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Effect of *Coriolus versicolor* polysaccharides on the hematological and biochemical parameters and protection against *Aeromonas hydrophila* in allogynogenetic crucian carp (*Carassius auratus gibelio*)

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Abstract The effect of dietary intake of *Coriolus versicolor* Polysaccharides (CVP) on the hematological and biochemical indices of Allogynogenetic crucian carp (*Carassius auratus gibelio*) was investigated. Fish were fed CVP supplemented diets (0, 0.25, 0.5, 1.0, 2.0 or 4.0 g CVP kg^{-1}) for 56 days. The RBC, WBC counts, hemoglobin content, ESR in blood and TP, ALT, AST, ALP, GLU, CHO, TG, and BUN in serum were measured on day 0, 14, 28, 42, and 56. After feeding of 56 days, fish were infected with *Aeromonas hydrophila* and mortalities were recorded. The results indicated that feeding crucian carp with suitable dose of CVP enhanced the RBC, WBC counts, hemoglobin and TP content, ALP activity, and decreased the ESR, ALT, AST, GLU, CHO, TG and BUN. There was no effect in fish at low dose (0.25 g kg^{-1}). Unexpectedly, the higher CVP dose used here (2.0 and 4.0 g kg^{-1}) has a negative effect in fish. The results of challenge experiment indicated that a moderate level of CVP in the diet (1.0 g kg^{-1}) was the most effective to enhance the survival of fish after infected with *A. hydrophila*. In summary, the use of CVP, as dietary supplements, can improve the innate defense of crucian carp providing resistance to pathogens.

Keywords *Coriolus versicolor* Polysaccharides · *Carassius auratus gibelio* · Hematological and biochemical parameters · Protection · *Aeromonas hydrophila*

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Introduction

Allogynogenetic crucian carp, *Carassius auratus gibelio*, is one of the main freshwater aquaculture species in China. The total output was 2.055 million tons in 2009 (Liu and Cui 2010). Disease outbreaks have been causing severe losses. Several *Aeromonas*, such as *A. hydrophila*, *A. sobria,* and *A. salmonicida,* are the causative agent of bacterial septicemia in aquaculture. The mortality rate caused by *Aeromonas* infection could be over 95 % (Zhan 2004). Although motile *Aeromonas* are fish pathogens, it is important to note that these bacteria also compose part of the normal intestinal microflora of healthy fish and consequently stress is often considered to be a contributing factor in disease outbreaks caused by *A. hydrophila* (Yin et al. 2009).

The outbreak disease caused by *Aeromonas* is a common problem in the intensive culture of fishes, and to overcome the disease problem, a number of approaches have been applied including sanitary prophylaxis, disinfection, and chemotherapy with a particular emphasis on the use of antibiotics (Chevassus and Dorson 1990). But there are detrimental side-effects associated with the over use of chemicals to treat disease, such as effects on public health associated with residual contaminants in the flesh and environmental hazards associated with waterborne applications (Harper 2002). The use of antibiotic therapy is also under criticism due to the potential for enhanced microbial resistance and accumulation of residues in tissues of fishes. Consequently, alternatives to chemotherapy are being investigated include using natural immunostimulants (Immunel et al. 2009; Harikrishnan et al. 2011).

Vaccines are being developed against *A. hydrophila* but these are not yet commercially available. *A. hydrophila* is such a heterogeneous species, having variable antigens, in which vaccine development is extremely complex (Yin et al. 2009).

Chinese herbs have been used as traditional medicine and immune booster for human beings for thousands of years in China. Recently, growing interest has been paid to the immune stimulating function of some herbs in aquaculture, non-specific immunity such as bacteriolytic activity, and leukocyte function was improved by mixtures of Chinese herbs in shrimp and fishes (Bricknell and Dalmo 2005; Ortuno et al. 2002; Yuan et al. 2007; Zhang et al. 2009). *Coriolus versicolor* is an important medicinal herb containing polysaccharides. *Coriolus* polysaccharides have been reported to be effective in modulating immune functions, inhibiting tumor growth, preventing oxidative damage, protecting liver, reducing serum glucose levels, while producing no toxic effects (Cui and Chisti 2003).

The *C. versicolor* polysaccharides recorded ability to improve non-specific immune activity of Crucian Carp (Pang et al. 2008). Recently, *C. versicolor* supplementation diet has reported to enhance the immune response and disease resistance against *Listonella anguillarum* (Harikrishnan et al. 2012). In this study, crucian carp (*C. auratus gibelio*) were fed with different doses of *C. versicolor* polysaccharides to investigate the effect on hematology and determine resistance against *A. hydrophila*.

Materials and methods

Experimental fish

Crucian carp (*C. auratus gibelio*) (58.3. ± 4.60 g) were provided by a commercial fish farm and held in a recirculation water system with aeration. Fish were kept in tanks (4 m^2) and acclimatized to the experimental conditions for 15 days. Water temperature and pH were constant (25–28 °C; pH 6.8) during the experimental period, and dissolved oxygen was maintained at 6–7 mg L^{-1}. Water flow was maintained at 7 L min^{-1}.

