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EFFECT OF BETA-GLUCANS ON HEMATOLOGICAL, IMMUNOGLOBULINS AND STRESS PARAMETERS OF STRIPED CATFISH (*Pangasianodon hypophthalmus*) FINGERLING

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ABSTRACT

The study aimed to investigate the effect of beta-glucans on hematological, immunoglobulins and stress parameters of striped catfish. The study included two experiments. In the first experiment, experimental fish (16.2±0.74 g) was fed for 30 days with feed containing different beta-glucans concentrations (0, 0.5, 1.0, 1.5, 2 and 2.5 g/kg). Fish blood were collected after 0, 1, 3, 7, 14 and 30 days of feeding for analysis of hematological parameters, whereas glucose, cortisol concentration and total immunoglobulins (Ig) were analyzed before initiating experiment and after 7, 14 and 30 days of feeding. In the second experiment, experimental fish (18.5±0.65 g) was fed beta-glucans (1.0 g/kg feed) for one, two and three weeks. After feeding beta-glucans with different periods, fish was crowded stressing at high density (3000 fish/m^3) for four hours then transferred into tanks (500 L) at lower density (60 fish/m^3) and blood samples were collected after 24, 48 and 72 hours for analysis of glucose, cortisol concentration and Ig. The result of experiment 1 showed that fish fed 1 g beta-glucan/kg feed had significantly higher red blood cells, white blood cells, hemoglobin, hematocrit and Ig compared to other treatments. Blood glucose and cortisol concentration in treatment fed 1.0 g/kg feed were significantly lower compared to other treatments. In the second experiment, the concentration of glucose and cortisol in fish fed beta-glucans (1g/kg feed) for three weeks were significantly lower compared to fish fed beta-glucans for one and two weeks when fish being stressed at high density. In fish fed beta-glucans for three weeks, total immunoglobulins was significantly higher than those of other treatments. In conclusion, the optimal level of beta-glucans adding into diet of striped catfish fingerlings should be 1.0 g/kg which improved fish health and stress resistance.

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1 INTRODUCTION

Nowadays, striped catfish is mainly farmed in the provinces in the Mekong Delta (De Silva and Phuong, 2011). The industry has significantly grown in recent years, reaching 1.1 million tons in 2014, making striped catfish production to become one of the most intensive aquaculture industries in the world (Directorate of Fisheries, 2015). Striped catfish is exported to more than 140 countries and was

worth US$ 1.77 billion in 2014 (Directorate of Fisheries, 2015). Highly intensive production in open farming systems led to frequent disease outbreaks and high mortality rates, mainly caused bacterial disease e.g. Bacillary necrosis of Pangasius (BNP) caused by *Edwardsiella ictaluri* (Crumlish *et al*., 2002; Dung *et al*., 2008; Phan *et al*., 2009). Typical mortality rates are up to 30% from after stocking to the mid-production cycle and less than 10% in later months (Phan *et al*., 2009). Research on immune-stimulants may enhance the fish health, especially in the transfer stage between nurseries to grow-out ponds.

Among different immune-stimulants used in aquaculture practices, beta-glucans is one of the promising immune-stimulant (Meena *et al*., 2012). Beta-glucans are glucose polymers which have been widely used in aquaculture sector in order to improve the fish health and stress resistance. Studies on the effectiveness of beta-glucans have been reported for several fish species e.g. sea bass (*Dicentrarchus labrax*) (Bagni *et al*., 2005), Nile tilapia (*Oreochromis niloticus*) (Barros *et al*., 2014) and orange-spotted grouper (*Epinephelus coioides*) (Chang *et al*., 2013). Feeding beta-glucans enhanced white blood cells in *Labeo rohita* (Misra *et al*., 2006), giant snakehead (Tam and Nguyet, 2012), common carp (*Cyprinus carpio*) (Ayyaru *et al*., 2010), salmon (Weinreb, 1958). Administration of beta-glucans had been found to enhance fish resistance to environmental stress (Vetvicka *et al*., 2013; Soltanian *et al*., 2014) or reduced transportation stress in rainbow trout (Jeney *et al*., 1997). Striped catfish injected with *E. coli* lipopolysaccharide enhanced innate immune responses (Hang *et al*., 2014). However, information on the effectiveness of beta-glucans on fish health improvement and stress resistance on striped catfish are limited.

