Bidens pilosa AS A MULTIFUNCTIONAL FEED ADDITIVE TO PROMOTE THE INNATE IMMUNITY OF PACIFIC WHITE SHRIMP AGAINST WSSV

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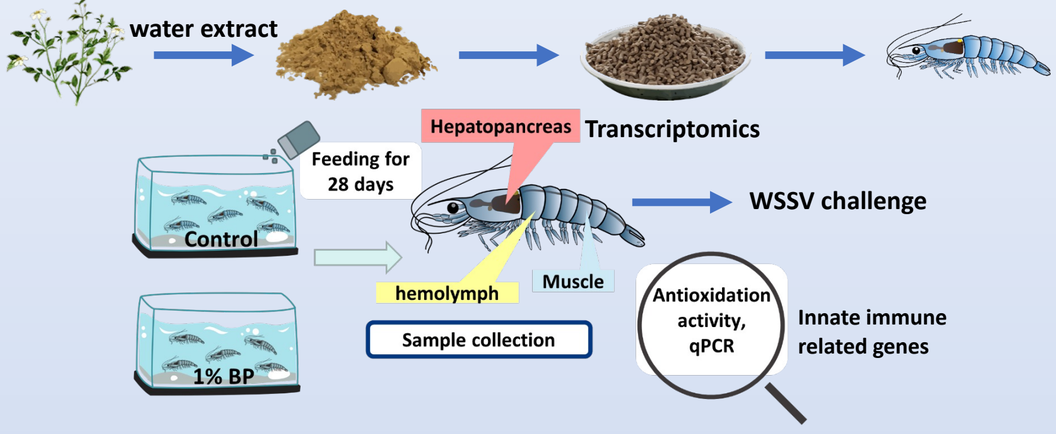
Introduction

Bidens pilosa is an asteraceae perennial plant, native to South America. Above two hundred compounds have been isolated from *B. pilosa*, especially polyacetylenes and flavonoids. It has been proven to prevent and treat type 1 and type 2 diabetes. There were several functions in the extractant of *Bidens pilosa* including anti-inflammatory, antioxidant, antibacterial, immunomodulatory, antifungal, anti-diabetic, anti-hyperglycemic, anti-malarial, antitumor, anti-ulcerative, and so on. *Bidens pilosa* could cure the gastrointestinal disease such as coccidiosis, enhanced the gut microbiota, and promoted growth rate in chicken. In our previous study, dietary *B. pilosa* can regulate endocrine IGF1 signaling and autocrine/paracrine MSTN signaling to activate the expression of MRFs to promote muscle growth in tilapia. In this study, we investigated the effect of dietary supplementation with an edible herb *Bidens pilosa* on the expression of non-specific immunity, and innate immunity-related genes in Pacific white shrimp (*Litopenaeus vannamei*).



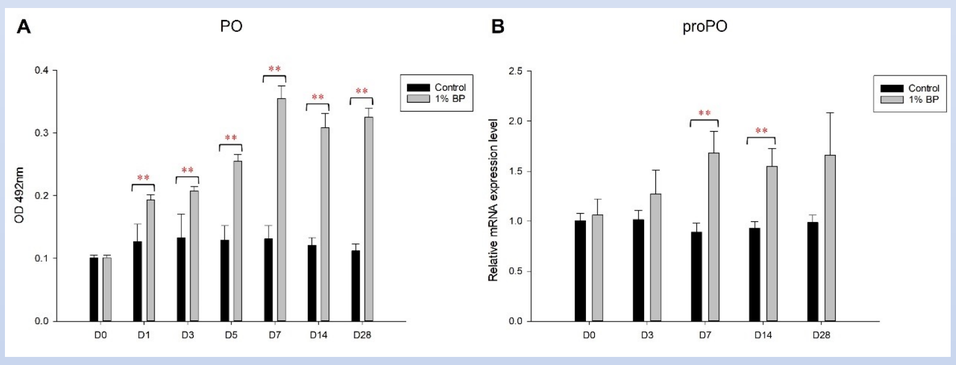


Fig. 1. ProPO system activity of white shrimp fed with 1% *B. pilosa*. (A) PO activity and (B) *proPO expression*. Control: shrimp fed with commercial feed; 1% BP: shrimp fed with 1% *B. pilosa*. The data are expressed as mean ± standard deviation of the mean (n = 3). **, $p < 0.01$.

