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Effect of dietary β-glucan on growth and immunity of *Ancherythroculter nigrocauda*

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ABSTRACT

The *Ancherythroculter nigrocauda* juveniles had been feeded with β-glucan from brewer's yeast for 50 days in order to observe their growth performance and serum immunoglobulin levels. With the addition level of β(1,3) -glucan from 0 to 1000 mg/kg, the weight gain rate (WGR), the specific growth rate (SGR) and protein efficiency ratio (PER) increased (< 500 mg/kg) first, and then appeared to decline (> 500 mg/kg), while the feed conversion ratio (FCR) showed the opposite trend. Moreover, A2 group (500 mg/kg) presented the highest WER, SGR and PER, and the lowest FCR. There were significant differences (P <0.05) between the control and treatments in lysozyme (LZM) activity, superoxide dismutase (SOD) activity, acid phosphatase (ACP) activity, alkaline phosphatase (ALP) activity, plasma complement C3 and the phagocytic index (PI), and also the lowest immune parameters were found in the control. For LZM, SOD, ACP, ALP and PI, the highest values (P <0.05) were obtained at the addition level of 500 mg/kg (A2) or 750 mg/kg (A3). In addition, although the highest plasma complement C3 was in A4 (1000 mg/kg), no significant difference (P >0.05) appeared between A3 and A4. In conclusion, lower doses of β(1,3)-glucan addition to the diet could increase appetite, promote growth, improve the efficiency of feed utilization, and so reduce the feed conversion ratio. And Immune parameters indicated that β (1,3) -glucan could boost their immune systems and the immune enhancement was dose-dependent. Therefore a suitable addition of β(1,3)-glucan to the diet might be inferred: 500 mg/kg.

KEYWORDS

β-glucan;
Ancherythroculter nigrocauda;
Growth;
Immunity.

INTRODUCTION

Ancherythroculter nigrocauda, with the delicious meat and belonging to the *Cypriniformes* order, the Culterinae family and the *Acherythroculter* genus, is an endemic species and lives in the upper reaches of the Changjiang River[24]. The fish is usually dominant in the fish assemblages of estuary habitats of small tribu-

taries and also abundant in lentic habitats of most reservoirs located in the region of its original distribution. However, due to long-term overfishing, water pollution and habitat degradation, the natural population of the species has decreased noticeably since the early 1990s and cultivation has only recently been developed[24].

With the success of artificial propagation of *Ancherythroculter nigrocauda* and the rapid increase in large-scale and intensive farming systems, stress factors in the breeding process, such as crowding, nutritional, environmental, metabolic, and other factors tend to weaken its immune system and produce disease, thus leading to the extensive use of antibiotics, and also the decline in product quality and the growth in food insecurity. So, the development and application of immune-stimulating agent, which can stimulate the immune response and strengthen the immune system against infection and disease, has become an effective way to improve health status and reduce disease for breeding fishes.

Among potential immunostimulants, β-glucan is a promising candidate, and has commercial product available for aquaculture[5]. Many feeding trials and in vitro tests have shown that β-glucan is able to enhance the immune capacities of aquatic animals such as phagocytosis, superoxide anion production and lysozyme activity in shrimps and fishes[2,16]. β-glucans, consisting of a backbone of β-1,3-linked β-D-glucopyranosyl units with β-1,6-linked side chains of varying distribution and length[25], are widely present in the cell wall of bacteria, yeast, mushrooms and cereals.

Although, in recent years researches on *Ancherythroculter nigrocauda* mainly focuse on the biological characteristics, biochemical composition, and artificial propagation[19,20,24], effects of dietary β-glucan on the *Ancherythroculter nigrocauda* have been scarcely reported. In present study, the *Ancherythroculter nigrocauda* juveniles had been feeded with β-glucan from brewer's yeast for 50 days in order to observe their growth performance and serum im- munoglobulin levels.

MATERIAL AND METHODS

Experimental design and diets

The basal practical diet was formulated to mainly contain fish meal, corn oil and fish oil, and dextrin (TABLE 1), which have been shown to be sufficient to support the optimal growth of *Ancherythroculter nigrocauda*. β(1,3) -glucan (purity, >99.0%; Haijie Connaughton Biotechnology Co., Ltd., China) was derived from brewer's yeast. The corresponding levels of dietary β (1,3)-glucan were 0 (the control diet, A0), 250 (A1), 500 (A2), 750 (A3), and 1000 (A4) mg/kg diets, respectively (TABLE 1).

