# Dietary Hypericum perforatum L. extract improves growth performance, antioxidant capacity, immune performance and intestinal flora of Koi carp (Cyprinus carpio)

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## Abstract

This study aimed to evaluate the effects of *Hypericum perforatum* L. extract (HPLE) on the growth, antioxidant, non-specific immunity, and intestinal flora of Koi carp (*Cyprinus carpio*). We randomly divided fish into four groups: CON (basal diet group with 0% HPLE), HPLE.1 (basal diet plus 0.1% HPLE), HPLE.3 (basal diet plus 0.3% HPLE), and HPLE.5 (basal diet plus 0.5% HPLE). After the 10-week feeding trial, blood, liver, kidney, and brain samples were taken to assess serum non-specific immunity, antioxidant and immune gene expression, and gut microbial community. The results indicate that the WG, WGR, and SGR were increased in the HPLE.5 treatment, the SI was reduced in the HPLE.3 treatment and the VSI was reduced in the HPLE.1 treatment. Dietary HPLE decreased FCR. Dietary HPLE decreased the concentration of serum TP, ACP, and ALP, and increased the concentration of serum IGF-1, LZM, and GH. Serum levels of DA, 5-HT, and NE were elevated in the HPLE.5 treatment. The mRNA expression of *tnf-α, il-6*, and *il-1β* were downregulated, while that of *sod*, *ghr1a*, and *ghr1b* were upregulated. At the phylum level, Bacteroidota was more abundance in the HPLE.5 treatment, whereas Acidobacteriota was less abundance in the HPLE.3 and HPLE.5 treatment. Intestinal bacteria, indicated by Chao 1 and observed_otus index, was more diverse in the HPLE.5 group. The principal coordinate analysis (PCoA) revealed that HPLE.1, HPLE.3, and HPLE.5 treatments had distinct bacterial communities from the control group. According to LEfSe analysis at the genus level, *Ralstonla* and *Clostridium_sensu_stricto_1* were enriched in the HPLE.1 group, while *Aeromonadales* was enriched in the HPLE.3 group and *Vibrio* and *Dielma* were enriched in the HPLE.5 group. In conclusion, dietary supplementation with HPLE considerably improved koi growth performance, antioxidant capacity, immunological response, and intestinal flora, with 0.5% being the optimum inclusion rate of *Hypericum perforatum* L. extract.

## Methods

**Table 1 Composition and nutrient levels per kg of diet**

| Ingredients（Per kg） | CON | HPLE.1 | HPLE.3 | HPLE.5 |
|---|---|---|---|---|
| Soybean meal% | 30.00 | 30.00 | 30.00 | 30.00 |
| Fish meal% | 20.50 | 20.50 | 20.50 | 20.50 |
| Wheat flour% | 20.00 | 20.00 | 20.00 | 20.00 |
| Wheat bran% | 15.00 | 14.90 | 14.70 | 14.50 |
| *Hypericum perforatum* L. extract % | 0.00 | 0.10 | 0.30 | 0.50 |
| Rapeseed meal % | 10.00 | 10.00 | 10.00 | 10.00 |
| Soybean oil% | 3.50 | 3.50 | 3.50 | 3.50 |
| Vitamin and trace mineral premix | 1.00 | 1.00 | 1.00 | 1.00 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Nutrient | | | | |
| Dry Matter% | 89.19 | 89.19 | 89.19 | 89.19 |
| Crude Protein% | 35.45 | 35.45 | 35.45 | 35.45 |
| Crude Fat% | 6.08 | 6.08 | 6.08 | 6.08 |
| Crude Ash% | 7.04 | 7.04 | 7.04 | 7.04 |
| Neutral Detergent Fiber% | 10.18 | 10.18 | 10.18 | 10.18 |
| Acid Detergent Fiber% | 4.14 | 4.14 | 4.14 | 4.14 |

## Conclusions

- *Hypericum perforatum* L. extract resulted in notable enhancements in the growth, antioxidant capacity, gut microbiota, and immune response of Koi.
- Dietary supplementation with 0.5% *Hypericum perforatum* L. extract was found to be the most effective.
- Thus, *Hypericum perforatum* L. extract is suggested to be a potential feed additive for aquatic species.

