



The Fishery Value of Licorice (*Glycyrrhiza uralensis* Fiseh): Current Situation, Problems and Suggestions

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Abstract: More and more herbal medicines have been applied to aquaculture. It is reported that herbal medicines can effectively improve immune functions of fish and shrimp, increase their non-specific killing activities to kinds of pathogens and enhance their resistance to diseases, which is very beneficial to prevention and cure of fish diseases and development of natural fish medicines. Licorice (*Glycyrrhiza uralensis* Fiseh), a perennial herb of the genus *Glycyrrhiza* in *Leguminosae*, is mainly distributed in Northeast China, North China and Northwest China. In aquaculture practice, if licorice can be used reasonably, not only can the resistance of pathogens caused by chemical drugs and antibiotics be avoided, but also because of its pure natural characteristics, the pollution of water and fish is relatively less than usual, thus it will avoid the problem of drug residues exceeding the standard. This opens up a new way for the prevention of fish diseases and the development of green fish medicine. However, there are still some problems to be solved in the use of licorice, such as the quality, dosage, specialized prescriptions and professional terms. In this paper, the fishery value of licorice was summarized from its active ingredients, application situation, problems and suggestions.

Keywords: Application, Aquaculture, Disease, Immune, Licorice

1. Introduction

With the increasing emphasis on food safety and ecological protection, the aquaculture industry has gradually turned to the development of new environmentally friendly and safely used fishery drugs. Compared with chemical drugs, plant-based fishery drugs are safer for aquaculture animals and the environment, and they are rich in resources, easy to obtain, and low in cost, so they are used more and more in aquaculture [1-3]. Many studies have shown that Chinese

herbal medicine has many beneficial effects on aquatic animals, including promoting growth, enhancing disease resistance, improving meat quality, promoting feed intake, increasing digestive enzyme activity, and regulating animal specific and non-specific immunity [1-3]. Besides China and other Asian countries, the United States and Europe have also reported the use of medicinal plants to successfully control aquatic diseases [4-6], many of which use licorice (*G. Fiseh*) [5, 6]. The reason why licorice can be widely used together with other herbs, and its own "harmony", "suppression" and other efficacy can not be neglected [1]. In the "*Shen Nong's*

Herbal Classic", licorice is listed as the top grade. *"Compendium of Materia Medica"* also lists licorice as the first of 100 medicines. It is described in the *Pharmacopoeia of the People's Republic of China* as "sweet, reconciling various medicines." Therefore, this article focuses on the review of licorice out of many Chinese herbal medicines. Licorice, one of the traditional Chinese herbal medicines, is a perennial herbaceous plant of the genus *Glycyrrhiza*. It is also known as sweet grass root, red licorice, pink licorice and powder grass. *Glycyrrhiza* is a generic term for licorice species. Worldwide, there are about 30 species of licorice, which are mainly distributed in Northeast China, North China and Northwest China [7]. In the European and Chinese Pharmacopoeia, many different licorice species names can be used interchangeably, which has caused the ambiguity of licorice species ambiguity to some extent.

Studies have shown that licorice has a variety of effects, for example, licorice root preparation has significant anti-inflammatory, anti-viral, anti-cancer, anti-allergic, liver protection and estrogen, and can regulate the immune function of fish [8]. Licorice can also increase the appetite of animals and reduce their energy consumption [9], promote the conversion rate of T lymphocytes and enhance their cellular immune function [10]. We have also carried out a special study on licorice, and evaluated its immunomodulatory effects on *Carassius auratus*. Through the evaluation of various parameters of humoral immunity and cellular immunity, and the analysis of the immune protection rate after artificial bacteria attack, licorice has a good regulatory effect on the immune function of *C. auratus*, which can significantly enhance the resistance of fish to *Aeromonas hydrophila* [11, 12]. Therefore, in the practice of aquaculture, if licorice can be used reasonably, not only the resistance of pathogenic bacteria caused by chemical drugs and antibiotics can be avoided, but also because of its pure natural characteristics, it has less pollution to water and fish. Thereby, the problem of excessive drug residues can be avoided. These characteristics of licorice are in line with the current trend of developing pollution-free aquaculture and producing green aquatic products.

