Lat. Am. J. Aquat. Res., 45(4): 690-698, 2017
DOI: 10.3856/vol45-issue4-fulltext-5

Research Article

Effect of β-glucan dietary levels on immune response and hematology of channel catfish *Ictalurus punctatus* juveniles

**Jesús Genaro Sánchez-Martínez[1], Jaime Luis Rábago-Castro[1], María de la Luz Vázquez-Sauceda[1]
Roberto Pérez-Castañeda[1], Zeferino Blanco-Martínez[1] & Flaviano Benavides-González[1]**
[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas
Victoria, Tamaulipas, Mexico
Corresponding author: Flaviano Benavides-González (flbenavides@docentes.uat.edu.mx)

ABSTRACT. Biological response modifiers such as pro and prebiotics have been recently used in aquaculture showing abilities as immunostimulants, enhancing resistance to different fungal, bacterial and parasitic pathogens and tolerance to environmental stress. This experiment evaluated three different concentrations (0.05, 0.1 and 0.5%) of β-1,3/1,6 glucans on nonspecific immune parameters of channel catfish *Ictalurus punctatus*. Results showed that intake supplementation with 0.05% of β-1,3/1,6 glucans provides a greater immunostimulation over the fourth treatment week. Significant higher differences ($P < 0.05$) were obtained on parameters related to phagocytic activity on blood cells, also other assays showed more reactive cells to nitroblue tetrazolium (NbT) stain on spleen and head kidney analysis. We conclude that the use of 0.05% of β-1,3/1,6 glucans added to a commercial diet improves nonspecific immune system of channel catfish *I. punctatus*.

Keywords: β-glucans, *Ictalurus punctatus*, channel catfish, fish, immunostimulation, *Saccharomyces cerevisiae*, aquaculture, Mexico.

INTRODUCTION

Catfish culture is a worldwide industry encompassing different culture methods. Fish cage culture is a highly efficient intensive system that is a potentially stressful environment due to overcrowding, inducing fish susceptibility to pathogen infection due to immune system alterations, which may result in fish disease (Villamil *et al.*, 2003). In recent years, immunostimulants have been included in the feed of cultured fish in order to improve their immune system (Meena *et al.*, 2013; De *et al.*, 2014; Vaseeharan & Thaya, 2014; Yang *et al.*, 2016), stress resistance, antioxidant activity (Zhao *et al.*, 2012; Guzmán-Villanueva *et al.*, 2014; Zanuzzo *et al.*, 2015; Zeng *et al.*, 2016), inflammatory response (Boltaña *et al.*, 2011), and resistance to bacterial (Bonaldo *et al.*, 2009; Meshram *et al.*, 2015), virus (Parmar *et al.*, 2012) and parasite infections (Jaafar *et al.*, 2011). Glucans are polysaccharides with different primary molecular structures that vary depending on their source, which have different implications on their immunostimulant activity (Akramienė *et al.*, 2007); glucans are commonly found in mushrooms, algae, yeast, cereals and cell walls of bacteria (Ringø *et al.*, 2011; Meena *et al.*, 2013). Glucans called β-glucans have glycosidic linkages β-1,3 and β-1,6 and can be found on baker's yeast; this type of glucans exhibit the greatest ability to increase immune ability (Ringø *et al.*, 2011).

β-glucans (1,3/1,6) have been widely used to stimulate innate immune response, and to improve parameters like phagocyte activity, respiratory burst activity, nitric oxide, complement and lysozyme activity, as well as to increase the number of leukocytes (Ringø *et al.*, 2011; Bridle *et al.*, 2005) on salt and freshwater fish, such as Atlantic salmon *Salmo salar* (Bridle *et al.*, 2005); rose snapper *Lutjanus guttatus* (Del Rio-Zaragoza *et al.*, 2011); sea bass *Dicentrarchus labrax* (Bonaldo *et al.*, 2009); grass carp *Ctenopharyngodon idella* (Kim *et al.*, 2009); rainbow trout *Oncorhynchus mykiss* (Jaafar *et al.*, 2011) and Nile tilapia *Oreochromis niloticus* (Cain *et al.*, 2003). In this regard, channel catfish *Ictalurus punctatus* also seems to respond to β-glucan immunostimulation when whole yeast cells have been used to improve its immune system (Welker *et al.*, 2007).

Corresponding editor: Jesús Ponce-Palafox

Channel catfish *Ictalurus punctatus* is endemic of North America. It is an important cultured fish species in Mexico (Lara-Rivera *et al.*, 2015), susceptible to bacterial or parasite diseases (Rábago-Castro *et al.*, 2011) endemic to this area; this has increased the use of chemicals on water bodies as treatment against these pathogens, which in turn can result in water contamination. This has led to the quest to find alternatives to enhance fish resistance to disease, and at the same time, to decrease the use of water-contaminating chemotherapeutants. Little is known about the specific effect that β-1,3/1,6 glucans may have over the nonspecific immune system of channel catfish; therefore, the present study was designed to assess the influence of purified β-1,3/1,6 glucans on hematological parameters, focusing on those associated to the nonspecific immune response.