Feed ingredients were ground into fine powder through 250 μm mesh and thoroughly mixed with fish oil and corn oil, and water was added to produce stiff dough. Then the dough was pelleted with a granulator and dried at 42 centigrade for about 2 h in a ventilated oven. Finally the diets were broken up and sieved into

TABLE 1 : Composition and nutrient level of foundation diets (air-dry basis.%)

Ingredients	Control A0	A1	A2	A3	A4
Fish meal	66.29	66.29	66.29	66.29	66.29
Fish oil	2.18	2.18	2.18	2.18	2.18
Corn oil	2.14	2.14	2.14	2.14	2.14
Choline chloride (50%)	0.50	0.50	0.50	0.50	0.50
Dextrin	14.46	14.435	14.41	14.385	14.36
Vitamin premix[1)]	1.00	1.00	1.00	1.00	1.00
Mineral premix[2)]	2.50	2.50	2.50	2.50	2.50
α-cellulose	9.93	9.93	9.93	9.93	9.93
β(1,3)- glucan	0	0.025	0.05	0.075	0.10
Adhesives	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Proximate composition					
Moisture	10.61	10.61	10.61	10.61	10.61
Crude protein	42.76	42.74	42.74	42.72	42.72
Crude fat	9.61	9.59	9.58	9.58	9.57
Ash	12.86	12.85	12.85	12.84	12.84
Gross energy ($kJ \cdot g^{-1}$)	17.43	17.42	17.41	17.41	17.40
EAA equilibrium correlation	0.8853	0.8853	0.8853	0.8853	0.8853

1) Vitamin provides for per kg diet: VA 5 000 IU, VD1 000 IU, VE 30 IU, VK 2.5 mg, VB_1 5 mg, VB_2 8 mg, VB_6 7 mg, VB_{12} 0.01 mg; niacin 30 mg; pantothenicacid 25 mg; folic acid 0.5 mg; biotin 0.2 mg; VC 35 mg; inositol 50 mg; cholinechlorids 700 mg; 2) Mineral provides for per kg diet: Mn 10 mg, Zn 30 mg, Fe 60 mg, Cu 3 mg, I 1 mg, Se 0.2 mg; 3) α- cellulose was adjusted to maintain all diets gross energy

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1.0 mm pellets, and were stored in sealed bags at -20 centigrade until used.

Experimental procedure

Ancherythroculter nigrocauda juveniles were obtained from Yangqiao fish station of Luzhou research work base, Institute of Hydrobiology, Chinese Academy of Sciences (Sichuan Province, China). Prior to the start of the experiment, 2000 healthy juveniles with similar size were reared in square fiber tanks (3.5 × 1.6 × 1.0 m, barrel gray) with recirculating systems filter in the aquaculture laboratory of Henan University of Technology (Henan Province, China), and fed the control diet twice daily (08: 30 and 17: 30) for 2 weeks to adapt to the experimental diet and conditions.

At the beginning of the experiment, fish (initial weight about 3.12-3.34 g) of similar size (7.2-7.6 cm) were randomly distributed into 15 square fiber tanks (1.0 × 1.0 × 1.0 m). Each tank was stocked with 50 fish, and provided with a continuous flow of water (2 L/min) and continuous aeration through air stones to maintain dissolved oxygen levels. Each diet was randomly assigned to three replicate groups of fish. Fish were hand-fed to apparent satiation twice daily (08: 30 and 17: 30). The feeding trial lasted for 50 days. During the experimental period, the temperature ranged from 25.0 to 26.5 centigrade, pH was 7.2-7.4, total hardness of water was 2.2 mmol/l, and the dissolved oxygen was 6.1-6.6mg/l. At the termination of the experiment, the fish were fasted for 24 h before harvest. Total number and body weight of fish in each tank were measured.

Sample collection

Following the feeding trial, after being fasted for 24 h, fish in each tank were individually weighed and sampled for tissue analysis. Blood samples were collected from the caudal vein of 20 representative fish from each tank using a 1-mL syringe with 27-gauge needle and allowed to clot at room temperature for 4 h and then at 4 centigrade for further 6 h. The clot was removed and residual blood cells were separated from the straw-colored serum by centrifugation (836×g, 10 min, 4 centigrade). The serum was frozen at -80 centigrade for later analysis

Functional immune assay

Lysozyme (LZM) activity

The lysozyme activity in serum was measured according to the method of Ellis (Ellis, 1990). Briefly, Add 0.05 mL serum to 1.4 mL of a 0.2 mg/mL suspension of Micrococcus lysodeikticus (Sigma) in a 0.1 mol/L sodium phosphate buffer (pH 6.8). Then, carry the reaction at 25 centigrade and measure the absorbance at 530 nm after 0.5 and 4.5 min in a spectrophotometer. The amount of sample causing a decrease in absorbance of 0.001 per minute is defined as each unit.

