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Effects of Dietary Avilamycin on Disease Resistance, Immune Responses in Juvenile Jian Carp (*Cyprinus carpio* Var.Jian)

Yao-Peng Liu, Zhi-Qiong Li and Chao-Wei Zhou
College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, Ya'an, China

Abstract: This experiment was conducted with juvenile Jian carp to evaluate the effects of avilamycin on growth performance and body immunity. In 60 days feeding trial, a total of 1200 juvenile Jian carp (*Cyprinus carpio* var.Jian) (27.26±0.04 g, mean±SD) were fed diets containing six levels of avilamycin: 0, 25, 50, 75, 100 and 125 mg kg^{-1}. After feeding experiment, a challenge trial was conducted by injection of *Aeromonas hydrophila* for 17 days to determine the effect of dietary avilamycin on disease resistance and immune response of juvenile Jian carp. Results indicated that dietary avilamcyin significantly improved weight gain, specific growth rate, feed efficiency and protein efficiency ratio ($p<0.05$). Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Hemoglobin (HGB), Glucose in serum and Total Protein in serum (TP) improved with increasing dietary avilamycin concentration up to 50~75 mg kg^{-1} diet ($p<0.05$) and further increase of dietary avilamycin level decreased these indexes. Survival rate challenged with *Aeromonas hydrophila* were improved ($p>0.05$) with increasing dietary avilamycin concentration up to 50 mg kg^{-1} and no differences were found with further increase of dietary avilamycin level ($p>0.05$). Haemagglutinaiton titre (HA), Lysozyme Activity (LA), Acid Phosphatase Activity (ACP), Antibody titre (Ab titre) and Immunoglobulin M (IgM) content of juvenile Jian carp challenged with *Aeromonas hydrophila* all appeared first significantly improved ($p<0.05$) then slightly decreased ($p>0.05$).

Key words: Jian carp, avilamycin, disease resistance, immune responses, RBC, China

INTRODUCTION

Avilamycin is called digestive enhancer and metabolic modulator which means it can greatly improve animal growth (Treede *et al*., 2003). Avilamycin is one kind of nutritional antibiotics and animal feed with avilamycin supplement can prevent disease, improve nutrients metabolism and growth performance (Biely and March, 1951). Nutritional status is an important factor influencing immune defence mechanisms of animals. Enhance disease resistance and immune response by supplement of adequate nutrient is an important way to prevent disease infection. The growth boost function of avilamycin is related with intestinal bacteria inhibition metabolism. Avilmaycin could promote chicken growth, more obvious at the early growing phase of broiler (Dzapo and Reiner, 1991). A total of 12 experiments conducted on 1710 swines and 20000 broilers in 8 farms indicated all testing avilamycin improved animal growth effect and feed conversion rate (Dai, 2001). In the toxicology study, there was no death and toxicity symptom in mice and rats used avilamycin and no drug related damage and harmful effect to growth and propagation. It only produced minimum toxicity in oral area of rats and in skin of rabbits (Salles *et al*., 1994).

Diet preparation: The basal diet formulated to contain approximately 378.8 g crude protein kg^{-1} diet, 50.1 g crude lipid kg^{-1} diet, the composition of the basal diet was shown in Table 1. Rice protein concentrate, fish meal and soya bean meal were used as dietary protein sources; fish

Table 1: Composition and nutrients content of experimental diets

Ingredients (%)	Diets (g kg^{-1}, dry diet)	Nutrient level	Dry weight (%)
Rice protein concentrate	15.20	Crude protein	37.88
Fish meal	13.20	Crude lipid	5.01
Flour	34.10	Ash	12.18
Soybean meal	28.80	Digestible energy	9.54
Soybean oil	2.00	Gross energy (MJ kg^{-1})	9039.87
Fish oil	1.70		
Monocalcium phosphate	0.80		
Choline chloride	0.40		
Ethoxyquin	0.05		
Mineral mixture	1.00		
Vitamin	0.10		

