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María del Carmen Monroy-Dosta
Universidad Autónoma Metropolitana-Xochimilco, Live Food Chemical Analysis, México

José Alberto Ramírez-Torrez
Universidad Autónoma Metropolitana-Xochimilco, Live Food Chemical Analysis, México

Dafne Itzel Orozco-Rojas
Universidad Autónoma Metropolitana-Xochimilco, Live Food Chemical Analysis, México

Luis Héctor Hernández-Hernández
Laboratory Universidad Nacional Autónoma de México, FES-Iztacala, Aquaculture Production Laboratory, México

Jorge Castro-Mejía
Universidad Autónoma Metropolitana-Xochimilco, Live Food Chemical Analysis, México

Daniel Becerril-Cortés
Universidad Autónoma Metropolitana-Xochimilco, Live Food Chemical Analysis, México

Correspondence
María del Carmen Monroy-Dosta
Universidad Autónoma Metropolitana-Xochimilco, Live Food Chemical Analysis, México

Functional foods in rainbow trout (*Oncorhynchus mykiss)* production

María del Carmen Monroy-Dosta, José Alberto Ramírez-Torrez, Dafne Itzel Orozco-Rojas, Luis Héctor Hernández-Hernández, Jorge Castro-Mejía and Daniel Becerril-Cortés

Abstract
Nowadays, diet optimize and disease control are two principal goals to guarantee aquaculture expansion. Considering that the organisms are in captivity, it is difficult to provide all nutrients that organisms need in culture systems, that is why it is important to add different substances to improve their nutritional condition and obtain better growth and reproduction rates. For this reason, alternatives to improve nutrition and reduce diseases, using different strategies applied until now, have been researched. Such is the use of functional foods including nutraceuticals, prebiotics, probiotics and symbiotic, as a tool which allow counteract use of antibiotics, hormones and chemotherapeutics because of adverse effects that they generate. In México, rainbow trout culture, for their economic value, occupies seventh place with an annual growth of 3.98% in last 10 years, which denotes the importance of the species. The goal of this study was to make a functional food review and their obtained benefits until now in *Oncorhynchus mykiss*.

Keywords: functional food, nutraceuticals, probiotics, *Oncorhynchus mykiss*

1. Introduction
In México, fish culture has acquired relevance in last few years, obtaining social and economic benefits, which were converted in food supply with high nutritional value for population [1]. One of the species with higher production in México is rainbow trout (*Oncorhynchus mykiss*), with an annual production above 6 892 tons, according to FAO in 2013. However, as a live organism, is susceptible to infection and nutritional diseases, which bring as consequences higher mortalities, in such way strict procedures and feeding strategies are demanded for their correct use, from breeding stage to commercialization phase [3]. Because of captivity, it is difficult to provide all the nutrients that a cultured fish requires, it is important to add different substances that improve their nutritional status and obtain higher growth and reproduction rates. In that sense, in recent years there has been a change in animal nutrition concept, because traditionally a "proper diet" were a function of protein contribution to cover their metabolic needs and satisfy hunger sense of cultured species, but nowadays, there is a great interest in the potential of some substances and microorganisms to promote health and improve physical well-being of species with high impact in aquaculture as rainbow trout, so new concepts like functional foods arise, defined as substances or food which have a positive effect in physiological function of one or more organs from cultured species, beyond their nutritional content, including nutraceuticals, prebiotics, probiotics and symbiotic [2]. The purpose of this review was to show a general view about the development of natural new products to improve diet nutritional value in aquaculture production and obtained benefits in diseases control and prevention, survival and growth of rainbow trout.

2. Functional foods
In recent years, it has been developed a new stage in food and animal nutritional science, where particular attention is taken in food-medicine interaction known as “functional foods” [4]. The functional food definition was proposed in Japan in the 80’s with publications of “Food for specific health use” or FOSHU and it refers to those foods that contain ingredients that play a positive effect in physiological functions of an organism, beyond their nutritional content.

In Europe, in 1999, a document was published about aspects to consider in scientific investigation, development and regulation of functional foods. This paper was the starting point to recognize this type of foods. In this paper, it was mentioned that a "functional food" is the one that contain a component, nutrient or not, with a selective effect over one or many organism functions, with an added effect upper their nutritional value and which positive effects justified their claim their functional character or even healthy [2].

