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# Prebiotics and their application in aquaculture. A review.

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## INTRODUCCIÓN

In Mexico, aquaculture activity was significantly increased during past years. However, to maintain the obtained status, it is important to consider that successful aquatic organisms production is conditioned by cultured species health, which is determined by the interaction between environment, pathogens, and hosts (FAO 2016). Due to the above, it is necessary the use of additives and complements which help to maintain healthy organisms without the need of chemical or antibiotic application, which have contributed to bacterial resistance and ecological imbalance (Dawood and Koshio 2016). In this sense several studies were made focused to search substances and microorganisms that can increase sickness resistance and improved the growth in fishes and crustaceans (Gainza and Romero 2017). Within the most developed strategies are the use of probiotic organisms and prebiotic substances (Carbone and Faggio 2016). In probiotic case, their exploitation was widely documented with positive results in most cases, but when we are talking about prebiotic substances, the study research is in its first development steps (Akhter et al. 2015). While several authors particularize that prebiotics by being no digestible food substances in diet, they can nourish and stimulate the growth of one or more several benefit bacteria which contribute to host intestinal balance to improve their health. It was observed that in many cases, the results were not satisfactory, so it is necessary to make more studies to evaluate the positive or negative effects for the use of different prebiotic sources obtained from vegetables that human consume and compared with obtained results from prebiotic sources from microalgae, which were nearest from fishes group (Mahious et al. 2006). This review has the goal to expand the panorama from obtained results of prebiotic exploitation in fishes and crustacean culture.

*Prebiotics*

Prebiotics in aquaculture are defined as not digestible food substances, that nourish and stimulate the growth or metabolic activity for one or more types of beneficial bacteria in the digestive tract of fishes, molluscs, and crustaceans (Lie et al, 2007; Kongnum and Hongpattarakere 2012), which indirectly limit the presence of potential pathogenic bacteria, like Vibrio, Aeromonas and Streptococcus (Zhou et al, 2010; Silva et al, 2014), improving the intestinal microbial balance and modulate immunology response (De Vrese and Schrezenmeir 2008; Bindels et al. 2015).

A key mechanism whereby it is considered that prebiotics perform benefits to host health, was the production of small chain fatty acids (scFAs) by bacteria used as substratum. This scFAs reduce intestinal pH and microbiota release acetic acid, hydrogen peroxide, and produce substances like biocine and bacteriocins, which exclude pathogenic bacteria (Bindels et al. 2013). Likewise, production of B group vitamins increases, and the availability of certain minerals, like calcium and magnesium, improving host nutrition. So, the prebiotic activity is influenced by used carbohydrates and fermentation process by specific microbial taxes (Bindels et al. 2015).

So that a substance can be considered as prebiotic, must comply with following criteria:

1. Must be from vegetable origin.
2. Be part of a highly heterogeneous set of complex molecules.



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3. Not be digested by digestive enzymes.
4. Need to be osmotic active.
5. Need to stimulate the grow or metabolic activity from beneficial bacteria present, naturally, in colon organ.

The main used prebiotic are polysaccharides and oligosaccharides, like: fructooligosaccharides (FOS), galactooligosaccharides (GOS), maltoligosaccharides (MOS), and xilooligosaccharides (XOS). The FOS and inulin are considered as typical prebiotics, and are widely commercialized for human, monogastric animals, and recently, in culture of aquatic organisms. It is important to show that obtained results with prebiotic use in fishes are variable. This can be explained with respect to the prebiotic effect that can be variable, depending on used carbon source, diet prebiotic concentration, solubility, fish's specie, water temperature, and duration of fed period (Dawood and Koshio 2016).

*Inulin as prebiotic*

Inulin is an energy reserve carbohydrate present in more than 36,000 plant species, of which it stands out the chicory, dandelion, onion, garlic, and agave, among others. It is constituted by fructose molecules joined by β-(2→1) fructosyl-fructose, being the "fructans" term used to denominate these type of compounds (Fig.1) (Ringo et al. 2010). Fructans, by their chemical configuration cannot be hydrolyzed by digestive enzymes of consumer organisms, that's why it can go intact in their way to upper part of gastrointestinal tract, but they are hydrolyzed and fermented for bacteria from lower part of gastrointestinal tract (Madrigal and Sangronis 2007).

