

Evaluation of Virulence Genes (*aerolysin*, *elastase*, *lipase*) in the Pathogenesis of *Aeromonas hydrophila* in Common Carp (*Cyprinus carpio*)

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Abstract

Aeromonas hydrophila is the causative agent of hemorrhagic septicemia in freshwater fish, especially in carp. Several factors are involved in the pathogenesis of the disease by this bacterium but the mechanism is yet to be investigated. Virulence genes are the most important pathogenicity factor in *A. hydrophila*. In this study, 30 isolates were identified as *A. hydrophila* by common biochemical methods and detection of 16S rDNA molecular marker using PCR method. PCR were then used to identify *Aerolysin* (*aerA*), *Elastase* (*ahyB*) and *Lipase* (*lip*) virulence genes using specific primers for each gene. The highest frequency belonged to the *elastase* gene (70%) and the *aerolysin* gene had the lowest abundance with (53.3%). For these three virulence genes eight different profiles were considered that the *lip*⁺/*ahyB*⁺/*aerA*⁺ isolates (33.3%) was the most frequent profile and *lip*⁺/*ahyB*⁻/*aerA*⁺ (3.3%) was the lowest frequency obtained. Mortality was measured and determined after injecting *A. hydrophila* containing each of eight different profiles to the common carp fry. The mortality rate of infected fish with *lip*⁺/*ahyB*⁺/*aerA*⁻ and *lip*⁺/*ahyB*⁺/*aerA*⁺ profiles was higher from other groups and control group. In diseased fish, signs of darkening skin, shortening of gills and abdominal and vent petechial hemorrhages were observed. These results indicate that *aerolysin*, *elastase* and *lipase* virulence genes increase the pathogenicity of the *A. hydrophila*.

Key words: *Aeromonas hydrophila*, *Aerolysin*, *Elastase*, *Lipase*, PCR, Common carp

The Effects of Dietary Nucleotide and *P. acidilactici* on Growth Parameters, Serum Biochemical and Immunity Parameters, and Disease Resistance of Rainbow Trout (*Oncorhynchus mykiss*) Against *Aeromonas hydrophila*

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Abstract

This study was conducted to investigate the effects of dietary nucleotide and *P. acidilactici* on the growth performance, some innate immune responses and serum biochemical parameters as well as the resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas hydrophila*. Nine experimental diets were formulated to contain three dietary nucleotide levels (0, 1 and 3 g kg⁻¹ diet) and three *P. acidilactici* levels (0, 0.1 and 0.3 g kg⁻¹ diet) according to a 3×3 factorial design. Each diet was randomly assigned to triplicate groups of 30 fishes (with the average initial weight of 63.4±1.62 g) per tank. At the end of an eight week feeding trial, several innate immune responses and serum biochemical parameters including serum total protein, lysozyme, serum alternative complement (ACH₅₀), cholesterol, triglycerides, albumin, and glucose levels and also fish performance (survival, weight gain, specific growth rate (SGR) and feed conversion ratio (FCR)) were measured. The results indicated that lysozyme and ACH₅₀, were all increased significantly ($P<0.05$) due to the interaction between dietary nucleotide and *P. acidilactici*. Also, the results showed that total protein, cholesterol, triglycerides, glucose, albumin, and globulin were not affected by dietary nucleotide and *P. acidilactici* ($P>0.05$). The results also showed that the interaction between dietary nucleotide and *P. acidilactici* significantly improved fish performance in the rainbow trout. Furthermore, at the end of the feeding trial, ten fish per each replicate were intraperitoneally injected with *A. hydrophila* to determine the disease resistance. The results showed that the supplementation of the rainbow trout diet with dietary nucleotide and *P. acidilactici* remarkably increased resistance to *A. hydrophila* infection; the highest resistance was also observed in the interaction groups (dietary nucleotide × *P. acidilactici*). Overall, the combination of dietary nucleotide and *P. acidilactici* showed more advantages than the administration of individual ones. The best combination of them for the rainbow trout was 3 g kg⁻¹ dietary nucleotide and 0.1 g kg⁻¹ *P. acidilactici*.

Keywords: Dietary nucleotide, *P. acidilactici*, Rainbow trout, Innate immune response, Disease resistance.