2.1 Nutraceuticals

Some authors mentioned that nutraceuticals are chemical and biological active substances that can be found as natural food components or added to it. It is presented in a no nutritional matrix (pills, capsules, powder, among others), which applied in higher doses than the one in foods, presume a favorable effect on health, greater than a normal food possess [5]. Therefore, nutraceutical products have capacity to strengthen healthy conditions, serving as assistant to health care and maintenance, such as disease prevention and improvement of organism functions. Within nutraceuticals most used in fishes, it can be found *Spirulina*, *Chlorella* and *Dunaliella* microalgae, which can be produced trough low cost technologies and are marketed as dry powder, pills and in liquid form; likewise, microalgae of genus *Laminaria*, *Undaria*, *Durvillea* and *Ascophyllum*, that are used as source of iodine, aluminum and iron as additive in aquatic food [6]. In salmonids case, like trout, their diets were enriched with different carotenoids pigments extracted from different microalgae to increase fish pigmentation [63]. Also as antioxidants source it generated better cultured fish welfare [9]. The essential polyunsaturated fatty acids of long carbon chain (PUFA), particularly "w3" type, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), were widely studied with significant results on survival of fishes. Flavonoids as nutraceuticals were evaluated [7] and showed their antioxidant capacity, also their interaction with protein phosphorylation, iron chelation and their capacity to interfere with different enzyme reactions [8], being the most relevant their antioxidant function [7]. Another component highly used are vitamins, especially ascorbic acid, due to many fish species cannot produce it and their deficiency slows growth and immune response, also stimulate malformations and parasite incidence [10]. So, their diet addition allows a positive response to lymphocytes activity, also an increase of red blood cells and better growth in length and weight. Some proved nutraceuticals are shown in Table 1.

Table 1: Nutraceutical used in trout culture

Author	Nutraceutical employed	Results
[9]	Red yeast and astaxanthin	Tissue pigmentation increase and oxidative stress reduction
[10]	Vitamin E and ascorbic acid	Growth and disease resistance
[11]	α -tocopherol + ascorbic acid, selenium (se) and iron (Fe)	Stress reduction; adequate protection against lipid peroxidation
[12]	Flavonoids	Antiestrogenic or androgenic effects as substitution of steroids
[13]	Lycopene	Oxidative stress reduction
[14]	Vitamin E + selenium	Oxidative stress reduction
[15]	Flavonoids	Handling stress resistance
[16]	*Laminaria*	Growth and survival
[17]	Linoleic acid	Growth and stress reduction

2.2 Prebiotics

Prebiotics are not digestible diet food substances, which nurture and stimulate growth and/or metabolic activity of one or more bacteria type in digestive tract of organisms. These substances have the proper to increase host health [18]. The principal used prebiotics are polysaccharides and oligosaccharides like: fructooligosaccharides (FOS), galacto-saccharides (GOS), mannan-oligosaccharides (MOS) and xilo-ologosaccharides (XOS) [19]. The FOS and inulin are considered typical prebiotics and were widely marketed for human and monogastric animals use [20]. The inulin is a storage carbohydrate who is present in many plants, but specially in chicories roots, while FOS are an inulin partial enzyme hydrolysis product [21], which show long oligosaccharides and polysaccharides chains with a polymerization grade about 2 to 65 units; instead, substances like FOS, MOS, XOS and β- Glucans are considered short chains prebiotics. Recently it was added a new prebiotic, β-glucans, obtained from partial hydrolysis of barley arabinoxylan-saccharides, produced by hydrolysis of cereals with high content of arabinoxylans [22]. Also, it was studied prebiotic properties of carbohydrates provenance of marine macro and micro algae [23].

Some studies with rainbow trout and other aquatic species, have demonstrated some advantages, within it can highlight the capacity to create specific modifications in microbial community present in digestive tract, also in their activity, observed effects on growth increasing fermentation products, improve mineral or essential micronutrients absorption and optimize food conversion indexes [20, 24] ; likewise it has been observed an effect over intestinal morphology increasing length and density of tract microvilli, with consequence potentization of adherence and proliferation protection against pathogens genus like: *Aeromonas*, *Vibrio* and *Flavobacterium*, using substance like MOS [25; 26]. Also, it was registered an increase of innate immune response, also complement system, by stimulating macrophages production, lysozyme activity, natural hemagglutination activity, oxidative explosion and phagocytic activity [24; 27].