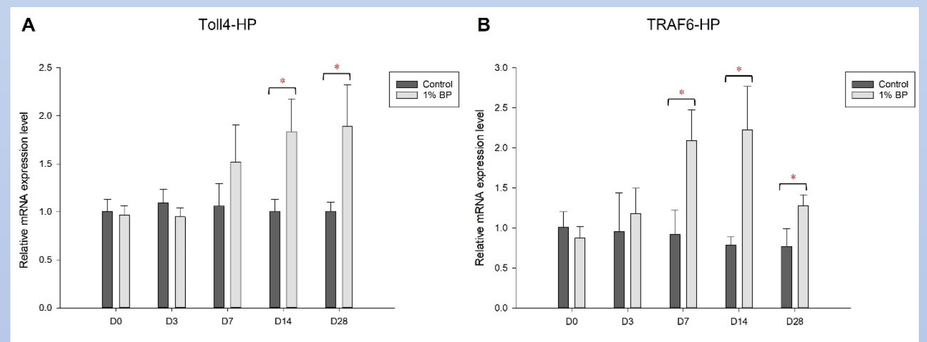


Fig. 2. Expression of PRR genes in the hepatopancreas (HP) of white shrimp fed with 1% *B. pilosa*. (A) *Toll4* and (B) *TRAF6*. Control: shrimp fed with commercial feed; 1% BP: shrimp fed with 1% *B. pilosa*. The data are expressed as mean ± standard deviation of the mean (n = 3). *, $p < 0.05$.

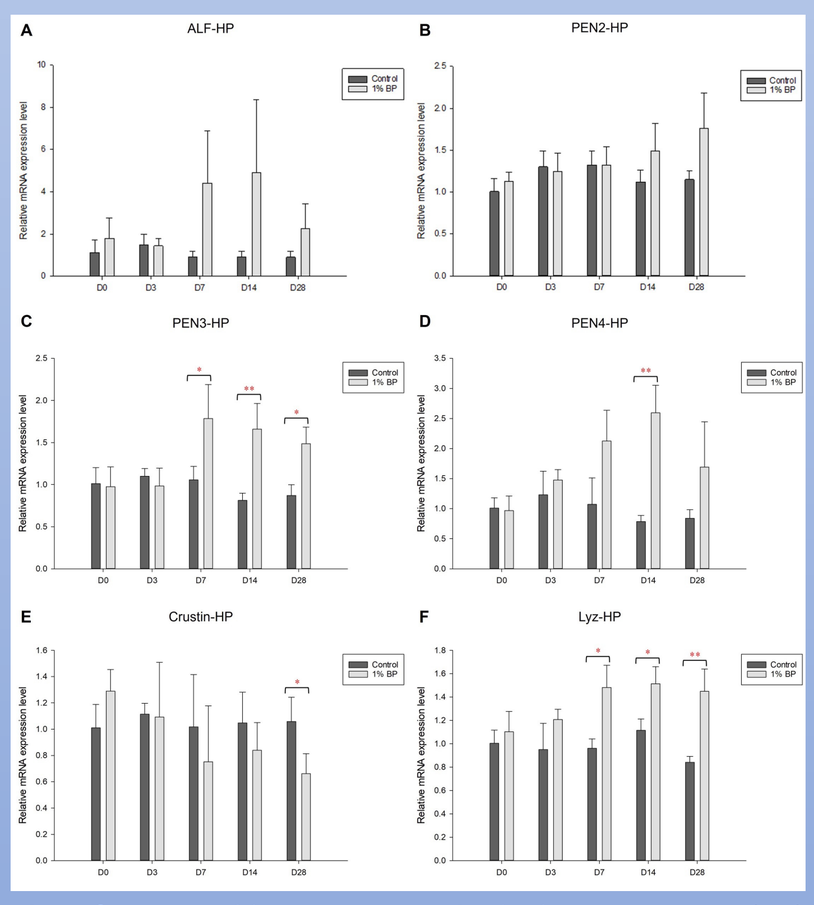


Fig. 3. Expression of AMPs and lysozyme genes in the hepatopancreas (HP) of white shrimp fed with 1% *B. pilosa*. (A) *ALF*, (B) *PEN2*, (C) *PEN3*, (D) *PEN4*, (E) *crustin*, and (F) *Lyz* Control: shrimp fed with commercial feed; 1% BP: shrimp fed with 1% *B. pilosa*. The data are expressed as mean ± standard deviation of the mean (n = 3). *, $p < 0.05$; **, $p < 0.01$.