Evaluation of different edible film effect on rainbow trout fillet stability

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Abstract

Fish meat is so perishable than other meat and during storage its quality decreased rapidly. The aim of this work was to evaluate the effect of chicken feet gelatin and carrageenan edible film with chitosan and cellulose nanoparticles on rainbow trout stability during refrigerator storage period. In present study edible film prepared with 2.5 and 5% extracted chicken feet gelatin and carrageenan. Edible film sprayed on fish fillet and their quality and stability during 16 days refrigerator storage evaluated. Results of this work depicted low antibacterial effect of gelatin 5% and carrageenan 5%. Bacterial count of Gelatin 2.5% at day 12 was 7.04 Log cfu/g and control treatment at day 8 was 7.17 Log cfu/g that were exceeded the limit. PH had decreasing and subsequently increasing trend ($P<0.05$). TBA factor showed increasing and subsequently decreasing trend in experimental group ($P<0.05$). Control group showed higher changes in moisture and fat content ($P<0.05$). Expressible moisture had correlation with trout fillet moisture ($P<0.05$). Sensorial analysis results depicted experimental group with edible film has better quality than control. Chicken feet gelatin and carrageenan in 5% could be applied to fish edible film but they do not have remarkable antibacterial properties.

Keywords: Edible film, Carrageenan, Chicken feet gelatin, Rainbow trout, Stability.

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Optimization of Energy Consumption in the Production of Warm Water Fish Using the Data Envelopment Analysis and Genetic Algorithm

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Abstract

Technical management in agricultural productions is a key factor for increasing the yield and reducing the cost of production. For this purpose, the technical efficiency of warm water fish farms was assessed during growing season 2014. The required information was collected from 57 growers by using questionnaires. Data envelopment analysis and genetic algorithm were applied to analyze the information. The results of data envelopment analysis showed that the total energy consumption in optimum situation is 158515.5 MJ ha⁻¹. The highest save of energy among inputs in optimum situation (without any output reduction) belongs to fish input (9.27%). Based on the genetic algorithm results in optimum situation the total energy need is 43766.51 MJ ha⁻¹ (with 26% energy saving).

Keywords: Warm water fish, Optimization, Data envelopment analysis, Genetic algorithm, Guilan Province.

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Nutritional Effects of Black Powder Seeds (*Nigella sativa*) on Antibacterial Activity and Safety Mucus Indicators Caspian Roach (*Rutilus caspicus*)

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Abstract

In this study, the effect different levels of *Nigella sativa* feed on mucus, antibacterial activity of some enzymes' mucus were examined the baby fish roach (*Rutilus caspicus*). 420 carp with average weight of $4/49 \pm 0/11$ gr on 4 group control, 0.5, 1 and 2 percent seed powder per kg of diet were fed with three replications for 60 days. At the end of levels of soluble protein mucus, antibacterial activity and phosphatase alkaline by microdilution methods were measured against Gram-positive bacteria *aureus Staphylococcus*, *Streptococcus faecalis* and Gram-negative bacteria *Escherchia coli* and *Pseudomonas aeruginosa*. The showed results that the antibacterial activity of mucus with increasing levels of black seed increased and the diameter of the growth in fish fed with %2 black seed significantly increased compared with the control group ($P < 0/05$) and the most diameter of inhibition against was observed to the bacteria *Streptococcus faecalis*. Also the levels soluble protein of mucus, lysozyme enzyme and phosphatase alkaline in fish fed up *Nigella sativa* 0.5 Ratio to the control group significantly increased ($P < 0/05$). Minimum the growth inhibitory concentration (MIC) on a concentration of 50 microliter on ml and concentration higher concentrations of mucus, was higher the antibacterial activity and the concentration 200 microlitre per ml the most was seen antibacterial activity. Overall results that showed diet of black seed has a positive effect on the antibacterial activity is the mucus roach fish.

Keywords: Antibacterial activity, Mucus, Roach, Black seeds.