MATERIALS AND METHODS

Fish

Sixty channel catfish *I. punctatus* of 35.04 ± 6.95 g body weight (mean ± SD) and 14.62 ± 1.25 cm of fork length were obtained from a local hatchery (Abasolo, Tamaulipas, Mexico), and were transferred to the Aquaculture Assay Laboratory in Veterinary and Zootechnic College (Victoria, Tamaulipas, Mexico) for acclimation during two weeks before the experiment, during this period all fish were fed with control diet with 32% of protein (Purina®, México). Photoperiod duration during experiment was natural (~689 min) according to the National Oceanic & Atmospheric Administration solar calculations from the U.S. Department of Commerce. Fish naturally infected with ectoparasites were treated with 570 mg L^{-1} of 3% hydrogen peroxide for four min (Benavides-González *et al.*, 2015). Fish were placed in twelve 40 L aquaria filled with 37 L ground well freshwater; a constant water flow (18 L h^{-1}) was maintained in aquaria during the acclimation and experimental periods. Air supply was provided with two (2") airstones connected to a ¼ HP blower; water temperature was kept at 26 ± 1ºC; aquarium water pH were 7.5 and dissolved oxygen averaged 6 ± 0.5 mg L^{-1}; these parameters were measured with a freshwater kit (La Motte®, Chertestwon, MD, USA).

Experimental design and diet preparation

Fish were randomly divided into four groups (15 fish per group, each group in triplicate). Groups were labeled CD, BG 0.05, BG 0.1 and BG 0.5, according to the concentration of the β-1,3/1,6 glucans added to the feed diet (0, 0.05, 0.1 and 0.5% respectively). The trial was run for five weeks and fish were fed 6 day a week. Fish were fed carefully until satiation twice a day (09:00 and 16:00 h).

Highly purified *Saccharomyces cerevisiae* β-1,3/1,6 glucan (Beta G® Nutribem, SA de CV, Guadalajara, Jalisco, Mexico) was added to the experimental diets. Experimental diets were prepared according to Del Rio-Zaragoza *et al.* (2011) with slight modifications using a commercial pelleted catfish food with 32% of protein (Purina®, México) that was ground to powder using a manual grain stone grinder. Afterwards, particles were mixed for 15 min using a powder mixer carefully adding β-1,3/1,6 glucan previously weighed to ensure the correct final concentration. Once the diets were homogenized, 700 mL of distilled water at 70°C were added to each kg of feed, this moisture allowed the binding of the product to the commercial food; then each mixture was pelletized using a butcher grinder equipped with a 3 mm diameter sieve. Pellets were dried at room temperature for 72 h, and stored at -4°C until required. Control diet was similarly processed without the addition of β-1,3/1,6 glucan.

Sample collection

All fish were carefully managed to minimize stress and were fasted 24 h before sampling. Fish were anesthetized with benzocaine (40 mg L^{-1}); one fish from each aquarium (three fish per group) was randomly sampled each week, weighed and measured. Heart blood was collected (0.05-1 mL) using insulin syringes and immediately placed in 1 mL micro-centrifuge tagged tubes containing 0.01 mL of EDTA (1%); one drop of whole blood was placed in a coverslip to determine glucose levels with a glucometer. Once blood sampling was complete, fish were euthanized with an overdose of benzocaine (120 mg L^{-1}), placed in tagged bags and stored at 4°C for 24 h. Blood parameters were obtained on the sampling day and spleen and head kidney were carefully dissected and measured, then they were processed as described below. All sample processing was blind.

Blood, spleen and head kidney analysis

Hematocrit (Ht), reported as the percentage of cell package, was calculated by filling 2/3 of a capillary tube (Corning®, México) with blood. The tube was sealed and centrifuged for 5 min at 14,000 g. Total Protein (TP) blood plasma was determined placing a drop of plasma on a hand refractometer (ATAGO® SPR-Ne, Japan), and reported as g 100 mL^{-1}. White blood cells (WBC) and red blood cells (RBC) count were determined using Natt & Herrick (1952) method; where blood samples were diluted with Natt-Herrick solution in a Thoma pipette, and this dilution was placed in a Neubauer chamber to count the number of

WBC and RBC using an optical microscope and multiplying by the dilution factor. Glucose was determined by placing a whole blood drop on the edge of a quick glucose strip using a One Touch® Ultra Meter reader (Lifescan, Milpitas, California) (Evans, 2003). Two nitroblue tetrazolium (NbT) assays were performed: The first assay is based on the method described by Yildirim *et al.* (2003), where NbT stains blue the respiratory burst of phagocytic cells due to superoxide anion (O_2^-) production. Five drops of collected blood were gently mixed with 50 μL of NbT (0.2% w v^{-1} in saline) over a glass coverslip and then incubated during 45 min in a moist chamber to allow the reduction of NbT by O_2^- into an insoluble blue formazan. Finally, the coverslips were washed gently with phosphate buffered saline (PBS) and fixed with methanol. The coverslips were placed upside down over a drop of NbT in a microscope slide. Blue staining cells were counted in 20 fields using a light microscope (40x).