Digestive energy = Protein+Lipid+Carbohydrate (Protein 23.9 kJ g^{-1}, Lipid 39.8 kJ g^{-1}, Carbohydrate 17.6 kJ g^{-1}), numerical value out of bracket is the theory value and in the bracket is practicaly determined; minerals premix: Fe ($FeSO_4.7H_2O$): 44 mg, Zn ($ZnSO_4.H_2O$): 80 m, Cu ($CuSO_4.5H_2O$): 4 mg, Mg ($MgSO_4.7H_2O$): 100 mg, KI: 0.65 mg, Se (Na_2SeO_4): 0.25 mg, Co ($CoCl_2.6H_2O$): 0.07 mg; Vitamins premix: V_A 4400 IU kg^{-1}, V_{D3} 2200 IU kg^{-1}, V_k 44 IU kg^{-1}, V_{B1} 11 IU kg^{-1}, V_{B2}; 13.2 IU kg^{-1}, V_{B6} 11 IU kg^{-1}, V_{B12} 0.01 IU kg^{-1}, V_H 0.5 IU kg^{-1}, pantothenate 35.2 IU kg^{-1}, V_{PP}88 IU kg^{-1}, PTGA 22 IU kg^{-1}, becholine 275 IU kg^{-1}, V_c 40 IU kg^{-1}

Corresponding Author: Yao-Peng Liu, College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, Ya'an, China

oil and soya bean oil and flour were used as dietary lipid and carbohydrate sources, respectively. Diets were prepared by thoroughly mixing all the ingredients. Distilled water was included to achieve a proper pelleting consistency and the mixture was further homogenized and extruded through a 2 mm die. The diets were cut mechanically into 5 mm long and dried using an electrical fan at 28°C for 24 h then stored at -20°C until used.

Experimental animals and husbandry: Juvenile Jian carp obtained from the Ya'an fisheries were used in this experiment and acclimated to the laboratory conditions for 4 weeks. During this period, Jian carp were fed with a diet containing 370 g crude protein kg^{-1} diet and 490 crude protein kg^{-1} diet which was similar to that of test diets. At the end of the acclimation period, a total of 1200 Jian carp (mean initial weight 27.26±0.04 g) were randomly distributed into each of 24 fishpond (200 cm W* 120 cm L* 20 cm H). Each fishpond was randomly assigned to one of four replicates of the six dietary treatments. The fishpond was connected to a closed water and oxygen auto-supplemented system. Water change rates in each fishpond were maintained at 2.5 L min^{-1} and water was drained through biofilters to remove solid substances and reduce ammonia concentration. The water temperature and pH were 24±1°C and 7.1±0.3, respectively. The experimental units were under natural light and dark cycle. After 80 days feeding, 10 fish were randomly collected from each aquarium and adapted to the culture condition for 5 days. All fish collected were infected by intraperitoneal injection with *Aeromonas hydrophila* by the modified method of Yang *et al*. (2008) at concentration of 10^{10} cfu 0.5 mL $fish^{-1}$ which is according to the preliminary study data. The control group was injected with 0.5 mL $fish^{-1}$ 0.9% NaCl solution. Then, the fish in each treatment group were randomly divided to one of four replicates. The challenge trial was conducted for 17 days at that time antibody content was highest and experiment conditions were the same to feeding trail except water temperature maintained at 25°C.

Sample collection and analysis: Fish in each fishpond were weighed at the initiation and termination of the study. Before the experiment, 30 fish from the same population were randomly selected to determine initial body composition. At the end of the study, 5 fish pooled from each fishpond were frozen, later frozen-dried, ground and analyzed for protein, lipid and ash composition (AOAC, 2002). The amount of feed casting and remnant feed collected were recorded each day. Blood was collected from caudal vein by syringe with heparin as anticoagulant from five fish of each aquarium for determining RBC count, WBC count, HGB and TP.

