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Hemato – Immunological and biochemical parameters, skin antibacterial activity, and survival in rainbow trout (*Oncorhynchus mykiss*) following the diet supplemented with *Mentha piperita* against *Yersinia ruckeri*

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ABSTRACT

This study was aimed to assess the potential effects of *Mentha piperita* on the hemato – immunological and biochemical parameters, skin antibacterial activity and protection against *Yersinia ruckeri* infection in rainbow trout *Oncorhynchus mykiss*. Fish were divided into 4 groups before being fed diets supplemented with 0, 1, 2 and 3% of *Mentha piperita* (MP) plant extract for 8 weeks. Dose-dependent increases immune (both in skin mucus and blood serum) and hematological parameters (number of red and white cells, hematocrit and hemoglobin contents), as well as in respiratory burst activity, total protein, albumin, and neutrophil levels in fish fed supplemented diets compared to the control fish. Furthermore, dietary MP plant extract supplements have no significant effect on blood biochemical parameters and enzymatic activities of liver determined in serum of rainbow trout. After 8 weeks the cessation of feeding with MP plant extract, survival rates of 54.4%, 63.6% and 75.2% were recorded in groups which received 1, 2 and 3% of MP plant extract of feed, respectively, compared to 34.6% survivals in the control. This study underlying several positive effects of dietary administration of MP plant extract to farmed fish.

1. Introduction

Yersinia ruckeri was initially isolated from rainbow trout, *Oncorhynchus mykiss* (Walbaum), in the Hagerman valley of Idaho, USA, in the 1950s and is now widely found in fish populations throughout North America, Australia, South Africa, Asia and Europe [1]. This bacterium is the causative agent of yersiniosis or enteric red mouth disease (ERM), and causes significant economic losses in the salmonid farming industry. Although infection with this agent has been reported in other fish species, salmonids and especially rainbow trout are most susceptible to ERM [2].

The use of antibiotics is the most common method to bacterial diseases control but it is often very expensive and leads to the selection of antibiotic-resistant bacterial strains, immunosuppression, environmental pollution and the accumulation of chemical residues in fish tissues, which can be potentially harmful to public health [3].

Today, the demand for fish products free of pollutants/antibiotics is steeply increasing and it is caused the use of medicinal plants in aquaculture has attracted a lot of attention globally as an alternative and has become a subject of active scientific investigations. In many studies, herbal plants are known to have an important role in disease control due to their antioxidant, antimicrobial and immunomodulatory activities [4–6].

Mentha piperita is a perennial herbaceous plant belonging to the family Laminacea. It is widely grown in temperate areas of the world, particularly in Europe, North America and North Africa but nowadays cultivated throughout all regions of the world [7] and it grows abundantly in Iran [8]. It has been confirmed to have antiviral, antimicrobial, anti-fungal and antioxidant effects [9–13], effects on biochemical and haemato – immunological parameters [14–16], anti-ulcer, anti-mutagenic and anticancer effects [17,18] and antispasmodic activity [19].

Recently, the positive effects of several herbal plants such as garlic (*Allium sativum*) [20,21], black cumin seed (*Nigella sativa*)

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[22–24], watercress (*Nasturtium nasturtium*) [25], green tea (*Camellia sinensis*) [26], Silymarin (*Silybum marianum*) [27], fennel (*Foeniculum vulgare*) and thyme (*Thymus vulgaris*) [28,29], mistletoe (*Viscum album*), nettle (*Urtica dioica*), ginger (*Zingiber officinale*) [30,31], lupin, (*Lupinus perennis*), mango (*Mangifera indica*) [31] and aloe vera (*Aloe barbadensis*) [32] are observed in immune system improvement, resistance to bacterial diseases, intestinal microflora and growth performance of rainbow trout.

It should be noted the positive effects of *Mentha piperita* have been shown on hemato-biochemical parameters, growth rate, immunity responses and bacterial infection resistance of common carp (*Cyprinus carpio*) [33], tambaqui (*Colossoma macropomum*) [34], cat fish (*Ictalurus punctatus*) [35], Asian seabass (*Lates calcarifer*) [36], Caspian white fish (*Rutilus frisii kutum*) [37] and Caspian brown trout (*Salmo trutta caspius*) [38], but to our knowledge, there is no information about the use of *Mentha piperita* in rainbow trout feed.

The aim of the present study was to investigate the effect of *Mentha piperita* on the growth, biochemical and hemato-immunological parameter, skin protection and survival rate against pathogen *Yersinia ruckeri* in rainbow trout. The potential use of this plant as natural growth promoter and immunostimulant in fish is discussed.

2. Materials and methods

2.1. Experimental diets

M. piperita (MP) plants were collected from natural habitat in Mazandaran province (North of Iran) and its identification was done according to standard methods by Shahrekord University Botany section [39]. One kilogram of aerial parts of *M. piperita* was dried in a well aired and dark room and then the plants were ground into fine powder using a grinder. Acquired powders were mixed in 1 L volumetric flask by 1:5 proportions with 80% ethanol for 48 h by using a shaker. The mixture was then filtered by Büchner funnel and filter paper. Primary extracts were distilled in rotary distillation in 80 °C for 4 h.

