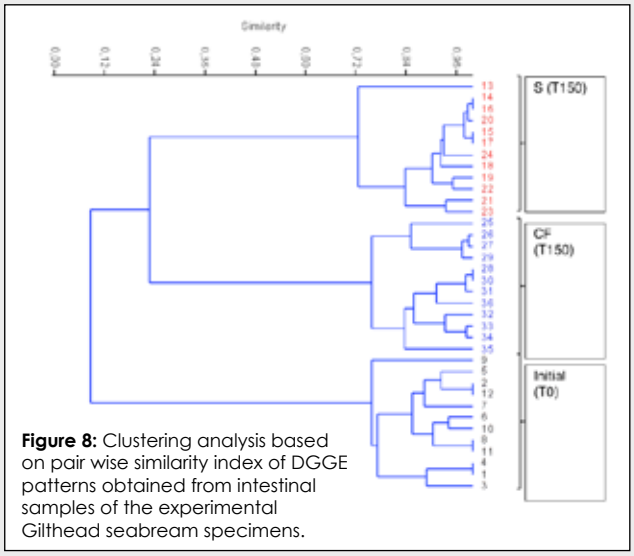


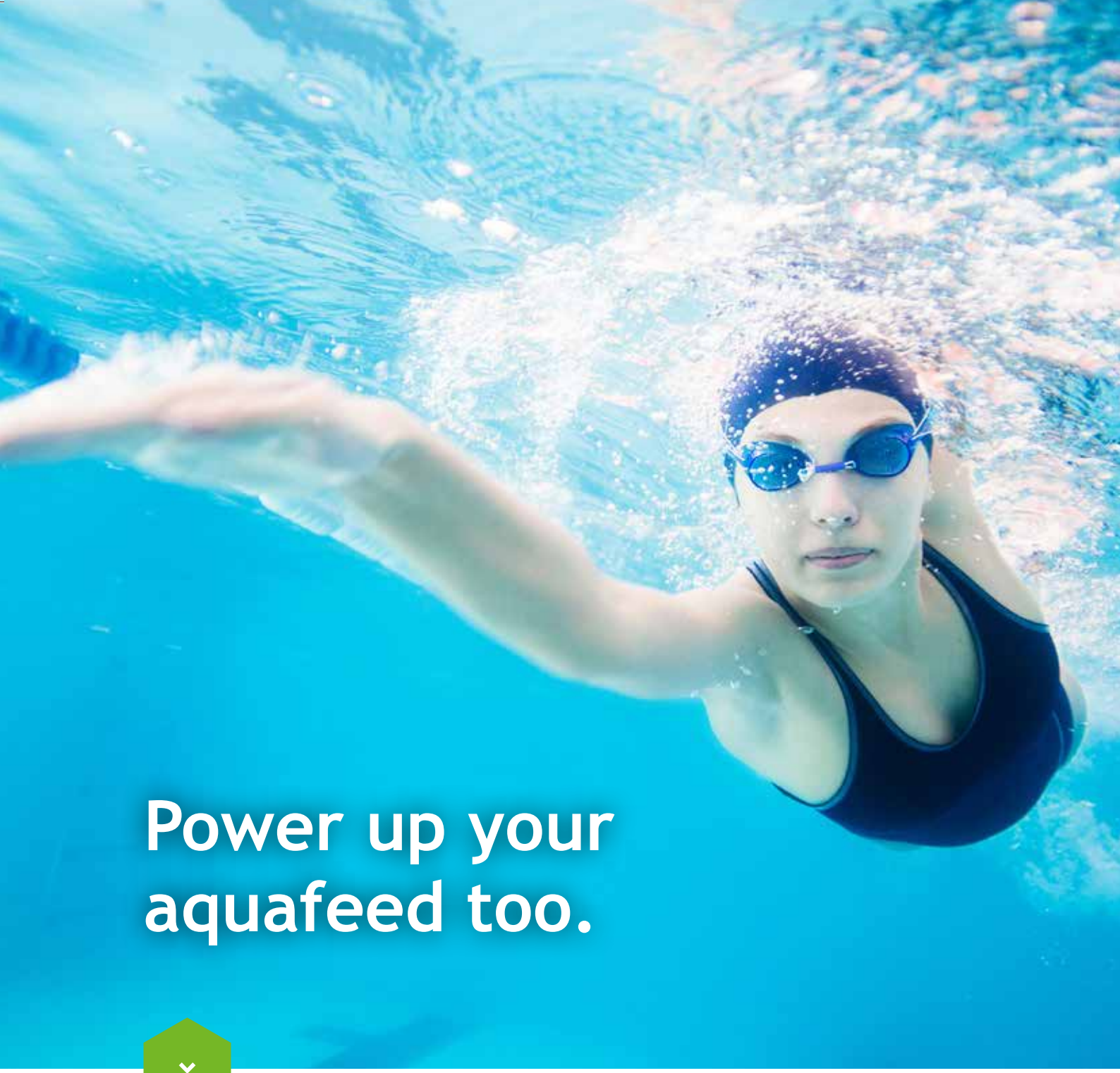
Figure 8: Clustering analysis based on pair wise similarity index of DGGE patterns obtained from intestinal samples of the experimental Gilthead seabream specimens.

Concerning the microbial analyses, Richness (R) and diversity (H') indexes showed an increased diversity of the microbial population present in the S group, for both European seabass and Gilthead seabream (Table 4). Richness (R) and Shannon Index (H') are higher in both species for the fish group Sanacore GM (S) compared to control feed diet (CF) at the 150day sampling point. The increased diversity of gut microbiota is considered as a robustness of the population if confronted to any kind of insult or abnormal event that could disturb the microbial community. This could be an explanation of the success of the S group fish when coping with the gills fluke outbreak.

Regarding the clustering analyses based on pair wise similarity index of DGGE patterns, the blind clustering of the 36 individuals analysed from each species (12 from T0 commercial feed; 12 from T150d commercial feed (CF) and 12 individuals from T150 Sanacore GM (S) group) show interesting results (Figure 7 and 8). The clustering analyses show two different groups clearly differentiated corresponding to T0 and T150 days sampling points. There is a well-defined clustering at T150 reflecting the different diet group (CF and S).

The results show that the dietary supplementation with Sanacore GM induced a substantial variability of the DGGE patterns compared with the profiles observed in fish fed the control diet. This proves the ability of Sanacore GM to modulate the gut microbiota in fish.

(References are available upon request to the authors)



Power up your aquafeed too.

DISCOVER NUTRIAD'S AQUA HEALTH PROGRAM

Nutriad's aqua team works together with researchers and producers around the globe to develop an innovative range of health promotors and optimize their application under today's challenging production conditions. Based on natural ingredients, these specialty additives reduce the impact of diseases and parasites on the productivity of fish and shrimp. Powered by scientifically proven anti-bacterial and anti-parasitic activities, immune-modulators, hepato-protectors as well as novel modes of action such as Quorum Sensing Inhibition. Today, our aqua-specific product lines SANACORE®, APEX®, AQUASTIM® and BACTI-NIL®, are applied in premium brands of functional feeds for fish and shrimp. Feed is much more than just nutrition.

Interested? Find your local contact at nutriad.com

nutriad
we add more