Therefore, the study aimed to determine the effect of beta-glucans feeding at different concentrations and periods on hematological, total immunoglobulins and stress parameters of striped catfish fingerling.

2 METHODS

2.1 Effects of different beta-glucans concentrations on hematological, total imunoglobulins and stress parameters of stripped catfish fingerling

2.1.1 Experimental setup

The experiment included six different dietary beta-glucans treatments. Beta-glucans (1.3/1.6 beta-D-glucans, 40% in the powder form) were obtained from Vemedim Company (Can Tho, Vietnam) which is imported from Germany. Beta-glucans was diluted into 200 mL water at the calculated dose for each treatment. The solution was sprayed onto the commercial pellet feed (26% crude protein, 7% crude lipid, 11% moisture, 1.5 mm). Fish oil (15 mL/kg feed) was added in all experimental diets to reserve the beta-glucans. The feed was mixed by hand to achieve homogenously. Experimental feed containing 0, 0.5, 1.0, 1.5, 2.0, 2.5 g beta-glucans was prepared in one kg each time and kept at -20°C until feeding.

Fish (16.2±0.74 g) were randomly assigned into 18 composite tanks (500 L) at stocking density of 50 fish/tank. Tanks were equipped with aeration. Each treatment was triplicated. Fish were fed twice a day at 8:00 am and 4:00 pm to satiation. The experiment lasted for 30 days. During the experiment, water exchange was done weekly at the rate of 50% tank volume.

2.1.2 Sample collection

Fish blood (three fish/tank) was collected one day before starting feeding beta-glucans. Fish blood was then collected after feeding beta-glucans 1, 3, 7, 14 and 30 days. Fish blood (0.4-0.6 mL) was withdrawn by using a 1 mL syringe with needle from the tail artery. Blood samples were stored in ice during sampling. Samples were divided into two parts. The first part of fish blood (0.3 mL) was used to analyze hemoglobin, hematocrit, the number of red blood cells and white blood cells while the remaining blood (0.3 mL) was centrifuged at 6000 rpm in 6 min at 4°C to obtain the plasma, stored at -80°C until analysis of cortisol, glucose and total Ig. It is noted that analysis of cortisol, glucose and total Ig was done for initial sampling and 7, 14 and 30 days after feeding

Water temperature (27.1-28.1°C), dissolved oxygen (above 5 mg/L) and pH (7.3-7.8) ranged on acceptable values throughout the experimental period.

2.1.3 Analytical methods

Red blood cell (RBC) counting

Total RBC was determined using a Neubauer hemocytometer with Natt-Herrick solution (pH 7.3) as a diluent stain (Natt and Herrick, 1952). Dilute 5 µL of each blood sample into 995 µL of Natt and Herrick's solution and mixed gently. The RBC was counted in five of the 25 small areas under microscope.

White blood cell (WBC) counting

A small drop of whole blood was smeared on a microscope slide by using a smearing slide. The

slide smear was dried quickly, fixed in methanol (95%, M1775, Sigma) for 1-2 min and stained with Wright's and Giemsa (Rowley, 1990). Results of WBC were calculated according to Hrubec *et al*. (2000).

Hemoglobin determination

Hemoglobin was determined by Drabkin reagent (Oser, 1965). Drabkin's reagent was used for quantification of hemoglobin concentration through spectrophotometer measurement in whole blood at 540 nm.

Hematorit determination

Hematocrit value was determined by the standard micro-hematocrit method, and expressed in percentage. Blood-filled heparinized micro-hematocrit capillary tubes were centrifuged at 12000 rpm for 5 min. And the hematocrit values were read directly by measure the length of the column of the packed red cells and divide it by the length of the whole column of blood (cells and plasma).

Total Ig assay

The total immunoglobulins concentration of sample was measured following the method described by Siwicki and Anderson (1993), modified by Milla *et al.* (2010). Briefly, immunoglobulins were precipitated with 10,000 kDa polyethylene glycol (PEG, Sigma). Serums were mixed with 12% PEG solution (v:v) for 2 hrs at room temperature under constant shaking. After centrifugation at 1000 *g* for 10 min, the supernatant was collected and assayed for its protein concentration. The total immunoglobulins concentration was calculated by subtracting this value from the total protein concentration in the plasma before precipitation with PEG.