2. Active Ingredients of Licorice

There are more than 200 kinds of known Chinese herbal medicines with immune activity, more than 400 kinds of Chinese herbal medicines with bactericidal and bacteriostatic effects, and more than 50 kinds of Chinese herbal medicines which can inactivate or inhibit the virus [7-10]. In the practice of traditional Chinese medicine, licorice is usually added to the compound medicine because it has the effect of "harmony" and "suppression", which is equivalent to a "buffering agent". The active ingredients in licorice are mainly polysaccharides, terpenoids, alkaloids, organic acids and volatile oils. In addition, there are many nutrients, including oils, resins, sugars, proteins, amino acids, vitamins, macro elements, trace elements and some unknown growth-promoting active substances [7]. Among all the

components, immunologically active ingredients are mainly polysaccharides, terpenoids and alkaloids [7]. Licorice polysaccharide is a high molecular carbohydrate composed of several monosaccharides in the form of glycosidic bonds. It is the most important substance with immunological activity [7]; Terpenoids are also called "glycosides" and "glycosides", and they are a cyclic acetal derivative obtained by dehydration of a hemiacetal hydroxyl group of a sugar or a sugar derivative with a hydroxyl group of another non-saccharide substance by an acetal bond (or "oxime bond"), which is widely present in licorice. It can effectively enhance the phagocytic activity of reticular cells of the reticuloendothelial system, promote antibody production, accelerate antigen-antibody reaction, and transform lymphocytes [8]. The licorice saponin extracted from licorice can improve the function of macrophages in the body. The activity of deoxyribonucleic acid, acid phosphoketol and amber dehydrogenase in cells is enhanced, which can better regulate humoral immunity and cellular immunity [9, 13]; Alkaloids in licorice are a variety of basic organic compounds containing nitrogen, synthesized from different amino acids or direct derivatives, and they are one of the secondary metabolites, having a strong regulating effect on the body. In a certain range, they can enhance the humoral and cellular immune responses [14].

3. Application of Licorice

3.1. Administration Method

In aquaculture, licorice usually has several common methods of administration, including feeding, filling, injecting, soaking and splashing. The first method is feeding. Wash the fresh licorice and chop it, then mix it with a certain percentage of the mass and mix the binder to make a palatable drug. If dry licorice is used, it is necessary to chop the boiled juice and mix the medicinal juice together with the dregs and the feed. The commonly used medicine baits are mainly three kinds: floating medicine baits, sinking medicine baits and saccharification medicine baits. This method can be administered on a large area without touching the fish body, and it is feasible and generally applicable to the prevention and treatment of fish diseases. The second method is filling. The licorice is chopped and boiled, then irrigated to fish mouth according to a certain dilution. This method is mainly used for acute diseases of precious aquatic products. It absorbs quickly and has good curative effect, but it is easy to cause fish body's stress reaction, which may create an invasive condition for the pathogenic bacteria, resulting in the body's susceptibility to rise high. In addition, the liquid medicine of this method is easy to overflow and cause loss, and the operation is also time consuming and laborious. The third method is injection. The licorice is chopped, and boiled, then introduced into the fish body by intraperitoneal injection or intramuscular injection according to a certain dilution. It is also possible to extract the active ingredients of licorice and then intra-abdominal or intramuscular injection into the body. During the abdominal

injection, the needle is inserted obliquely toward the pectoral fin along the base of the ventral fin of the fish body. When the intramuscular injection is made, the needle is inserted into the needle from the base of the dorsal fin to the head in a certain angle. Common methods and separation techniques for licorice extraction include traditional reflux method, microwave extraction method, ultrasonic extraction method, chromatographic technique and liquid chromatography technology [14]. The dosage of the injection method is relatively accurate, and there is no pollution to the aquaculture water, but the workload is large, and the needle is prone to infection with pathogenic bacteria. The fourth method is soaking. The licorice is bundled into bundles, placed in the water inlet of the fish pond or near the food field for soaking, and the immersed medicinal juice is spread to the whole pool to achieve the purpose of controlling diseases. This method is simple and easy to operate, but it is easy to leave a large area of the drug in water. In addition, there is a method of small area soaking, or medicated bath method, which concentrates the fish in a small container for a short-term high-concentration medicated bath to kill the *in vitro* pathogens. This method uses less medicine, does not affect the growth of other bait organisms, but does not kill the bacteria in the water. The last method is splashing. After mashing the licorice, soak the juice with water, and slag with juice to splash the whole pool. If it is a dried herb, it must be chopped and boiled for a while before use. The medicine should be taken after feeding. Splash the licorice juice from upwind to downwind and observe the behavior of the fish all the time. When the fish is difficult to bear and begin to float, immediately add new water to suspend the medicine. This method can avoid fish damage or stress reaction caused by fishing, but the dosage is large, and the efficacy will be unstable due to the water quality, water temperature and PH value in the pool.