Coriolus versicolor polysaccharide

Coriolus versicolor polysaccharide (CVP, containing 60 %) was a commercial product from the Microbiology Institute of Guangdong, China (Cat NO. 061027).

Diets and experimental design

The composition of the experimental diet is given in Table 1. Five diets with *C. versicolor* polysaccharide feed were prepared, viz., $C_{0.25}$ (0.25 g CVP kg^{-1} feed), $C_{0.5}$(0.5 g CVP kg^{-1}), $C_{1.0}$(1.0 g CVP kg^{-1}), $C_{2.0}$(2.0 % g CVP kg^{-1}), and $C_{4.0}$(4.0 g CVP kg^{-1}). Diets were prepared by thoroughly mixing the ingredients, and pellets were made by a hand pelletizer. Pellets were dried in an oven at 60 °C and stored at 4 °C until use.

Crucian carp were randomly distributed in 5 treatments, each of 3 replicates (60 fish per replicate). Control fish were fed basal diets without supplements of CVP. The feeding trial was conducted for 56 days. Feeding was done at 3–5 % body weight, with the daily ration being subdivided into two equal parts and fed twice a day at am 9:00 and pm 16:00.

Sampling procedure

Blood samples were collected day 0, 14, 28, 42, and 56 after the feeding. Five fish per replicate, a total of fifteen fish per group, were anaesthetized with MS-222 (1:2,500), and blood samples were drawn from caudal vein using a medical syringe with heparin as an anticoagulant. Each fish blood sample (about 1 mL) was divided into two parts. One was heparinized used

Table 1 Composition of basal diet

Ingredients	Percentage (%)	Proximate composition	Percentage (%)
Fish meal	12.0	Crude protein	33.28
Soybean meal	25.0	Crude lipid	6.89
Rapeseed meal	30.0	Moisture	10.59
Flour	27.0	Ash	7.52
Soybean oil	2.2		
CaH_2PO_4	2.0		
Vitamin premix[a]	0.1		
Mineral premix[b]	0.3		
Salt	0.2		
Choline chloride	0.2		
Sodium carboxymethyl cellulose	1.0		

[a] Vitamin premix provided (g^{-1}): VA 6500 IU, VD_3 4500 IU, VC 120 mg, VE 25 mg, VK_3 5 mg, VB_1 12.5 mg, VB_2 12.5 mg, VB_6 15.0 mg, VB_{12} 0.025 mg, niacinamide 50 mg, pantothenate 40 mg, inositol 75 mg, folic acid 2.5 mg, biotin 0.08 mg

[b] Mineral premix provided (%):NaCl 1.0, $MgSO_4$ 15.0, $NaH_2PO_4{\cdot}2H_2O$ 25.0, $AlCl_3{\cdot}6H_2O$ 0.06, KH_2PO_4 32.0, $Ca(H_2PO_4)_2{\cdot}H_2O$ 20.0, $C_6H_5FeO_7{\cdot}10H_2O$ 2.5, $CaC_6H_{10}O_6{\cdot}5H_2O$ 3.5, $ZnSO_4{\cdot}7H_2O$ 0.353, $MnSO_4{\cdot}4H_2O$ 0.162, $CuSO_4{\cdot}5H_2O$ 0.031, $CoCl_2{\cdot}6H_2O$ 0.001, $KIO_3{\cdot}6H_2O$, 0.003, Fiber 0.39

immediately for analysis of hemoglobin, total red blood cell count (RBC), white blood cell count (WBC), and erythrocyte sedimentation rate (ESR), and other was transferred immediately to dried centrifugal tubes to clot at 4 °C for 2 h, then centrifuged (3,000rps, 15 min). The serum samples collected were stored at −70 °C and analyzed for total protein (TP), blood glucose (GLU), alanine amino transferase (ALT), aspartate amino transferase (AST), blood urea nitrogen (BUN), alkaline phosphatase (ALP), cholesterol (CHO), and triglyceride (TG).

Hematological parameters and plasma biochemical analyses

RBC, WBC, and ESR were measured by use of standard methods (Yang and Xiao 2009). For determinations, serum enzyme activities (ALT, AST, and ALP) and concentrations of GLU, BUN, CHO, and TG were measured with commercially available reagent kits (Jiancheng Bioengineering Institute, Nanjing, China) based on colorimetric reaction in an automatic analyzer (ACTA, Italy). The hemoglobin and TP content were determined using a diagnostic kit (Sichuan Maker Science And Technology Co., Ltd. China).

Challenges with virulent pathogen

At the end of the experiment, a challenge test was performed on 30 fish from each experimental group with *A. hydrophila*. Each fish in test group was injected intraperitoneally with 0.2 mL per fish containing 1×10^6 *A. hydrophila*. Fish injected with sterilized 0.65 % sodium chloride at the same volume were used as the control. Cumulative mortalities were noted daily for 28 days. The cause of death was confirmed by re-isolating the organism from the kidneys of dead fish using conventional methods.

Statistical analysis

Values were expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Duncan's test was used to determine the levels of difference between all doses groups over time, using the Statistica software package (Version 6.0, Statsoft). Differences were measured and considered to be statistically significant at $P < 0.05$.