As mentioned above, the prebiotics exert their action trough positive impact that have to intestinal microbiota, although there exist other indirect effects like show in Table 1.

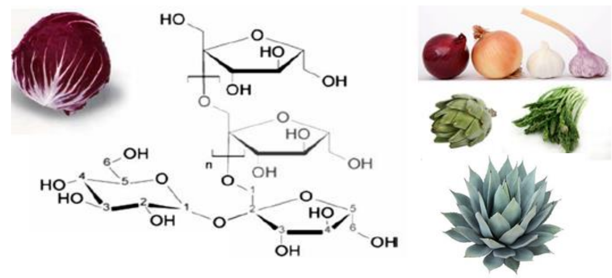

**Fig. 1.** Inulin chemical configuration

*Studies made with Inulin as prebiotic source in aquatic organisms*

Several studies have evaluated the growth of several strains of Lactobacillus using agave Inulin with positive results, checking that function as prebiotic to promote reproduction and bacterial grow, inclusive with better results with respect glucose as carbon source (Castillejos et al. 2006; Deney et al. 2009; Pinheiro de Sousa et al. 2012; García et al. 2012; Urías 2008), because a great number of probiotic bacteria own the β- fructofuranans enzyme, which is necessary to hydrolyze glucoside β (1-2) which is present at Inulin and in this way can metabolize the resultant monomers (Cerda 2014; Pinheiro de Souza et al. 2012). Other studies show the prebiotic importance in bacterial pathogen control principally in Vibrio genus. By reducing the presence of such bacteria, also digestive tract pathogen colonization decrease, what in turn decrease, collaterally, the exotoxins and bacterial translocation over the intestinal epithelium strengthen the integrity and functionality of intestinal epithelium barrier (Torrecillas et al., 2014). This process was reflected over the intestinal morphological changes, directed to be faster and efficiency of nutrient absorption, promoting the increase of availability and energy reserves that induce changes in crustacean physiology conditions.

According with Daniels et al. (2010) MOS action stabilize microbiota composition and suppress, to some extent, variations and affluence of new bacteria strains coming of culture medium. Studies



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**Table 1.** Intestinal functions attributed to prebiotics (adapted from Gaggía et al. 2010).

| | |
|---|---|
| Effect on front gastrointestinal tract | Digestion resistance<br>Retarded gastric emptied<br>Time increase in orocecal transit<br>Reduction of glucose absorption and low glycemia<br>Epithelium hyperplasia of small intestine<br>Stimulation of intestinal peptide hormone secretion |
| Effect in posterior gastrointestinal tract | Substratum for microflora fermentation<br>Final fermentation products production<br>Stimulation of saccharolytic fermentation<br>Intestinal epithelium hyperplasia<br>Stimulation of intestinal peptide hormone secretion<br>Faces production regulation (frequency and consistence)<br>Acceleration of coloanal transit |

