



Luteolin protects against acute hepatopancreatic necrosis disease-causing strain of *Vibrio parahaemolyticus* in *Litopenaeus vannamei*

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ABSTRACT

The survival, disease resistance, and immunity of *Litopenaeus vannamei* infected with *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis syndrome (VP_{AHPND}), and treated with luteolin were evaluated. Compared with the infected only group, the shrimps in the luteolin groups showed significantly lower cumulative mortality over 5 days after infection ($p < 0.05$). In the luteolin groups, the vibrio density were consistently lower and the immune parameters were consistently higher than those in infected only group ($p < 0.05$). In the luteolin groups, the hepatopancreatic tubule structure and integrity were better than those in the infected only group. Moreover, with the dose of luteolin increased, the therapeutic effect was also enhanced. Thus, the use of luteolin improved the survival rate, disease resistance, and immunity of shrimp challenged with VP_{AHPND}.

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 reduce or avoid the use of antibiotics, and to achieve good preventive or treatment effects, we focused on the utilization of traditional Chinese herbs.

RESULTS

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

	Groups				
	Control	Infected Only	Luteolin-L	Luteolin-M	Luteolin-H
Fish meal (g/kg)	200.0	200.0	200.0	200.0	200.0
Wheat gluters (g/kg)	300.0	300.0	300.0	300.0	300.0
Wheat meal (g/kg)	200.0	200.0	200.0	200.0	200.0
Cellulose (g/kg)	180.0	180.0	179.95	179.9	179.8
Fish oil (g/kg)	25.0	25.0	25.0	25.0	25.0
Soybean oil (g/kg)	25.0	25.0	25.0	25.0	25.0
Soybean phospholipids (g/kg)	20.0	20.0	20.0	20.0	20.0
Gelatin (g/kg)	20.0	20.0	20.0	20.0	20.0
Choline chloride (g/kg)	10.0	10.0	10.0	10.0	10.0
Vitamin mix (g/kg)	10.0	10.0	10.0	10.0	10.0
Mineral mix (g/kg)	10.0	10.0	10.0	10.0	10.0
Luteolin (mg/kg)	0	0	50	100	200
Proximate nutrient composition (as fed)					
Crude protein (g/kg)	431.0	431.0	431.0	431.0	431.0
Crude fat (g/kg)	73.0	73.0	73.0	73.0	73.0
Crude ash (g/kg)	68.0	68.0	68.0	68.0	68.0
Total energy (kJ/g)	16.44	16.44	16.44	16.44	16.44