Superoxide dismutase (SOD) activity

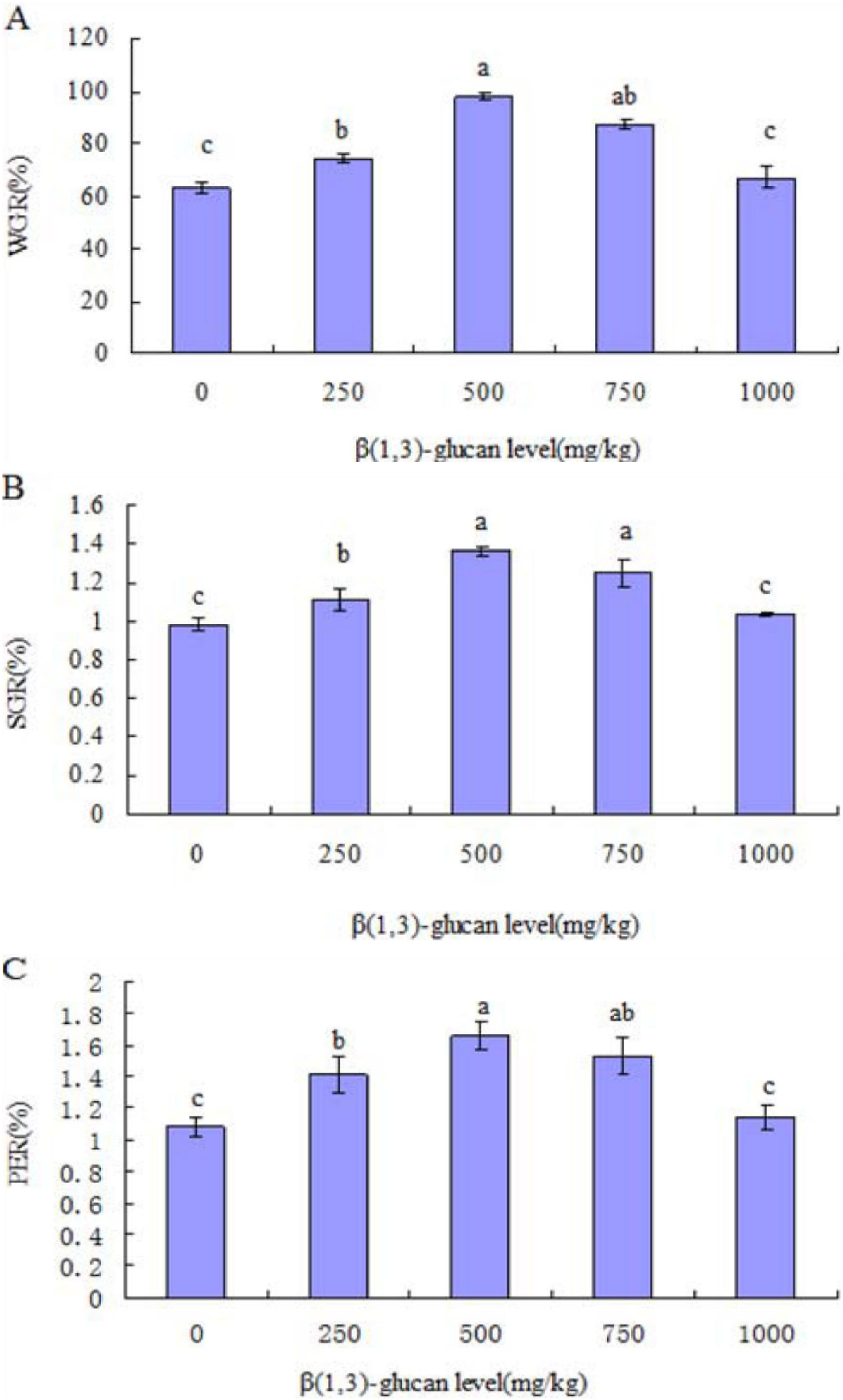
Superoxide dismutase activity was measured by its ability to inhibit superoxide anion generated by xanthine and xanthine oxidase reaction system[12] using a SOD detection kit (Nanjing Jiancheng Bioengineering Institute, China). The optical density was measured at 550 nm. One unit of SOD activity is defined as the amount of required for inhibiting the rate of xanthine reduction by 50% in 1 mL reaction system, specific activity was expressed as SOD unit per mL serum.

