

# Effects of combined florfenicol and quercetin to treat acute hepatopancreatic necrosis disease in *Litopenaeus vannamei* caused by *Vibrio parahaemolyticus*

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## ABSTRACT

The survival, disease resistance, and immunity of *Litopenaeus vannamei* infected with *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis syndrome (VPAHPND), and treated with quercetin and florfenicol, alone or in combination were evaluated. Compared with either drug alone, the shrimps in the florfenicol and quercetin combination groups showed significantly lower cumulative mortality over 5 days after infection ( $p < 0.05$ ). Moreover, in the drug combination groups, the vibrio density were consistently lower and the immune parameters were consistently higher than those in single drug groups ( $p < 0.05$ ). In the drug combination groups, the hepatopancreatic tubule structure and integrity were better than those in the single drug groups. Thus, the use of florfenicol and quercetin combined improved the survival rate, disease resistance, and immunity of shrimp challenged with VPAHPND compared with either drug alone. The use of florfenicol could be reduced to gain an improved therapeutic effect.

## INTRODUCTION

With the development of intensive culture and the deterioration of the ecological environment, the occurrence and spread of acute hepatopancreatic necrosis disease (AHPND), have emerged as one of the most serious threats for shrimp aquaculture worldwide today, causing massive mortality and marked production loss. For a long time, antibiotics has been widely used to prevent and control diseases. However, with excessive use of these drugs, issues such as the emergence of drug-resistant microbial strains, environmental pollution, and drug residue accumulation have exacerbated. Medicinal herbs have attracted a lot of attention, because they are safer to aquatic animals and ecofriendly. Thus, to achieve better preventive or treatment effects, and to reduce the use of antibiotics, we focused on the combined utilization of traditional Chinese herbs and antibiotics.

Table 1. Formulation and chemical proximate composition of the experimental diets

Ingredients	Groups						
	Control	Infected Only	Quercetin	Florfenicol	Low-dose combination	Mid-dose combination	High-dose combination
Fish meal (g/kg)	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Wheat glutens (g/kg)	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Wheat meal (g/kg)	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Cellulose (g/kg)	180.0	180	179.6	179.99	179.79	179.59	179.17
Fish oil (g/kg)	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Soybean oil (g/kg)	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Soybean phospholipids (g/kg)	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Gelatin (g/kg)	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Choline chloride (g/kg)	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin mix (g/kg)	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mineral mix (g/kg)	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Quercetin (mg/kg)	0	0	400	0	200	400	800
Florfenicol (mg/kg)	0	0	0	15	7.5	15	30
Proximate nutrient composition (as fed)							
Crude protein (g/kg)	431.0	431.0	431.0	431.0	431.0	431.0	431.0
Crude fat (g/kg)	73.0	73.0	73.0	73.0	73.0	73.0	73.0
Crude ash (g/kg)	68.0	68.0	68.0	68.0	68.0	68.0	68.0
Total energy (kJ/g)	16.44	16.44	16.44	16.44	16.44	16.44	16.44