Cortisol assay

Plasma cortisol was assayed in duplicate using a cortisol ELISA kit (DRG Instruments GmbH, Germany) following manufacturer's instructions.

Glucose assay

Plasma was measured as follows: plasma (25 µL) was deproteinized by adding 50 µL of perchloride acid 0.33 M, and centrifuged at 3000 *g* (for 10 min at 4°C). Glucose concentration was determined in the supernatant with reactive solution (adding glucose oxidase, peroxidase and ABTS with phosphate buffer 0.1M) according to method of Hugget and Nixon (1957).

2.2 Effects of different beta-glucans feeding periods on total immunoglobulins and stress parameters of stripped catfish fingerling

Fish (18.5±0.65 g) were stocked at 60 fish/m^3 in nine tanks (500 L). Fish was fed beta-glucans (1 g/kg, optimal concentration found in the first experiment) for one, two and three weeks. Each treatment was triplicated. After beta-glucans feeding with different periods, fish was crowded stressed at high density (3000 fish/m^3) for four hours. The fish blood (3 fish/tank) was collected before and after 4 hours of stressing. After stressing, fish was transferred into tanks (500 L) at density of 60 fish/m^3 (normal pond condition, Phan *et al*., 2009) and blood samples were collected after 24, 48 and 72 hours. Survival rate was observed. Blood sampling of each fish and analysis methods were applied the same as the first experiment. Analysis of cortisol, glucose and total Ig was done for this experiment.

Water temperature (27.4-29.2°C), dissolved oxygen (above 5 mg/L) and pH (7.0-7.1) ranged on acceptable values throughout the experimental period.

2.3 Data analysis

Data were calculated the mean value and standard error by using Microsoft Excel 2010. Difference in mean between experimental parameters was compared statistically by Oneway-ANOVA following by Duncan test using SPSS 16.0 (USA) software at significant level of 95%.

3 RESULTS

3.1 Effects of different beta-glucans concentrations on hematological, total immunoglobulins and stress parameters of stripped catfish fingerling

3.1.1 Red blood cell (RBC) and white blood cell (WBC)

Red blood cells (RBC) of experimental fish fed different beta-glucans concentrations are presented in Table 1. RBC before feeding beta-glucans was $1.80x10^6$ – $2.29x10^6$ cells/mm^3 and there was no significant difference between treatments ($P>0.05$). During feeding of beta-glucans from day one to day seven, there was no significant difference in RBC between treatments. However, RBC in treatment fed 1 g/kg was significantly higher than that of other treatments ($P<0.05$) after 14 ($2.81x10^6$ cells/mm^3) and 30 days ($2.71x10^6$ cells/mm^3) feeding beta-glucans. There was no significant difference between other treatments in RBC after 14 and 30 days of feeding ($P>0.05$), except treatment feeding 1 g/kg beta-glucans.

Table 1: Red blood cell (RBC) (10^6 cells/mm^3) and white blood cell (WBC) (10^3 cells/mm^3) of experimental fish fed different beta-glucans concentrations