The use of prebiotics in aquaculture field seems to be a promising strategy due to their multiple advantages which grant, also by being natural substances (Table 2). However, it is important to determine concentrations and specific prebiotics for each organism, since not all substances act in the same way under same characteristics. According some authors [20; 28], the FOS effect is influenced by organism culture temperature, because in crops made at 18°C it was observed better prebiotic efficiency with respect to higher temperatures than 25°C; focusing in specific case of rainbow trout, the culture temperature, is between 13 and 18°C [29], so

prebiotic use like inulin and FOS can give positive results. Finally, it is important to determine administration periods or prebiotics tests [30], because substances like inulin which content an average polymerization grade of 10 units, requires higher fermentation time to produce a response, nevertheless it arises that this substance can produce better effects in proliferation and maintenance of beneficial bacteria in host digestive tract, in comparison with low chain prebiotics [31].

Table 2: Effect of *in vivo* administration of different prebiotic sources in aquaculture.

Prebiotic	Doses	Studied specie	Effect	Reference
GOS	10 gKg^{-1} 4 months	*Salmo salar*	Higher food efficiency was registered.	[30]
	0.5 to 10.0 gKg^{-1}/45 days	*Oncorhynchus mykiss*	Increased of weight of fish, increase in absorption in calcium in bone tissue and at intestinal microbial level, *Vibrio sp*. decrease their concentration.	[20]
	2,000 ppm 42 and 90 days	*Oncorhynchus mykiss*	Prebiotic added on rainbow trout diet increase 13% growth rate and decrease mortality in fishes when response of complement system increase.	[27]
	0, 0.2, 0.4 and 0.6% of weight /2 months	*Oreochromis niloticus*	MOS supply did not modified growth parameters, instead was registered a increase in high and wide of intestinal muscular layer at 0.4 and 0.6 % concentration.	[32]
	38.0, 52.0 y 82.0 g kg^{-1} 9 weeks	*Oncorhynchus mykiss*	An increase was registered in organisms' survival exposed against virus, which produce hematopoietic necrosis at 52 and 82 gKg^{-1} concentrations (43% and 29%).	[33]
	0, 2.0 % de peso /20 weeks	*Oncorhynchus clarkii lewisi*	Prebiotic incorporation levels did not show effect on organisms' growth. However, was observed a shield protection reducing mortality of trout against *Flavobacterium psychrophilum* pathogen.	[34]
MacroGard®	2.0 g Kg^{-1}/9 weeks	*Oncorhynchus mykiss*	To expose organisms against prebiotic was decrease mortality at 36%.	[35]

2.3 Probiotics

Probiotics by definition are live microorganisms that when they are applied in proper amounts, it confers to host a benefit [29]. The main difference between probiotic concept in terrestrial organisms with respect to aquatic organisms are that benefits are not only conferred to host, but also to environment [36; 37]; because aquatic organism's dynamic with their environment is different, because they share same niche and therefore show a direct relationship [38]. Probiotics provides of different benefits to cultured species trough different action mechanisms, assuring best use of food and increasing their nutritional value and disease response or enhance environmental quality [39; 40]. Action mechanisms that were described in probiotics microorganisms is shown in Table 3.

Table 3: Action mechanism of probiotics over aquatic organisms

Action mechanisms	Process
Colonization and adhesion	Once probiotic has adhered to epithelium improves mucus production by goblet cells, distributed throughout epithelium, the cells close junctions forming a continuous biological barrier that prevents macromolecules and pathogenic bacteria entry [29].
Antimicrobial substances production	The probiotics produce of antimicrobial substances, siderophores, bacteriolytic enzymes, lactic acid, acetic acid and other short-chain acids of low-molecular-weight, metabolites such as hydrogen peroxide, carbon dioxide, diacetyl and bacteriocin's which inhibit growth of not only bacterial as well as viral and fungal pathogens [30].
Immune system modulation	They have influence over immune system improving humoral response and controlling the balance between cytokines pro and anti-inflammatory, as well as an increase of immunoglobulin production, increase of mononuclear cells and lymphocytes [31].
Competition	The existence basis of any microbial population is him capacity to competition for chemical products and available energy. The benefic intestinal microbiota use nutrients, that in other way, are consumed by pathogen microorganisms [26; 29].
Nutrient sources	Bacteria can supply or contribute to substances production that were disabled in some diets, synthetized vitamin group B, particularly B_{12}, also calcium, magnesium and iron [32].
Increase of enzymatic digestive activity	One characteristic of probiotics is to excrete exoenzymes which increase host enzymatic activity, contributing with compounds hydrolysis that fishes cannot make alone, for example, chitin, cellulose, among other molecules [33].
Water quality improvement	Probiotic bacteria have demonstrated their capacity to transform nitrogen compounds, which were high toxics for fishes. Especially *Bacillus* sp. genus was demonstrated their efficiency to controlled these compounds [34; 35].
Quorum sensing	Was defined as bacterial communication process cell to cell. Rupture of this condition was recognized as new antiinfection strategy in aquaculture, which was carried out for probiotic microorganisms, although the process was not totally described, it was observed that avoid cell communication between pathogen cells [36].