## RESULTS

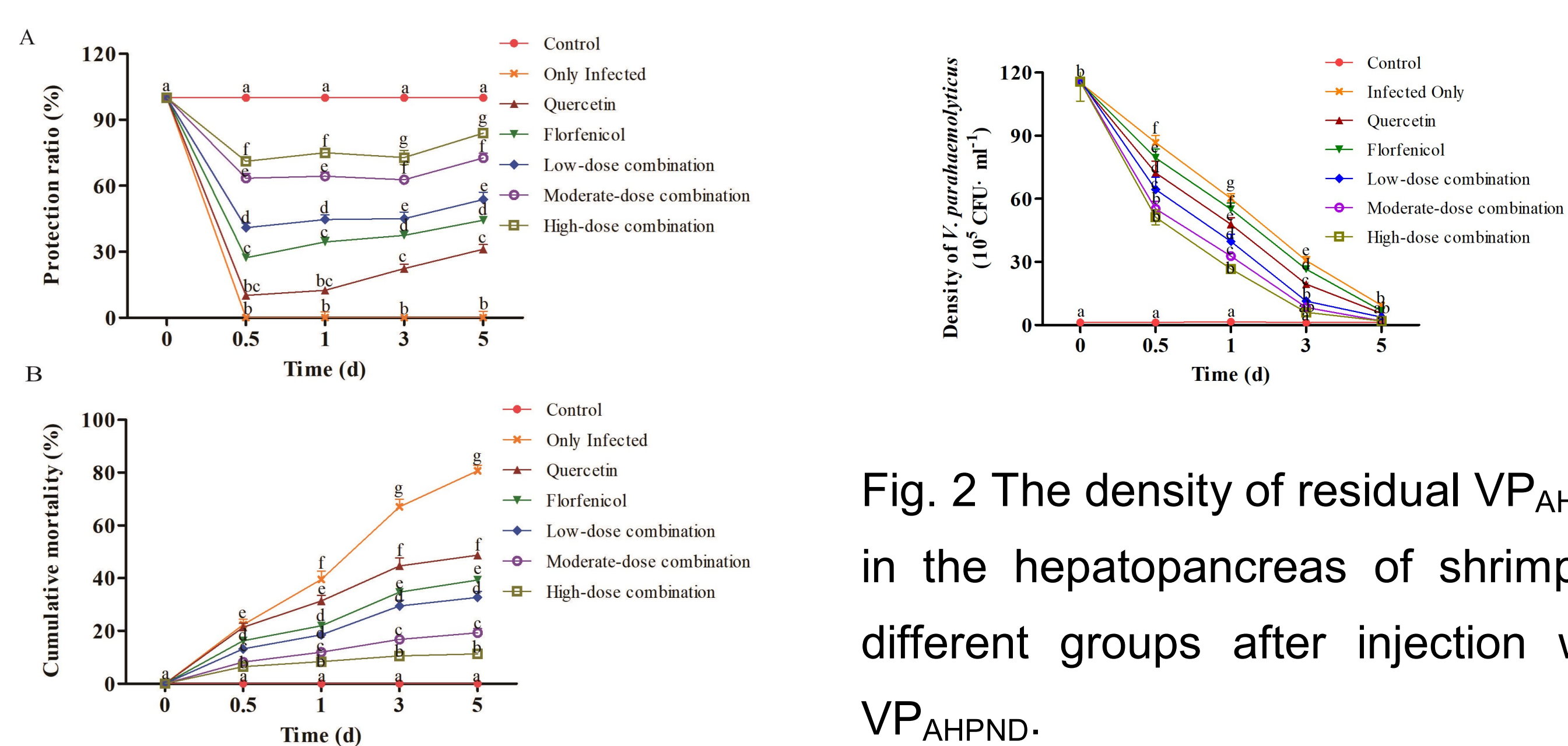


Fig. 1 Cumulative mortality (A) and protection rates (B) of *L. vannamei* in different groups after infection with VPAHPND.

Fig. 3 The total hemocyte counts (THCs) (A), hemocyanin (HEM) concentration (B), and antibacterial activity (C) of shrimp after injection with VPAHPND.