Acid phosphatase (ACP) and Alkaline phosphatase (ALP) activity

Acid phosphatase (ACP) activity and alkaline phosphatase (ALP) activity were assayed as described by the reference method[18]. ACP activity unit is defined as per 100 mL of serum at 37 centigrade with the substrate for 60 min, resulting in l mg phenol as a unit of enzyme activity; the ALP activity unit is defined as 15min per 100 mL of serum at 37 centigrade and substrate effects, produce l mg of phenol by a unit of enzyme activity.

Phagocytic index (PI)

Phagocytic activity was determined by a modified method[8,15]. The 100 mm cell suspensions of head kidney leucocytes (1×10^7cells mL^{-1}) was placed into a sterile slide and the cells allowed to attach for 30 min at 25 centigrade. Following attachment, 100 mL yeast suspension (Bakers yeast, Type II, Sigma, USA, 1×10^8 cells mL^{-1}) was added to the cell monolayer, and the slide was incubated for 45 min at 25 centigrade. Then unattached cells were washed off with phosphate buffered saline. After air-drying, the slides were fixed in ethanol, redried and stained with Giemsa. Then, 200 cells were examined by microscopy to determine the


A
WGR(%)
120
100
80
60
40
20
0
c
b
a
ab
c
0
250
500
750
1000
β(1,3)-glucan level(mg/kg)
B
SGR(%)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
c
b
a
a
c
0
250
500
750
1000
β(1,3)-glucan level(mg/kg)
C
PER(%)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
c
b
a
ab
c
0
250
500
750
1000
β(1,3)-glucan level(mg/kg)

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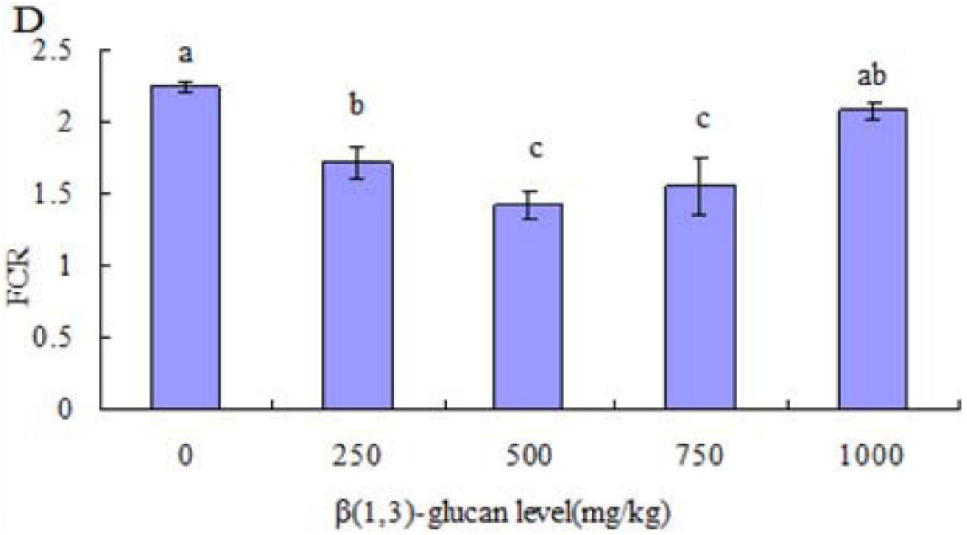


Figure 1 : The effect of β(1,3)-glucan supplementation on growth performance (A. WGR; B. SGR; C. PER; D. FCR) of *Ancherythroculter nigrocauda* juveniles. Different letters represent significant differences ($P < 0.05$)

percentage of cells with phagocytic activity.