2.3.1 The use of probiotics for the prevention and control of bacterial infections in rainbow trout

One of the most frequent uses that has been given to probiotics is for disease control, as alternative of antibiotics [36]. It is registered that probiotics can compete with pathogenic bacteria, based on competitive exclusion principle [37]. The investigation in this respect follows the next process, initially, fish microbiota is characterized and then those that can have probiotic potential are chosen to be subjected to *in vitro* antagonistic tests and subsequently *in vivo* [38]. Pérez-

Sánchez *et al*. (2011) [39], isolated and characterized bacteria of rainbow trout, to determine its capacity to inhibit *Lactococcus gerviae.* From 335 obtained strains, only 11 showed *in vitro* antagonistic activity against *L. gerviae*, which belong to genus *Lactobacillu*s, *Lactococcus* and *Leuconostoc*. In Table 4 it is shown some of the works done for the control of infectious processes in trout.

Table 4: Studies focused on development of probiotics and control of pathogens in rainbow trout

Used microorganism	Obtained results	Author
Bacillus subtilis AB1	Stimulation of immune response and improvement of survival after an experimental infection with *Aeromonas* sp. ABE1	[2]
Brochothrix thermosphacta BA211	Stimulation of immune response and improvement of survival after an experimental infection with *Aeromonas bestiarum* and *Ichthyophthirius multifiliis*	[40]
Lactobacillus plantarum CLFP 238, *Leuconostoc mesenteroides* CLFP 196	Competitive exclusion and improvement of survival after an experimental infection with *Lactococcus garvieae*.	[41]
Lactic acid bacteria	From 335 strains of *Lactobacillus plantarum*, *L. lactis* and *Leuconostoc mesenteroides*, 11 showed antagonistic activity against evaluated pathogen.	[39]
Bacterial strains isolated from gastrointestinal tract of rainbow trout	Bacteria of genus *Aeronomas* spp., *Plesiomonas* sp., *Enterobacter* spp., *Citrobacter* sp., were able to inhibit *F. psychrophilum in vitro.*	[42]
Bacterial strains isolated in culture water	From 79 isolations, *Vibrio* spp. showed antagonism against *V. anguillarum* and *Lactococcus garvieae*, while strains of genus *Aeromonas* spp. where able to inhibit *Lactococcus garvieae*.	[43]
Enterococcus casseliflavus	Activivty of immune system increased, indicated by the values of IgM, leukocyte and neutrophils. Used strain, improved the resistance against infection by *S. iniae*.	[44]

As an alternative of indiscriminate use of antibiotics, probiotics have shown benefits without secondary effects that antibiotics present. After treatment with antimicrobial, microbiota is severely affected, losing much of commensal microorganisms and in many cases resistant bacteria have been generated [45], medicated rainbow trout with oxalinic acid and subsequently administrated *Bacillus subtilis*, *B. licheniformis* and *Enterococcus faecium* probiotics to determine its effect on growth, utilization of food and bacterial load of rainbow trout after application of antibiotic. The beginning of experiment, it was registered a low count of viable cells in order of <log 4 UFC g^{-1}, nevertheless, at the end this raised to order of log 6-7 UFC g^{-1}[45].

2.3.2 Effect of probiotics on growth of rainbow trout

In relation to the use of probiotics to improve the growth of fish, other authors [53], associated health condition and growth of rainbow trout, weight gain, food conversion rate, lysozyme activity and leucocyte density, to addition of *B. subtilis*, *B. licheniformis* and *E. faecium* for ten weeks. It was observed an increase in biomass of 300% in all fishes treated with probiotics; best efficiency with respect to food conversion factor was recorded in treatments with *E. faecium*. Probiotic bacteria can have proved high production of short chain fatty acids. Also, they are an important source of group B vitamins, minerals as iron and zinc [45; 43]. It has been determined that these microorganisms also produce exoenzymes and enzymes, which contributes better assimilation of nutrients.

