

Research Title :	Dietary propolis complementation relieves the physiological and growth deterioration induced by <i>Flavobacterium columnare</i> infection in juveniles of common carp (<i>Cyprinus carpio</i>).
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Abstract: The current study was proposed to explore the role of dietary propolis (PR) supplementation in alleviating the negative effects of columnaris disease (CD) challenge on the growth performance, plasma biochemicals, antioxidant activity, stress indicators, and immunological reactions of common carp (*Cyprinus carpio*) fish. Five hundred forty common carp juveniles were evenly placed in thirty-six 100-L tanks and stocked for acclimatization to the lab conditions with a control diet within a started period of 14 days. Fish (average initial weight of 7.11 ± 0.06 g) were randomly distributed into one of six treatment groups (6 replicate tanks \times 15 fish per tank in each treatment group). Fish in the first group was assigned as a negative control without CD challenge or PR supplementation. Fish in the other five groups were challenged with CD by immersion of fish for 60 min into a 10-L water bath supplemented with 6×10^6 CFU/mL (median lethal dose, LD₅₀) of pathogenic *F. columnare* bacteria. After infection, the fish were restored to their tanks and fed on a basal diet supplemented with PR at 0, 3, 6, 9, or 12 g/kg diet. The experimental period continued for 6 consecutive weeks in which the feed was introduced twice a day (8:00 and 15:00 h) at a rate of 2% of the fish biomass. Ten percent of water was siphoned and renewed after each meal every day, in addition to 50% of water refreshment after cleaning the tank every three days. The tanks were continuously aerated and provided with standard rearing conditions for carp fish ($24.0 \pm 1.12^\circ\text{C}$, 7.7 ± 0.22 pH, 6.3 ± 0.16 mg/L O₂, and 14L/10D photoperiod). The growth performance traits such as feed intake (FI), weight gain (WG), final weight (FW), specific growth rate (SGR), feed efficiency (FE), and cumulative mortality rates (CM) were recorded during the experimental period. At the end of the trial, blood samples were obtained from the fish to evaluate some plasma biochemicals, including aspartate aminotransaminase (AST), alanine amino- transferase (ALT), creatinine (CRE), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), antioxidant biomarkers, including total antioxidant capacity (TAOC), total superoxide dismutase (TSOD), reduced glutathione (rGSH), and catalase (CAT), stress indicators, including heterophil to lymphocyte (H/L) ratio, cortisol (COR), malondialdehyde (MDA), and myeloperoxidase (MPO), and

immunological reactions, including peripheral blood leukocyte proliferation (PBLP), phagocytosis activity (PHG), lysozyme activity (LYS), alternative complement hemolytic action (ACH50), and total immunoglobulin concentration (TIG). In addition, samples of infected fish gills were taken to quantify the number of *F. columnare* in the PR-supplemented groups using the quantitative real-time polymerase chain reaction (qPCR) technique. The results showed that incorporating PR into the dietary ingredients of common carp has a protective effect against the challenge with *F. columnare* infection. There were linear and quadratic positive trends ($P < 0.05$) in most parameters of growth performance, plasma biochemicals, antioxidant activity, stress indicators, and immunological reactions with the increased PR-supplemented levels in the diet of infected fish. The best results were obtained when using PR at 9 g/kg in the diet, while higher levels (12 g/ kg PR) showed an adverse trend in the evaluated parameters. The FI, WG, FW, SGR, and FE were improved by approximately 37, 104, 34, 73, and 49% in the fish treated with 9 g/kg PR compared to none-PR-infected fish. In addition, adding PR at the 9 g/kg diet level was the best dose that reduced the H/L ratio, COR, MDA, and MPO by about 14, 52, 48, and 29%, respectively, in the infected fish. Furthermore, the mortality rate was reduced by 94%, and the number of pathogenic bacteria cells adherent to the fish gills was lowered by 96% in the infected fish treated with 9 g/kg PR compared to none-PR infected fish. Our results concluded that dietary supplementation with 9 g/kg PR could be a promising nutritional approach for improving the growth performance, physiological profile, and health status of common carp fish, particularly when challenged with *F. columnare* or similar bacterial infections.