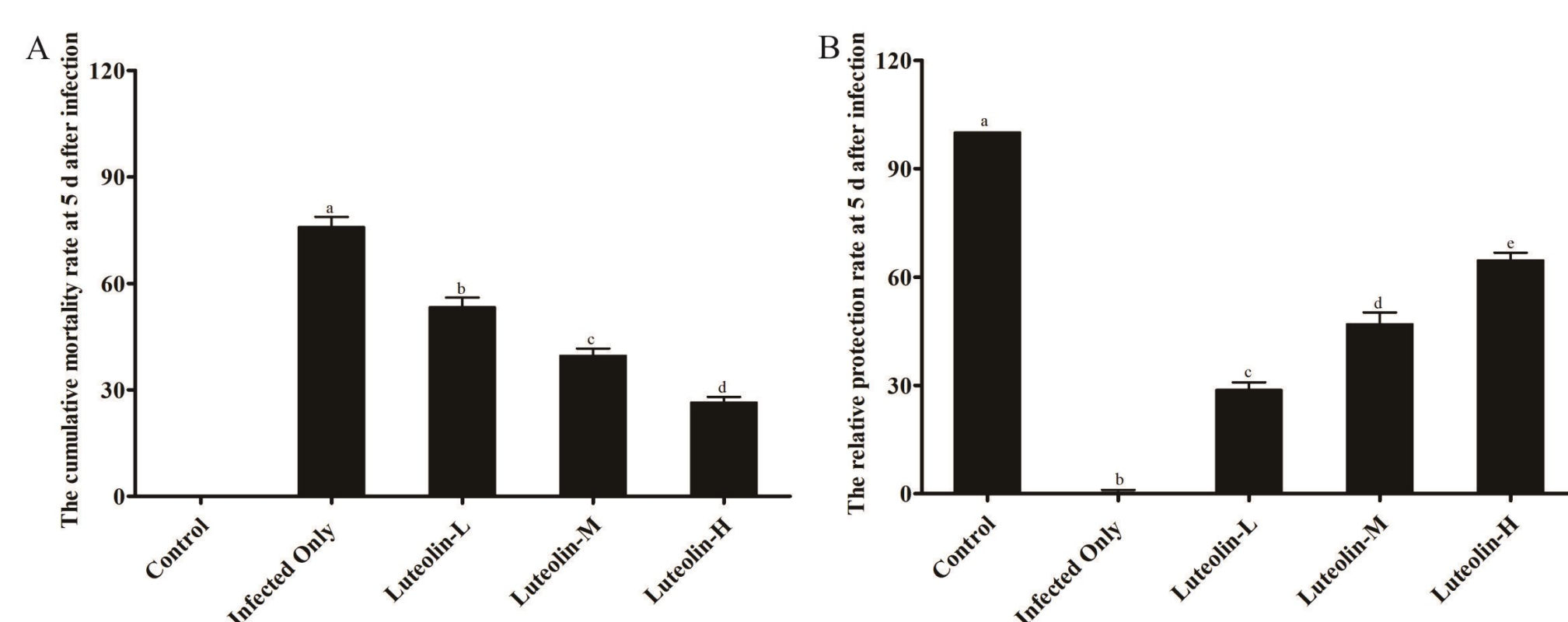


Fig. 1 Cumulative mortality (A) and protection rates (B) of *L. vannamei* in different groups after infection with VP_{AHPND}.