like Burr and Gatkin (2007), evaluated the supplementation effect of GroBiotic®-A and Inulin prebiotics over grow efficiency and gastro intestinal microbiota of *Sciaenops ocellatus*. Prebiotics addition in any feeding trials did not modified the weight increase, feeding efficiency relation or efficiency protein relation of corvina fish. Electrophoresis gel denaturing gradient analysis (DGGE) of gastro intestinal microbiota community did not show prebiotic effect on diet, because microbial community seemed inactive by only one organism with low diversity in comparison with other animals and fish species. The DGGE of biofilter microbiota community of independent aquariums show a diverse microbiota community, which were not affected for diet prebiotics. Mahious et al. (2006), made an experimental study to probe the effect of three prebiotics (Inulin (Raftiline ST), oligofructose (Raftilose P95) and lactosacarose) in growth, survival and microbiota content of halibut (*Psetta maxima*) larvae. Experimental diets were formulated to 2% content of each prebiotic (commercial), control diet was added with 2% of cellulose powder as carbon source. Twenty-nine days after egg hatching, halibut larvae stage was fed with the experimental diets for one month; At 29 and 55 sample days, biometric values were taken and gastrointestinal samples to analyze bacteria presence. The results shown that fishes which were fed with oligofructose supplement show the highest weight mean value (0.50 g), followed by lactosacarose diet with 0.43 g; the lowest values were for control and Inulin diet, both with 0.41 g with a significant difference between them ($p<0.05$). With respect to survival, it did not show significant difference between treatments and in bacterial counts was not possible to observe any difference between bacterial abundance, but it was possible to observe that Vibrio spp. presence was lowest in lactosacarose treatment (5%), at same with Vibrio ordalli; with Bacillus subtilis only was registered in oligofructose treatment.

Making a review in Biological Science data base (CABI full text), was made a result analysis obtained by different studies regarding prebiotic use like Inulin and vegetable cellulose, where it could be verified that results have not been successful and on contrary it was observed adverse effects, like it is observed in Fig. 2.

It can observe the result inconsistence related with the improvement of fishes and crustaceans culture parameters fed with prebiotics, its mainly due in most cases the dosage was not optimal, because was different depending the specie and their life stage of cultured organisms, hence the not significant different results obtained in many studies, like were mentioned by Ringo et al. (2010) and Torecillas et al.



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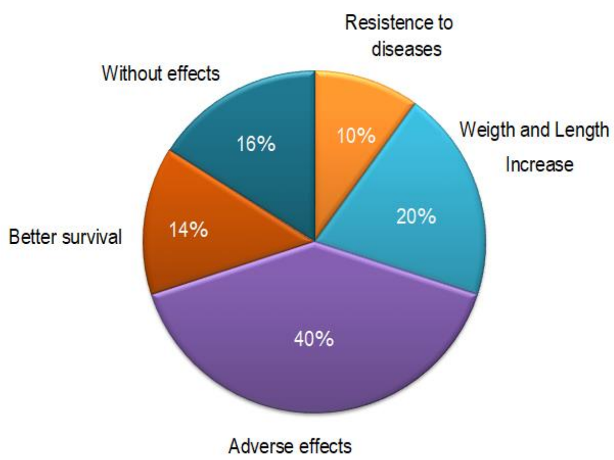


**Fig. 2**. Result of studies research about prebiotics used in aquaculture.

(2014). It is necessarily to make more studies about doses, supply time and carbon sources as aquaculture prebiotic sources (Romero et al. 2014). Likewise, the recent advantages of massive sequencing have opened a broad directly studies the effects of gastrointestinal microbiota modulation of fishes and crustaceans for prebiotics and diet components.

The above make us think about that Inulin use and other prebiotic sources obtained from vegetables used to human consumption can affect over results, since be directed to colonic bacteria, some fraction can intervene in digestive enzymatic activity in negative way, because there were carbohydrates sources not proper from aquatic environment. It exists another carbohydrates source with probiotic application obtained from microalgae that can obtain better benefits, because there were more closed to aquatic organisms.

*Marine and freshwater algae as prebiotic sources to aquaculture activity*

Algae were used in many countries as fertilizer, biofuel, hydrocolloids sources, and for live food source for many species of fishes and crustaceans by their high protein, polyunsaturated lipids, vitamins, and minerals content. Recently, it was broad their application as functional food, because they were a dietetic soluble fiber source phenolic compounds, and pigments.

Inside this algae group was found the macroscopic algae classified in three groups: Chlorophyta or cloroftas, Phaephyta or feoftas, and Rhodophyta or rodoftas, which correspond to green, brown, and red algae respectively (Quitral et al. 2012).