Number of died fish was noted during challenge trial to calculate vaccinated survival rate. At the end of the challenge trial, 5 fish per dietary treatment were anaesthetized with benzocaine (50 mg L^{-1}) 12 h after the last feeding. Blood was collected from caudal vein of carps, stored at 4°C overnight and centrifuged at 3000*g for 10 min then, stored at -20°C until it was analyzed for immune parameters.

RBC and WBC count, HGB, TP were measured by automated hematology analyzer (KX-21, Sysmex, Japan). Serum agglutination activity was determined using haemagglutination assay which was modified from Barracco *et al*. (1999) and Sritunyalucksana lysozyme activity of the serum followed immunization was measured by the method of Ellis (1990). Acid Phosphatase activity (ACP) of the serum followed immunization was sectrophotometrically measured according to Pipe (1990). Antibody titre determined using a microagglutination test by the method of Vivas. Immunoglobulin M level were determined by the method of Takemura (1993). Serial two fold dilutions of serum followed immunization from all groups were diluted using Phosphate Buffered Saline (PBS, pH 7.2) in U-shaped bottom microtitre wells to which an equal volume of freshly prepared 2% erythrocyte suspension (rabbit in PBS) was added and incubated for 2 h at 25°C. Titres were recorded as the reciprocal of the highest dilution showing agglutination.

Statistical analysis: All data were subjected to one-way analysis of variance followed by the Duncan method to determine significant differences among treatment groups and $p<0.05$ was considered to be statistically significant. The quadratic regression model was used to calculate the dietary lysine optimal level.

RESULTS

IW (g*$fish^{-1}$), FW (g*$fish^{-1}$), WG (g*$fish^{-1}$), FI (g*$fish^{-1}$), SGR, FE of juvenile Jian carp is shown in Table 2. Results indicated dietary avilamycin improved FI, SGR and FE significantly ($p<0.05$) until adding level up to 75 mg kg^{-1} and further increase of avilamycin level made no significant differences.

Table 2: IW, FW, WG, FI, SGR, FE of fish fed diets containing graded levels of dietary Avilamycin

AV	IW	FW	WG	FI	SGR	FE
A0	23.4±0.0^{a}	34.3±2.3^{a}	10.9±2.3^{a}	24.8±3.2^{a}	0.9±0.2^{a}	43.5±4.4^{a}
A25	23.4±0.1^{a}	49.0±2.8^{b}	25.6±2.8^{b}	39.1±1.7^{b}	1.8±0.1^{b}	65.4±4.4^{b}
A50	23.4±0.1^{a}	58.9±0.7^{c}	35.3±0.8^{c}	44.7±1.3^{c}	2.2±0.0^{c}	79.5±0.3^{c}
A75	23.4±0.1^{a}	62.2±2.4^{c}	38.8±2.4^{c}	44.8±2.8^{c}	2.3±0.1^{c}	86.8±4.0^{d}
A100	23.4±0.1^{a}	58.9±0.8^{c}	35.8±0.2^{c}	43.9±1.5^{c}	2.2±0.0^{c}	81.7±2.3^{c}
A125	23.4±0.0^{a}	60.7±2.2^{c}	37.4±2.2^{c}	43.2±2.1^{c}	2.3±0.1^{c}	86.7±6.1^{d}

Values are means±SD of three groups of fish (n = 3). Values within the same line having different superscripts are significantly different ($p<0.05$)

Table 3: Survival rate of juvenile Jian carp challenged with *Aeromonas hydrophila* and fed diets containing graded levels of avilamycin (AV, mg kg^{-1})

AV	0	25	50	75	100	125
Survival rate	56.6±4.68^{a}	69.28±2.15^{b}	75.54±1.12^{bc}	80.00±0.00^{c}	85.21±2.45^{d}	82.12±3.14^{c}

Values are means±SD of three groups of fish (n = 3). Values within the same row having different superscripts are significantly different ($p<0.05$)

