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Phyto-additives in rainbow trout (*Oncorhynchus mykiss*) nutrition

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Abstract. Fodder additives have been used for a long time with notable effects. Most of them are chemically synthesized and are antibiotics derivatives. The biggest problem following their use is the development of drug-resistant bacteria. In order to overcome this downside and to abide to the new food safety regulations, a search for natural alternatives has begun and the attention has been focused on plants or plants extracts, some used for millennia as natural remedies. As they are natural substances the negative impact on fish health and environment is negligible. Phyto-additives are a relatively new class of additives and the knowledge regarding their use or mode of action is sometimes partially or totally lacking. They have multiple effects; most notable being the antimicrobial, antioxidant, immunostimulating and bioproductive effects. Phyto-additives are a heterogeneous group of additives being extracted from bulbs, roots, leaves or fruits of different plants species; they are available in many forms: dry and/or crushed or as liquids (essential oils). Research has been carried out world-wide, focusing on cultured species (rainbow trout, tilapia, and catfish) using a wide variety of plants (garlic, onion, ginger, Echinacea, nettle, sage, cinnamon) demonstrating the positive effects of the phyto-additives used. Even if they exhibit a positive influence on many parameters, their use is dose dependent, as higher or lower doses could have inhibitory effect or no effects at all.

Key words: phyto-additives, fish nutrition, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Fishing in world seas and oceans appears to reach a maximum productivity level, at least for the present stage, reason for the specialists to direct their attention to inland fish farming, which registers constant progress and increasing dynamics (Bud et al. 2009).

Salmon farming is the second branch as importance in fishfarming and it acquired amplitude without precedent in the past 30 years thanks to the scientific research in Salmonids breeding, in countries like: USA, Denmark, Norway, Japan, Holland, France and Italy, where the annual average production has increased to hundreds or even thousands of tons (Bud et al. 2009).

The rainbow trout, *Oncorhynchus mykiss,* is native to areas of the Pacific Ocean in Asian and North America, which in relatively short time has been introduced into at least 45 countries and territories. The Salmonids of the order Salmoniformes are found in fresh, brackish and marine water, widely distributed in Northern Hemisphere. There are some 11 genera with about 66 species. In addition to China, some salmonid species have been widely introduced and are cultivated on all the continents for sports and aquaculture. The *Oncorhynchus mykiss* is easy to propagate, grows rapidly, and therefore has gained popularity amongst fish farmers. However, like the other introduced species, this species may cause irreparable harm to native fish species through disease transmission, predation and competition (Zeng 2011).

One of the most important aspects in trout farming is the nutrition factor. It can influence the growth performances as well as the health status of the cultured fish. In order to optimize feed utilization and conversion and to improve growth performances a series of additives are used. The most notables are the antibiotics or antibiotics based additives. The main problem derived from their use is the occurrence of drug-resistant bacteria. In order to overcome this issue, attention has been focused on natural alternatives – phyto-additives. Among their numerous effects, the most notable are the antimicrobial, antioxidant, immunomodulator and bioproductive effects. Their main advantage is that they are of natural origin and do not pose any threat to human or fish health or to the environment.

Phyto-additives in fish nutrition

Fish are exposed to pathogenic microorganisms since they live in an unfavorable environment. The use of antibacterial drugs in aquaculture is hazardous due to cross resistance against pathogens and residues in tissues (Dorucu et al. 2009). Then again, of course, the growing tendency for food safety led to the ban of antibiotics. Vaccination may prevent fish disease outbreaks, but the development of vaccines against many intracellular pathogens has not yet been successful (Fazlolahzadeh et al. 2011). To replace their effects, the search for natural alternatives has begun. Thus, strong candidates to replace antibiotics are phyto-additives. Phyto-additives are fodder additives obtained from medicinal plants or plants extracts. Only recent, the study on the possibility of phyto-additives usage in aquaculture has begun and researches conducted in this field have shown the diverse effects of these phyto-additives used in fish as immunomodulators, immunostimulants, antioxidants, antimicrobials, stimulants of the enzymatic equipment, and stimulants of nitrogen absorption. A major advantage in the use of phyto-additives is the fact that they are natural substances and do not pose any threat to fish, man or environment (Gabor et al. 2010).