Treatments	Day 0	Day 1	Day 3	Day 7	Day 14	Day 30
Red blood cell (10^6 cells/mm^3)						
0	2.13±0.69^a	2.15±0.50^a	2.32±0.45^a	2.35±0.70^a	2.34±0.55^a	2.31±0.20^a
0.5	1.80±0.36^a	2.36±0.41^a	2.32±0.33^a	2.39±0.54^a	2.47±0.37^{ab}	2.43±0.36^a
1.0	2.11±0.50^a	2.23±0.32^a	2.50±0.29^a	2.52±0.79^a	2.81±0.40^b	2.71±0.13^b
1.5	2.12±0.71^a	2.04±0.33^a	2.42±0.77^a	2.51±0.53^a	2.41±0.30^a	2.44±0.37^a
2.0	2.29±0.66^a	2.13±0.28^a	2.11±0.30^a	2.28±0.70^a	2.27±0.39^a	2.25±0.20^a
2.5	1.91±0.65^a	2.11±0.31^a	2.17±0.19^a	2.09±0.35^a	2.08±0.27^a	2.26±0.29^a
White blood cell (10^3 cells/mm^3)						
0	69.5±11.6^a	71.3±14.0^a	70.6±12.8^a	72.8±13.5^a	74.1±12.8^a	78.5±18.1^a
0.5	75.0±10.5^a	80.0±9.10^a	81.5±11.4^a	84.7±10.6^a	87.9±11.0^b	92.0±9.90^{ab}
1.0	72.8±13.9^a	80.7±3.90^a	84.2±9.10^a	100±9.60^b	114±16.5^c	119 ±19.1^c
1.5	73.4±19.2^a	73.4±10.4^a	77.4±8.40^a	79.0±9.50^a	89.9±8.40^b	98.0±7.90^b
2.0	70.6±16.2^a	76.6±8.20^a	77.4±20.8^a	79.8±10.9^a	86.4±4.20^{ab}	91.9±15.6^{ab}
2.5	71.9±10.1^a	76.9±15.7^a	76.0±14.6^a	80.1±18.8^a	83.8±15.6^{ab}	85.4±16.0^{ab}

Data are presented as mean±standard error

The different letters in the same column indicated the significant difference between treatments (P<0.05)

White blood cells (WBC) counts are presented in Table 1. There was no significant difference between treatments (P>0.05) in WBC before feeding beta-glucans, ranged 69.5x10^3 – 75x10^3 cells/mm^3. During feeding of beta-glucans from day one to day three, there was no significant difference in WBC between treatments (P>0.05). However, there was an increase in WBC after seven days of feeding beta-glucans in all treatments. WBC in treatment fed 1 g/kg was significantly higher than that of other treatments (P<0.05) after seven (100x10^3 cells/mm^3), 14 (114x10^3 cells/mm^3) and 30 days (119x10^3 cells/mm^3) feeding beta-glucans. After 30 days of feeding beta-glucans, the lowest WBC was found in control treatment (78.5x10^3 cells/mm^3).

3.1.2 Hemoglobin and hematocrit

Hemoglobin and hematocrit of experimental fish fed different beta-glucans concentrations are shown in Table 2. Similar to RBC and WBC, hemoglobin and hematocrit in treatment fed 1 g beta-glucans/kg feed had the higher value than those of other treatments after 14 days of feeding. Hemoglobin in treatment fed 1 g beta-glucans/kg feed was significantly higher than that of other treatments (P<0.05) after 30 days (10.4 g/100 mL) of feeding. Hematocrit in treatment fed 1 g beta-glucans/kg feed was significantly higher than that of control treatment (P<0.05) after 30 days (39.7%) feeding.

Table 2: Hemoglobin (g/100 mL) and hematocrit (%) of experimental fish fed different beta-glucans concentrations

Treatments	Day 0	Day 1	Day 3	Day 7	Day 14	Day 30
Hemoglobin (g/100 mL)						
0	8.07±1.41^a	7.86±1.83^a	8.20±1.46^a	8.18±1.02^a	8.03±1.15^a	7.94±1.11^a
0.5	7.98±0.94^a	8.16±1.31^a	8.00±1.32^a	7.96±1.25^a	8.70±1.73^{ab}	8.08±0.85^a
1.0	8.30±0.94^a	7.75±1.20^a	7.66±1.50^a	7.41±1.74^a	9.82±0.91^b	10.4±1.03^b
1.5	7.73±0.91^a	7.69±1.11^a	8.19±1.38^a	8.34±1.87^a	8.73±1.13^{ab}	8.76±0.88^a
2.0	8.44±1.18^a	8.51±0.90^a	8.54±1.85^a	8.29±1.46^a	8.54±1.39^{ab}	8.26±1.42^a
2.5	7.86±0.98^a	7.32±1.19^a	7.53±0.75^a	7.34±1.13^a	7.87±1.17^a	7.76±1.22^a
Hematocrit (%)						
0	33.7±2.58^a	31.4±2.61^a	30.7±3.93^a	30.6±4.54^a	31.9±3.79^a	32.7±4.31^a
0.5	33.1±4.46^a	34.2±4.73^a	35.1±2.81^a	34.3±3.09^{ab}	33.3±2.80^a	35.0±4.78^{ab}
1.0	31.8±2.29^a	34.4±2.81^a	35.1±5.05^a	36.8±2.80^b	39.3±2.71^b	39.7±2.32^b
1.5	31.8±2.29^a	34.4±2.81^a	35.1±5.05^a	35.1±3.35^{ab}	35.7±2.85^{ab}	35.5±3.41^{ab}
2.0	32.9±3.22^a	31.7±5.07^a	32.4±3.00^a	32.8±1.68^{ab}	32.1±4.12^a	34.7±2.63^{ab}
2.5	31.7±2.10^a	34.7±5.00^a	33.3±3.69^a	34.3±2.30^{ab}	34.5±4.15^a	35.3±1.86^{ab}