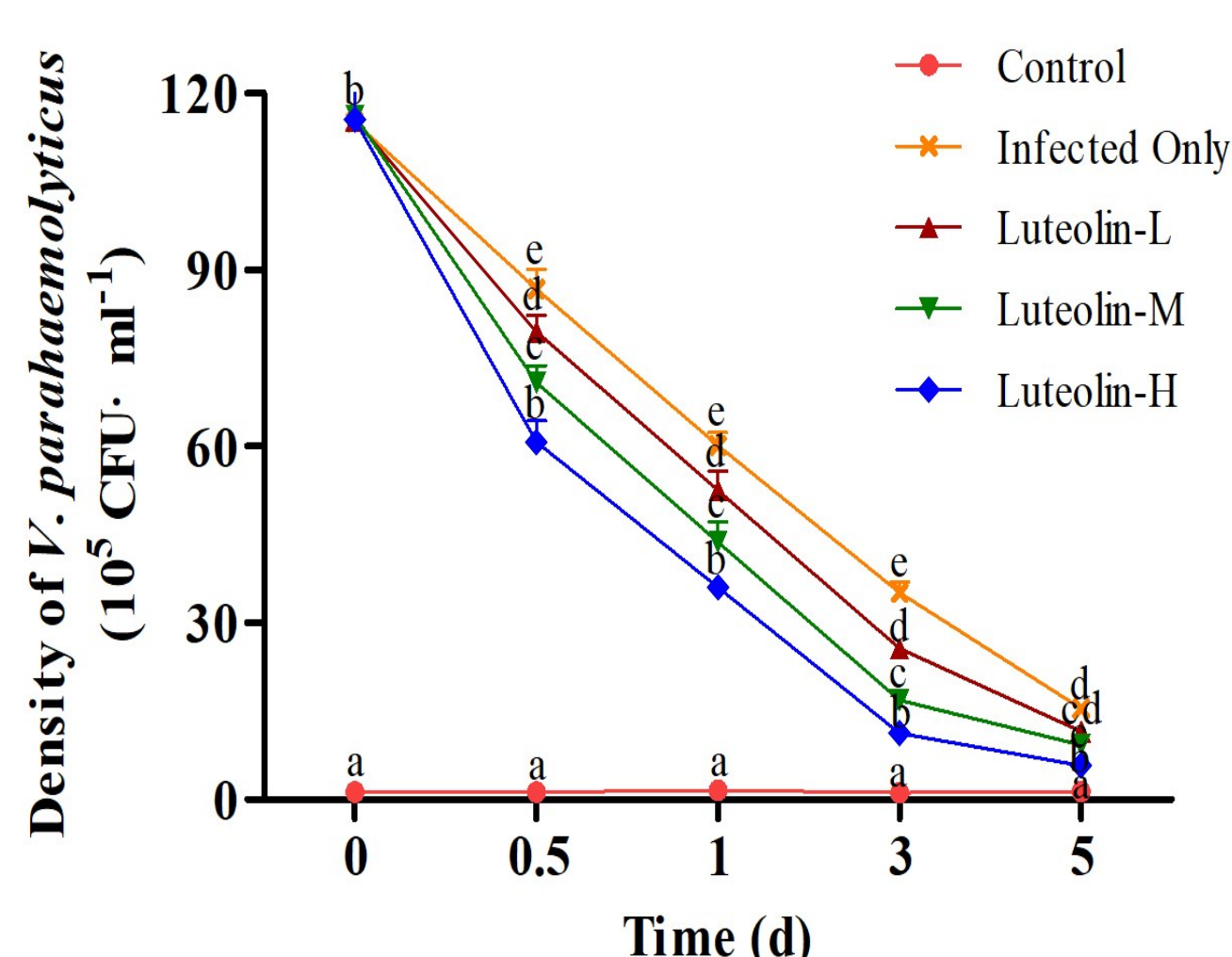


Fig. 2 The density of residual VP_{AHPND} in the hepatopancreas of shrimp in different groups after injection with VP_{AHPND}.

Fig. 3 The total hemocyte counts (THCs) (A) and antibacterial activity (B) of shrimp after injection with VP_{AHPND}.

