Original Article

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Comparative evaluation of the antioxidant potential of rainbow trout exposed to Yersiniosis vaccine and non-vaccinated fish

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ARTICLE INFO

Article history:
Received 13 Jun. 2022
Received in revised form 06 Nov. 2022
Accepted 19 Nov. 2022

Keywords:
Yersiniosis vaccine;
Antioxidant activity;
Cartenoids;
Liver

ABSTRACT

The antioxidant capacity of liver in fish is a major property which could prevent the diminished quality and safety of fish resulted from oxidation reactions. The aim of this study was to compare the antioxidant capacity of the liver of rainbow trout exposed to the yersiniosis vaccine and non-exposed fish. The amounts of carotenoids, glutathione, cupric assay, and lipid peroxidation levels of liver were determined. Then, the samples were normalized by Bradford test. The results showed that significant differences may exist in lipid peroxidation level ($p<0.05$). The total antioxidant capacity, glutathione, and carotenoids content showed no significant difference in both group ($p\geq0.05$). The use of vaccine, in addition to increasing resistance to yersiniosis, could prevent the lipid oxidation of fish liver.

Citation: Sadighara P, Araghi A, Molaee-Aghaee E, Erfanmanesh A, Pirhadi M, Farkhondeh T. **Comparative evaluation of antioxidant potential of rainbow trout exposed to Yersiniosis vaccine and non-vaccinated fish.** J food safe & hyg 2022; 8(4): 264-268. DOI: 10.18502/jfsh.v8i4.11959

1. Introduction

The genus Yersinia belongs to the Enterobacteriaceae family with 18 species including *Y. ruckeri, Y. enterocolitica, Y. pestis, Y. Pseudotuberculosis, Y. aldovae, Y. aleksiciae, Y. bercovieri, Y. entomophaga, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. massiliensis, Y. mollaretii, Y. nurmii, Y. pekkanenii, Y rohdei, Y. similis* and *Y. wautersii* (1).

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Y. ruckeri is a fish pathogen which imposes a huge amount of losses in the aquatic products (2). *Y. ruckeri* can establish and maintain subclinical infection, which lead in asymptomatic carriers. When stressed, these carriers instigate horizontal transfer of the pathogen, subsequently producing clinical infection within a population (3).

The cause of yersiniosis or red mouth disease is *Yersinia ruckeri*. This complication is one of the serious diseases with high economic losses in the rainbow trout industry. *Y. ruckeri* belongs to the family Enterobacteriaceae, rod-free, without spores, negative oxidase and positive and mobile catalase, grows at 37°C, and does not lose its motility (4). In recent years, biotype 2 species have played a significant role in the prevalence and mortality of yersiniosis in vaccinated fields, as in Chile, the United States, Spain, the United Kingdom, and a number of other European countries. Commercial vaccines are often made against biotype 1 (5). Enterobacter cloacae and Bacillus mojavensis were isolated from rainbow trout gut and was shown that they can be used to prohibit and control yersiniosis disease. These organisms as a dietary supplementation can protect fish from yersiniosis and improve digestibility and utilization of feed (6). Bioactive compounds have an increasing effect on the growth of fish species and improve the antioxidant and immune defense pathways (7).

The yersiniosis vaccine, which is a killed bacterium in an oil emulsion, is made to prevent disease and used in salmon farms. It can be given by bath or injection. Safety studies show that the use of the vaccine does not cause side effects in fish. However, the antioxidant capacity of fish has not been studied.

This study aimed to evaluate the antioxidant capacity of rainbow trout liver exposed to yersiniosis vaccine and compare it with fish without vaccination.

2. Materials and Methods

2.1. rocedure of animal experiments

The fish were splited into two groups of 10 samples and all conditions were equalized except injecting the vaccine. The samples of liver were collected and homogenated.

2.2. Measurement of protein content

The results should be normalized with the amount of protein. Bradford test is used for normalization. In this method, bovine serum albumin protein is used as a standard. In Bradford method, coomassie dye reacts with protein and changes color. The color change is proportional to the amount of protein.

2.3. Determination of carotenoids

The solution absorbance was read on spectrophotometer at 470 nm. Its carotenoids content was calculated on the standard curve of Β carotene. The adsorption of standard solution concentrations and the sample obtained will be read by a spectrophotometer. The line curve is drawn using standard concentrations (10).

2.4.Determination of Cupric Ion Reducing Assay (Cupric)

This method quantifies the cupric reducing capacity. The solutions of $CuCl_2$ and neocuproine reagent in ammonium acetate buffer were used for mixing the

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samples. The absorbance of solutions was read at 450 nm after incubation at 50°C for 20 min (11).

2.5. Measurement of lipid peroxidation in liver tissue by TBARS method

The samples were mixed with 20% trichloroacetic acid and the mixture was centrifuged. Then, thiobarbituric acid was added to the supernatant and heated. The absorbance of the supernatant was read at 532 nm. The values were expressed in micro moles of malodialdehyde (MDA) (12).

