Use of Apiculture Products in Aquaculture - Review

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Abstract
Apiculture products are natural substances secreted or produced by bees, which can be used in aquaculture through food, intramuscular administration or direct in water, in order to improve health due to the antioxidant, antifungal and antimicrobial effect that these products have and also improve growth and reproductive performance of the fish species of economic interest. In this review, we aim to highlight the main studies conducted so far on the use of bee products in aquaculture in order to be able to present an overview of this topic of interest.

Keywords: apiculture products, aquaculture, productive performance

1. Introduction

Apiculture products, especially honey and pollen, have been used by humans for thousands of years due to their many healing benefits as well as their valuable chemical properties [1].
They are mainly used in the human pharmaceutical industry for the prevention and treatment of various types of diseases but can also be used successfully in animal therapy and aquaculture [1, 2].
Apitherapy is part of alternative medicine and consists of the use of bee products in the treatment of various diseases [2, 3].
The bee products used must be carefully chosen according to the clinical symptoms of each organism in order to be as optimal as possible for individual needs, which is why it requires the mastery of knowledge about each individual [2].
Their composition can stop the progression of the disease, can improve it or even treat it completely, but also requires increased attention, as it can cause various side effects, especially various allergies manifested by inflammations at local level [2].
On the whole, products obtained from bees, such as pollen, propolis, honey, royal jelly, drone larvae extract, bee venom and wax, are characterized by a strong antiviral and antibacterial activity against pathogens that cause various diseases and not only, but can also present other essential characteristics beneficial to the human and animal immune system by developing immune cells and stimulating their innate and adaptive responses, but also by stimulating the production of antibodies [3].
Bee products such as honey and bee pollen can prevent or even completely eliminate the oxidative damage caused to various species of fish in aquaculture, thus being a good natural treatment option, as they are sources with a high content of polyphenols, which is known to have antioxidant properties in the literature [4].
Bee products such as pollen and larvae as a residual product in the process of obtaining royal jelly can be used successfully as a food supplement in aquaculture due to their many chemical characteristics such as high content of amino acids, fatty acids and minerals, for example phosphorus and magnesium.

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Bee larvae have these characteristics due to the fact that they have a standard nutrition based mainly on royal jelly, and this is a great advantage to be used in aquaculture as feed supplements alternative to the classic supplements already used [5].
Apiculture products such as drone larvae extract and royal jelly have a stimulating effect on the reproductive function of various species of fish. Royal jelly is used to stimulate the production of eggs, but also to achieve sexual inversion, and drone larvae extract is used to stimulate the production of semen in males.

2. Discussions

There are currently not many studies and research on the influence of bee products in aquaculture. However, we will further highlight some research done so far.

Royal jelly is a product with antimicrobial action, resulting from the secretion of the hypopharyngeal and mandibular glands, has a white-yellow color, the taste is sour, the viscous-gelatinous consistency, and the smell is slightly similar to phenol. It is produced by young bees between the ages of 3 and 12 days and is used to feed adult larvae and queens. Its secretion is closely related to the existence of pollen and nectar collection. Royal jelly has an acidic pH with values between 3.6-4.2, being composed of water (60%-70%), proteins (12%), lipids (5%-6%), sugar (12%-15%), and relatively small amounts of vitamins and minerals, but also a significant number of amino acids [3].
By administering freeze-dried royal jelly in the diet of zebrafish (after hatching) (0.02 and 0.04 g in the structure of the feed) it was shown that sexual inversion can be made, obtaining a percentage of 70.76% females and 29.24% males at 0.02 g dose and 81.19% females and 18.19% males at double dose, compared with control group (50.7% females and 49.3% males) [6].
Royal jelly administered in different concentrations 0.0; 0.1; 0.4; 1.6 and 6.4%, has direct effects on the growth performance and immunity of the zebrafish, *Danio rerio*, finding that the royal jelly included in the feed positively influences the growth hormones, muscle and liver RNA levels and were positively adjusted these components in fish fed with 1.6% royal jelly [7].
Studies by Ferreira et al., (2018) [8] have shown that royal jelly counteracts the oxidative damage caused by exposure in water containing TEB (tebuconazole) to *Rhamdia quelen* (silver catfish). The high levels of protein carbonyl observed in fish exposed to TEB were completely counteracted when water inoculated with tebuconazole was supplemented with royal jelly.
Researches conducted by Aksakal et al., (2021) [9] have focused on the effects of different doses of royal jelly administered over a period of 8 weeks in rations of zebrafish (*Danio rerio*), and the results of these researches have shown a relationship between the administration of royal jelly and enzymes that cause oxidative stress in this species. The inclusion of royal jelly in rations positively modulated both mRNA expression levels and dose-dependent enzyme activity and the tissue at which it acts.