Complement C_3(C_3)

Complement C_3 kit (Zhejiang Yili Kang Biotechnology Co., Ltd. China.) was used and the operation was as follows: the composition of a working solution was the anti-serum complement C_3: application of liquid = 1:50 (v/v), and three test tubes labeled blank tube, standard tube and measuring tube were placed in an ice bath with the addition of 2μL saline, 2μL different concentration of standard solution and 2μL serum to be measured, respectively. Then 400μL working solution was added to each tube and mixed, after incubated at 37 ° C for 15min, OD value of each tube was measured at 340 nm. According to a standard curve and equation by the concentration and the OD value of the standard solution, the serum complement C_3 content of the sample was calculated by the following formula: complement C_3 content in the sample (g / L) = ΔAU / ΔAS × CS (g / L). ΔAU, OD value of sample tube: ΔAS, tube OD value of standard tube; CS, the C_3 concentration in the standard solution.

Calculations and statistical methods

The parameters were calculated as follows:

Weight gain ratio (WGR, %) = $(W_t - W_0) \times 100 / W_0$

Specific growth ratio (SGR, % /day) = $(\ln W_t - \ln W_0) \times 100 / t$

Protein efficiency ratio (PER) = weight gain (g) /protein intake (g)

Feed conversion ratio (FCR)= feed consumed (g, dry weight)/ weight gain (g)

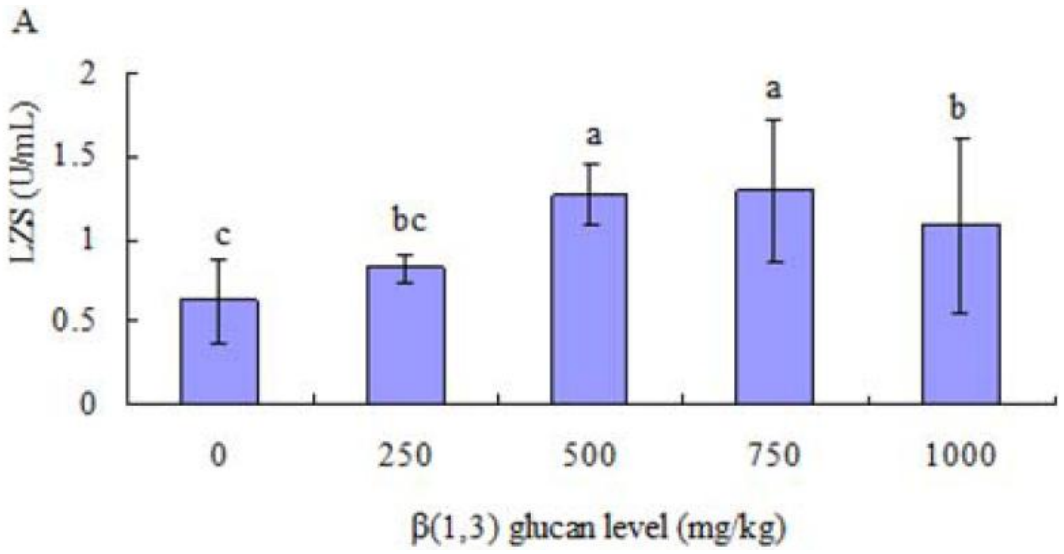
where W_t is final body weight (g), W_0 is initial body weight (g); t is experimental duration in days. Results are presented as means ± S.E.M.(standard error of the mean).

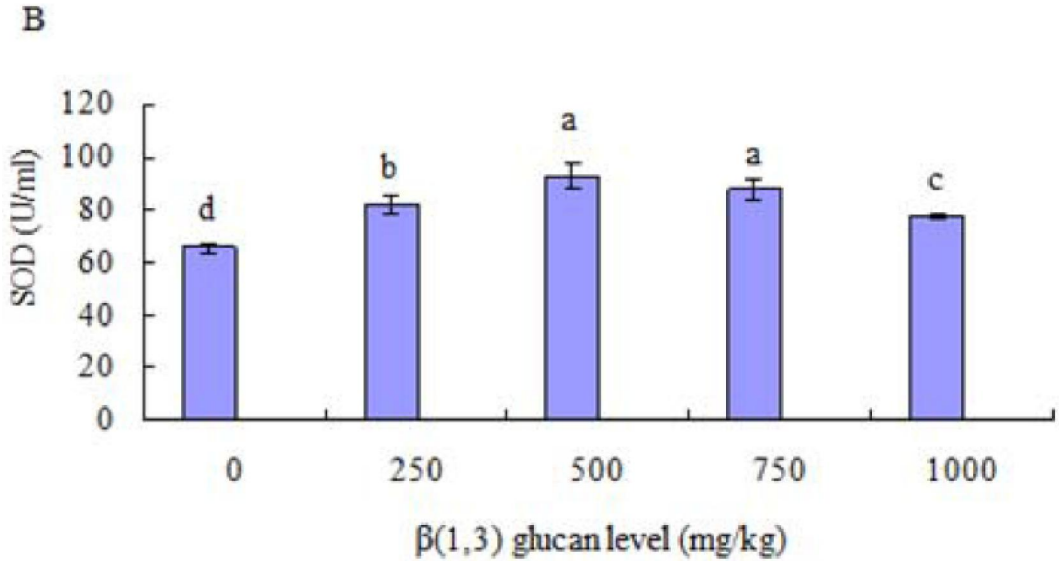
All data were subjected to one-way ANOVA using SAS 9.0. When there were significant differences ($P < 0.05$), the group means were further compared with Tukey's multiple range test.