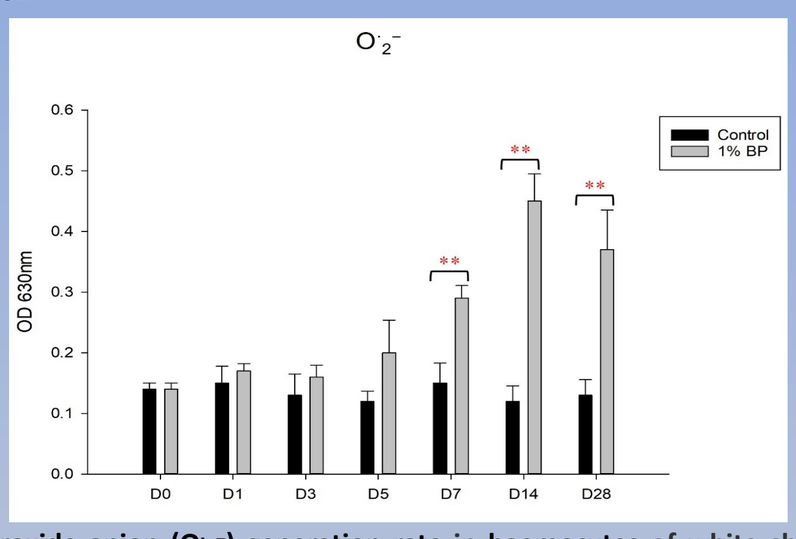


Fig. 4. Superoxide anion ($O^{\cdot}_2{}^-$) generation rate in haemocytes of white shrimp fed with 1% *B. pilosa*. Control: shrimp fed with commercial feed; 1% BP: shrimp fed with 1% *B. pilosa*. The data are expressed as mean ± standard deviation of the mean (n = 3). **, $p < 0.01$.

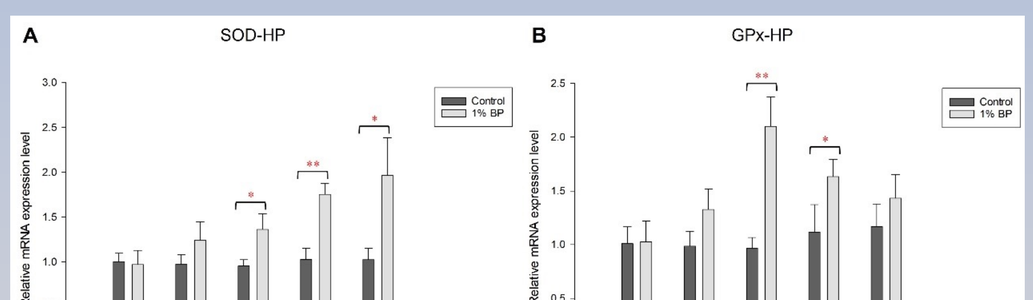


Fig. 5. Expression of antioxidant defence mechanism genes in the hepatopancreas (HP) of white shrimp fed with 1% *B. pilosa*. (A) *SOD* and (B) *GPx*. Control: shrimp fed with commercial feed; 1% BP: shrimp fed with 1% *B. pilosa*. The data are expressed as mean ± standard deviation of the mean (n = 3). *, $p < 0.05$; **, $p < 0.01$.

Fig. 7. Gene expression of immune-related pathways in transcriptome analysis. (A) Toll pathway, (B) IMD pathway, and (C) AMPs. Each transcript is presented as a bar. Control: shrimp fed with commercial feed; 1% BP: shrimp fed with 1% *B. pilosa*. The data are expressed as FPKM (1% BP/Control). *, $p < 0.05$.

Table 2. The differentially expressed genes from comparative transcriptomics of hepatopancreas of white shrimp fed with 1% BP and control diet for 14 days.