Effect of Genistein and β -sitosterol on Some Reproduction Indicators of Caspian Kutum (*Rutilus kutum*)

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Abstract

Phytoestrogens are natural compounds with the potential to elicit negative effects on the endocrine systems and reproduction of aquatic life. In this study, the effect of Genistein and β -sitosterol on some reproduction indicators of Caspian Kutum (testosterone, 17β -estradiol, aromatase and EROD) were studied. A total of 49 none-ripened Kutum's exposed to 3 different levels of Genistein and β -sitosterol (10, 50, 500 ng/l respectively). After 21 days, serum, liver and ovary were sampled and mentioned parameters were measured according to exist protocols. The result showed high level of Genistein lead to increased 17β -estradiol and aromatase activity. Also, treated fish with β -sitosterol showed high level of testosterone and EROD induction. While, both of them had no significant effect on Ca^{++} level. According to this study, high concentration of Genistein (500 ng/l) could act as a stimulant endocrine system and might cause changes in steroid hormone levels and aromatase activity. It was also found that the same concentration of β -sitosterol could induce EROD activity and increasing testosterone level. It could alter biosynthesis of sex hormones and disrupt the function of endocrine system. It seems that these compounds could effect on the endocrine system of Kutum and reduce reproduction performance of Kutum in the long period.

Keywords: Genistein, β -sitosterol, Caspian Kutum, Steroid hormones, Ca^{++} , EROD, Aromatase activity.

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Evaluation and Comparison of Effects of N-hexane and Acetone Extracts Derived from Marine Geysers (*Phaulasia nigra*), Marine Sponge (*Cliona* spp.), Carpet Anemone (*Sarcophyton* spp.) and Starfish (*Pentaceraster* spp.) on *E.coli* Bacteria

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Abstract

To study the pharmacological characteristics of marine natural products has led to the discovery of bioactive substances. The source of bioactive natural products biological and chemical structural properties marine environment cannot be seen of other natural products terrestrial plants and animals. This study aimed to evaluate and compare the antibiotic properties of n-hexane and acetone extracts were taken from four species of invertebrates. Geysers (*Phaulasia nigra*), sponge (*Cliona* spp.), carpet anemone (*Sarcophyton* spp.) and starfish (*Pentaceraster* spp.) species were collected from the Island lark. Then extraction was carried out by n-hexane and acetone solvents soaking. Antibacterial effects on *Escherichia coli* bacteria dilution method were at first Agar diffusion method and then performed to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). N-hexane extracts derived from any of the four species studied had antibacterial properties. Estonia extracts derived from sponge and starfish in concentration of 10 and 40 mg/ml was bacterial growth inhibition and a concentration of 20 mg/ml acetone extracts derived from the sponge has antibacterial properties and cytotoxicity. Maximum zone of inhibition was 16.2±1.5 mm by the sponge acetone extract and minimum zone of inhibition was 0.2±0.3 mm by the starfish acetone extract. The results show Estonia sponge and starfish extracts contains compounds with antibacterial effect but the cytotoxicity of Estonia sponge extracts in this study much more.

Keywords: Marine sponge, Marine geysers, Marine starfish, Carpet anemone, MIC.

Synergistic Effect of Nisin and *Mentha piperita* Essential Oil on Growth of *Streptococcus iniae* in Fillet of Rainbow Trout

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Abstract

In this study, the antibacterial effects of *Mentha piperita* (0, 0.4 and 0.8%) and nisin (0, 0.25 and 0.75 µg/ml) alone and together for control of *Streptococcus iniae* in rainbow trout (*Oncorhynchus mykiss*) fillets were evaluated at 4 and 8°C. The results showed that *M. piperita* essential oil had a higher inhibitory effect in both temperatures of 4 and 8°C compared to nisin. At 4°C, the essential oil of *M. piperita* and nisin in comparison to the control treatment had a better effect and in combined treatment, 0.75 µg/ml nisin and 0.8% *M. piperita* essential oil, growth of *Streptococcus iniae* from day third stopped. Antimicrobial activity was also increased by increasing the amount of nisine and *M. piperita* essential oil and at 8 °C, this combination was able to prevent bacterial growth until the ninth day. The results of this study showed that *M. piperita* essential oil had more antibacterial activity compared to nisin against *S. iniae*. In both treatments, with increasing levels of nisin and *M. piperita* essential oil, the inhibitory activity increased at a temperature of 4 and 8 °C in comparison with control and treatments with lower amounts of nisin and *M. piperita* essential oil. At both storage temperatures, the best performance was observed in treatment with the highest level of 0.75 µg/ml nisin and 0.8% *M. piperita* essential oil.