Data are presented as mean±standard error

The different letters in the same column indicated the significant difference between treatments (P<0.05)

3.1.3 Total immunoglobulins

Total immunoglobulins (Ig) of experimental fish are presented in Table 3. There was no significant difference between treatments (P>0.05) in total Ig before feeding beta-glucans, ranged 5.24 – 6.96 mg/mL. There was an increase in total Ig after seven days of feeding beta-glucans in all treatments. Total Ig in treatment fed 1 g/kg was significantly higher than that of other treatments (P<0.05) after 7, 14 and 30 days of beta-glucans feeding.

Table 3: Total immunoglobulins (mg/mL) of experimental fish fed different beta-glucans concentrations

Treatments	Day 0	Day 7	Day 14	Day 30
0	5.24±1.58^{a}	7.86±1.21^{a}	9.45±1.64^{a}	9.94±0.51^{a}
0.5	6.96±1.65^{a}	11.3±1.2^{b}	12.1±0.41^{b}	12.3±1.22^{b}
1.0	5.35±0.99^{a}	15.0±2.01^{c}	15.5±1.55^{c}	16.1±1.41^{c}
1.5	5.85±1.31^{a}	12.1±1.53^{b}	12.5±0.08^{b}	13.8±1.61^{b}
2.0	6.33±1.17^{a}	10.9±0.94^{b}	11.1±1.77^{ab}	11.8±2.04^{ab}
2.5	5.75±0.52^{a}	11.8±1.71^{b}	12.2±1.40^{b}	12.1±1.26^{ab}

Data are presented as mean±standard error

The different letter in the same column indicated the significant difference between treatments (P<0.05)

3.1.4 Cortisol and glucose

Cortisol and glucose level in plasma of experimental fish fed different beta-glucans concentrations are presented in Table 4. At the beginning, there was no significant difference in cortisol (57.4 to 60.8 ng/mL) and glucose (78.3-84.5 mg/100 mL) levels between treatments. Results showed that there was a reduction of cortisol and glucose level after 30 days of feeding beta-glucans. At this sampling time, the level of cortisol (36.2 ng/mL) and glucose (60.5 mg/100 mL) in fish fed 1 g/kg was significantly higher than those of other treatments (P<0.05).

Table 4: Cortisol (ng/mL) and glucose (mg/100 mL) of experimental fish fed different beta-glucans concentrations

Treatments	Day 0	Day 7	Day 14	Day 30
Cortisol (ng/mL)				
0	58.9±1.9^{a}	54.6±1.8^{b}	51.9±2.2^{c}	49.0±0.6^{c}
0.5	58.8±2.7^{a}	50.7±2.7^{b}	47.8±2.5^{b}	45.5±0.8^{b}
1.0	60.8±3.7^{a}	40.7±2.1^{a}	37.9±2.5^{a}	36.2±2.3^{a}
1.5	59.4±1.2^{a}	50.4±1.2^{b}	48.5±1.4^{bc}	46.5±0.9^{bc}
2.0	60.6±4.9^{a}	51.2±1.7^{b}	49.5±1.2^{bc}	47.7±2.0^{bc}
2.5	57.4±4.6^{a}	51.2±3.2^{b}	50.7±1.5^{bc}	46.6±1.1^{bc}
Glucose (mg/100 mL)				
0	76.9±6.1^{a}	71.3±5.9^{b}	71.2±7.0^{b}	70.3±6.8^{b}
0.5	82.3±4.2^{a}	73.4±6.1^{bc}	73.1±6.6^{b}	70.5±5.3^{b}
1.0	84.5±6.4^{a}	62.2±2.5^{a}	61.0±2.5^{b}	60.5±3.1^{a}
1.5	78.3±5.3^{a}	72.8±2.4^{bc}	70.0±4.6^{b}	67.9±5.0^{b}
2.0	82.2±5.2^{a}	77.3±5.9^{c}	75.2±7.9^{b}	70.8±4.8^{b}
2.5	83.9±4.8^{a}	74.0±4.9^{bc}	74.1±4.8^{b}	70.9±4.7^{b}