Result

Hematology

The RBC, WBC counts, blood hemoglobin, and ESR of different groups are given in Table 2. The RBC, WBC counts, hemoglobin percentage, and ESR were not significantly different ($P > 0.05$) between $C_{0.25}$ and control groups during the whole experiment. The RBC, WBC counts, and hemoglobin percentage were elevated ($P < 0.05$) in the $C_{0.5}$ group on day 28 and 42 and in the $C_{1.0}$ group on day 14, 28, and 42 compared to the control, but that were lower ($P < 0.05$) in the $C_{4.0}$ group on day 42. Compared with the control, the reduction of the ESR was noticed on day 28 and 42 in the $C_{0.5}$ group and on day 14, 28, and 42 in the $C_{1.0}$ group, whereas significant elevation ($P < 0.05$) of the ESR was observed on day 42 in the $C_{4.0}$ group compared to control. At the end of the experiment,

Table 2 Effect of CVP on RBC, WBC, hemoglobin content and ESR of *C. auratus gibelio* (mean ± SD)

Treatments	Days				
	0	14	28	42	56
RBC (10^6 mm^{-3})					
C_0	1.05 ± 0.04^a	1.06 ± 0.02^a	1.05 ± 0.02^a	1.07 ± 0.02^b	1.10 ± 0.03^b
$C_{0.25}$	1.07 ± 0.05^a	1.09 ± 0.04^{ab}	1.08 ± 0.04^a	1.07 ± 0.02^b	1.09 ± 0.04^b
$C_{0.5}$	1.08 ± 0.02^a	1.16 ± 0.06^{ab}	1.20 ± 0.03^b	1.16 ± 0.03^c	1.12 ± 0.02^b
$C_{1.0}$	1.06 ± 0.03^a	1.21 ± 0.04^b	1.23 ± 0.03^b	1.19 ± 0.03^c	1.14 ± 0.03^b
$C_{2.0}$	1.06 ± 0.05^a	1.13 ± 0.02^{ab}	1.06 ± 0.02^a	0.99 ± 0.03^{ab}	0.92 ± 0.05^a
$C_{4.0}$	1.04 ± 0.05^a	1.10 ± 0.05^{ab}	1.04 ± 0.06^a	0.97 ± 0.03^a	0.89 ± 0.02^a
WBC (10^4 mm^{-3})					
C_0	0.65 ± 0.03^a	0.66 ± 0.02^a	0.71 ± 0.03^a	0.73 ± 0.02^b	0.68 ± 0.03^b
$C_{0.25}$	0.63 ± 0.03^a	0.69 ± 0.04^a	0.73 ± 0.02^{ab}	0.73 ± 0.02^b	0.67 ± 0.04^b
$C_{0.5}$	0.64 ± 0.03^a	0.76 ± 0.04^{ab}	0.83 ± 0.03^{bc}	0.81 ± 0.01^c	0.70 ± 0.02^b
$C_{1.0}$	0.64 ± 0.03^a	0.85 ± 0.04^b	0.89 ± 0.03^c	0.83 ± 0.03^c	0.72 ± 0.03^b
$C_{2.0}$	0.63 ± 0.03^a	0.73 ± 0.02^{ab}	0.71 ± 0.04^{ab}	0.65 ± 0.03^{ab}	0.50 ± 0.05^a
$C_{4.0}$	0.64 ± 0.04^a	0.70 ± 0.05^a	0.68 ± 0.06^a	0.61 ± 0.03^a	0.47 ± 0.02^a
Hemoglobin content (mg mL^{-1})					
C_0	78.67 ± 1.20^a	79.00 ± 2.31^a	78.33 ± 2.03^a	79.67 ± 2.19^b	81.33 ± 1.86^b
$C_{0.25}$	79.67 ± 2.96^a	81.67 ± 4.37^a	82.33 ± 3.53^a	80.67 ± 1.76^b	81.67 ± 1.45^b
$C_{0.5}$	79.67 ± 1.86^a	89.00 ± 2.65^{ab}	93.33 ± 2.73^b	89.33 ± 3.18^c	82.67 ± 1.86^b
$C_{1.0}$	79.00 ± 1.15^a	94.00 ± 3.61^b	96.33 ± 2.91^b	92.00 ± 3.46^c	84.67 ± 3.18^b
$C_{2.0}$	79.33 ± 2.19^a	86.33 ± 1.86^{ab}	79.33 ± 2.19^a	72.00 ± 2.52^{ab}	65.33 ± 2.85^a
$C_{4.0}$	78.67 ± 2.40^a	83.00 ± 4.73^{ab}	77.00 ± 3.61^a	69.67 ± 3.28^a	62.33 ± 2.33^a
ESR (cm h^{-1})					
C_0	0.34 ± 0.02^a	0.33 ± 0.01^b	0.34 ± 0.01^c	0.36 ± 0.02^b	0.32 ± 0.02^a
$C_{0.25}$	0.32 ± 0.03^a	0.32 ± 0.03^{ab}	0.32 ± 0.02^{bc}	0.35 ± 0.02^b	0.32 ± 0.02^a
$C_{0.5}$	0.33 ± 0.03^a	0.29 ± 002^{ab}	0.24 ± 0.01^{ab}	0.26 ± 0.01^a	0.30 ± 0.02^a
$C_{1.0}$	0.32 ± 0.02^a	0.26 ± 0.02^a	0.21 ± 0.02^a	0.23 ± 0.02^a	0.27 ± 0.03^a
$C_{2.0}$	0.33 ± 0.02^a	0.30 ± 0.02^{ab}	0.33 ± 0.04^{bc}	0.40 ± 0.03^{bc}	0.45 ± 0.03^b
$C_{4.0}$	0.35 ± 0.03^a	0.31 ± 0.01^{ab}	0.36 ± 0.05^c	0.43 ± 002^c	0.50 ± 0.05^b