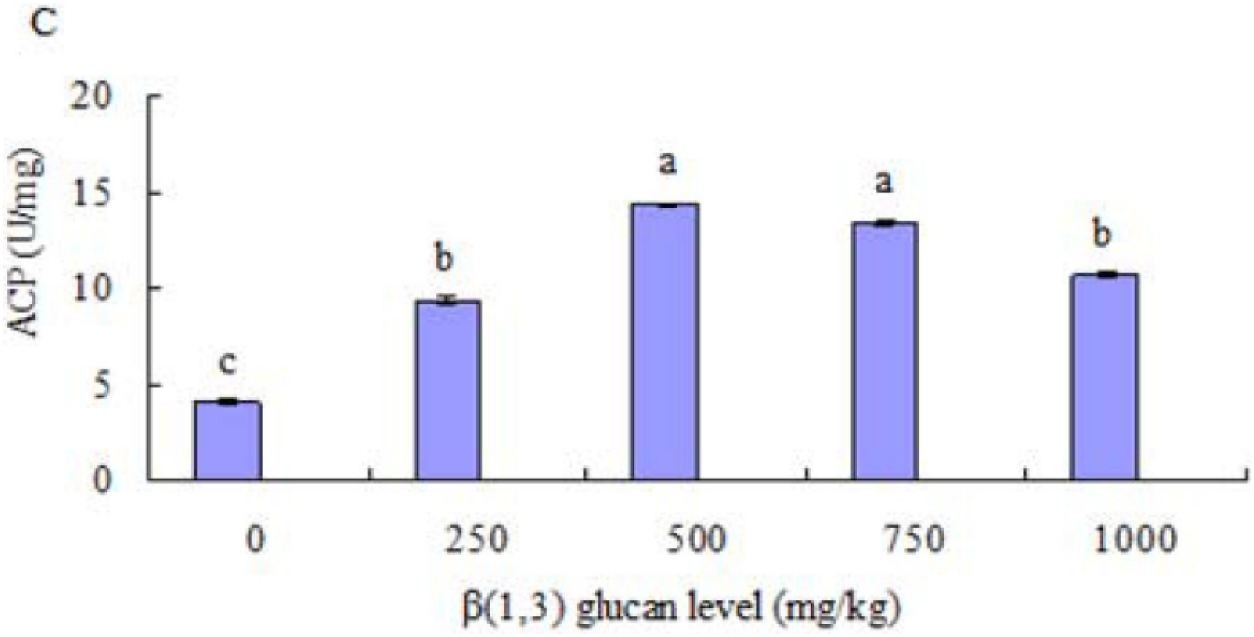
RESULTS

Growth performance

Figure 1 presents the effect of β(1,3)-glucan supplementation on growth performance of *Ancherythroculter nigrocauda* juveniles. With the β(1,3) -glucan added from 0 to 500 mg/kg, the weight gain rate (WGR, Figure 1 (A)), the specific growth rate (SGR, Figure 1 (B)) and the protein efficiency ratio (PER, Figure 1 (C)) increased first, and then at addition level from 500 mg/kg to 1000 mg/kg, the three parameters above appeared to decline; WGR, SGR and PER of A1 (250 mg/kg), A2 (500 mg/kg) and A3 (750 mg/kg) treatments were higher ($P < 0.05$) than those of the A0 (0 mg/kg, control) and A4 (1000 mg/kg) treatments. But the feed conversion ratio (FCR, Figure 1 (D)) showed the opposite trend: first decreasing from 0 to 500 mg/kg and then increasing from 500


A
LZS (U/mL)
2
1.5
1
0.5
0
c
bc
a
a
b
0
250
500
750
1000
β(1,3) glucan level (mg/kg)