2.6. Determination of glutathione

The homogenized sample mixed with 20% trichloroacetic acid (TCA). The proteins of samples were precipitated. Then, after centrifugation (5000 rpm in 5 min), 0.5 mL of the centrifuged sample was mixed with 0.5 mL of DTNB solution and read at 412 nm. The DTNB reagent measures the amount of glutathione produced in the tissue (13).

2.7. Statistical analysis

Statistical analyzes were performed by SPSS software. The data represent the mean ± standard deviation for samples. Kolomogorov-smirnov test was used to determine the parametric or nonparametric state of the data. Carotenoid and lipid peroxidation data were normal. T test was used to determine the significance. Total antioxidant and glutathione data were not normal; So Mann- Whitney test was used.

3. Results

The results are shown in Tables 1. In order to eliminate variances from the samples, results of individual samples were normalized to the protein content.

The concentration of carotenoid pigments in the extracts was calculated using the standard curve obtained by a commercial β-carotene reagent.

The formula used for the calculation was as follows; R^2, $y=8.3303x+0.0031$

The values presented in Fig. 1 show that, the level of lipid peroxidation changed significantly ($p=0.05$). The changes in other parameters; glutathione content ($p=0.42$), carotenoids ($p=0.26$) and cupric assay ($p=0.84$) were not significant (Table 1).

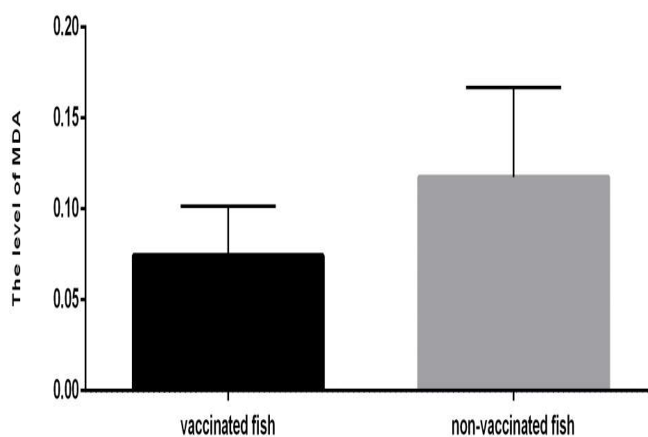

Figure 1. A comparison between MDA content in liver samples of vaccine and non-vaccinated samples

Table 1. The antioxidant capacity of vaccinated and non-vaccinated fish

Antioxidant capacity parameters	Vaccinated fish	Non- Vaccinated fish
Lipid peroxidation	0.074±0.06*	0.11±0.11*
Glutathione content	0.17±0.14	0.08±0.06
Carotenoids	0.1±0.03	0.089±0.01
Cupric assay	0.24±0.05	0.34±0.24

*P=0.05, each value represents the Mean ± SD per group. Lipid peroxidation data were significantly

4. Discussion

In this study the total carotenoids, lipid peroxidation, cupric assay and glutathione of liver were measured and compared in two groups. The cause of yersiniosis, which is the same as septicemia-yersinia or enterobacterial red mouth disease, is Yersinia rocker. This complication is one of the serious diseases with high economic losses in the rainbow trout breeding industry (14). To date, there are no studies about the antioxidant capacity of liver in vaccinated fish. This assay is necessary because the vaccine and its exposure doses may have side effects in addition to preventing yersiniosis. The oxidative stability was assayed by measuring content of malondialdehyde (15). In present study the level of malondialdehyde was lower than vaccinated fish (Fig. 1). Lipid oxidation leads by attracting reactive oxygen species (ROS) to lipid. The lipid oxidation in rainbow trout due to its relatively high unsaturated fatty acid profile can be an important factor in spoilage and product shelf life. The total antioxidant capacity is usually investigated by cupric ion reducing capacity assay (Cupric assay). The antioxidant power of a product can protect it against oxidation and oxidative chemical decay. In this study, data obtained of cupric assay was nonparametric and there was no difference between two groups (16).

Glutathione is a small protein compound made up of the three amino acids cysteine, glutamic acid and glycine. Due to the use of three amino acids in the structure of this protein, it is also called tripeptide. This substance occurs naturally in the liver due to the combination of these three amino acids.

Glutathione is a powerful antioxidant and protects important cellular components against reacting with oxygenated functional groups such as free radicals. Free radical toxins are usually combined with glutathione and excreted from the body. Therefore, the increase in glutathione production in tissues is the reason for the production of free radicals and the toxicity of the substance entering the body. In this study the level of glutathione did not change in two groups (13).

5. Conclusions

The use of the vaccine, in addition to increasing resistance to yersiniosis, also could prevent the lipid oxidation of fish liver. The present evaluation can bring practical results for decision makers and breeders in fish farms.

Conflict of interest

Authors declare to have no conflict of interest.

Acknowledgments

This study was supported by Amol University of Special Modern Technologies, Amol.

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