

## Effect of oral consumption of *Aloe vera* gel on intestinal microflora and liver tissue of rainbow trout

Parsa A.<sup>1\*</sup>; Bahramian S.<sup>2</sup>; Sharifpour I.<sup>3</sup>

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1- Department of Aquatic Animal Health and Diseases, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.

2- Department of Food Science and Technology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.

3- Department of Aquatic Animal Health & Disease, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran

\* Corresponding author's Email: [a.parsa@iausdj.ac.ir](mailto:a.parsa@iausdj.ac.ir)

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### Introduction

Rainbow trout as an important breeding fish has a special place in the food basket of human societies. Statistical survey shows that in the period between the years of 1995 to 2011, the number of trout breeding farms and the amount of salmon production in Iran have increased respectively by more than 23 and 70 times. Ghorbanzade *et al.* (2013). Most of the fish pathogens include short rod gram-negative bacteria belonging to the families of Enterobacteriaceae, Pseudomonadaceae and Vibrionaceae. Among these, the Enterobacteriaceae is extremely important in terms of formation of gastrointestinal normal flora and on gastrointestinal function, digestion and absorption of food, production of some useful material for the body and

providing immunity against foreign pathogens Soltani *et al.* (2004). On the other hand, these microorganisms by creating initial predisposing factors can spread their growth extremely and be pathogen. Ghorbani-Choboghlo *et al.* (2014). Medicinal plants due to having different active ingredients can be used in the treatment of many diseases. Ayoub (1984). In addition, these active ingredients due to their comrade compound have a biological equilibrium and therefore they do not accumulate in the body and do not leave side effects. Ramos (1991). These materials can have beneficial effects on modification of intestinal function, intestinal secretary, gastrointestinal motility, absorption of materials, gastrointestinal safety, pH change, inflammation control and so on. Scolz

(2007). In recent years, several studies have been conducted on adding the medicinal plants to the feed and using their beneficial effects in aquaculture, while the diversity of medicinal plants and their treatment effects require extensive research. Jump (2009). *A. vera* plant belongs to Liliaceae family which in traditional medicine, various effects such as rejuvenating, anti-inflammatory, antifungal and germicidal have been listed for it. Balch (2008). Sixty percent of the dry matter of *A. vera* gel is polysaccharides and if they have prebiotic material specifications, they can affect intestinal bacteria populations. Alishahi and Abdy (2013) examined the effect of oral consumption of *A. vera* on non-specific and specific immune of common carp. Haghighi *et al.* (2014). Another researcher studied the effect of crude extract of *A. vera* plant on growth indices and some of the rainbow trout immunity indicators. Gabriel *et al.* (2015). By considering human approach for providing organic food and on the other hand, the role of natural materials in animal husbandry, the aim of this study is addition of *A. vera* gel in rainbow trout feed and investigate its effect on intestinal microbial flora population and liver tissues of fish.

### Materials and methods

In this study, 90 healthy rainbow trout fish weighing approximately  $107 \pm 3.5$  were randomly selected. Location of the study was a rainbow trout breeding farms in West of Iran. Fish were

randomly divided into treatment and control groups, with three replications. For this purpose, 6 fiberglass fish pond with a capacity of 200 liters was used. Physical and chemical parameters of water were set at the optimum condition for this fish. Dissolved oxygen was between 8.5-9.0 mg L<sup>-1</sup> and water pH was about 7.2-8.3. The average temperature of water was 14 °C. Water flow was 5 L min<sup>-1</sup> for each outdoor fish pond. The fish were kept there for 10 days to adapt with the environment. *A. vera* plant were provided from Havin Company, Kurdistan, Iran. Plant gel was extracted manually and then diluted with distilled water at a ratio of 1:1. It was sprayed into the daily feed of fish at amount of 1% meal weight. The required daily feed for fish was GFT1 grad of extruded feed and was calculated and consumed based on the standard table. Vilaki (2007). The intervening period was three months then all fish euthanized and necropsy samples was used for the examination of intestine microorganism and liver tissue. Sasani (2008). In order to microbial examination, all content of the intestine was poured into suitable and sterile container with a cap and its weight was determined. By adding sterile distilled water, serial dilution was prepared and by pour plate or surface culture techniques was inoculated in culture mediums. Lactobacillus was cultured in MRS agar (DeMan Rogosa Sharpe Agar, Merck, Germany) medium and incubated anaerobically at 37 °C for 48 hours. Total count of bacteria was determined