## Funds

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## Results

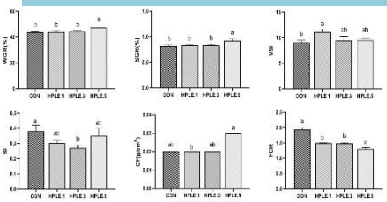

**Figure 1.** Effects of Different Contents of HPLE on the Growth Indexes of Koi Carp.

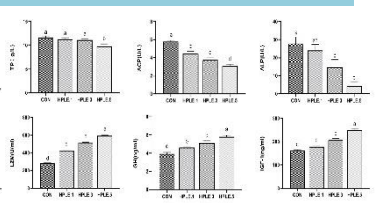

**Figure 2.** Effects of Different Doses of HPLE on serum TP , ACP , ALP, LZM , GH , and ALP levels of Koi Carp.

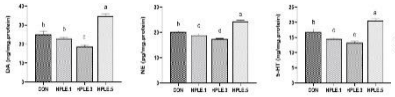

**Figure 3.** Effects of Different Doses of HPLE on DA, NE, and 5-HT levels in brain of Koi Carp.

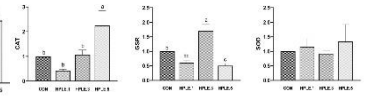

**Figure 4.** Effects of Different Doses of HPLE on *cat* , *gsr* , and *sod* gene expression in liver of Koi Carp.

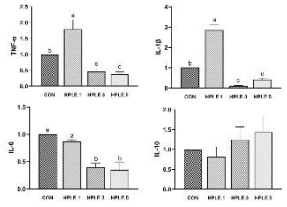

**Figure 5.** Effects of Different Doses of HPLE on *tnf-α*, *il-1β*, *il-6*, and *il-10* gene expression in head kidney of Koi Carp.

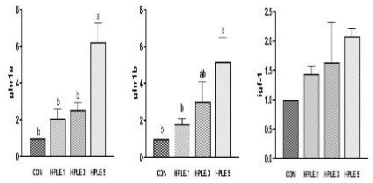

**Figure 6.** Effects of Different Doses of HPLE on *ghr1a*, *ghr1b*, and *igf-1* gene expression of Koi Carp.

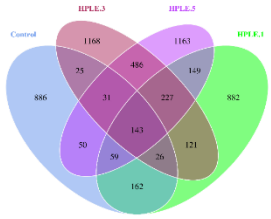


**Figure 7.** Venn diagram of intestinal flora.

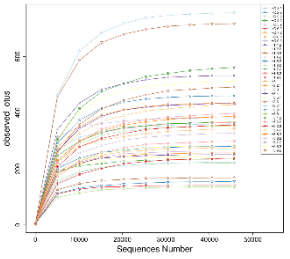


**Figure 8.** Rarefaction curves

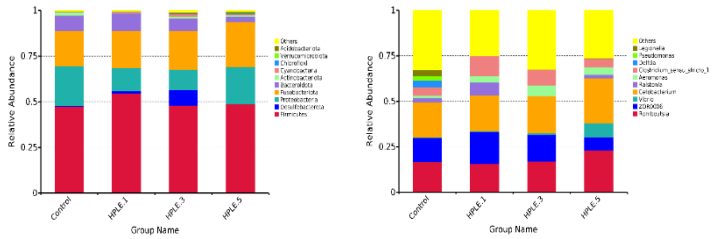


**Figure 9**. The relative abundance of gut bacteria at the level of phylum and genus.

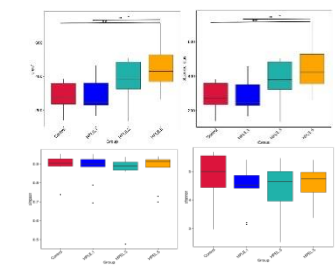

**Figure 10.** Alpha-diversity analysis

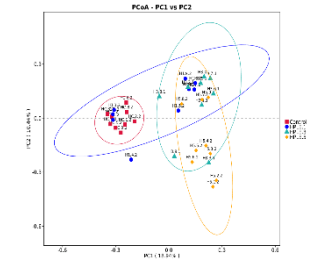


**Figure 11.** PCoA plot based on Unweighted unifrac distances among the dietary groups

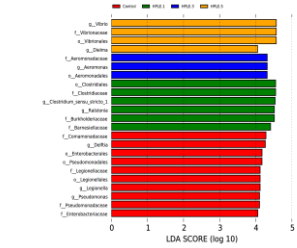


**Figure 12.** Linear discriminant analysis coupled with effectsize (LEfSe) of gut microbiota among treatment groups

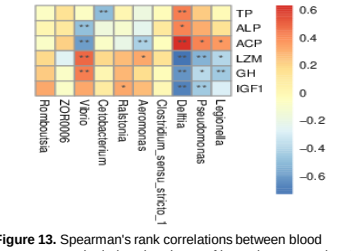


**Figure 13.** Spearman's rank correlations between blood parameters and relative abundance of bacteria at genus level. The 10 most abundant genera are included in the correlation analysis. Blue and red represent negative and positive correlations, respectively. Significant difference was detected by *t*-test between the HPLE-treated groups and control as *, ** at $P < 0.05$, $P < 0.001$, respectively.