3.2. Immune Regulating Effect of Licorice

Like higher animals, aquatic animals rely on specific and non-specific immune mechanisms to protect themselves from pathogens. In fish, non-specific defenses are mainly done by skin and mucus. When the pathogen enters fish body, non-specific defenses of cells and body fluids are activated. The immune function of licorice on fish is mainly reflected in the non-specific immunity of the body. For example, Jian and Wu [15] smashed several Chinese herbal medicines such as licorice, scutellaria, and angelica, and added them to the bait with different mass fractions to feed the carp (*Cyprinus carpio* var. Jian). The results showed that 1.0% of the drug group could significantly improve the lysozyme activity and the number of macrophages and neutrophils of fish. Other studies have shown that the innate immunity and physiological response of yellow perch (*Perca flavescens*) can be changed by eating licorice and astragalus. During the whole experiment, the growth performance, antioxidant and immune response of fish can be significantly improved ($P < 0.05$), indicating that licorice is a natural immune enhancer [8]. Wang et al. [11, 12] carried out crude

extraction of licorice, and then studied the immunomodulatory effects of crude extract of licorice on *C. auratus* by mixed feeding and intraperitoneal injection. Through the detection of indicators of humoral immunity and cellular immunity, as well as the immune protection rate and cortisol level after artificial challenge, it is believed that the crude extract of licorice has immunomodulatory effects on *C. auratus* and enhanced the fish's resistance to *A. hydrophila*. The immune protection rates of the high drug group and the low drug group were 21.4% and 35.7%, respectively, while the control group was 0. Other studies have shown that licorice can significantly increase the ability of cells to induce interferon, enhance the phagocytosis of the reticuloendothelial system and the phagocytic power of macrophages, and protect the liver [16, 17]. Chen et al. [18] showed that a certain amount of licorice was added to the feed of Chinese soft shelled turtle (*Pelodiscus sinensis*). After feeding for a period of time, the immune response of Chinese soft shelled turtle was enhanced, and the mortality rate after infection with *A. hydrophila* decreased. Elabd et al [8] evaluated the potential immunomodulatory and growth-promoting effects of *Astragalus membranaceus* and licorice on yellow perch (*Perca flavescens*). The results showed that the addition of *Astragalus membranaceus* and licorice to the diet significantly improved superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), lipid peroxidase (LPX) and lysozyme activity of yellow perch, and can regulate biochemical enzyme activities, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity. In addition, compared with the control group, the drug group's expression of related genes, such as insulin-like growth factor-1 (IGF-1), serum amyloid A (SAA), complement component C3 (CcC3), $\alpha 2$ macroglobulin (A2M), SOD and GPX increased significantly. It was concluded that during the whole experiment, feeding of *Astragalus membranaceus* and licorice can significantly improve ($P < 0.05$) the growth performance, antioxidant capacity and immunity of yellow perch, indicating that they are natural immune regulators. It can be seen from the above studies that the effect of licorice on the non-specific immune function of fish is mainly manifested in the activation of non-specific enzyme activities of fish and the phagocytic activity of immune cells.