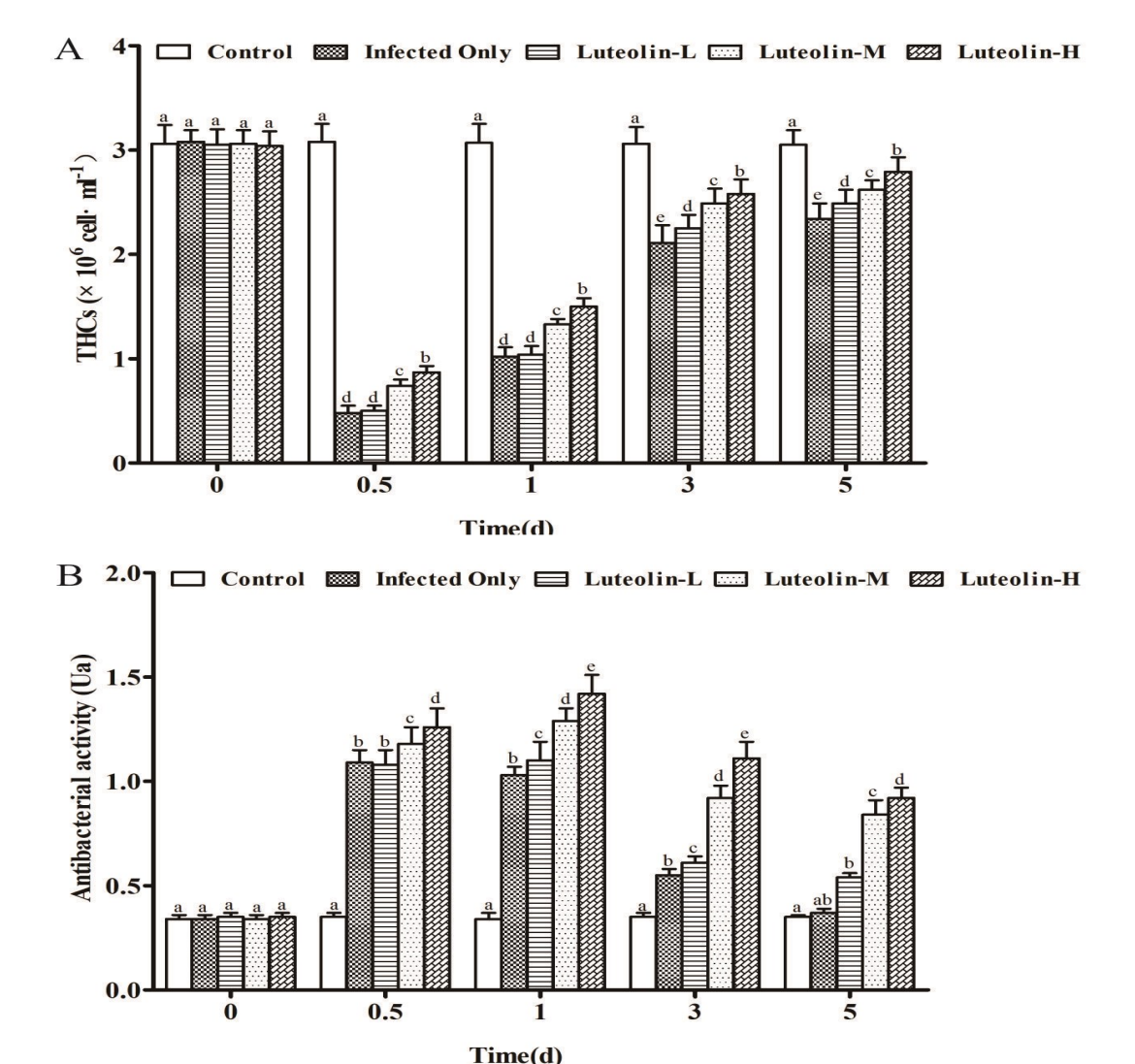


Fig. 4 The immune enzyme activities in cell-free hemolymph of shrimp after injection with VP_{AHPND}: SOD (A), LZM (B), ACP (C), and AKP (D).