Description	Gene ID	Length	FPKM Control	FPKM BP	$\log_2$ Fold Change (BP/C)
Prophenoloxidase 1	Unigene4959_All	2296	4.75	20.42	2.1040
Prophenoloxidase 2	Unigene20354_All	2590	5.38	11.67	1.1171
Prophenoloxidase 3	Unigene20848_All	880	2.36	13.4	2.5054
Anti-lipopolysaccharide factor 4	Unigene16252_All	1092	0.57	2.53	2.1501
Penaeidin 2b	Unigene3087_All	746	9.24	25.37	1.4572
Penaeidin 3a	CL3005.Contig2_All	1699	13.87	42.23	1.6063
Penaeidin 3b	CL3005.Contig1_All	1047	24.54	152.72	2.6377
Penaeidin 4a	CL3005.Contig4_All	715	8.26	32.68	1.9842
Crustin	CL463.Contig1_All	685	68.97	222.68	1.6909
Mn Superoxidase dismutase	CL4196.Contig2_All	1214	0.01	27.31	11.4152
Glutathione peroxidase	CL2250.Contig3_All	2093	38.42	89.47	1.2195
Glutathione peroxidase 2	Unigene20703_All	1332	92.36	232.99	1.3349
Glutathione peroxidase 7	Unigene9546_All	1110	7.75	16.59	1.0980
myostatin	Unigene11729_All	1395	0.97	0.28	-1.7926
Activin receptor Type 2A	Unigene9246_All	3088	0.44	0.01	-5.4594

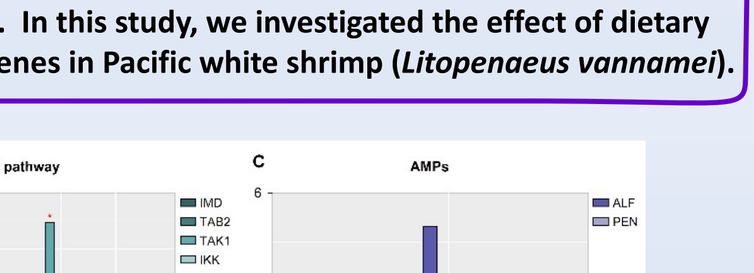


Fig. 8. Toll and IMD signaling pathways activated in white shrimp fed with *B. pilosa*. The activation of *Toll*, *TRAF6*, *Dorsal* in Toll pathway, and *IMD*, *TAB2*, *TAK1*, *IKK*, *Relish* in IMD pathway, and the inhibition of *Cactus* in Toll pathway promote the expression of downstream AMPs including *ALFs*, *PENs*.

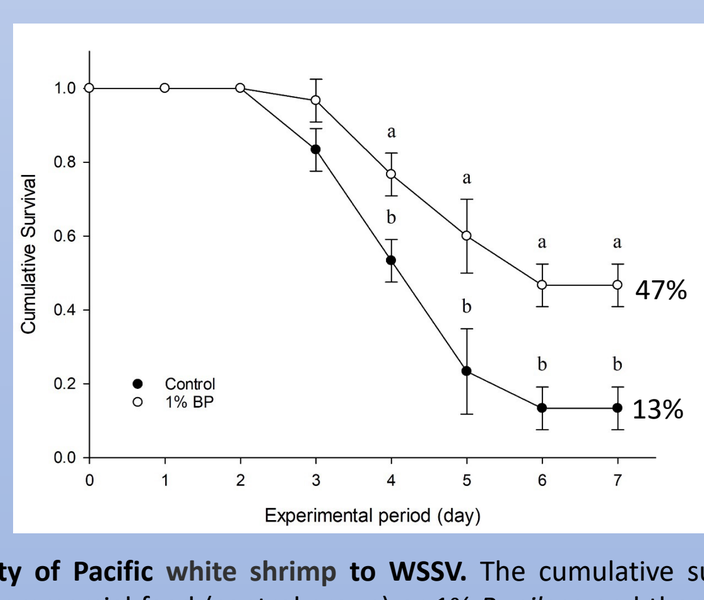


Fig. 9. Susceptibility of Pacific white shrimp to WSSV. The cumulative survival rate of white shrimp fed with commercial feed (control group) or 1% *B. pilosa* and then infected with WSSV. The data are expressed as mean ± standard deviation of the mean (total n = 30 for triplicates per group). Different letters indicate significant differences ($p < 0.05$).