2.3.3 Probiotic effect about digestive enzyme activity in rainbow trout

Growth of animals is in function of their capacity to digest and assimilate nutrients available in diets, which in turns is determined by efficiency of digestive system [48], which in concrete it involves digestive enzymes secretion and activity to unfold proteins, lipids, and carbohydrates In an experimented with *Saccharomyces cerevisiae var*. *Boulardii* and *Debaryomyces hansenii*, as probiotics to determined their effect in three digestive enzymes from brush edge membrane (alkaline phosphatase, γ-glutamil-transpeptydase and leukino-aminopeptydase). The group with probiotics showed significant differences, compared with control test, however, it was not clearly distinguished effect tendencies of yeast over enzymatic evaluated activity. Another approach that has been given to probiotic use, is when it is sought to improve origin of vegetal protein assimilation that has begun to be evaluated as complement or substitute of fish flour. However, vegetal protein generates non-pathogenic clinical conditions, due to the presence of anti-nutritional factors [50], decreasing efficiency of various digestive enzymes, affecting growth in fishes [49].

2.4 Symbiotics

Symbiotics are prebiotic and probiotic mixture which affect, in benefic way, to host, by promoting selectively modulation, survival and metabolism of bacteria which promote host health [63]. The symbiotic supply in aquaculture activity is a recent practice that promises to be very helpful strategy, because unlike administrating by itself each supplement, this alternative offer a competitive probiotic advantage on rest present microbiota [65]; in this way, it was determined that a successful symbiotic for aquaculture, must fulfill a synergy effect, that is to say, combined response of products must be higher that benefit obtained by each one [63; 66]; so some authors have dedicated to evaluate response of their addition in different aquatic organisms which include rainbow trout; registering benefits with higher nutrient absorption, which was attributed to digestive enzyme participation that host did not have capacity to produce, which increase degradation rates, fermentation and substances absorption present in food, which was reflected increasing host growth rates [62; 58]; at same time, it was observed that it improves better survival rates increasing immune gene expression and with production of participant cells in innate and humoral response [69]. However, in some recent studies [70; 71] this synergy was not reported, because variables like cultured specie, compounds selection, compound doses and supply period, must be affect significantly symbiotic activity [64], so it is necessary to make more research in this field because studies in this scientific field are scarce. In Table 5, are shown some studies made with diverse symbiotic supplements to aquatic organisms.

Table 5: Effect of symbiotic supply in aquaculture

Probiotics	Prebiotics	Supply period	Studied specie	Effect	Reference
Debaromyces hansonii L2	Inulin	one month	*Sparus aurata*	Improve immunity	[57]
Enterococcus faecalis	MOS	three months	*O. mykiss*	Weight gain increase of 30.2%. Decrease of mortality until 50% exposed to *V. anguillarum*.	[54]
Bacillus subtilis	FOS	10 weeks	*Larimichthys crocea*	Did not show positive results with combination.	[53]
Bacillus megaterium	IMO	28 days	*Litopenaeus vannamei*	Better survival, however, did not exist a significant interaction between symbiotics compounds.	[31]
Bacillus spp	MOS	one month	*Homarus gammarus*	Weight gain and food conversion relation, while bacterial species richness decreases in digestive tract.	[70]
Lactobacillus sakei	Inulin	Two months	*Mycteroperca rosácea*	Humoral immune parameters increased.	[66]
Biomin IMBO®	FOS	Two months	*Cyprinus carpio*	Better growth, hematocrit levels, leucocytes, and hemoglobin increase, while serum cholesterol decrease	[67]

3. Conclusion

In this review, it was observed that the development and implementation of functional foods and specifically the use of probiotics in the rainbow trout culture has been widely documented and the 70% positive results have been observed. However, few studies have sought to elucidate the compounds that are determinants to considered a food as functional. Although its application is a reality, it is necessary to be sure about different cellular processes, genetic, among host, microorganism and substances used and thus improve the efficiency of nutraceuticals, prebiotics, probiotics and symbiotics in aquaculture.

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