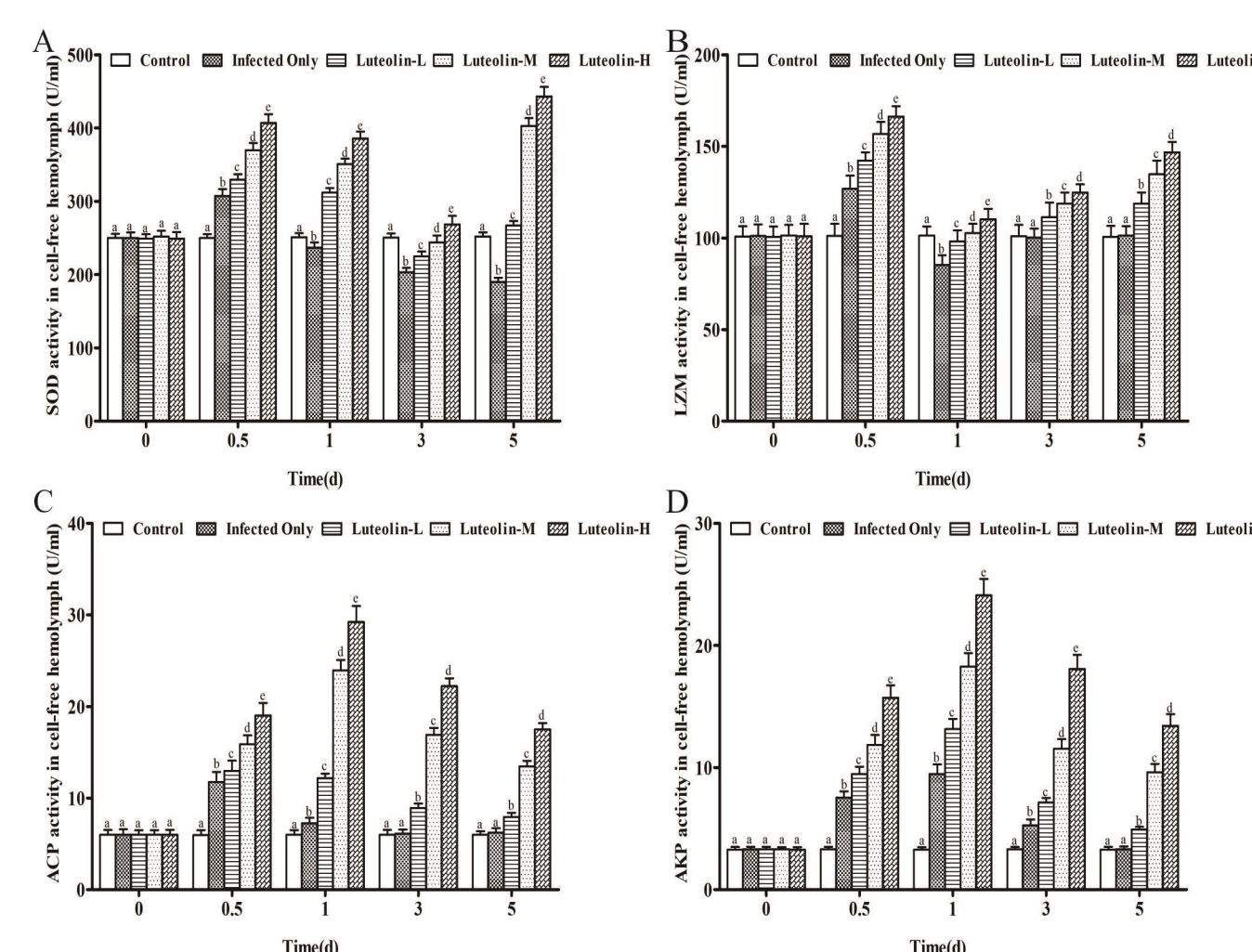


Fig. 5 Expression profiles of immune-related genes in hemocytes of shrimp after infection with VP_{AHPND}: anti-lipopolysaccharide factor (*Aif*) (A), cathepsin B (*CatB*) (B), crustin (*Cru*) (C), lectin (*Lec*) (D), lysozyme (*Lzm*) (E), and Toll-like receptor (*Tlr*) (F).