Drone larvae extract is a natural bee product, obtained from drone larvae harvested at the age of 7 days together with the contents of the cell.
Of all the ontogenetic process of development of drones, only the embryonic and larval stage is necessary to obtain drone larvae extract. Environmental conditions must be conducive to efficient production, but bee families must also be strong and well-developed. The harvested drone larvae extract is stored in low capacity, tight containers at a storage temperature between -5 and -20°C.
It has many energizing, toning, vitalizing and psychotonic properties, thus helping to stimulate the immune system.
Studies conducted by Farag et al., (2021) [10] have shown that feeding the brood of *Labidochromis caeruleus* with drone larvae extract, as a third meal, did not produce changes in the weight of the studied specimens, but influenced the reproductive activity.

Propolis is a bee product produced by honey bees from resinous substances collected from different parts of plants, such as flowers, leaves and buds to which are added glandular secretions, residues from pollen digestion or wax [3]. Bees harvest the raw material for the production of propolis in general from the following plants: conifers, willow, beech, poplar, oak, ash, chestnut, alder, but also others. Annually, about 100-300 g of propolis can be obtained from a bee family by cleaning the frames and the interior of the hives. Propolis is composed of about 50% resins, 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds, including polyphenols, ethanol, amino acids, minerals, vitamin A, vitamin E complex and vitamin B [3]. Because propolis has over 70 pharmaceutical properties, it is used as an antimicrobial agent against bacteria, viruses and other pathogenic microorganisms.

Research has been conducted on the functional actions of propolis and the prospects for its use as an antimicrobial, antioxidant, immuno-modulatory, antiseptic, antiparasitic, anti-inflammatory and food additive in aquaculture, demonstrating that propolis could be a natural supplement that has the potential to improve the health and immunity of fish, increasing the productivity and economic efficiency of a fish farm. Propolis can be used to improve feed efficiency, growth performance and immune responses of different fish species, by decreasing microbial activity and improving disease resistance and can be administered in both raw and liquid extract form [11].

Research by Ariyanto et al., (2012) [12] has shown that propolis is an alternative product in sexual reversal. Oral administration of 5 ml of propolis in 1 kg of food resulted in the highest percentage of *Tilapia* males -76.67%. At the same time the study showed that there were no negative influences on the survival rate of the brood and by the administration of propolis not fish development abnormalities have occurred. In this study, experimental batches with quantities of 3, 4, 5 and 6 ml of propolis per kg of feed for a period of 35 days in the aquarium were tested, and the growth of the brood will be carried out for another 2 months in specially arranged ponds.

Studies by Gulhan et al., (2012) [13] have shown the effects of propolis on the microbiological and biochemical parameters of rainbow trout after exposure to a toxic pollutant (cypermethrin). Lactic acid, base nitrogen, and total mesophilic bacteria were shown to be increased in the cypermethrin batches compared to the control batch, and in those treated with cypermethrin + propolis all of the above were significantly reduced compared to batches exposed only with cypermethrin. The results also showed that the fillet has some modified biochemical and microbiological functions in groups that are exposed to cypermethrin.

Research has shown [14] that cypermethrin has increased oxidative stress parameters in the brain tissue of rainbow trout, and propolis is beneficial in reversing the negative effects in the brain of rainbow trout exposed to cypermethrin, as it has antioxidant and neuroprotective effect and can prevent cerebral dysfunction in fish.

Studies by Velotto et al., (2010) [15] have shown that propolis has a direct effect on the muscular development of fish and their histomorphometric characteristics, on the mortality of fish eggs due to mycoses, but also on its effectiveness on the growth rate of fish. The studies were performed with two different experimental groups: traditional feeding and feeding with the addition of propolis. The study was conducted on two species of fish, namely rainbow trout and brown trout. The utilization of propolis reduced fish spawn mortality, and propolis-fed fish showed faster muscle growth compared to the control group with a standard feed ration.

Studies by Ferreira et al., (2013) [8] have shown that propolis counteracted the oxidative damage caused by exposure to TEB (tebuconazole) on *Rhamdia quelen* (silver catfish). High levels of protein carbonyl observed in fish exposed to TEB were completely counteracted when water with tebuconazole was supplemented with propolis.