Data are presented as mean±standard error

The different letters in the same column indicated the significant difference between treatments (P<0.05)

3.2 Effects of different beta-glucans feeding periods on stress parameters, total immunoglobulins and survival rate of stripped catfish fingerling

3.2.1 Cortisol and glucose

In this experiment, after feeding beta-glucans (1 g/kg) with different feeding periods, cortisol and glucose levels was not significant difference between treatments (P>0.05) (Figure 1 and 2). After four hours crowding stress, cortisol and glucose levels increased in all treatments. Cortisol and glucose levels in treatments fed beta-glucans for three weeks was significantly lower compared to other treatments (P<0.05). The same trend was found after transferring fish into normal conditions.

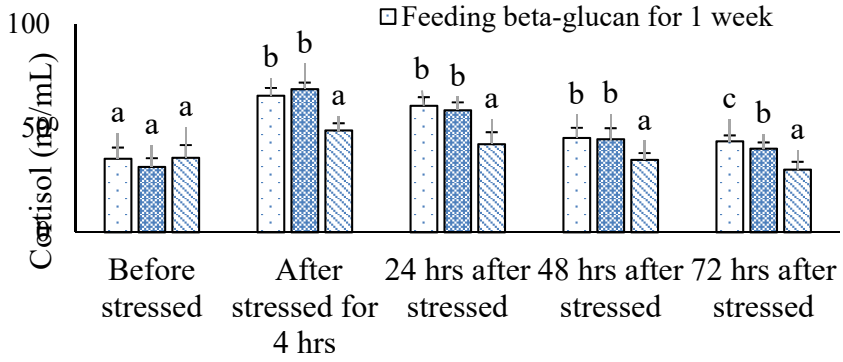

Fig. 1: Cortisol (ng/mL) level of experimental fish fed different beta-glucans feeding periods

Different letters above bars indicated significant difference (P<0.05) between sampling times

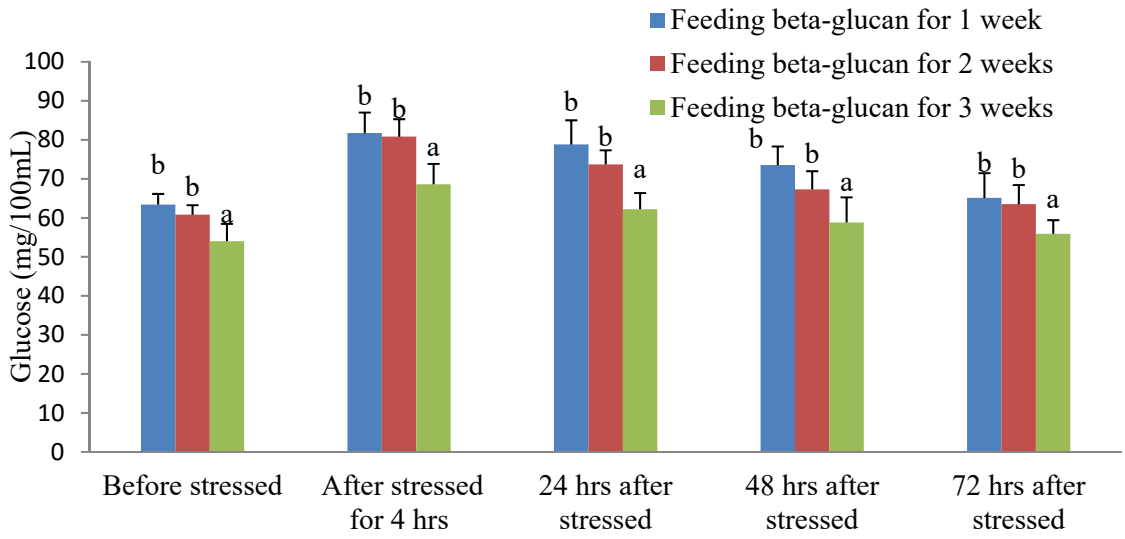

Fig. 2: Glucose (mg/100 mL) level of experimental fish fed different beta-glucans feeding periods