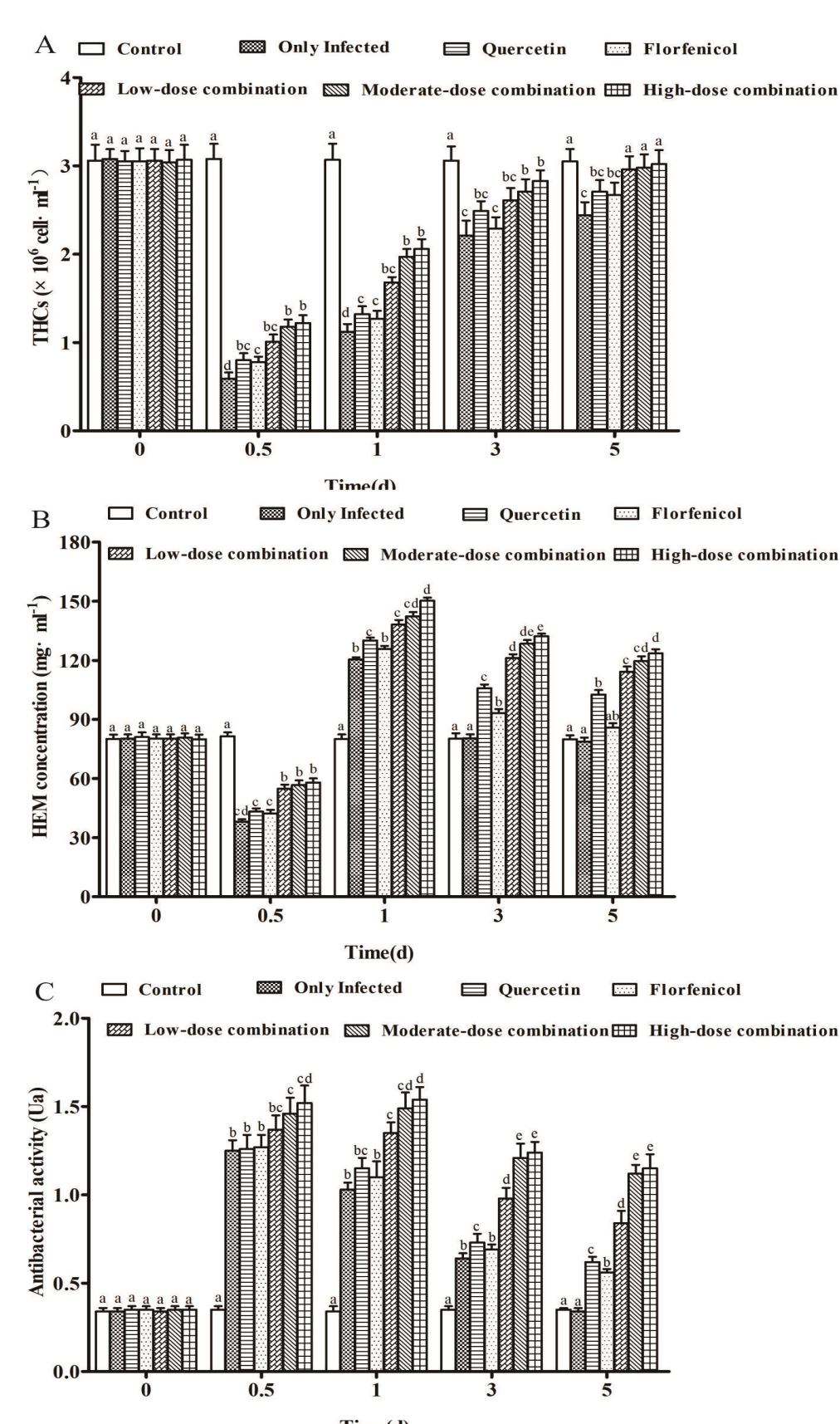


Fig. 2 The density of residual VPAHPND in the hepatopancreas of shrimp in different groups after injection with VPAHPND.