in PCA (Plate Count Agar, Merck, Germany) medium and plates were incubated at 37°C for 48 hours. In the case of Aeromonas, MacConkey Agar medium (Merck, Germany) was used at 25 °C for 24 hour. Enterobacteriaceae, was cultured in VRBGA (Violet Red Bile Glucose Agar, Merck, Germany) medium at 37 °C for 48 hours. Kalbassi *et al.* (2012). Liver sample was collected from each fish and after fixing with buffered formalin, the tissue sections were prepared by autotechnicon. After cutting, they were painted with specific staining of PAS (Periodic Acid Schiff) and were examined by light microscopy. Pousti (2003). In PAS staining, positive PAS materials (glycogen) were painted with red to dark red. Gonzalez (1993). Since one of the main activities of liver is storing glycogen, the liver cells due to existing glycogen will be dark red and more glycogen causes darker red. Humansan (1997). This study was conducted in a completely randomized design with using average comparison method. The obtained results were analyzed by statistical software of SPSS 16.0 and two- sample t-test method was used for the hypothesis test. Mirzaei (2006).

### Results and discussion

By comparison of total population of trout intestinal bacteria in treatment and control samples, there was not seen any significant difference ( $p>0.05$ ) between two groups. In addition, changes in the number of Lactobacilli spp., Enterobacter spp. and Aeromonas spp.

were not significant ( $p>0.05$ ). However, enterobacter and aeromonas which usually are not useful bacteria in the trout intestine, have more reduction compared to lactobacillus (Table 1).

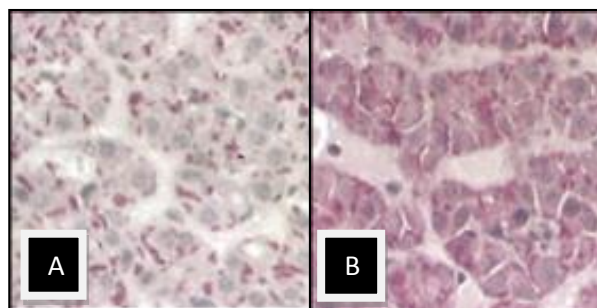
Microscopic examination of liver tissue stained with PAS indicated that in treatment group more glycogen stores were in liver hepatocytes compared with control samples. It can be concluded that by application of *A. vera* with this dosage the amount of glycogen in liver cells and their metabolism was effected (Fig. 1).

The main objectives of aquaculture industry are the maintaining health and improving fish performance. These have caused to emergence over 100 new functional supplements in commercial fish feeding. These compounds include probiotics, prebiotics and special herbal products that are known as phytochemicals. Furthermore, following the ban of growth promoters such as antibiotics in the European Union in 2006, these compounds have been further considered. In addition, consumers demand environmentally friendly farm production operations and food products with no chemical compound. Over the past decades, several researches have been conducted on the application of plant extracts, essential oils and herbal products as phytochemical compound, instead of antibiotic growth promoters in animal feed. Giannenas (2012).