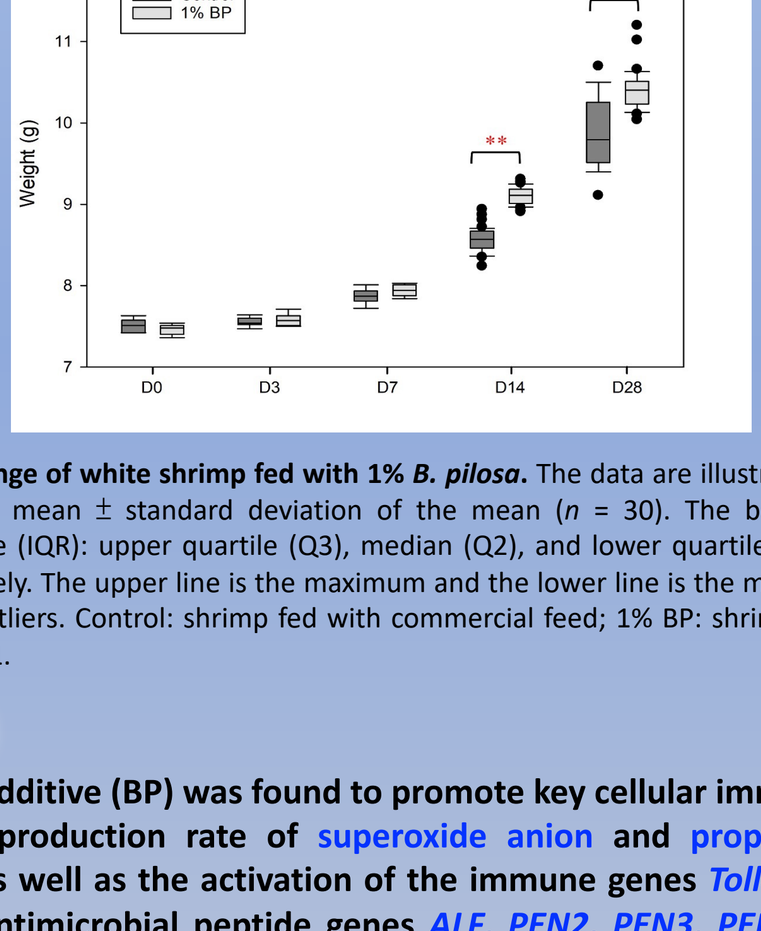


Fig. 10. Weight change of white shrimp fed with 1% *B. pilosa*. The data are illustrated as a box plot and expressed as mean ± standard deviation of the mean (n = 30). The box represents the interquartile range (IQR): upper quartile (Q3), median (Q2), and lower quartile (Q1) from top to bottom, respectively. The upper line is the maximum and the lower line is the minimum; the black dots represent outliers. Control: shrimp fed with commercial feed; 1% BP: shrimp fed with 1% *B. pilosa*. **, $p < 0.01$.

Conclusion

- ***Bidens pilosa* additive (BP) was found to promote key cellular immune responses, including the production rate of superoxide anion and prophenoloxidase in haemocytes, as well as the activation of the immune genes *Toll4, TRAF6* and its downstream antimicrobial peptide genes *ALF, PEN2, PEN3, PEN4, Lyz*, and the antioxidant enzyme genes *SOD* and *GPx* in the hepatopancreas.**
- **Similar results of differentially expressed genes were identified by transcriptome analysis of hepatopancreas in white shrimp after 14 days of feeding with 1% BP additive and control diet.**
- **Moreover, the immune-related Toll and IMD signaling pathways were upregulated in KEGG enrichment analysis after 14 days of feeding with 1% BP.**
- **Shrimp were then artificially infected with white spot syndrome virus (WSSV) to determine whether *B. pilosa* additive can improve disease resistance against WSSV. The *B. pilosa* supplemented group exhibited a significantly higher survival rate from 4 days after WSSV challenge.**
- **In addition, there was a significant difference in body weight between the 1% *B. pilosa* treatment group and the control group without additives after 14 and 28 days of feeding.**
- **Our findings thus demonstrated that dietary supplementation with *B. pilosa* as a functional feed additive can promote both immune function and growth in white shrimp.**