In order to prepare the diets, a commercial pellet diet (Bayza Feed Com. Iran) (containing 44.50% protein, 14.20% lipid, 7.62% ash and 21.90 MJ kg^{-1}GE) was crushed, mixed with the appropriate MP plant extract and water and remade into the pellets, which were allowed to be dried for 18 h at 45 °C by air circulation and stored at 4 °C until it was used. The control diet was prepared by adding only water [40]. The dietary MP plant extract was supplemented at the levels of 0 (control), 1, 2 and 3% dry food for the three experimental groups. The control group received no MP plant extract supplement. The approximate chemical composition of formulated diet was determined according to standard methodology (AOAC) [41].

2.2. Feeding and culture system

Rainbow trout (32.2 ± 2.2 g) were obtained from a commercial fish farm in Sari (Mazandaran province, north of Iran). The health of the fish (changes in behavior and physical appearance) was checked for 2 week before starting the experiment. Fish were randomly distributed into 12 fiberglass tank (900 L) at 80 fish per tank density (3 tanks per treatment) and acclimatize for 7 days before experimental feeding regime. During the 8 weeks of experimental feeding, the fish were fed 3–4% body weight twice on daily basis. Water temperature, dissolved oxygen, pH and electrical conductivity were monitored daily and maintained at 14.2 ± 1.2 °C, 7.8 ± 0.6 mg L^{-1} and 5736.4 ± 127.2 MM cm^{-1} respectively. Continuous aeration was provided to each tank through an air stone connected to a central air compressor.

2.3. Blood sample collection

At the end of the experiment, 6 fish were sampled randomly from each tank and were anaesthetized by clove solution and about 2 ml of blood was drawn from the caudal vein, using a non-heparinized syringe, after they were starved for 24 h. One half of each blood sample was transferred to micro tube containing heparin anti-coagulant and immediately used for respiratory burst assay and hematological examination, while the other half was transferred to non-heparinizes micro tube, placed at room temperature and allowed to clot for 2 h. Sera were separated by centrifugation at 1500*g* for 20 min and stored at −20 °C until use.

2.4. Hemato − biochemical assays

The total red blood cell (RBC: 10^6 mm^{-3}) and white blood cell (WBC: 10^4 mm^{-3}) were counted manually by haemocytometer. The haemoglobin level (Hb: g dL^{-1}) of blood was analyzed spectrophotometrically at 540 nm by the cyanmethaemoglobin method. Hematocrit (Ht%) was determined by microcentrifuge technique, using standards heparinized microhaematocrit capillary tubes (75 mm at 7000 g for 10 min). Thin blood smears slides were prepared and stained withWrighte Giemsa. A total of at least 100 leucocytes were counted under a light microscope, and the percentages of leukocyte types were calculated [42]. The hematological indices of mean cell haemoglobin concentration (MCHC: gdL^{-1}), meancell haemoglobin (MCH: pg) andmeancell volume (MCV: fL) were calculated using the total RBC count, Hb concentration and Ht [43].

Albumin, glucose, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated using commercial kits (Pars Azmoon, Tehran, Iran) and a biochemical auto analyzer instrument (Eurolyser, Belgium) [44].

2.5. Immunological assays

2.5.1. Lysozyme activity

Serum lysozyme activity was measured using a modified turbidimetric method described by Ellis [45]. Briefly, aliquots (1.75 ml^{-1}) of Micrococcus lysodeikticus (Sigma, USA) suspension (0.375 mg mL^{-1}, 0.05 M PBS, pH 6.2) were mixed with 250 ml^{-1} of each sample and optical density was measured after 15 and 180 s by spectrophotometer (Biophotometer Eppendorf) at 670 nm. One unit of lysozyme activity was defined as reduction in absorbance of 0.001/min. The units of lysozyme present in serawere obtained from a standard curve made with hen egg white lysozyme (Sigma, USA).

2.5.2. Respiratory burst activity

The oxidative burst produced by leukocyte of the blood samples was measured by Chemiluminescent assay (CL) (measuring of light emission) as described by Khoshbavar-Rostami et al. [46] with using an automated system for CL analysis (LUMI skan Ascent T392, Finland). The results of light emission are expressed in the form of relative light units per second (RLU s^{-1}) recorded by the luminometer. CL assays were performed in duplicate and mean of replicate assays was used in subsequent evaluations.

2.5.3. Serum total protein and total immunoglobulin

Serum total immunoglobulin (Ig) levels were determined according to the method described by Siwicki and Anderson [47]. Briefly, serum total protein (TP) content was measured using a microprotein determination method (C-690; Sigma), prior to and after precipitating down the immunoglobulin molecules, using a