3.3. Disease Control Effect of Licorice

Licorice has been widely used in the prevention and control of aquatic animal diseases in China and abroad because of its low cost, good curative effect and environmental protection. In terms of viral diseases, grass carp (*Ctenopharyngodon idellus*) hemorrhagic disease (Bacterial hemorrhagic septicemia) is very common. The medicines used to prevent this disease mainly include licorice, rhubarb, *Phellodendron amurense*, green leaves, verbena, etc. [19, 20]. Through indoor and outdoor experiments, Zou and Pan [21] proved that liquorice, rhubarb, seaweed iodine and other Chinese herbal medicines had better curative effect on grass carp hemorrhagic disease, and the survival rate could be increased by 17.7% - 30%. Licorice also

has a preventive and therapeutic effect on bacterial diseases. Guo *et al.* [22] used liquorice, rhubarb, *Scutellaria baicalensis*, phellodendron, tulip, *Coptis chinensis* and *Radix Isatidis* to treat streptococcal disease of rainbow trout (*Oncorhynchus mykiss*). Compared with the control group, the curative effect was very obvious. The number of dead fish decreased significantly on the third day after administration, and stopped dead on the seventh day and basically returned to normal level. Zhang *et al.* [23] reported that licorice, *Forsythia suspensa*, *Radix Isatidis* and more than ten other herbal medicines could prevent and treat white spot disease, red neck disease and red floor disease of turtle (*Amyda sinensis*). The experiment of Duan [24] showed that the survival rate of grass carp (*C. idellus*) and carp (*C. carpio*) was increased from 30% - 50% to 70% - 80% and the yield was increased from 150 kg to 450 kg by adding liquorice, *Polygonum hydropiper*, *Acorus tatarinowii* and pine needles 1% (mass percentage), rhubarb, *Coptis chinensis*, *Phellodendron amurense* 0.5% (mass percentage) into the feed. Hassan *et al.* [16] evaluated the protective effect of licorice plant extract (LPE) on carbon tetrachloride (CCl₄) induced hepatotoxicity in carp (*C. carpio*) with 50 adult carps. The experimental group was exposed to CCl₄, while the control group was treated with normal saline. The fish in all groups were anesthetized one hour after CCl₄ exposure and blood samples were collected. Liver specimens were dissected and stored in 10% formalin for further pathological study. Serum test showed that the poisoning level of acute CCl₄ exposure group increased significantly ($P < 0.05$). Pretreatment of fish with Licorice Plant Extract (LPE) for 3 days could reduce the degree of poisoning, which indicated that Licorice Plant Extract (LPE) had good antioxidant effect and had protective effect on liver poisoning induced by CCl₄. In conclusion, licorice or its extracts as immune stimulants can stimulate cellular and humoral defense mechanisms against pathogens, such as viruses, bacteria and fungi, which are mainly caused by skin damage into fish body. Therefore, if licorice and other immune stimulants were used before the outbreak of the disease, a large number of deaths of aquatic animals could be avoided. The reason that licorice can improve fish resistance to viruses, bacteria, fungi and parasites is that it enhances the fish's self-immunity, especially non-specific immunity. Polysaccharides, glycosides, alkaloids, organic acids, volatile oils and various trace elements in licorice provide an important material basis for disease control.

3.4. Other Effects of Licorice

Licorice contains a variety of natural nutrients and active substances, which can effectively promote the body's metabolism and protein and enzyme synthesis, thereby improving production performance, reducing the feed coefficient and shortening the breeding cycle [23]. Like other animals, fish also have preferences for the color, aroma and taste of food. Licorice and other Chinese herbal medicines can induce some fish to eat [24]. Studies have shown that liquorice, parsley, cardamom and mint have strong attraction to the mud spade (*Misgurnus anguillicaudatus*) and crucian carp (*C. auratus*). The degree of attraction is positively