Biochemical and histopathological studies performed by Talas et al., (2014) [16] on the antioxidant effects of propolis on *Cyprinus carpio* carp exposed to arsenic showed that MDA levels in fish exposed to 0.01 mg l-arsenic increased significantly compared to the control group without exposure, and MDA levels in the arsenic + propolis group were significantly lower compared to the arsenic group. Histopathological changes in the liver, gills and muscle tissues of carp were examined by light microscopy and various changes were observed in all fish tissues in the group submitted to arsenic, and propolis showed significant antioxidant effects against arsenic toxicity in all fish tissues.

The study by de la Cruz Cervantes et al., (2018) [17] highlighted the functional properties attributed to propolis in various scientific publications, as well as advances in understanding how it works and its use as an immunostimulant, food additive in feed rations, but also antimicrobial in aquaculture, concluding that propolis is a 100% natural functional additive, which used in appropriate doses results in better health of the fish, thus improving productivity.

Research by Yonar et al., (2015) [18] has shown the beneficial effect of propolis on the biochemical and haematological changes induced by Trichlorfon (one of the most common products used to treat fish parasites in aquaculture) on *Cyprinus carpio*. Fish specimens were subjected to sublethal concentrations (11 and 22 mg l-1) of trichlorfon and propolis (10 mg kg-1 of fish weight). Blood and tissue samples (liver, kidneys, gills) were collected at the end of their administration. Hematological changes (red and white blood cell count, hemoglobin concentration, hematocrit level and erythrocyte levels) were determined in the blood samples, while antioxidant parameters were assessed in the liver, kidney and gill samples. Trichlorfon has led to negative changes in the haematological parameters and antioxidants investigated, but the administration of propolis has attenuated this effect and suggests that fish treated with trichlorfon improve their physiological state when fed a diet supplemented with propolis.

Research by Yonar et al., (2012) [19] on the protective role of propolis in chlorpyrifos-induced changes in the haematological parameters and oxidative/antioxidant status of *Cyprinus carpio* has been evaluated in the blood and various tissues of carp exposed to chlorpyrifos (CPF). Fish samples were exposed to sublethal CPF concentrations (0.040 and 0.080 mg/l) for 10 days, and propolis (10 mg per kg body weight of fish) was administered concomitantly. Blood and tissue samples (liver, kidneys and gills) were collected at the end of the experiment and analyzed for their oxidative-antioxidant status, but also for changes in hematological parameters, such as the number of red blood cells and white blood cells, hemoglobin concentration, hematocrit level and erythrocyte indices. The results of this study showed that CPF had a negative effect on the hematological parameters and antioxidant enzymatic activities of fish, but the toxic effect was neutralized by the administration of propolis, thus demonstrating that propolis can be effective in protecting CPF-induced toxicity in fish.

Another study by Orun et al., (2014) [20] again refers to the role of propolis on the hematological and biochemical parameters of *Oncorhynchus mykiss* exposed to cypermethrin (CYP), demonstrating the protective effects of propolis. Specimens were subjected to three sublethal concentrations of CYP (0.0041, 0.0082 and 0.0123 ppm) and propolis (10 ppm) for 96 hours. Samples were analyzed for changes in biochemical parameters, such as metabolites: glucose, total protein, creatinine, urea, triglycerides, total cholesterol and enzymes, while testing for fish blood hematological parameters, including total leukocytes, granulocytes, agranulocytes and erythrocyte counts, and the results show that propolis may be effective in preventing CYP-induced toxicity.

A study on the antioxidant effect of propolis against exposure to the toxic effects of chromium in *Cyprinus carpio* was performed [21] by examining markers of oxidative damage, such as lipid peroxidation and antioxidant defense components in carp. The fish were exposed to sublethal chromium concentrations, and propolis was administered simultaneously for 28 days, and at the end of this period blood and tissue samples (liver, kidney, spleen and gills) were collected. Malondialdehyde levels, as an index of lipid peroxidation, increased in the blood and tissues. The activities of the antioxidant enzymes in the blood and tissues were altered in the chromium groups compared to the control group, but the simultaneous administration of propolis improved these parameters, the results suggesting that the administration of propolis could alleviate the oxidative stress induced by chromium.