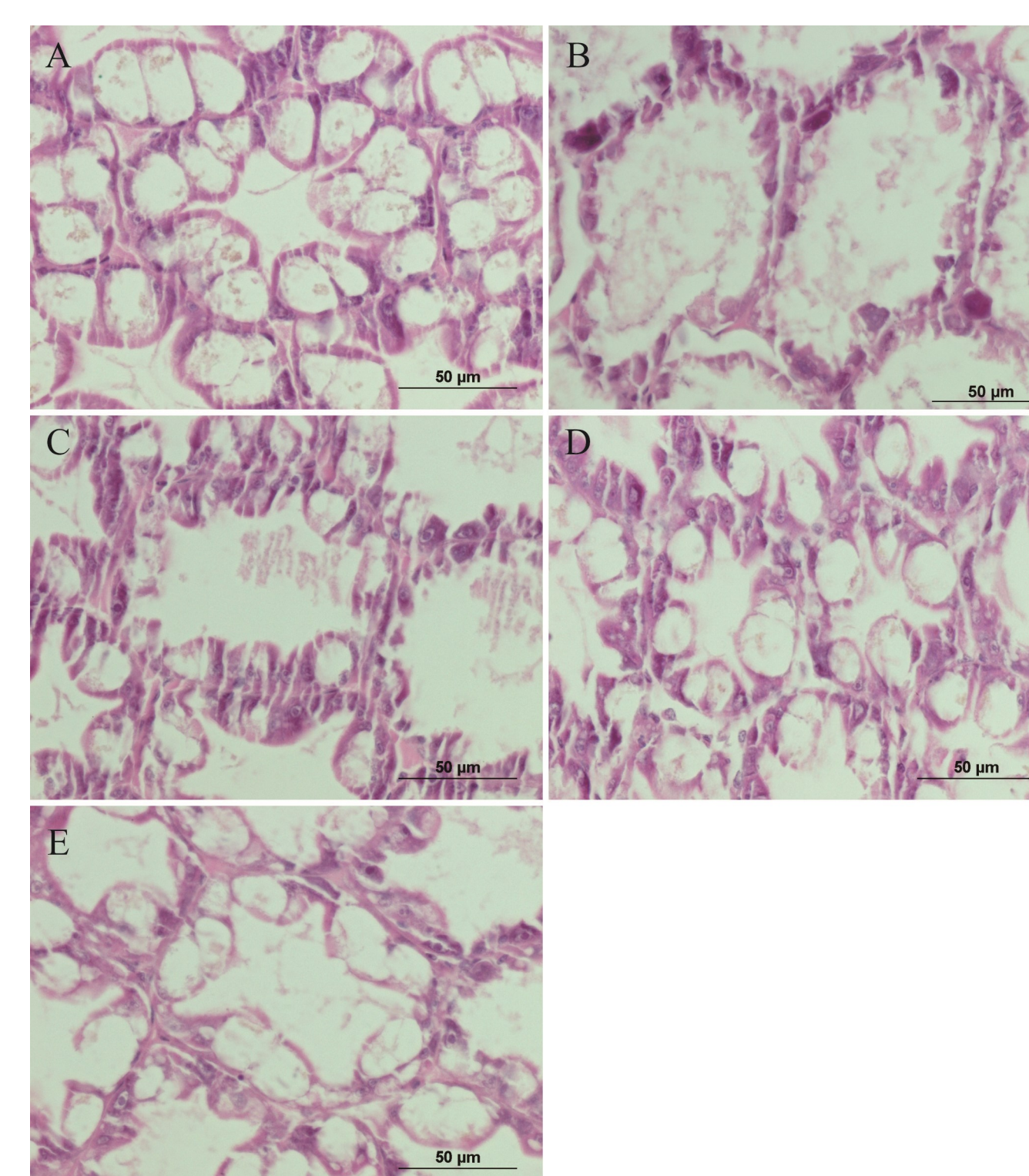
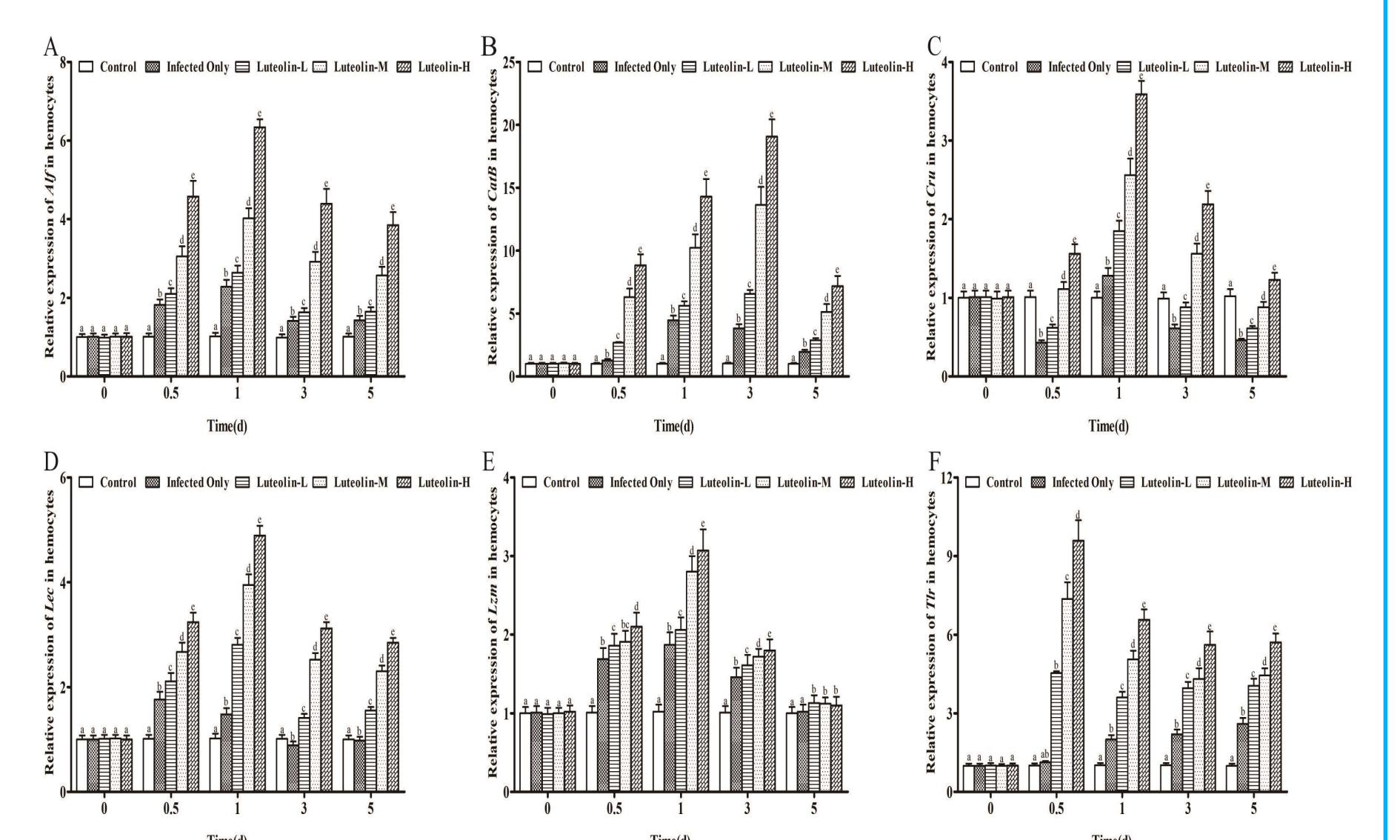


Fig. 6 Histological sections of the hepatopancreases obtained from shrimp in the control group (A), infected only group (B), luteolin low dose group (C), luteolin moderate dose group (D), luteolin high dose group (E).

CONCLUSIONS

1. The used of luteolin improved the immunity of VP_{AHPND}-infected shrimp.
2. The used of luteolin enhanced the disease resistance of VP_{AHPND}-infected shrimp.
3. The immunity and disease resistance of shrimps were improved with the increase of the luteolin dose.