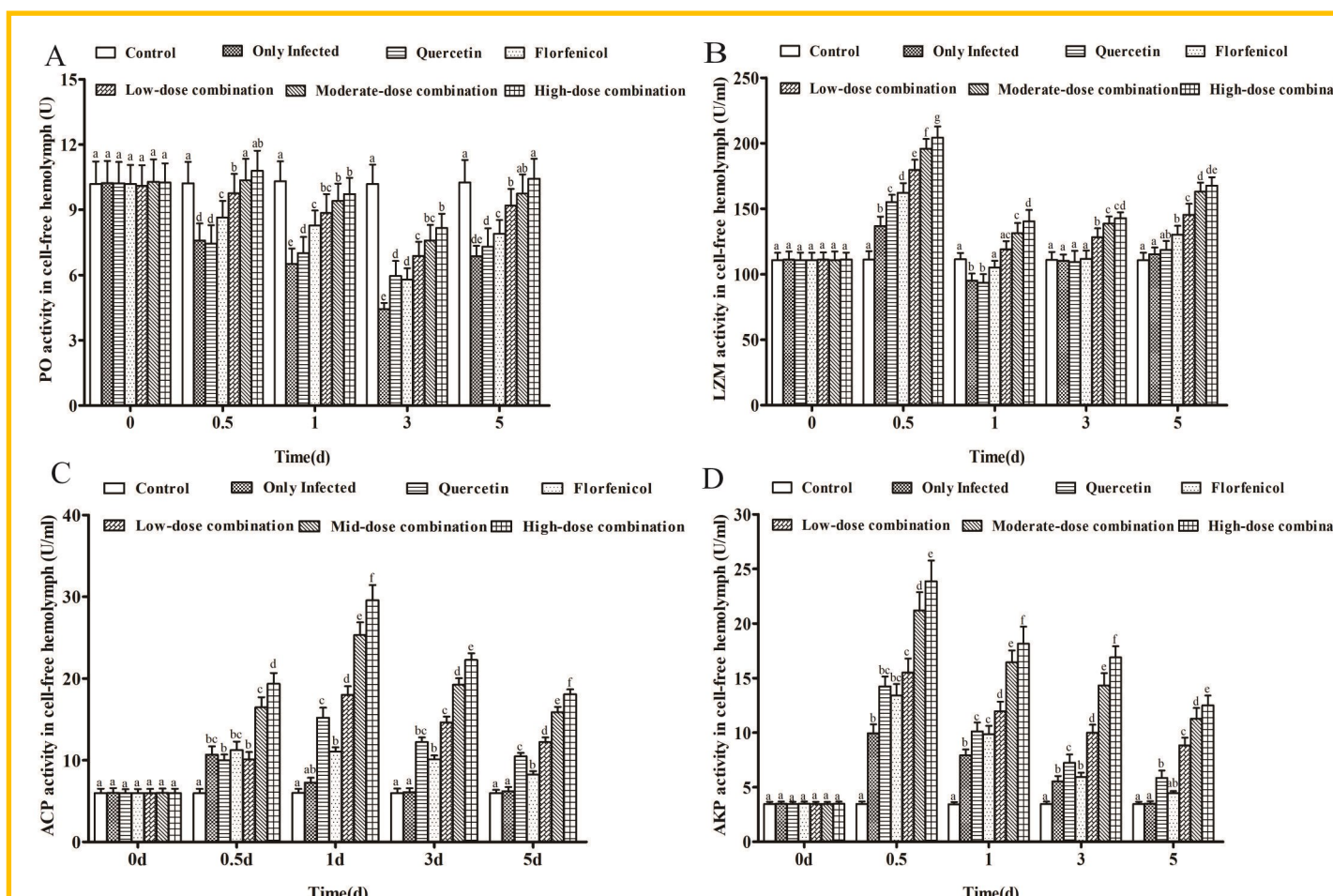
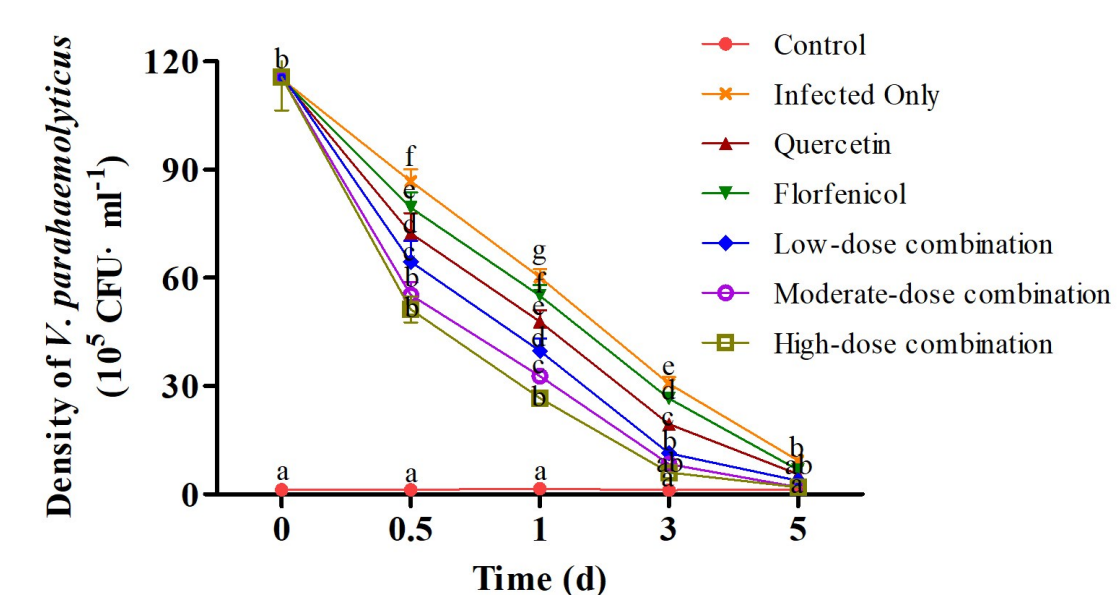


Fig. 4 The immune enzyme activities in cell-free hemolymph of shrimp after injection with VPAHPND: PO (A), LZM (B), ACP (C), and AKP (D).