Values with different superscript in a column differ significantly ($P < 0.05$)

there were no effect ($P > 0.05$) on the RBC, WBC counts, blood hemoglobin, and ESR among C_0, $C_{0.25}$, $C_{0.5}$, and $C_{1.0}$, whereas there were significantly lower ($P < 0.05$) RBC, WBC, and hemoglobin in $C_{2.0}$ and $C_{4.0}$ groups with significantly higher ($P < 0.05$) ESR. The highest RBC, WBC count, and hemoglobin were observed in $C_{1.0}$ group on day 28 and the lowest in $C_{4.0}$ group on day 56. The lowest ESR was observed in $C_{1.0}$ group on day 28 and the highest in $C_{4.0}$ group on day 56.

Biochemical analysis

Total serum protein of various treatments is given in Table 3. The $C_{0.5}$ and $C_{1.0}$ groups showed significantly ($P < 0.05$) higher serum protein than the control group during the whole experiment. There was no significant ($P > 0.05$) effect on total protein in $C_{0.25}$ and $C_{2.0}$ groups; however, significantly lower ($P < 0.05$) protein was recorded in $C_{4.0}$ group at the end of experiment.

There was significantly lower ($P < 0.05$) blood glucose level in $C_{0.5}$ and $C_{1.0}$ groups compared to control group during the whole experiment; there was no significant change in the blood glucose in the C2.0 and C.4.0 between 14 and 42 days. The fish in C2.0 and C4.0 groups showed significantly higher ($P < 0.05$) blood glucose level than control group at the end of experiment. There was no significant ($P > 0.05$) effect between C0.25 and control groups (Table 3).

The CHO, TG, and BUN of different groups are given in Table 3. The CHO, TG, and BUN were not

Table 3 Effect of CVP on TP, GLU CHO, TG and BUN in serum of *C. auratus gibelio* (mean ± SD)