Garlic (*Allium sativum*)

A member of the *Liliaceae* family, garlic was used for centuries as a spice and also in popular medicine. It is a rich source of calcium and phosphorus; it has a high content of carbohydrates and as a consequence a high nutritive value. Garlic also contains iodine salts which have positive effects on the circulatory system and rheumatism, silicates which

have a positive effect on the skeletal and circulatory system and sulfur salts with positive effects on the skeletal system, cholesterolemia and liver diseases. Garlic also contains vitamin complex B, vitamins A, C and F. Another substance with a major role is aliicin, which has anthelmintic effects.

Studies conducted by several researchers, mainly on Nile Tilapia (*Oreochromis niloticus*), reported that after administering garlic, an increased final weight and an improved growth rate compared to the control group were noticed (Shalaby et al. 2006; Metwally 2009). Also, Ndong and Fall (2011) studied the effect of garlic on growth and immune response of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*). Their study documented that 0.5 g/kg supplementation of garlic had significantly improved leukocyte count, respiratory burst, phagocytic activity, phagocytic index and lysozyme activity, indicating the immunostimulant properties of garlic in juvenile hybrid tilapia (Ndong and Fall, cited by Gabor et al. 2011).

Nya and Austin (2009b) used garlic to control an *Aeromonas hydrophila* infection in rainbow trout (*Oncorhynchus mykiss*), recording at the same time the growth and consumption indices. They reported an increased weight gain and an improved feed conversion ratio in the group fed on garlic containing diets (Gabor et al. 2011).

Onion (*Allium cepa*)

Onion is also a member of the *Liliaceae* family and it is used as a medicinal plant, vegetable and spice. Onion has antibiotic, antiseptic and antiinfectious properties and antimicrobial effects showed against two bacteria (*Staphylococcus aureus, Salmomella enteritidis*) and three fungi (*Aspergillus niger, Penicillium cyclopium and Fusarium oxysporum*) by Benkeblia (2003). Onion contains small quantities of sugar, fats and vitamins A, C and B complex; it has a high content of magnesium, potassium and copper.

Al-Salahy (2002) administered onion and garlic juices on *Clarias lazera* once a day for 5 days. Contents of free amino-acid, total lipids, cholesterol, total protein and urea levels in blood serum, liver and muscles where determined. Both experimental groups presented a rise in liver free amino-acids. Moreover, the garlic fed group presented a rise in muscle free amino-acid levels; an enhanced muscle uptake of free amino-acids may enhance protein synthesis (Gabor et al. 2010).

Oregano (*Origanum vulgare L*)

A member of the *Lamiaceae* family, oregano is well known in the whole world for its special aroma and for its antioxidant and antimicrobial qualities. The main active substances in oregano are thymol and carvacrol with antioxidant properties.

Zheng et al. (2009) evaluated the effect of the oregano essential oil on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). He reported an increase in weight gain and an improvement of the feed conversion ratio in the oregano feed group, compared with the control group (Gabor et al. 2010).

Ginger (*Zingiber officinale*)

Ginger is a member of the *Zingiberaceae* family and it is being used mainly as a spice due to its sweet-spicy taste. It is being used successfully in eastern popular medicine as an antioxidant and anti-inflammatory agent.

Nya and Austin (2009a) studied the possibility of using ginger to control an *Aeromonas hydrophila* infection in rainbow trout, while recording the growth and consumption indices. The ginger fed group showed a greater body weight gain and a better feed conversion ratio compared to the control group. Also there was a proliferation in the numbers of neutrophils, macrophages and lymphocytes and enhanced phagocytic and lysozyme activity.

Echinacea (*Echinacea purpurea*)

The Echinacea plant is a part of the *Asteraceae* family, endemic to central end eastern North-America. The active components in the Echinacea plants are phenols, represented by cichoric and caftaric acid. Echinacea has anti-tumoral and immunostimulator effects, especially in the case of non-specific immune system.