Green algae or Chlorophyta group around of 1,200 species, they shown starch at reserve polysaccharide, their wall cell show a cellulose matrix (Dominguez 2013), generally embedded in hemi-cellulose matrix, in other species by cellulose or xylose, so they can be interesting prebiotic sources in aquaculture activity (Peña-Rodríguez et al, 2011).

Some studies show that alginate applied to fish's diet in marine aquaculture produce better grow and fed efficiency (Conceição et al. 2001; Yeh et al. 2008; Ahmadifar et al. 2009; Jalali et al. 2009). Also, Conceição et al. (2001), observe increase in new synthesis protein retention three times higher in halibut organisms which were fed with diets with alginate addition.

Other studies demonstrated the immune stimulant activity of algae and their polysaccharide compounds in aquaculture (Bagni et al. 2005; Cheng et al. 2007; Chiu et al. 2008; Ahmadifar et al. 2009; Harikrishnan et al. 2010). In vitro studies with halibut phagocytes have shown that polysaccharides extracted in algae like *Ulva rigida*, and *Chondrus crispus* (Castro et al. 2006) produce a better response of immune system.

On the other hand, more and more in vivo studies reiterate the capacity to increase immune system response of polysaccharides compounds from different algae. The immune activity of alginate was demonstrated in different marine organisms like: *Hippoglossus hippoglossus* L. (Skjermo and Bergh 2004), bass *Dicentrarchus labrax* (Bagni et al. 2000) and different mero species like *Epinephelus coicoides*, *Epinephelus fuscoguttatus*, *Epinephelus brneus* (Cheng et al. 2007, 2008; Chiu et al. 2008,



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Yeh et al. 2008, Harikrishnan et al. 2011), producing an increase on fish survival. The carrageenans and ulvan like modulators of immune system response in marine fishes are little studied; even so, the obtained results until now are promising. Two studies made with different carrageenans in *E. coicoides* and *E. fuscoguttatus* obtained positive results in Vibrio alginolyticus infection resistance (Cheng et al. 2007).

*Freshwater macroalgae*

The freshwater micro and macroalgae have in common a high protein percentage (50-70%) of higher assimilation biological value. Also, have a high content in B group vitamins, minerals like: iron, magnesium, copper, and zinc, and higher quantity of chlorophyll. Lower Content values of iodine that marine algae but are excellent natural prebiotic source for their high content of easy assimilation polysaccharides (Nicoletti 2016). Several studies have evaluated the addition of *Spirulina* sp. and Chlorella sp. as additive to aquaculture feeds with positive results. Capelli et al. (2010) evaluated the addition of fresh Spirulina sp. in fed of *Litopanaeus schmitti* larvae and were obtained an increase in nutritional larval index, showing that these values can be attributed to microalgae influence on intestinal microbial community and digestive enzyme secretion, that facilitates nutrient assimilation, enhance the survival and grow of shrimp larvae and post-larvae stages.

In macrophyte case, it was checked that *Lemna* sp. has 40% of soluble matter (sugars and amino acids), 15% of proteins, 5% of starch, 5% of ashes, and 35% of cellulose polymers as carbon source, which can be used for microbial growth (Arroyave 2004). Because of his nutritive value, it was ideal source for fishes and crustaceans feed. Recently, the interest is higher for this aquatic plant because it has the capacity to grow fast on nutrient rich residual waters and produce protein rich biomass that can be used to animal nutrition (Ponce-Palafox et al. 2005). Fresh *Lemna minor* was used to replace 50% of fish powder in fed *Oreochromis niloticus* diet, obtained positive results with respect survival and length at the end of productive cycle. The same was observed with commercial culture of *O. hornorum* and *O. mossambicus* in Morelos State, México (Ponce and Fitz 2004).

Due to all above, we can see that there are natural sources of prebiotics that can be used in aquaculture in order to minimize the low significant results regarding to prebiotics sources like inulin and other MOS which are obtained from terrestrial plants, with the idea to apply aquatic plants closer to fishes and crustaceans, so it is required to make relevant researchs to stablish their viability.

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