B
SOD (U/ml)
120
100
80
60
40
20
0
d
b
a
a
c
0
250
500
750
1000
β(1,3) glucan level (mg/kg)


C
ACP (U/mg)
20
15
10
5
0
c
b
a
a
b
0
250
500
750
1000
β(1,3) glucan level (mg/kg)

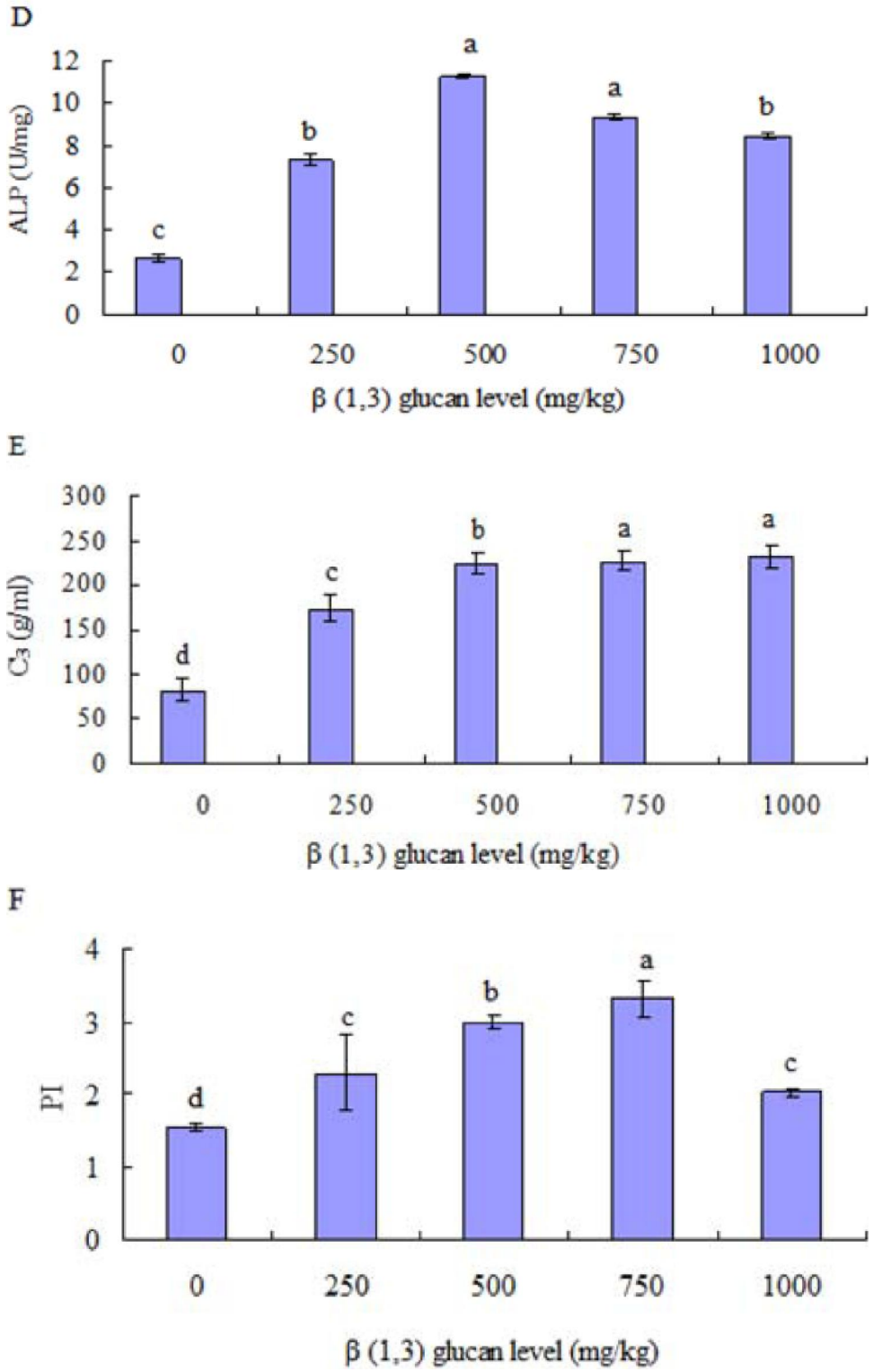


Figure 2 : The effect of β(1,3)-glucan supplementation on immune parameters (A. LZS; B. SOD; C. ACP; D. ALP; E. C_3; F. PI) of *Ancherythroculter nigrocauda* juveniles. Different letters represent significant differences ($P < 0.05$)

to 1000 mg/kg. Moreover, A2 group (500 mg/kg) presented the highest WER, SGR and PER, and the lowest FCR, and had a significant difference ($P < 0.05$) with the control (A0) and the treatment groups of A1 and A4. However, there was no significant difference ($P > 0.05$) between A2 and A3, or between A0 and A4

on growth performance *Ancherythroculter nigrocauda* juveniles.