Salah et al. (2008) studied the effect of Echinacea on growth rate and disease resistance in Nile tilapia. A controlled infection with *Pseudomonas fluorescens* was also carried out by intraperitoneal inoculation. The test group showed a significant increase in body weight gain, specific growth rate, hematocrit values, lysozime activity and total leukocyte count. The survival rate was significantly enhanced in the experimental group prior and post inoculation (Gabor et al. 2010).

Oniszczuk and Mościcki took a closer look on the physical properties and energy consumption of the manufacture of extrusion-cooked carp feed enriched with Echinacea and concluded in their study that the addition of 3% of Echinacea proved to be a critical value and, if exceeded, caused the process efficiency to diminish. Considering the healthful properties of Echinacea and its impact on the physical characteristics of extruded carp feed, the optimum content of this ingredient should be maintained at 2 to 3% (Oniszczuk & Mościcki 2009).

Kasiri studied the positive effects of supplemented diets by levamisole and Echinacea purpurea on growth and reproductive parameters in angelfish (*Pterophyllum scalare*). The fishes were fed by three diets which included commercial extruder diet (control), commercial extruder diet supplemented by 0.25 ppt levamisole (T1) and commercial extruder diet supplemented by 0.25 ppt *Echinacea purpurea* extract (T2). The weight gain in T2 was significantly higher than other groups (Kasiri et al. 2011). This study indicates that growth performance can be improved by dietary *Echinacea* extract supplementation.

Cinnamon (*Cinnamomum verum*)

Cinnamon is a member of the *Lauraceae* family and comes from tropical Asia and Africa. Cinnamon essential oil has antibacterial, antifungal, antiviral and antioxidant properties. Cinnamon is also used in food industry for its capacity to inhibit the development of bacteria, molds and yeasts.

Pongsak and Parichat (2010) have studied the cinnamon oil potential to control a *Streptococcus iniae* infection on Nile tilapia. They studied and compared inhibitory capacity of four essential oils: leech lime (*Citrus hystrix*), lemon grass stems (*Cymbopogon citratus*), tumeric (*Curcuma longa*) and

cinnamon (*Cinnamomum verum*). The highest inhibitory activity was observed in cinnamon oil. As for growth parameters, there was no significant difference observed between the control group and the experimental groups. The group that received cinnamon oil did not have any losses prior to *Streptococcus iniae* infection. After the infection, mortality rate at the same group was reduced significantly, no adverse effects being observed as a result of cinnamon administration (Gabor et al. 2010).

Nettle (*Urtica dioica*)

Nettle is a plant from *Urticaceae* family and spontaneously grows in Europe, Asia, Africa and North America. Nettle is rich in vitamins C, B2 and K, β-carotene, calcium, magnesium, iron, silicon and phosphorus.

Süheyla et al. (2003) studied the effects of dietary intake of nettle, mistletoe and ginger in rainbow trout and then determining various parameters of non-specific defense mechanisms. The addition of these plants led to an enhancement of cellular activities and rise in plasma protein levels (Gabor et al. 2010).

Phyto-additives in rainbow trout (*Oncorhynchus mykiss*) nutrition

Feed and feeding are among the most important factors influencing growth, feed utilization and tissue composition of the fish in intensive culture (Farahi et al. 2010). The primary objective of intensive aquaculture is to minimize costs and maximize growth, understanding that feed is the largest cost in salmonid culture. Overfeeding mostly leads to feed spillage, decreasing feed efficiency and polluting the environment. Likewise, underfeeding results in reduced growth as well as decreased feed efficiency. The amount of the daily feed ratio, frequency and timing of the feedings are the key factors of feed management strategies, influencing the growth and feed conversion. Optimal feeding frequency may vary depending on species, age, size, environmental factors, husbandry, and food quality (Dediu et al. 2011). Researches allow us to conclude that breeding and reproductive performances are influenced by food and all its variables (Bud et al. 2009), including phyto-additives.