Different letters above bars indicated significant difference (P<0.05) between sampling times

3.2.2 Total immunoglobulins

Total immunoglobulins (Ig) of experimental fish fed different beta-glucans feeding periods are shown in the Table 5. After feeding beta-glucans for two and three weeks, the total Ig was significantly higher than that of feeding beta-glucans for one week (P<0.05). There was no significant difference in total Ig between feeding beta-glucans for two and three weeks (P>0.05). After four hours crowding stress the experimental fish, total Ig level decreased in all treatments but the total Ig in treatment fed beta-glucans for three weeks was significantly higher than that of other treatments (P<0.05). There was no significant difference in total IG between treatments after transferring fish into normal pond condition, 60 fish/m^3.

Table 5: Total immunoglobulins of experimental fish fed different beta-glucans feeding periods

Treatments	Before stressed	After stressed for 4 hrs	24 hrs after ending of stressing	48 hrs after ending of stressing	72 hrs after ending of stressing
Feeding beta-glucans for 1 week	12.2±1.2^a	10.0±1.4^a	10.3±1.7^a	10.4±1.6^a	10.6±1.6^a
Feeding beta-glucans for 2 weeks	14.6±1.8^b	9.77±1.5^a	9.95±2.2^a	10.2±1.3^a	10.6±1.2^a
Feeding beta-glucans for 3 weeks	14.9±1.3^b	11.6±1.9^b	11.9±1.9^a	12.1±1.4^a	12.6±1.4^a

Data are presented as mean±standard error

The different letters in the same column indicated the significant difference between treatments (P<0.05)

3.2.3 Survival rate

Feeding beta-glucans for different periods did not affect on survival rate before shocking, survival rate was 100% in all treatments. After four hours crowding stress the experimental fish, the significant lowest survival rate (93.3%) was found in treatment fed beta-glucans for one week while the survival rate in other treatments were higher than 98%. There was no significant difference in survival rate between treatments after transferring fish into normal condition, survival rate ranged 96.4-98.9%.

4 DISCUSSION

4.1 Hematological parameters

Results showed that after feeding striped catfish with beta-glucans at level of 1g/kg for 30 days, red blood cell, white blood cell (WBC), hematorit and hemoglobin significantly increased. This finding is in agreements with other fish species which feeding beta-glucans enhanced hematological parameters. Misra *et al.* (2006) reported that feeding beta-glucans to *Labeo rohita* at level of 250 mg/kg for 42 days enhanced WBC, phagocytes and lysozyme activities. Tam and Nguyet (2012) also reported that feeding beta-glucans to giant snakehead at level of 0.5 g/kg increased WBC. Ayyaru *et al*. (2010) concluded that feeding beta-glucans (1% in feed) to common carp (*Cyprinus carpio*) for 60 days enhanced the WBC. Weinreb (1958) reported that supplementation of immune-stimulant enhanced WBC in salmon blood. Supplementation of immune-stimulant in striped catfish diets increased hematological parameters. Addition of 0.8 g *Saccharomyces cerevisiae* per kg feed in diet for striped catfish enhanced the WBC compared to control treatment (Thuy *et al*., 2012). In striped catfish, lipopolysaccharide injection significantly increased white blood cell number and quantity of monocyte (Hang *et al*., 2013). WBC are important cells of the non-specific immune pathways in fish, thus, the increase of WBC may be considered as a good indicator of activatory response of fish cellular immunity by beta-glucan stimulation (Dalmo *et al*., 1997). The increased beta-glucans inclusion concentration in diet above 1 g/kg in the first experiment did not enhance the hematological parameters which is consistent with findings of Hang *et al*. (2013) who reported that lipopolysaccharide (LPS) injection and oral feeding at high dosage did not enhance the WBC. Moreover, supplementation of immune-stimulant in striped catfish diets would help to reduce stress during the transportation. The number of erythrocyte and white blood cell of the striped catfish fed vitamin C diets (20, 30 and 40 mg/kg fish) did not change during four hours of transportation (Huong *et al*., 2012).