Fig. 5 Expression profiles of immune-related genes in hemocytes of shrimp after infection with VPAHPND: anti-lipopolysaccharide factor (ALF) (A), cathepsin B (catB) (B), crustin (C), lectin (Lec) (D), lysozyme (LZM) (E), and Toll-like receptor (TLR) (F).

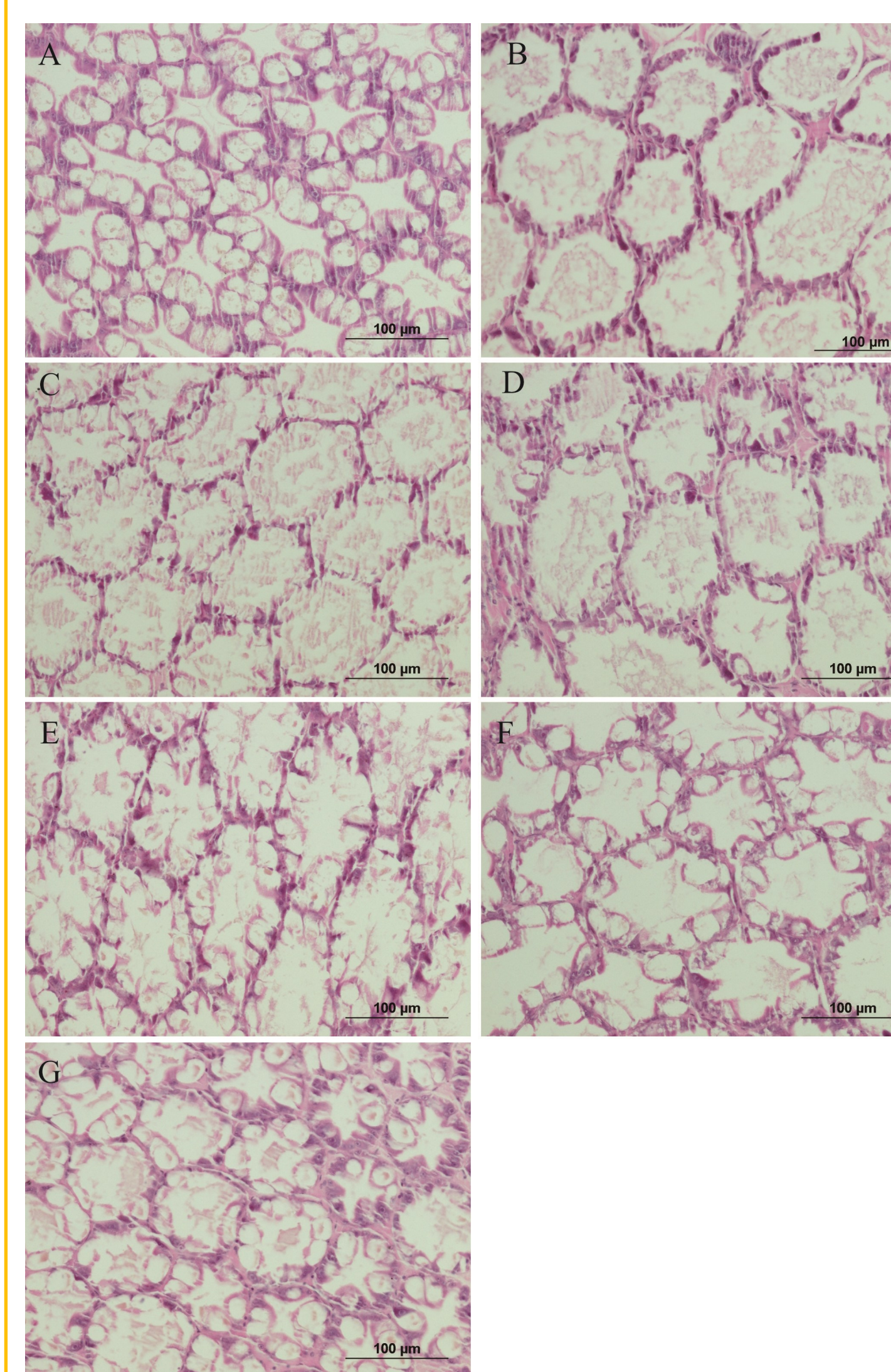
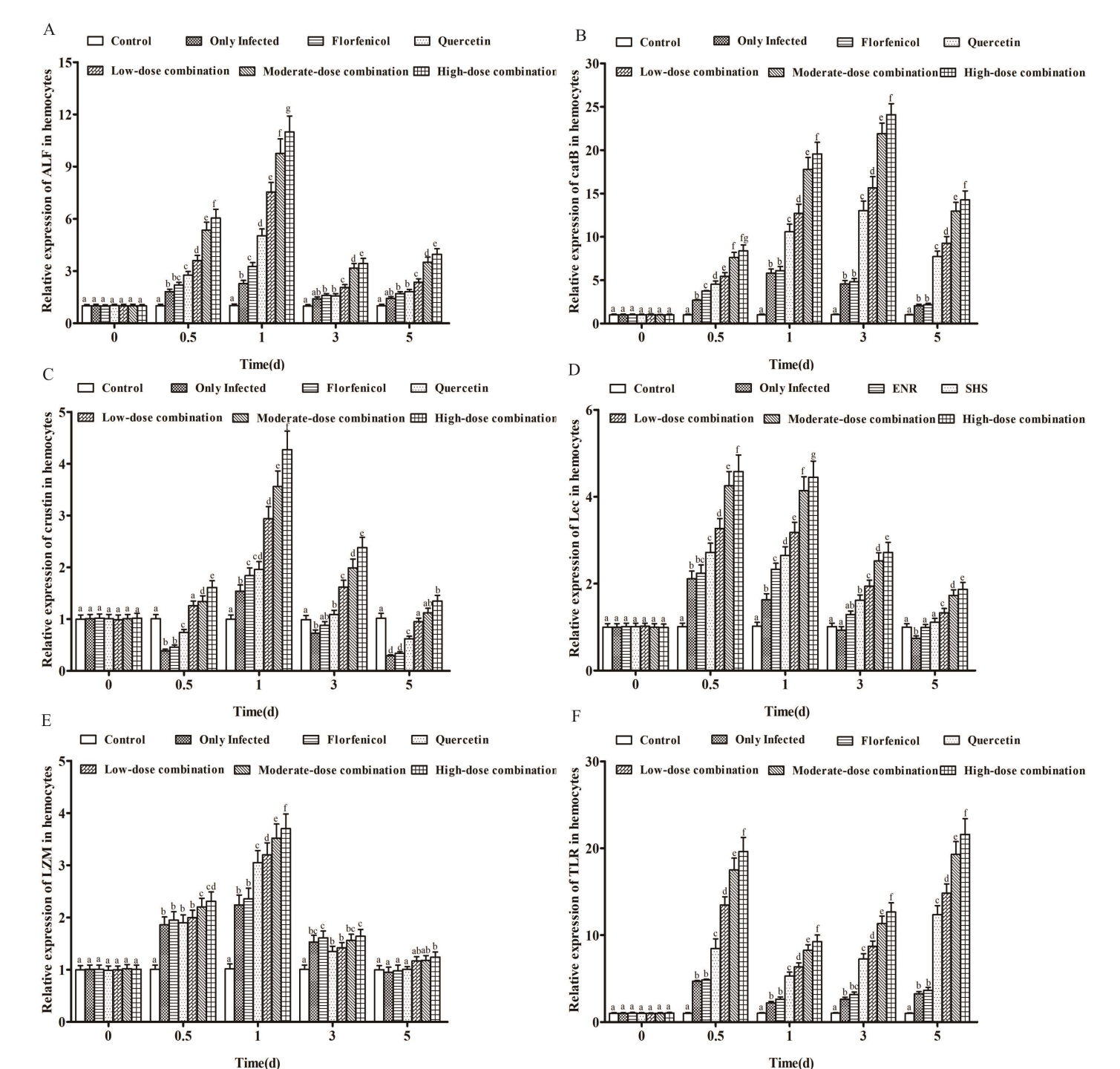


Fig. 6 Histological sections of the hepatopancreases obtained from shrimp in the control group (A), only infected group (B), quercetin group (C), florfenicol group (D), low-dose combination group (E), moderate-dose combination group (F), and high-dose combination group (G).

## CONCLUSIONS

1. The combined used of florfenicol and quercetin improved the immunity of VPAHPND-infected shrimp.
2. The combined used of florfenicol and quercetin enhanced the disease resistance of VPAHPND-infected shrimp.
3. The immunity and disease resistance of shrimps were improved with the increase of the florfenicol + quercetin dose.