The main advantage of using phyto-additives is that they are natural substances that don't pose any threat to fish health, human health or to the environment. Researches are still in progress to determine their way of action and the possible side effects that can appear as a result of their use, and to determine the possibility of using other plants as phyto-additives (Gabor et al. 2010).

For example, the aim of one of the researches was to determinate the influence of some phyto-additives (garlic, ginger, oregano and Echinacea) on growth and consumption performances in rainbow trout (*Oncorhynchus mykiss*). The additives where added into rainbow trout feed in proportions of 2% garlic, 1% ginger, 1% oregano and 0.5% Echinacea. The results obtained confirm the effects of these phyto-additives on the growth and consumption indices in rainbow trout: better food assimilation and improvement of the growth performance, better feed conversion ratio, increasing survival rate (effect of garlic and its compound allicine) (Gabor et al. 2011).

Feeding rainbow trout with 1% lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, for 14 days led to reductions in mortality after challenge with *Aeromonas hydrophila*, which is a significant problem in freshwater fish in many countries and is associated with a wide range of diseases from bacterial hemorrhagic septicemia to ulceration and fin/tail rot. In addition, there was significant enhancement in serum bactericidal activity, respiratory burst and lysozyme activity in the treatment groups compared to the controls (Awad & Austin 2010).

Plant materials, including the soapbark tree, *Quillaja saponaria*, European mistletoe, *Viscum album*, stinging nettle, *Urtica dioica*, ginger, *Zingiber officinale* and garlic, *Allium sativum*, have been increasingly reported to have health benefits, specifically enhancing disease resistance and immunity of fish. The results this studies reinforced the idea that plant materials have a role in fish disease control strategies. Indeed, the reduction in mortalities after challenge with A. hydrophila coincides with previous work, including the use of leaf extracts of false daisy, *Eclipta alba*, devil's horsewhip, *Achyranthes aspera*, and the Chinese herb, *Astragalus* (Awad & Austin 2010).

Garlic (*Allium sativum*)

Garlic is an important vegetable extensively cultivated in many countries. It is used as food for humans as well as some animals and as remedy for several diseases, as reported in folk medicine (Shalaby et al. 2006).

Garlic contains sulfur containing compounds. Alliin is converted to the antimicrobial active allicin when the bulb is cut or crushed. The fresh bulb contains alliin, allicin and volatile oils. When the garlic clove is crushed, the odorless compound alliin is converted to allicin, via the enzyme allinase. Also, it contains vitamins and minerals and trace elements (selenium & germanium). Garlic has proven to be hypolipidemic, antimicrobial, antihypertensive, hepatoprotective and insecticidal. Garlic extract has also been shown to reduce serum cholesterol levels and increase blood clotting time.

The use of garlic in fish farming has become popular for enhancing the activity of non-specific defense systems, conferring protection against diseases, increasing body gain, feed intake, feed efficiency ratio and it was used as a growth promoter in *O. niloticus* culture.

Farahi's work was carried out to study the effect of different values of garlic on growth factors, some hematological parameters and body composition in rainbow trout (*Oncorhynchus mykiss*). He studied the growth performance in fish after dietary application of garlic, in doses of 10, 20 and 30 g/kg garlic. Feed intake increased with increasing *Allium sativum* levels. Feed conversion ratio decreased with increasing *Allium sativum* levels.

In the present study, results of *Oncorhynchus mykiss* body compositions showed that crude protein and ash increased significantly with diets containing 30g *Allium sativum*, although total lipid content decreased significantly with the same levels of *Allium sativum*. The present study demonstrated that administration of garlic induced significant increases in all blood parameters (erythrocyte count, haemoglobin content and hematocrit value) in treated fish. Also

plasma glucose concentration reduced significantly in fish fed on diets containing the *Allium sativum*. Total protein of plasma increased in treatments. The results of the study showed that use of garlic can effectively improve growth performance and fish health (Farahi et al. 2010).