Immune parameters

Figure 2 showed there were significant differences (P <0.05) between the control and treatments in lysozyme (LZM, Figure 2 (A)) activity, superoxide dismutase (SOD, Figure 2 (B)) activity, acid phosphatase (ACP, Figure 2 (C)) activity, alkaline phosphatase (ALP, Figure 2 (D)) activity, plasma complement C3 (Figure 2 (E)) and the phagocytic index (PI, Figure 2 (F)), and also the lowest immune parameters were found in the control. For LZM, SOD, ACP, ALP and PI, the highest values (P <0.05) were obtained at the addition level of 500 mg/kg (A2) or 750 mg/kg (A3), but there was no significant difference (P >0.05) between A2 and A3 except PI. In addition, although the highest plasma complement C3 was in A4, no significant difference (P >0.05) appeared between A3 and A4.

DISCUSSION

Results of growth performance showed that lower doses of β(1,3)-glucan increased appetite, promoted growth, improved the efficiency of feed utilization, and so reduced the feed conversion ratio[14]. On the other hand, higher doses of β(1,3)-glucan increased intestinal digesta viscosity could enhance the thickness of immobile water layer of the intestinal mucosal surface and block diffusion of nutrients, so the cell capsule was formed, thus affecting the digestion and absorption of nutrients. And β (1,3) - dextranthe carbohydrase eliminate hinder nutrient digestion and absorption factors, thereby increasing the digestibility of feed and fish weight gain rate[11]. Moreover, it was reported that high doses of β-glucan as a good immune enhancer, could make the body in the state of immune activation, result in the redistribution of nutrients, and thereby reduce the weight gain rate[22].

Yeast β-glucan, as an important biological response modifier[1], can selectively bind to specific receptors of macrophages, granulocytes and natural killer cells, and cytokines released play key roles in the activation of T and B cells, and thus β-glucan can increase the specific and nonspecific immunity[3,21,23]. β(1, 3)-glucan receptors has now been confirmed as the complement receptor 3, lactosylceramide, scavenger receptors and C-type lectin receptors. The complement receptor 3 of macrophages is involved in macrophage recognition and phagocytosis of pathogenic microorganisms[6]. It was reported that carp (Cyprinus carpio) infected with Aeromonas hydrophila injected with glucan on day 7 had a significant increase in total blood leucocyte counts and an increase in the proportion of neutrophils and monocytes, and the superoxide anion production by kidney macrophages was also elevated[17]. These findings indicated that increased resistance by β (1, 3)-glucan feeding was indirectly mediated by non- specific immune cells and humoral defense mechanisms.

Lysozyme plays an important role in the initiation and maintenance of the body's immune response, with the dissolution of the bacterial cell walls and inducing and regulating the synthesis and secretion of other immune factors. The study found that, to a certain extent, there was a positive correlation between the number of white blood cells in the circulatory system and the serum lysozyme activity, and superoxide dismutase could scavenge reactive oxygen species in vivo and also enhance the phagocytic capacity and the immune protein produced[13]. Moreover, as a lysosomal marker enzyme in macrophage, ACP is an important part of the lysosomal, and its release is accompanied by the immune response of crustacean blood cells by engulfing and surrounding foreign objects[10]. In addition, ALP is an important enzyme in vivo with metabolic regulation and catalyzing hydrolysis of phosphate monoesters, and it is directly involved in the transfer of phosphate groups and can accelerate the uptake and absorption of substances[7] to provide inorganic phosphate for the phosphorylation of ADP to form ATP[7].

In present study, lower doses of β(1,3)-glucan addition to the diet could increase appetite, promote growth, improve the efficiency of feed utilization, and so reduce the feed conversion ratio. And effects of β(1,3)-glucan addition to the feed on LSZ, SOD, ACP, ALP, C3 and PI of *Ancherythroculter nigrocauda* juveniles indicated that β (1,3) -glucan could boost their immune systems and the immune enhancement was dose-dependent. Therefore a suitable addition of β(1,3)-glucan to the diet could be inferred: 500 mg/kg.

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