Table 4: Red blood cell (10^{12} L^{-1}), white blood cell (10^{10} L^{-1}), HGB (g/100 mL) and TP (g L^{-1}) of juvenile Jian carp fed diets containing graded levels of avilamycin (AV, mg kg^{-1})

AV	0	25	50	75	100	125
RBC	1.06±0.15^{a}	1.21±0.21^{b}	1.26±0.22^{b}	1.24±0.18^{b}	1.02±0.34^{a}	0.98±0.45^{a}
WBC	0.60±0.25^{b}	0.73±0.53^{c}	0.96±0.75^{d}	0.78±0.86^{c}	0.57±0.46^{b}	0.36±0.35^{a}
HGB	5.81±0.64^{a}	5.93±0.65^{b}	5.91±0.98^{b}	5.81±1.23^{a}	6.04±0.24^{c}	6.15±0.64^{c}
TP	27.12±0.57^{a}	30.98±0.68^{c}	31.88±0.98^{c}	30.91±1.45^{c}	29.31±1.58^{b}	26.82±1.66^{a}

Values are means±SD of three groups of fish (n = 3). Values within the same row having different superscripts are significantly different ($p<0.05$)

Table 5: Non-specific immunity parameters of juvenile Jian carp challenged with *Aeromonas hydrophila* and fed diets containg graded levels of avilamycin (AV, mg kg^{-1})

AV	0	25	50	75	100	125
HA	31.000±0.000^{a}	37.50±21.310^{a}	68.700±25.15^{b}	75.200±13.34^{c}	70.100±35.03^{b}	78.300±28.35^{c}
LA	3.120±0.140^{a}	5.640±0.270^{b}	8.130±0.350^{c}	8.250±0.210^{c}	8.050±0.410^{c}	7.890±0.110^{c}
ACP	89.240±0.340^{a}	158.800±0.240^{b}	176.200±0.540^{b}	189.700±0.640^{b}	191.200±1.210^{bc}	188.300±0.840^{b}
C3	0.209±0.001^{a}	0.270±0.011^{b}	0.280±0.005^{b}	0.302±0.006^{c}	0.270±0.004^{b}	0.250±0.007^{b}

Values are means±SD of three groups of fish (n = 3). Values within the same row having different superscripts are significantly different ($p<0.05$)

Table 6: Specific immunity parameters of juvenile Jian carp challenged with *Aeromonas hydrophila* and fed diets containing graded levels of avilamycin (AV, mg kg^{-1})

AV	0	25	50	75	100	125
Ab titre	15.47±2.53^{a}	30.57±7.14^{b}	51.90±15.53^{d}	40.90±13.31^{bc}	48.90±15.56^{d}	35.30±13.45^{b}
IgM	89.04±4.45^{a}	157.23±6.75^{b}	190.54±8.430^{c}	179.24±13.34^{c}	174.06±8.780^{c}	183.30±9.980^{c}

Values are means±SD of three groups of fish (n = 3). Values within the same row having different superscripts are significantly different ($p<0.05$)

Survival rate (%) of juvenile Jian carp challenged with *Aeromonas hydrophila* is shown in Table 3. RBC, WBC, HGB and TP are shown in Table 4. Results indicated that with increasing dietary avilamycin levels up to 50 mg kg^{-1} diet, RBC was improved ($p<0.05$) and further increase of dietary avilamycin levels decreased RBC ($p<0.05$). WBC and TP also followed a similar pattern to that as observed with RBC. HGB appeared improved significantly all the way with increase of dietary avilamycin ($p<0.05$).

Haemagglutination titre, lysozyme activity, acid phosphatase activity and C3 of juvenile Jian carp challenged with *Aeromonas hydrophila* are shown in Table 5. Results indicated that with increasing dietary avilamycin, HA, LA (U mL^{-1} serum), ACP (U mL^{-1} serum) and C3 (g L^{-1} serum) were improved ($p<0.05$).