Keywords: *Streptococcus iniae*, *Mentha piperita* essential oil, Nisin, Rainbow trout, Temperature.

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The Protective Effect of Dietary Cinnamon Essential Oil (*Cinnamomum verum*) Supplementation in Reducing the Toxicity of Aflatoxin B₁ to Rainbow Trout (*Oncorhynchus mykiss*) Fingerlings

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Abstract

The present study was to scrutinize the protective effect of dietary cinnamon essential oil supplementation on reducing the toxicity of aflatoxin B₁ to rainbow trout fingerlings regarding hematological indices, serum biochemistry and liver histopathology. To that end, fish were randomly allotted into 6 distinct treatments composed of different combinations of three dietary aflatoxin (0, 25 and 50 ppb) and two dietary cinnamon essential oil (0 and 1 percent) inclusion levels with three respective replicates. The experiment lasted for 60 days. At the end of the experiment random samples were taken to study the aforementioned indices. Hematocrit, red blood cell count and hemoglobin concentration were positively affected by dietary supplementation of cinnamon essential oil ($P<0.05$). Dietary aflatoxin also resulted in increased red blood cell count and hemoglobin concentration ($P<0.05$). Activities of ALP and ALT were increased due to dietary aflatoxin contamination ($P<0.05$); however, supplementation of cinnamon essential oil lowered the activity of ALP in serum of aflatoxin exposed fish ($P<0.05$). Total protein and globulin content were only affected by aflatoxin and cinnamon essential oil ($P<0.05$), while, the interactive effect of aflatoxin and cinnamon essential oil significantly affected albumin and lysozyme activity of serum ($P<0.05$). Hepatosomatic index of fish were significantly decreased by dietary aflatoxin exposure ($P<0.05$). Histological observations also showed blood vessels dilation, cytoplasmic degeneration, blood congestion, immune cells infiltration and necrosis in a dose dependent manner. In conclusion, 1 % dietary supplementation of cinnamon essential oil resulted in decreased deleterious hepatic tissue changes and also improvement of hematological and serum biochemical indices of fish fed aflatoxin contaminated diet especially of those fish exposed to 25 ppb dietary aflatoxin.

Keywords: Aflatoxin B₁, Cinnamon essential oil, Hematological indices, Serum biochemistry.

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Effect of Different Ratios of CNP on Growth Indices and Frequency of Phytoplankton

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Abstract

A completely randomized experimental design in triplicates was conducted to evaluate the effects of different levels of nitrogen with the constant level of carbon and phosphorous on production of warm water fish in Chapakrod fish farm in Joybar. The treatments were use of three levels of C: N: P, respectively, 1: 5.5: 88.6 (T1) 1: 7.5: 88.6 (T2) and 1: 9.5: 6.88 (T3). The mixed chemical fertilizers and organic manures consisted of chicken, cattle feces, vermi-compost, phosphate and urea mixed together to get different levels of CNP. The effect of different concentrations of nitrogen on phytoplankton abundance and chlorophyll, were investigated as well. At the end of experiment, the highest number of phytoplankton, chlorophyll, relative growth indices were significantly different ($P<0.05$) among treatments. The highest primary production and phytoplankton number was recorded in treatment T2 with rate of 638 mg/m³/hr and 438,750 per/ml, respectively. The least rates of primary production and phytoplankton were recorded in T1. The mean individual fish weight was higher in T2 for all chines fish carp at the end of the experiment. This rate for silver carp attained to 40/875 gr at the end of the experiment. The most prominent of phytoplankton were recorded among treatments included: Ankyra, chlorella, Pediastrum, Tetrastrum, spirogyra, Navikula, diatoms, filamentous algae and the most benthos and zooplanktons were included: rotifers, Daphnia, copepoda, ceratopogonidae, and tippulidae.

Keywords: Fertilization, Vermicompost, Carp fishes, Primary production, Phytoplankton, Zooplankton, Benthos

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