correlated with the concentration of the attractant [25]. In addition, licorice can also regulate osmotic pressure in some marine fishes. It has been reported that licorice derivatives (LRDS), glycyrrhetic acid (GL) and glycyrrhetic acid (18beta GA) have effects on salt-water balance and gill ion transport in rainbow trout (*O. mykiss*), which can change the end point of ion migration in fish and improve salt-water balance [5]. Chen *et al.* [6] showed that dietary supplementation of glycyrrhizic acid (18beta GA) could change the end point of transepithelial ion transport and corticosteroid level in gill tissue of rainbow trout (*O. mykiss*). It was found that fish fed with glycyrrhetic acid (18beta GA) had better resistance to sudden stress at gill tissue and circulating cortisol level. Glycyrrhetic acid (18beta GA) could also improve the ionic regulation disorders of rainbow trout (*O. mykiss*) when exposed to poor ionic water. Li *et al.* [17] tested three extracts of licorice, evaluated the potential of interaction between licorice dietary supplements and drug metabolism. Fourteen isolated licorice compounds were used to inhibit nine cytochrome P450 enzymes. Their results showed that three kinds of licorice commonly used in plant dietary supplements as cytochrome P450 subtype inhibitors had different drug-plant interactions, and each licorice has its own characteristics and may interact with drugs. In addition, licorice can also regulate the level of cortisol secretion in fish after environmental stress, thereby improving the anti-stress ability of fish [11, 12]. The mechanism of action of licorice remains to be further studied in the future.

4. Problems and Suggestions of Licorice Application

Compared with antibiotics and chemicals, licorice has many advantages in the prevention and control of aquatic diseases. With the promotion of natural green food, licorice will be more and more widely used in aquaculture practice. However, like most Chinese herbal medicines, there are some problems to be solved in the use of licorice. Firstly, the quality of medicinal materials is not stable. Licorice is a generic term for many species of *Glycyrrhiza*. The genetic variation among species can lead to the difference in species and quantity of secondary metabolites (active ingredients), so there is the reference of "genuine medicinal materials". Even for the same licorice, the contents of its active ingredients are often different due to different seasons, origins and processing methods. Therefore, the quality monitoring system of licorice should be improved and standardized, and the production standard of finished products should be worked out. Secondly, the use method of licorice is extensive. At present, licorice is widely used in aquaculture, and ineffective ingredients account for a large proportion. The main active ingredients in licorice are polysaccharides, glycosides and alkaloids. If the effective ingredients of licorice can be extracted and concentrated, the dosage can be reduced and the efficacy can be improved. In terms of research methods, at present there are more crude licorice

feeding in China, while foreign countries have turned to the precise study of extracts. Therefore, with the improvement of aquaculture conditions, the use method of licorice should be more refined. Thirdly, there are fewer standardized prescriptions. Traditional Chinese medicine theory advocates dialectical treatment and comprehensive conditioning. Although licorice is most commonly used of all kinds of Chinese herbs, its ingredients and efficacy are very complex, and the compound preparations with multi-kind herbs are more complex. If it can not be compatible scientifically, the pharmacodynamics will not be "mutually reinforcing" but "mutually resisting" [13, 14]. Therefore, the use of licorice and other herbal medicines should be standardized, the rational compatibility should be strengthened, and the development and registration of relevant refined and specialized prescriptions should be strengthened. Fourthly, the naming and describing of licorice are not accurate enough. As mentioned above, in the European and Chinese Pharmacopoeia, many species of licorice belonging to the same genus and different species are cross-used, which is easy to cause confusion in both production practice and theoretical research. In terms of description of medicinal properties and pharmacodynamics, Chinese medicine terminology is still used, which is for human beings, and is obviously not applicable to aquatic animals. Therefore, in this regard, it is also necessary to strengthen the standardization of aquatic terminology for reference and exchange.

5. Conclusion

Chinese herbal medicine is the precious wealth of traditional Chinese medicine theory. It has been proved to have good disease treatment and prevention effects in clinic. Therefore, Chinese herbal medicine is widely used in animal husbandry and aquaculture. Licorice is a common kind of Chinese herbal medicine, and licorice is found in many traditional Chinese medicine prescriptions, which can balance the efficacy. It is known as the "king of herbs". In aquaculture, more and more studies have shown that licorice has the effects of immune conditioning, promoting growth and enhancing resistance to a variety of aquatic products. However, the following problems are also highlighted, such as non-standard medication, poor repeatability of experimental results, unstable efficacy and so on. Therefore, in the future application of licorice, we should strengthen the standardized use, and the experimental results should also carefully explain the conditions and parameters to facilitate the repetition of the experiment. Licorice shows various regulatory effects, which shows its good value and prospect in aquatic application.

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