Treatments	Days				
	0	14	28	42	56
TP (g L^{-1})					
C_0	30.88 ± 1.05^{a}	32.78 ± 0.62^{a}	30.84 ± 1.27^{a}	31.05 ± 1.03^{ab}	32.56 ± 0.70^{bc}
$C_{0.25}$	30.55 ± 1.00^{a}	33.93 ± 1.91^{ab}	34.01 ± 0.73^{a}	34.24 ± 1.87^{b}	35.09 ± 0.78^{c}
$C_{0.5}$	30.16 ± 1.28^{a}	37.73 ± 0.92^{bc}	39.23 ± 0.81^{b}	40.55 ± 0.68^{c}	41.15 ± 0.68^{d}
$C_{1.0}$	31.22 ± 0.94^{a}	39.17 ± 0.81^{c}	40.36 ± 1.99^{b}	41.60 ± 0.70^{c}	42.15 ± 0.95^{d}
$C_{2.0}$	30.75 ± 1.08^{a}	36.47 ± 0.50^{abc}	32.26 ± 0.44^{a}	32.24 ± 0.69^{ab}	30.75 ± 1.48^{ab}
$C_{4.0}$	31.06 ± 0.90^{a}	35.99 ± 1.67^{abc}	30.99 ± 0.90^{a}	30.35 ± 0.70^{a}	28.95 ± 1.03^{a}
Glu (mmol L^{-1})					
C_0	6.42 ± 0.18^{a}	6.29 ± 0.18^{c}	6.41 ± 0.16^{c}	6.28 ± 0.27^{b}	6.29 ± 0.07^{b}
$C_{0.25}$	6.51 ± 0.25^{a}	6.12 ± 0.21^{bc}	6.31 ± 0.31^{bc}	6.14 ± 0.10^{b}	6.20 ± 0.20^{b}
$C_{0.5}$	6.53 ± 0.13^{a}	5.60 ± 0.19^{ab}	5.13 ± 0.28^{ab}	4.90 ± 0.27^{a}	4.57 ± 0.27^{a}
$C_{1.0}$	6.41 ± 0.13^{a}	5.16 ± 0.20^{a}	4.81 ± 0.38^{a}	4.36 ± 0.19^{a}	4.21 ± 0.29^{a}
$C_{2.0}$	6.46 ± 0.10^{a}	6.08 ± 0.11^{bc}	6.46 ± 0.67^{c}	6.66 ± 0.23^{b}	7.24 ± 0.22^{c}
$C_{4.0}$	6.58 ± 0.13^{a}	5.92 ± 0.19^{bc}	6.51 ± 0.34^{c}	6.72 ± 0.24^{b}	7.57 ± 0.18^{c}
CHO (mmol L^{-1})					
C_0	4.31 ± 0.37^{a}	4.25 ± 0.25^{d}	4.13 ± 0.10^{c}	4.34 ± 0.24^{b}	4.42 ± 0.12^{a}
$C_{0.25}$	4.25 ± 0.21^{a}	4.14 ± 0.25 ^{cd}	4.13 ± 0.33^{c}	4.23 ± 0.40^{b}	4.44 ± 0.36^{a}
$C_{0.5}$	4.28 ± 0.14^{a}	3.77 ± 0.16^{bcd}	3.36 ± 0.27^{b}	2.66 ± 0.20^{a}	3.80 ± 0.17^{a}
$C_{1.0}$	4.36 ± 0.20^{a}	2.80 ± 0.33^{a}	2.67 ± 0.26^{a}	2.59 ± 0.17^{a}	3.77 ± 0.33^{a}
$C_{2.0}$	4.26 ± 0.08^{a}	3.18 ± 0.22^{ab}	3.57 ± 0.11^{bc}	4.41 ± 0.18^{b}	5.26 ± 0.17^{b}
$C_{4.0}$	4.34 ± 0.13^{a}	3.25 ± 0.42^{abc}	3.61 ± 0.09^{bc}	4.54 ± 0.23^{b}	5.52 ± 0.15^{b}
TG (mmol L^{-1})					
C_0	3.67 ± 0.24^{a}	3.70 ± 0.11^{b}	3.78 ± 0.19^{c}	3.55 ± 0.04^{b}	3.72 ± 0.04^{b}
$C_{0.25}$	3.63 ± 0.23^{a}	3.65 ± 0.23^{ab}	3.59 ± 0.18^{bc}	3.50 ± 0.10^{b}	3.72 ± 0.29^{b}
$C_{0.5}$	3.68 ± 0.24^{a}	3.27 ± 0.08^{ab}	3.03 ± 0.21^{ab}	2.92 ± 0.11^{a}	2.56 ± 0.05^{a}
$C_{1.0}$	3.64 ± 0.03^{a}	3.02 ± 0.11^{a}	2.82 ± 0.22^{a}	2.78 ± 0.20^{a}	2.53 ± 0.23^{a}
$C_{2.0}$	3.68 ± 0.31^{a}	3.47 ± 0.29^{ab}	3.52 ± 0.16^{bc}	3.58 ± 0.28^{b}	4.36 ± 0.23^{c}
$C_{4.0}$	3.68 ± 0.10^{a}	3.50 ± 0.27^{ab}	3.61 ± 0.09^{bc}	3.65 ± 0.13^{b}	4.42 ± 0.22^{c}
BUN (mmol L^{-1})					
C_0	1.74 ± 0.04^{a}	1.76 ± 0.08^{b}	1.71 ± 0.04^{b}	1.66 ± 0.16^{b}	1.68 ± 0.03^{b}
$C_{0.25}$	1.74 ± 0.13^{a}	1.67 ± 0.17^{ab}	1.63 ± 0.04^{b}	1.58 ± 0.09^{b}	1.69 ± 0.05^{b}
$C_{0.5}$	1.75 ± 0.02^{a}	1.47 ± 0.05^{ab}	1.33 ± 0.12^{a}	1.25 ± 0.09^{a}	1.20 ± 0.03^{a}
$C_{1.0}$	1.79 ± 0.07^{a}	1.35 ± 0.08^{a}	1.24 ± 0.03^{a}	1.20 ± 0.10^{a}	1.15 ± 0.03^{a}
$C_{2.0}$	1.80 ± 0.14^{a}	1.52 ± 0.05^{ab}	1.56 ± 0.03^{b}	1.72 ± 0.05^{bc}	1.97 ± 0.09^{c}
$C_{4.0}$	1.79 ± 0.09^{a}	1.60 ± 0.10^{ab}	1.64 ± 0.06^{b}	1.91 ± 0.06^{c}	2.08 ± 0.16^{c}

Values with different superscript in a column differ significantly ($P < 0.05$)

significantly different ($P > 0.05$) between $C_{0.25}$ and control groups during the whole experiment. There were significantly lower ($P < 0.05$) CHO, TG, and BUN in $C_{0.5}$ group on day 28, 42, in $C_{1.0}$ on day 14, 28, 42, and in $C_{2.0}$ and $C_{4.0}$ on day 14. At the end of experiment, the TG, and BUN were significantly lower in $C_{0.5}$ and $C_{1.0}$($P < 0.05$), whereas the CHO, TG, and BUN were significantly higher in $C_{2.0}$ and $C_{4.0}$ ($P < 0.05$). The lowest CHO was observed in $C_{1.0}$ group on day 42, and TG and BUN were observed in $C_{1.0}$ group on day 56, the highest of that were in $C_{4.0}$ group on day 56.