The venom is a bee product secreted by the venom glands of the worker bees and stored in their venom bags, being eliminated on the outside at the time of the sting, and once used it will not be able to recover. The maximum amount of venom accumulates at the age of 15-20 days and is influenced by several factors including the age of the bees and the abundance of food. The venom collector is used to collect bee venom, consisting of an electromagnetic pulse generator but also one or more collecting membranes. Bee venom is composed of peptides (mast cell degranulant peptide and adolapine), enzymes (such as phospholipase A2 and hyaluronidase), amino acids, sugars, phospholipids, biogenic amines, volatile compounds, pheromones and water (>80%). At high concentrations administered in various procedures, bee venom can induce inflammation, pain and allergic reactions, including death. The low concentrations of this natural bee product have caused several pharmacological effects on various animals, such as anti-inflammatory, nociceptive, antimicrobial and antitumor effects, but also effects in increasing immunity [3].

Research on bee venom harvested from *Apis mellifera* bees has shown antimicrobial effect against pathogenic bacteria in fish. Three bacteria were used for the study: *Edwardsiella tarda*, *Vibrio ichthyoenteri* and *Streptococcus iniae*, which were originally obtained from the gastrointestinal tract of Olive Flounder Paralichthys olivaceus, and bee venom showed antibacterial activity against all three pathogens. Its antimicrobial activity was not dependent on the pH value, as the inhibition zones were identical at pH levels between 2 and 11, practically the results obtained inhibit the growth and survival of bacterial strains and demonstrate that bee venom can be a complementary antimicrobial agent useful against pathogenic bacteria in fish [22].

Pollen is the most important source of protein and vitamins for the bee family, being a natural bee product collected by shaking the anthers or crushing them with the jaws at the level of flowering plants, to be transported to the hive with corbicules and stored on the sides of honeycomb frames with brood. After depositing the pollen grains in the honeycomb cells, the bees press them with their heads, removing the air inside the cell, thus compacting the pollen grains and turning them into beebread. Pollen has a complex composition with a total of about 185 nutrients, of which 22 amino acids, proteins (5%-60%), reducing sugars (13%-55%), lipids (4%-7%), nucleic acids (especially RNA), crude fiber (0.3%-20%), minerals (Ca, Mg, Fe, Zn, Cu, K and Na), vitamins (vitamin E), niacin, thiamine, biotin and folic acid, all vitamins, carbohydrates, lipids, enzymes, coenzymes and hormones [3]. Pollen has multiple therapeutic properties, such as: antiallergic, anabolic, antiarteroslerotic, antibacterial, anti-inflammatory, antioxidant and antiparasitic, being used in the prophylaxis of several infectious diseases. Studies by Ferreira et al., (2013) [8] have shown that bee pollen counteracts the oxidative damage caused by exposure in water containing TEB (tebuconazole) to *Rhamdia quelen* (silver catfish). High levels of protein carbonyl observed in fish exposed to TEB were completely counteracted when water inoculated with tebuconazole was supplemented with bee pollen.

The effect of pollen supplementation on the diet of zebrafish did not have a significant effect on reproductive rates, but it did positively influence the vertical immunity of zebrafish, due to the fact that in the early stages the larval immune system is not fully developed and may be influenced [23].

Another study by Sánchez et al., (2020) [24] demonstrated the effect of bee pollen as a source of natural carotenoids on rainbow trout growth and pigmentation - *Oncorhynchus mykiss*. A total of 144 rainbow trouts were fed over eight weeks using granular feed with the addition of pollen extract on a control batch and several experimental batches. At the end of the eight weeks of monitoring, there were no significant differences between the batches in terms of total growth parameters, but the diet of a batch in which pollen was administered had a positive effect on the growth parameters. Trout fillets fed with pollen-added rations had a higher carotenoid concentration ($p<0.5$) and a much lighter pink color compared to those fed a single carotenoid-free diet.

The study conducted by Asgari et al., (2020) [25] investigated the effects of dietary hydroalcoholic extract from bee pollen on growth yield and performance, as well as meat quality, immune stimulation and individuals' response to stress. Rainbow trouts weighing about 11.14 g were fed five rations of feed with increasing content of pollen extract, thus in the control group 0.25 g/kg, group I 0.5 g/kg, group II 1 g/kg and lot III 1.5 g/kg. The results were evaluated at 56 days, and the rainbow trouts fed with pollen rations showed a significantly higher weight gain compared to the control group. Significant results were also highlighted in the quality of the meat that had a higher protein content.

Research by Ren et al., (2021) [26] has shown that by supplementing the diets of common carp with peony pollen, some positive results can be obtained on growth, intestinal function, meat quality and fatty acids. The study was conducted over a period of 6 weeks, and the results showed that pollen supplementation improved growth performance and meat quality. At the same time, the administration of pollen in food rations significantly improved the height of enterocytes, microvillus, but also the thickness of the intestinal wall on common carp specimens tested, compared to the control group with normal ration. Supplementing the diet with peony pollen can regulate lipid metabolism and the composition of common carp fatty acids, but also improve the health of fish by increasing immunity and antioxidant capacity of the intestine. An optimum recommended concentration should be 30 g of peony pollen per kilogram of food.