**Table 1: Intestinal bacteria populations of trout in control and treatment samples.**

Bacteria	Number of bacteria in control sample cfu/g (Mean $\pm$ Std. Error)	Number of bacteria in treatment sample cfu/g(Mean $\pm$ Std. Error)
Total count	$4.9 \times 10^6 \pm 0.9^*$	$4.4 \times 10^6 \pm 0.8^a$
Lactobacillus spp.	$1.7 \times 10^4 \pm 0.2^a$	$1.4 \times 10^4 \pm 0.2^a$
Enterobacter spp.	$2.4 \times 10^4 \pm 0.6^a$	$1.8 \times 10^4 \pm 0.3^a$
Aeromonas spp.	$1.5 \times 10^4 \pm 0.3^a$	$1.1 \times 10^4 \pm 0.2^a$

\* Identical letters indicate that there is no significant difference in the rows.



**Figure 1: Histological section of liver, (A) Control group, dark red areas are less, (B) Treatment group, dark red areas are more (PAS Staining, 40X).**

Their potential in fish feeding is not limited to replacement of antibiotic growth stimulator, but it can be considered in other aspects such as control of disease, immune response and disease resistance of fish and improving the quality and durability of fish meat by preventing oxidation Gatlin (1992). Moreover, these active ingredients do not accumulate in body and do not leave side effects and despite slow action. Ayoub (1984). their effect is very stable compared to other drugs. Ramos (1991). Investigation of fish farming quality in Iran during the last ten years shows that proper and complete nutrition of fish particularly species which gain weight through artificial diets (Salmonidae) are especially important and should be carefully considered. It is necessary to

improve nutritional status and gastrointestinal function, strengthen its normal flora and improve the efficiency of other organs of body with consuming appropriate amount of feed materials with animal and plant origin and using compounds with synergism effect. Miar *et al.* (1993). The formation of intestinal bacterial flora is based on the feed and types of bacteria in the environment. Marian (1990). In this survey, it was tried to investigate the prebiotic effect of *A. vera* gel and its impact on intestinal microbial population, which play an important role in providing the biological needs of fish. However this study showed that the changes in bacterial population were not significant, but the less decrees in lactobacillus populations, which are beneficial bacteria in the

gastrointestinal tract, suggesting that the use of this plant's gel besides other beneficial effects can play a prebiotic role and by adjusting its dosage in fish feed, better effects can be achieved. The result of this study is in accordance with other researchers who confirmed this fact that prebiotic ingredients can reduce Enterobacteriaceae in fish digestive tract, and on the other hand can increase Lactobacillus spp. Gonca (2012). *Aeromonas* and Enterobacteriaceae as two main factors involve in fish health and by controlling their population in the intestine of fish and replacing them with Lactobacillus spp., beneficial effects may be observed. This is possible by consuming appropriate amount of suitable feeds and additives. Akhlaghi *et al.* (2009). Studies show that the *A. vera* consumption at this dosage can have a positive role in improving the performance of fish and reduction of feed conversion coefficient. Gabriel (2015). Other studies have shown that fish growth indices such as specific growth, food conversion ratio and percentage of weight gain are significantly increased with *A. vera* consumption. Alishahi *et al.* (2010). One of the important reasons of performance improvement can be equilibrium in intestinal microbial population and therefore improving the digestion and absorption performance. Regarding the effect of this plant on liver tissue, it was found that more glycogen stores are in hepatocytes of treatment fish and this was obvious with more red color centers called

positive PAS reaction in microscopic images. The liver as a storage organ of carbohydrates and fats has a fundamental role in destruction of blood cells, detoxification and blood chemistry. Surveying its cells can be a general scheme of body's metabolism. Peyghan *et al.* (2001). In this study, the minimum and maximum decreases in bacterial population were related to lactobacillus and enterobacteria, respectively. Although, the downtrend of number of bacteria was not uniform, but enterobacteria and aeromonas which play an essential role in the incidence of various diseases have been declined. Therefore, by proper use of prebiotic compound such as *A. vera* gel, intestinal bacterial population can be in safer position. Although further studies must be done about its dosage, its synergism with other herbs and evaluation its effect on other parts of the digestive system.

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