Alanine amino transferase (ALT) and aspartate amino transferase (AST)

The ALT and AST activities of different groups are given in Table 4. The ALT and AST activities were

Table 4 Effect of CVP on the activity of ALT, AST, and ALP in serum of *C. auratus gibelio* (mean ± SD)

Treatments	Days				
	0	14	28	42	56
ALT (U L^{-1})					
C_0	34.78 ± 0.77^a	34.45 ± 0.63^b	33.89 ± 0.89^b	34.62 ± 0.49^b	33.57 ± 1.33^b
$C_{0.25}$	34.86 ± 2.65^a	33.25 ± 0.66^{ab}	33.73 ± 0.95^b	34.54 ± 0.91^b	33.33 ± 1.24^b
$C_{0.5}$	35.18 ± 1.13^a	32.37 ± 0.64^a	30.36 ± 1.06^a	26.90 ± 0.92^a	29.15 ± 1.16^a
$C_{1.0}$	35.10 ± 1.40^a	31.32 ± 0.35^a	29.80 ± 1.08^a	25.38 ± 1.05^a	28.03 ± 1.19^a
$C_{2.0}$	34.94 ± 2.87^a	33.01 ± 0.49^{ab}	33.65 ± 1.04^b	35.34 ± 1.20^b	37.43 ± 0.91^c
$C_{4.0}$	34.45 ± 1.50^a	32.85 ± 0.64^{ab}	34.05 ± 1.28^b	38.95 ± 0.82^c	40.48 ± 1.11^c
AST (U L^{-1})					
C_0	22.68 ± 0.77^a	22.38 ± 0.63^b	21.73 ± 0.60^b	22.62 ± 0.58^b	21.47 ± 0.75^b
$C_{0.25}$	22.76 ± 0.93^a	21.08 ± 0.68^{ab}	21.53 ± 0.72^b	22.40 ± 0.77^b	21.23 ± 0.90^b
$C_{0.5}$	22.91 ± 0.97^a	20.20 ± 0.69^a	18.19 ± 0.44^a	14.70 ± 0.86^a	17.05 ± 0.84^a
$C_{1.0}$	23.00 ± 1.40^a	19.06 ± 0.51^a	17.46 ± 0.86^a	13.21 ± 0.99^a	15.93 ± 0.62^a
$C_{2.0}$	22.84 ± 1.45^a	20.91 ± 0.50^{ab}	21.55 ± 0.93^b	23.37 ± 1.08^b	25.66 ± 1.01^c
$C_{4.0}$	22.35 ± 1.50^a	20.75 ± 0.64^{ab}	21.99 ± 0.74^b	26.92 ± 0.78^c	27.95 ± 0.75^c
ALP (U L^{-1})					
C_0	69.67 ± 2.60^a	71.67 ± 0.88^a	71.67 ± 2.85^a	72.67 ± 2.40^b	73.33 ± 2.03^b
$C_{0.25}$	70.00 ± 3.51^a	72.67 ± 2.19^{ab}	73.00 ± 2.52^a	73.67 ± 3.18^b	73.00 ± 1.00^b
$C_{0.5}$	70.67 ± 1.86^a	76.33 ± 2.60^{ab}	80.00 ± 2.65^b	84.33 ± 2.84^c	75.33 ± 2.85^b
$C_{1.0}$	70.00 ± 0.58^a	79.67 ± 2.73^b	82.67 ± 1.86^b	85.67 ± 2.96^c	77.00 ± 1.73^b
$C_{2.0}$	71.00 ± 2.08^a	75.67 ± 2.03^{ab}	71.00 ± 2.08^a	66.00 ± 2.65^a	64.00 ± 3.06^a
$C_{4.0}$	70.00 ± 1.53^a	73.00 ± 1.53^{ab}	69.00 ± 1.00^a	63.33 ± 2.40^a	61.33 ± 1.76^a

Values with different superscript in a column differ significantly ($P < 0.05$)

found significantly lower ($P < 0.05$) in $C_{0.5}$ and $C_{1.0}$ than control during the whole experiments. The lowest ALT and AST activities were observed in $C_{1.0}$ on day 42. The significant elevation ($P < 0.05$) of the ALT and AST activities was observed on day 42 in the $C_{4.0}$ group compared to the control. At the end of experiment, there were significantly higher ($P < 0.05$) ALT and AST activities in $C_{2.0}$ and $C_{4.0}$ groups than control.

Alkaline phosphatase (ALP)

The ALP activity of different groups is given in Table 4. The ALP activity was not significantly different ($P > 0.05$) between $C_{0.25}$ and control groups during the whole experiment. The ALP was elevated ($P < 0.05$) in the $C_{0.5}$ group on day 28 and 42 and in the $C_{1.0}$ group on day 14, 28, and 42 compared to the control. At the end of experiment, there were no significant effect on the ALP activity among C_0, $C_{0.25}$, $C_{0.5}$, and $C_{1.0}$, whereas there was significantly lower ($P < 0.05$) ALP activity in $C_{2.0}$ and $C_{4.0}$ groups.

Challenges with virulent pathogen

After injected with *A. hydrophila*, fish started to die at 24 h and no mortality was observed at day 14–28. The survivability percentage of post-challenge treatment groups was indicated in Fig. 1. The survival rate of post-challenge was 68.9 % in group $C_{1.0}$ at day 14, which is higher than that in control group (24.4 %).