Another study, by Ren et al., (2022) [27], was based on the rationing of peony pollen to observe its effects on the growth performance and immunity of the common carp (*Cyprinus carpio*) in five different diets with 0% (control group), 1 %, 2%, 3% and 4% pollen, by feeding the fish for a period of 6 weeks, and the results of the study showed that pollen has beneficial effects on the growth characteristics of common carp. At the same time, the evaluation of the activity of hepatic antioxidant enzymes in the experimental group with a ration of peony pollen 3% showed a significant increase. The gene expression levels of tumor necrosis factor in groups of carp fed on peony pollen decreased significantly compared to the control group. Another important aspect is that the levels of heat shock protein have been significantly adjusted in batches of carp fed on peony pollen. Thus, it was found in this study that the supplementation of peony pollen in feed rations has the potential to improve the immunity of specimens by activating cellular antioxidant activity and regulating the expression of immunity-related genes, which may increase tolerance of carp to various pathological conditions.

Another study analyzed the effects of bee pollen, as well as its extract on the immunity of some specimens of the fish species *Sparus aurata*. The results showed that the pollen diet improved both the parameters related to the health of the fish, but at the same time did not affect the quality of the fillets [28].

Honey is a natural sweet substance produced by *Apis melifera* bees and contains different types of sugars, such as fructose and glucose, but also other substances such as organic acids, enzymes and solid particles that vary depending on the type of harvest and the environmental conditions. Honey has been used to treat microbial infections by ancient civilizations, such as the Egyptians, Greeks, Mayans, Romans, and Babylonians, dating back more than 5,500 years [3].

The chemical composition of honey is very complex and is influenced by the source of harvest, weather conditions, but also by the way it is stored. Honey has a content of 18% water and 82% dry matter (95% sugars, minerals, vitamins, enzymes, organic acids, protein substances, bacteriostatic substances but also bactericidal substances and pigments). Of the total carbohydrates, the simple ones (fructose and glucose) are represented by a percentage of 70-75%, and the disaccharides (sucrose, maltose) of only 5%. The ratio of fructose to glucose depends on the variety of honey, but in most cases the amount of fructose is clearly higher than glucose, and the pH of honey from nectar is variable between 3.3 and 4.6 [29]. Studies by Ferreira et al., (2013) [8] have shown that bee honey counteracts the oxidative damage caused by exposure in water containing TEB (tebuconazole) to *Rhamdia quelen* (silver catfish). The high levels of protein carbonyl observed in fish exposed to TEB were completely counteracted when the water inoculated with tebuconazole was supplemented with honey.

A study by Salosso, (2019) [30] analyzed the antibacterial activity and chemical composition of East Nusa Tenggara honey on fish pathogenic bacteria, the honey used in this research being collected specifically from the Semau and Timor Islands, and *Aeromonas hydrophila* and *Vibrio alginoliticus* were used as bacteria for testing. Antibacterial tests have shown that honey from Semau and Timor has shown antibacterial activity against *V. alginoliticus* and *A. hydrophila.*

Research has been conducted to evaluate the potential of Timor's wild honey as an antibacterial agent on the pathogenic bacteria *Aeromonas hydrophila* and *Vibrio alginolyticus*. The test of antibacterial activity of honey was done using the method of the disk without dilution, and the centralized results showed that all forest honey in the island of Timor had antibacterial activity against the two pathogenic bacteria *A. hydrophila* and *V. alginoliticus* with different zones of inhibition [31].

3. Conclusion

Research has shown that the effect of bee products used in aquaculture, such as royal jelly, drone larvae extract, propolis, bee venom, pollen and honey on various species of fish such as *Danio rerio*, *Labidochromis caeruleus*, *Rhamdia quelen* and other species, have had significant results in terms of increased reproductive capacity, weight gain, immunity and resistance to oxidative damage.

Although there are studies on the use of bee products in aquaculture, this topic still allows a lot of research, because the complexity of the properties of these natural products gives us an extensive framework of applicability, and fish species in aquaculture are also a great advantage in terms of their variability for the administration of bee products.

In the future of aquaculture, bee products can be used successfully to increase the reproduction, growth and development of different species of fish.

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