The effect of garlic on hematological parameters and plasma activities of ALT and AST of rainbow trout in temperature stress (Fazlolahzadeh et al. 2011) was evaluated in a short study carried out for 58 days. In this study, the effects of garlic, with three different doses 0.3, 0.45, and 0.6 g powder of garlic per kg dry diet consumption, were surveyed in two interrupted periods of time in heat stress condition on 120 Rainbow trout species and some hematological parameters related to nonspecific immunity in three treatment groups and one control group were studied. The erythrocytes count in all treatment groups in comparison with the control group showed no significant difference. There was a significant increase in the leukocyte count in fish fed a diet containing 0.6 g/kg garlic compared with the control group and the diets containing 0.3, 0.45g/kg. The mean leukocyte count in all treatment groups were more than the control group. The mean percent of neutrophils decreased significantly in fish fed on diets containing 0.45, and 0.6 g/kg doses compared with in fish fed on diets containing 0.3 g/kg doses. The results indicated that lymphocyte count increased significantly in fish fed on diets containing all garlic doses compared with the control group. In evaluation of the plasma parameters (ALT and AST) revealed a non-significant difference in all treatments compared to the control. In conclusion, use of garlic balanced and formulated in suitable doses can decrease mortality rate and increase immunity (Fazlolahzadeh et al. 2011).

Bacterial hemorrhagic septicemia, which is also referred to as motile Aeromonas septicemia, is caused by *Aeromonas hydrophila* and occurs in freshwater fish worldwide. To date, disease control strategies for this pathogen have centered on the use of antimicrobial compounds and to some extent vaccination. With the success of probiotics for the control of motile *Aeromonas* infections, attention has focused on immunostimulants and plant products which could have a beneficial effect in disease control, and in particular, garlic. Consequently, the aim of the study conducted by Nya and Austin (2009b) was to examine the effects of dietary garlic in disease control against *A. hydrophila* infections in rainbow trout, *Oncorhynchus mykiss*.

Feeding garlic to rainbow trout led to enhanced growth and reduced mortalities after challenge with *A. hydrophila*; 88% mortalities were recorded for the controls compared with 12% and 8% mortalities in the groups which were fed with 0.05 g and respectively 0.1 g garlic per 100 g feed. The number of erythrocytes and leucocytes was significantly higher than the controls in the groups that received garlic. Similarly, the number of leucocytes was enhanced following feeding with garlic and higher proportions of lymphocytes were recorded. Conversely, there was a significant reduction in the proportion of monocytes and neutrophils at the highest level of garlic. Eosinophils and basophils were not observed in the blood samples. The serum total protein content was different from the controls in all garlic fed groups and the phagocytic ratio was significantly higher in fish fed with garlic compared with the controls as the serum lysozyme activity and serum bactericidal activity also (Nya & Austin 2009b).

In another study Nya et al. (2010) proved that the garlic component, allicin, which has been credited with antimicrobial and immunostimulatory activities, prevents disease caused by Aeromonas hydrophila in rainbow trout. The mode of action of allicin may well include inhibition of cysteine protease, scavenging and trapping of free radicals (hydroxyl, superoxide anions and hydrogen peroxides) and initiation of the inhibition of thiol-containing protein in the cells of pathogens. However, it was previously demonstrated that dietary garlic enhances the non-specific immune mechanism of rainbow trout by stimulating the proliferation of immune cells, and enhancing phagocytosis, oxidative burst and lysozyme activities. The involvement of allicin in stimulating immune parameters would certainly suggest that this compound may be involved with the beneficial effect of garlic. These immune parameters are the proliferation of differential leucocytes, mostly monocytes and thrombocytes (Nya et al., 2010). Certainly, the results of this study partially explain the observed antibacterial activity of dietary garlic obtained in previous work (Nya & Austin 2009b) and suggest that allicin and its derivatives may be responsible for the beneficial effects of garlic.