Discussion

Ozaki (1982) indicated when the feed was not sufficient and the fish was malnourished, the red blood cell counts got lowered. Siegel et al. (1981) demonstrated that except carrying oxygen, erythrocytes possess important immune functions as well. Zhang (2006) reported that when *Cyprinus carpio* dieted with yeast mannan oligosaccharides, there was a significant elevation of the RBC, and a similar elevation of the RBC was observed in soft-shelled turtle (*Trionyx sinensis*) fed with Na_2SeO_3, selenoyeast

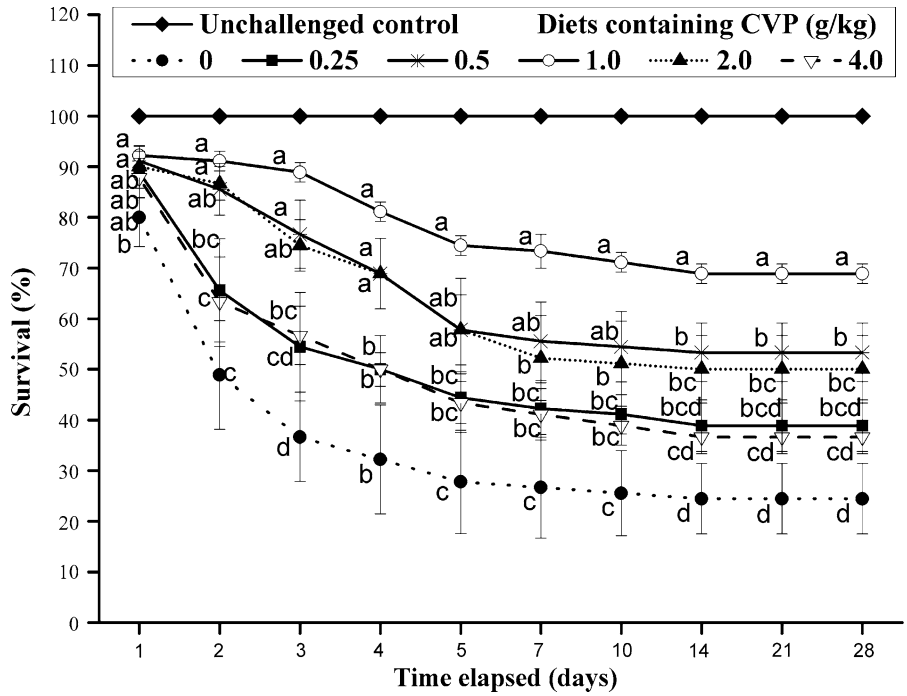

Fig. 1 Survival after artificial challenging with *Aeromonas hydrophila* in *C. auratus gibelio* fed diets containing CVP in the same time with different letters are significantly ($P < 0.05$) among treatments

fructo-oligosaccharides, and β-glucan for 100 days (Liu et al. 2006). Choudhury et al. (2005) reported no significant differences in the hemoglobin, and RBC levels were observed when dietary ribonucleic acid or chitin was supplied to rohu (*Labeo rohita*). However, in the present study, we observed were significant elevation in the hemoglobin and RBC levels on day 14, 28, and 42 in fish fed with 0.5 and 1.0 g kg^{-1} doses of CVP, whereas the RBC were reduced on day 56 in 2.0 and 4.0 g kg^{-1}doses groups.

Leukocyte count is considered an indicator for the health status of fish due to its role in non-specific or innate immunity (Kumar et al. 2007). Many studies have shown that immunostimulants, such as β-glucan, chitosan, chitin, and tuftsin, elevated WBC of fish and shrimp (Selvaraj et al. 2005; Choudhury et al. 2005; Wang and Chen 2005; Misra et al. 2006). The increase in the leukocyte count and its functions is quite likely to result in an enhancement of the non-specific defense, because macrophages and other phagocytic cells are the key elements in the immune system (Misra et al. 2006). In our study, the WBC count increased in 0.5 and 1.0 g kg^{-1} groups, whereas in high doses (2.0 and 4.0 g kg^{-1}), the WBC count decreased. On the other hand, no significant WBC count change was observed in the 0.25 0 g kg^{-1} dose group. These results suggested that a moderate level of CVP in the diet could provoke the immune response.

Alteration in the erythrocyte sedimentation rate (ESR) has been attributed to both plasma and red blood cell factors (Al-Homrany 2002). An increased ESR level indicated the increase of erythrocyte rouleaux which was detrimental to red blood cells through the microcirculation (Qian et al. 2002). *Angelica sinensis* decreased the ESR level in chicken (Hou et al. 2008). In this study, the ESR level decreased in the groups with 0.5 and 1.0 g kg^{-1} doses, while the RBC counts increased. In the groups with 2.0 and 4.0 g kg^{-1} doses, the ESR level decreased and the RBC counts increased during days 0–14, whereas the ESR increased when the RBC counts decreased during days 28–56.