4.2 Total immunoglobulins

Total immunoglobulins in striped catfish was significantly increased after feeding beta-glucans at level of 1g/kg for 30 days. Also, after two and three weeks feeding beta-glucans to striped catfish at level of 1g/kg in the second experiment, the Ig was significantly higher than that of feeding for one week. Hang *et al.* (2013) also reported that the innate immunity of striped catfish was responsive to all LPS treatments but the strongest stimulation was reached at the low dose of 3 mg/kg. Hang *et al*. (2014) also reported that feeding striped catfish with LPS at level of 0.01 mg/kg feed for four weeks significantly enhanced total Ig compared to treatment feeding 0.05 mg LPS/kg feed. The effectiveness of beta-glucans is depended on species, types of glucans, administration and combined with other stimulants (Couso *et al*., 2003; Bridle *et al*., 2005; Del Rio-Zaragozaet *et al*., 2011; Jaafar *et al*., 2011). Administration of beta-glucans through various routes including immersion, oral or injection have been found to enhance many types of immune responses, resistance to bacterial and viral infections and resistance to environmental stress (Vetvicka *et al*., 2013). In previous studies, enhanced activities of important parameters related to innate immunity were detected (Siwicki *et al*., 1993; Jeney *et al*., 1997; Volpatti *et al*., 1998).

4.3 Stress parameters

Feeding beta-glucans at level of 1 g/kg for three weeks significantly reduced level of glucose level in plasma of experimental fish while there was no significant difference in cortisol level between treatments. Moreover, after crowding stress for four hours, cortisol and glucose levels were significantly lower in treatment fed beta-glucans for three weeks. Upcoming study should elongate the feeding period to investigate if the longer beta-glucans feeding, the lower level of stress indicators. Plasma cortisol and glucose in striped catfish were not affected by LPS treatments (Hang *et al*., 2014). Plasma cortisol is an indicator of the primary stress response and is relevant to evaluate fish welfare. Administration of beta-glucans had been found to enhance fish resistance to environmental stress (Vetvicka *et al*., 2013). Supplementation of beta-glucans in diet (0.5 and 1%) for striped catfish in nine weeks resisted stress due to cold conditions by decreasing temperature from 28°C to 15°C. Also, survival rate of treatment fed 0.5% and 1% was significantly higher than those of fed 0 and 2% (Soltanian *et al*., 2014). Huong *et al*. (2012) reported that the significant difference of cortisol levels

of the striped fish fed high vitamin C diets (40 mg/kg) was not found during transportation. Plasma glucose concentrations of the fish during transportation fed with 40 mg/kg vitamin C diets were lower than those of without vitamins C and lower vitamin C levels (20 and 30 mg/kg). The lowest cortisol levels in plasma against transportation stress in rainbow trout feeding 0.1 % beta-glucans was confirmed (Jeney *et al*., 1997). Our findings stimulated stressing under crowded conditions in wet laboratory which promising applied in on-farm trial as feeding beta-glucans to striped catfish fingerlings before transportation to grow-out farms. Misra *et al.* (2006) reported that feeding beta-glucans to *Labeo rohita* fingerlings enhanced survival rate which is consistent with our findings in the second experiment.

5 CONCLUSIONS

Feeding beta-glucans at level of 1 g/kg for three weeks significantly reduced level of cortisol and glucose level in plasma of experimental fish. Moreover, after crowding stress for four hours, cortisol and glucose level was significantly lower in treatment fed beta-glucans for three weeks. In conclusion, the optimal level of beta-glucans addition into feed should be 1.0 g/kg which improved fish health and stress resistance of striped catfish fingerling. The upcoming study will confirm if beta-glucans can reduce stress during transportation between nursery to grow-out ponds and fish health improvement after stocking into grow-out ponds.

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