Formation of reactive oxygen species (ROS) is an inevitable consequence in most organisms due to aerobic metabolism. Oxygen supports life for organisms; however, oxygen consumption generates cytotoxic byproducts. Conversely, there is a multilayered strategy of defence against oxidative damage including enzymatic and nonenzymatic antioxidants as well as adaptive responses. Oxidative stress refers to the imbalance between generation of ROS and antioxidative defence mechanisms and it is an important cause of cell damage associated with the initiation and progression of many diseases. The antioxidative effect of dietary garlic on rainbow trout was examined. Trout fingerlings were fed on diets containing 10, 20, 30, 40 and 50 g garlic powder per 1 kg fodder. Serum lipid peroxides and activities of antioxidant enzymes were measured. The results showed a significant increase in superoxide dismutase in all of the garlic-treated groups compared with the control. Ingestion of 10, 20 and 30 g/kg dietary garlic resulted in a significant reduction in the catalase activity compared with all but the 10 g/kg group. There was no significant difference in glutathione peroxidase activity among the different groups. Serum alanine aminotransferase and aspartate aminotransferase levels increased significantly in trout-fed diets containing 40 and 50 g/kg garlic powder. These results suggest that dietary garlic may improve the antioxidant status of rainbow trout (Mohebbi et al. 2012). However, undesirable effects of higher doses of garlic should be considered, such as negative physiological consequences.

Ginger (*Zingiber officinale*)

Continuing with the theme of beneficial plants for disease control, attention focused on ginger, the rhizome of Zingiber officinale, which has been recognized to have broad-spectrum prophylactic and therapeutic functions. Previously, studies have indicated that ginger is effective for the control of a range of bacterial, fungal and parasitic conditions. Also, ginger has been reported to have anti-

inflammatory and anti-oxidative activity and to be effective as an immunomodulatory agent in animals, including fish. Against this background, ginger has been examined for its potential to control Aeromonas hydrophila infection in rainbow trout.

A recent study, conducted also by Nya and Austin (2009a), revealed that feeding ginger significantly enhanced the growth rate of rainbow trout and conferred resistance to challenge with A. hydrophila. Ginger, Zingiber officinale, which was fed at 0, 0.05, 0.1, 0.5 and 1.0 g per 100 g of feed for 14 days to rainbow trout, led to control of experimental infection with *Aeromonas hydrophila*. At 0.5 g ginger per 100 g of feed, there was a reduction in mortalities to 0% compared with the controls (64%). Moreover, there was a significant increase in growth, feed conversion and protein efficiency. There was proliferation in the number of neutrophils, macrophages and lymphocytes, and enhanced phagocytic, respiratory burst, lysozyme, bactericidal and anti-protease activities compared with the controls (Nya & Austin 2009a). In common with other plant products, such as *Achyranthes aspera* and *Ocimum sanctum* leaf extracts, ginger conferred health benefits in terms of a reduction in mortalities after challenge and a heightened effect on non-specific immune mechanisms. In short, ginger is recognized to have broad-spectrum activity including activation of phagocytic cells, which is an important component of the non-specific immune system of fish. A possible mode of action of ginger is in immunostimulation as a result of its bioactive constituent, gingerol, which has been reported to induce the activity of interleukin-6. Also, ginger has been regarded to have potent antioxidant properties, being an effective scavenger of superoxide radicals, and so has been proposed as a possible protective mechanism against autotoxicity and lethality.

Black cumin seeds (*Nigella sativa Linn*.)

Nigella sativa Linn., commonly known as the black cumin seed, is an annual herb that belongs to the botanical family of *Ranuculaceae*. It has been employed for thousands of years as spice and food preservative, as well as a protective and curative remedy for numerous disorders.

The diuretic, antihypertensive, antibacterial and anthelmintic effects of black cumin seed in human and animals have been investigated. Additionally, antidiabetic, antioxidant and anti-inflammatory characteristics of *N. sativa* have been proved.

The effect of black cumin seeds on immune system of rainbow trout was very scarce. Therefore, the aim of another study was to examine if black cumin seeds, *Nigella sativa,* extract would influence some immunohematologic parameters and specific as well as non-specific immune response of rainbow trout. The results showed that feeding with 1%, 2.5% and 5% black cumin seeds supplemented diets for three weeks stimulated the specific defence mechanisms of rainbow trout, revealed by a significant increase in total immunoglobulin levels of experimental fish groups (Dorucu et al. 2009).