The concentration of total proteins (TP) in blood serum is routinely used as a basic index for the health status of fish (Mulcahy 1971). It has been reported in various studies that TP is enhanced when the fish is treated with immunostimulants, for example, oral administration of yeast products for rainbow trout (Siwicki et al. 1994) and rohu (Choudhury et al. 2005), as well as the extracts of four medicinal plants (*Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera,* and *Zingiber officinale*) for tilapia (Immanuel et al. 2009) or crucian carp. In rainbow trout, plasma

total protein level significantly increased after feeding the fish with various herbal extracts (Dügenci et al. 2003). In the present study, there was a significant effect of CVP on TP level with 0.5 and 1.0 g kg^{-1}doses. Serum total protein content may also reflect hepatic protein metabolic status in responses to dietary treatments (Tang et al. 2005). Thus, dietary supplementation of CVP may have improved hepatic functions in fish.

Many polysaccharides can decrease the blood glucose in mice, pig, and fish. Kiho demonstrated the hypoglycemic activity of polysaccharides from *Cordyceps sinensis* in mice (Kiho et al. 1993, 1996). Polysaccharides has been reported to promote secretion of insulin, influence activities of nitrogen and sucrose metabolism enzymes, advance utilization of glucose in tissue and inhibit gluconeogenesis (Hikno et al. 1989). In this study, the blood glucose was reduced in the 0.5 and 1.0 g kg^{-1} groups after 14-day feed, while got reduced during days 0–14 and then elevated in the 2.0 and 4.0 g kg^{-1} doses groups.

Tang found that dietary supplementation of chitosan, galacto-mannan-oligosaccharide, and antibiotics all resulted in a reduction of the serum BUN in early-weaned piglets (Tang et al. 2005). In our study, dietary supplementation of CVP at 0.5 and 1.0 g kg^{-1} doses reduced the serum BUN in crucian carp during the whole feeding period, while with 2.0 and 4.0 g kg^{-1} doses, the serum BUN increased on day 42 and 56. The change in serum BUN concentrations reflects the whole body status of amino acid metabolism and utilization in animals. Thus, reduction of serum BUN concentration might suggest a potential enhancement of synthesis of protein in animals (Eggum 1970). Therefore, dietary supplementation of CVP improved whole body protein anabolism in crucian carp.

Immanuel reported that supplementary diets with four medicinal plants extracts reduced serum TG and CHO concentrations in tilapia (Immanuel et al. 2009). Supplementing the control diet with chitosan, galacto-mannan-oligosaccharide, or lincomycin reduced serum TG and CHO concentrations in weaned piglets (Tang et al. 2005, Burkey et al. 2004). Li reported that CVP decreased serum TG and CHO in experimental hyperlipoidemia rabbits and rats (Li 2002). In this study, dietary supplementation of CVP at 0.25–1.0 g kg^{-1} doses reduced the serum CHO and TG during whole feeding period, but at 2.0 and 4.0 g kg^{-1} the serum CHO and TG increased on day 42 and 56.

ALT and AST are two of the important amino acid catabolic enzymes in liver. The increases of serum ALT and AST levels associate with hepatocytes damages. Certain herbs have been reported to be able to protect liver and enhance liver function. Rao reported ALT and AST levels increased significantly in *Labeo rohita* infected with *A. hydrophila,* and elevated levels of ALT and AST were brought back to normal by *Achyranthes* treatment (Rao et al. 2006). Chen reported that yeast β-glucan decreased ALT and AST activities in *C. auratus gibelio* (Chen et al. 2003). In the present study, the ALT and AST activities decreased when fish were fed with CVP at a suitable dose (0.5 or 1.0 g kg^{-1}). On the contrary, the ALT and AST activities increased at high doses (2.0 and 4.0 g kg^{-1}).

ALP is one of the important phosphatase in phagocyte. It has been reported that the ALP level was drastically decreased in *Labeo rohita* infected by *A. hydrophila*, and *Achyranthes* treatment restored this level to normal in comparison with the uninfected fish (Rao et al. 2006). Also, the ALP activity has been observed to increase in *Litopenaeus vannmei* fed with β-glucan (Shen et al. 2007). In our study, ALP level significantly increased in crucian carp fed with moderate doses (0.5–1.0 g kg^{-1}) of CVP. However, fish fed with 2.0 and 4.0 g kg^{-1} CVP doses showed lower ALP levels on day 42 and 56, comparing with the control.

Polysaccharides as immunostimulants have been shown to reduce mortality against pathogenic challenges in aquatic animals (Yin et al. 2009; El-Boshy et al. 2010; Zhao et al. 2011; Harikrishnan et al. 2012). The present study also contributed to this literature and showed that crucian carp fed supplementally with CVP were less susceptible to the pathogen *A. hydrophila*. After challenging with *A. hydrophila*, survival was significantly increased in all groups comparing to control, and the survival percentage was the highest in 1.0 g kg^{-1} group. This indicated that polysaccharides can be used to protect the fish against *A. hydrophila.*

In conclusion, the results of this study showed that the supplement of CVP (0.5 and 1.0 g kg^{-1}) in the diet of crucian carp for 14–28 days could significantly enhance the RBC, WBC counts, hemoglobin content, TP in blood, and the activity of ALT, AST, ALP, and decrease the ESR, GLU, CHO, TG, and BUN. A moderate level of CVP in the diet (1.0 g kg^{-1}) was the most effective to enhance the survival of fish

after infected with *A. hydrophila*. In summary, the use of CVP, as dietary supplements, can improve the innate defense of crucian carp in providing resistance to pathogens.

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