Antibody titre and Immunoglobulin (mg L^{-1} serum) content of juvenile Jian carp challenged with *Aeromonas hydrophila* are shown in Table 6. Results indicated that Ab titre and IgM content were very low in low avilamycin concentration treatment group. With increasing dietary avilaycin levels up to 50 mg kg^{-1} diet, Ab titre and IgM content got to maximum and no differences were found with further increase of avilamycin levels ($p>0.05$).

DISCUSSION

The results of the present study clearly showed that the growth performance was enhanced with increasing levels of dietary avilamycin. WG, FI, SGR and FE were significantly increased up to 75 mg kg^{-1} diet and thereafter reached a plateau. These results were confirmed by the fact that RBC, HGB and TP enhanced with increasing dietary avilamycin levels. The results are in agreement with the findings of experiments conducted with swine (Dzapo and Reiner, 1991).

Resistance to pathogenic bacteria can represent immunocompetence of fish. The study showed that with increasing dietary avilamycin concentration up 100 mg kg^{-1} diet, vaccinated survival rate was improved and no differences were found with further increase of avilamycin concentration.

The fish defense system is basically similar to that described in mammals (Sakai, 1999). Teleosts also have various humoral defense components such as agglutinin, complements, lysozyme (Sakai, 1999; Dalmo *et al*., 1997). Agglutinin is proteins or glycoproteins that can be directed against various saccharide moieties on cell surfaces. So, it act as opsonins and cause aggregation by binding to proteins/glycoproteins and/or carbohydrate moieties that are free in solution or are constituents of microbes (Ingram, 1980). In the study, HA of the serum followed immunization was significantly affected by dietary avilamycin concentration.

Lysozyme of fish wildly distributes in surface mucus, serum, brankia, gastric secretion, lysosome, granulocyte and monocyte. Lysozyme can kill pathogen by enzymolysising cell wall. The concentration and activity of lysozyme directly related immune function and health of fish. In the present study, lysozyme activity was

significantly affected by dietary avilamcyin concentration. Complement is an important component of microbe-resisting system, comprising various globin with lysozyme activity. C3 is the basis of complement system which can be activated by classical and bypass way. The substance produced after the activation of complements can improve phagocytic function, neutralize and dissolve virus and remove immune complex. It can be learned in some degree about the effect of antibiotic. In the study, C3 was significantly affected by dietary avilamcyin concentration.

Antibody is a very important component of specific immune responses parameters. The study showed that antibody titre and immunoglobulin content of the serum followed immunization were significantly affected by dietary avilamycin concentration.

In the past, a lot of research was conducted to know the effect of antibiotic to the immune function of fish. It is reported injection or casting oxytetracycline can produce inhibition to the humoral immunity of carp. However, Grondel found OTC may generate temporary inhibition. Wishkovsky *et al.* (1987) found tetracycline and OTC would inhibit chemiluminescence function of head kidney phagocyte (Partanen *et al*., 2002; Siwicki *et al*., 1989). Anderson (1997) found oxilinic acid had no inhibition function to specific immunity of rainbow trout and 10 mg kg^{-1} OTC and levamisol would suppress specific immunity of rainbow trout. Lunden found activity of phagocytic cells could be stimulated by oxilinic acid and suppressed by OTC both could prohibit the generation of antibody and had no influence to immune protective rate of rainbow trout. Lunden also found that florfenicol could inhibit activity of phagocytic cells of rainbow trout, decrease RPS and had no influence to the generation of antibody in rainbow trout. Villamil *et al*. (2003) found different concentrations of antibiotic produced different immune stimulation function to turbot and low concentration improved and high suppressed.

According researches before, avilamycin can bound with 30 S subunit of bacteria ribosomes and prohibit the synthetization of bacteria proteins (Kofoed and Vester, 2002). In this view, avilamycin may directly prohibit phagocytic activity of leucocytes and generation of antibodyies and finally affect carp immune response level.

CONCLUSION

The study shows that growth performance, disease resistance, non-specific and specific immune responses of juvenile Jian carp can be enhanced by supplement of adequate dietary avilamycin concentration under the experimental conditions.

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