Although black cumin seed is known as an effective immunomodulator, it exhibits a moderate activity in this study. However, considering its low cost and immunostimulative effect, black cumin seed could be recommended to be used for farmed fish to decrease mortalities caused by pathogenic microorganisms.

Olive mill vegetation water

The antioxidative defence system capacity of cultured fish has been found insufficient. With regard to increasing water pollution and its stimulating effects on oxidative stress, improving antioxidative status of fish seems to be necessary. Therefore, any attempt to enhance this system may be associated with beneficial effects on health status of fish. In modern aquaculture, the principal food additives used to scavenge lipid peroxide in fish feeds and feed ingredients are synthetic antioxidants (Mohebbi et al. 2012).

On the other hand, these molecules have been investigated for carcinogenicity due to their potential for toxicological and adverse health effects in both fish and fish consumers through ''carry-over'' processes. In order to avoid these toxic effects, several natural compounds have been investigated to find valid alternatives as partial substitute of synthetic antioxidant molecules. Recent trends in prevention and treating diseases tend to prefer to use natural-based antioxidants rather than synthetic ones in degradation and scavenging of ROS. Natural antioxidants represent a wide class of compounds and among these, polyphenols contained in olive mill waste water (VW) have been studied for their antioxidant properties. VW is an olive oil by-product rich in polyphenols obtained by mechanical compression of olives during oil extraction. Recent studies on human diet demonstrated that virgin olive oil with a higher content of polyphenolic compounds shows protective effects in inflammation models in rats, and these effects have been largely attributed to the antioxidant effects of olive oil biophenols such as hydroxitirosol. At the same time, it is well known that some vegetal polyphenols have hormonal activity, which sometimes gives rise to significant endocrine effects in experimental animals. These effects could be linked to the presence of non-steroid plant constituents, phytoestrogens, which elicit estrogen-like effects in one or more target tissues in animals. Phytoestrogens presents similarities with estradiol, which has a critical impact on reproductive and sexual functions, but also affects other organs. Phytoestrogens have been found to affect the endocrine system of farmed fish and they are largely present in fish feed ingredients.

Considering both the interest in the investigation of novel natural antioxidants to be used in fish feed as well as the possible negative effects of some of these molecules on the fish physiology, there are two main aims for this research. Diets included of VW tested against a control diet. At the end of the trial, the growth performance traits were determined and sampling of blood and different tissues (brain, ovary, stomach, liver, and intestine) were carried out for hematology, endocrinology, histology, and digestive enzyme analysis. The main results of the experiment are that VW inclusion in rainbow trout feed slightly affects the productive traits and blood chemistry, while the histological structure of digestive organs and digestive enzyme physiology were not affected (Sicuro et al. 2010). Moreover, other results on similar experimentation on rainbow trout show that VW inclusion in feed partially improves the antioxidant parameters of fish fillets. These results confirm the possible agriculture by-products utilization as a source of natural antioxidants in

fish nutrition. This fact could contribute to the valorization of olive oil VW, which is the main olive oil by-product in all Mediterranean countries (Sicuro et al. 2010).

Conclusions

Phyto-additives represent alternative solutions to substitute antibiotics used in aquaculture. They can be used as growth promoters; recent studies showing their effects as immunostimulants, immunomodulators, antimicrobials and antioxidants.

Not only application of phyto-additives have more effective approach to controlling disease in aquaculture through the enhancement of natural disease resistance, but also contribute to health management in aquaculture through the enhancement of the activity of the non-specific defense mechanisms and increasing disease resistance.

There are large numbers of plants which have been used in traditional medicine for the control and treatment of diseases and it is speculative how many other useful plants remain to be evaluated, but clearly a search is justified. However, further researches are necessary, including total commercial cost and benefit analysis, before a large scale use in aquaculture.

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