The second assay with NbT uses the principle described above on phagocytic spleen and head kidney (HK) cells. Organs were carefully dissected and weighed, three imprints of the squashed organs were made (Jiménez-Guzmán, 2007) on a glass slide and covered with NbT solution (0.2% w v^{-1} in saline); slides were placed in a moist chamber for 45 min. Blue stain cells were observed in 20 fields under a light microscope (40x). Spleen somatic index (I_S) and nephro somatic index (I_N) were determinated as described by Rohlenová *et al.* (2011) obtaining organ weights (0.001 accuracy) expressed as: $I_S = 100$ [spleen weight (g) × body weight (g)$^{-1}$]; $I_N = 100$ [head kidney weight (g) × body weight (g) $^{-1}$]. Total immunoglobulin M (IgM) levels present on blood samples were determined following the methodology described by Rohlenová *et al.* (2011); the difference between total proteins determined using a commercial kit (Bio-Rad, USA) and the proteins in the supernatant after precipitation with zinc sulphate (0.7 mM ZnSO4.7H2O, pH = 5.8) and centrifugation, results was reported as g L^{-1}. Phagocytic activity (PA) was determined as described by Zhu *et al.* (2012) with slight modifications. *Staphylococcus aureus* (ATCC 25923) was grown on brain heart agar for 24 h at 37°C and inactivated at 121°C for 15 min. Using McFarland turbidimetric standards, *S. aureus* was concentrated at 30×10^8 cells mL^{-1} and stored at 4°C until needed. Subsequently, 50 μL of sampled blood and 50 μL of inactivated *S. aureus* (30×10^8 cells mL^{-1}) were mixed in microcentrifuge tubes and incubated at room temperature for 30 min; 150 μL of PBS were added and centrifuged at 300 *g* during 5 min at 4°C. Supernatants were removed and the pellets were smeared onto glass slides, air dried and stained with Giemsa (Sigma®). Three hundred phagocytes were counted using a light microscope (40x) to determine phagocytic rate (R_P) expressed as: $R_P = 100$ (phagocytic leucocytes × total leucocytes^{-1}), and phagocytic index (I_P) expressed as: I_P = phagocytic number of *S. aureus* x phagocytic leucocytes^{-1}.

Data analysis

All data are expressed as mean ± standard error (SE) and were processed using the commercial software Statistica® (StatSoft Inc., Tulsa, OK). When necessary data were transformed to Log_{10} and $Log_{10}(x + 0.5)$ functions to meet ANOVA assumptions: normality test (Kolmogorov-Smirnov) and variance homogeneity test (Bartlett) (Zar, 1999). Two-way ANOVA using diets and time as independent variables was performed; significant differences ($P < 0.05$) were identified using a Tukey honestly significant difference *post hoc* test.

RESULTS

No fish mortalities were observed during the duration of the experiment. Differences ($P < 0.05$) on the effect of the different diets over leucocyte count are showed on Figure 1. The β-1,3/1,6 glucan diet supplementation at 0.05% showed significantly higher leucocyte values than control and other diets, as observed in weeks 2, 3 and 4 (Table 1). No interaction was observed on diet x week analysis on two-way ANOVA.

β-1,3/1,6 glucan supplementation resulted in a significant ($P < 0.05$) decrement on weekly Ht and RBC values (Table 1). NbT reactive blood cells showed statistically significant differences ($P < 0.05$) on fish in different diets (Table 2), where higher values were mainly observed during the week 3 for BG 0.5 diet. No interaction was observed on diet x week analysis.

Weekly differences ($P < 0.05$) on NbT reactive spleen and HK cells were observed, with both parameters being higher on week 3 for control and BG 0.5 diet respectively. No influence ($P > 0.05$) of β-1,3/1,6 glucan intake was observed on I_S and I_N, but weekly analysis showed statistical differences ($P < 0.05$) with significantly higher indices observed on weeks 4 and 3 respectively, being the control diet which has a greater value on both parameters (Table 1). No interaction was observed on diet x week analysis.

Unpredictable weather conditions resulted in the loss of the first week samples for R_P and I_P; thus, Figure 2 shows data corresponding to weeks 2-5. I_P does not evince differences ($P > 0.05$) between diets, weeks or diet x week interaction (Fig. 2b); on the other hand, R_P indicates weekly difference ($P < 0.05$) with higher

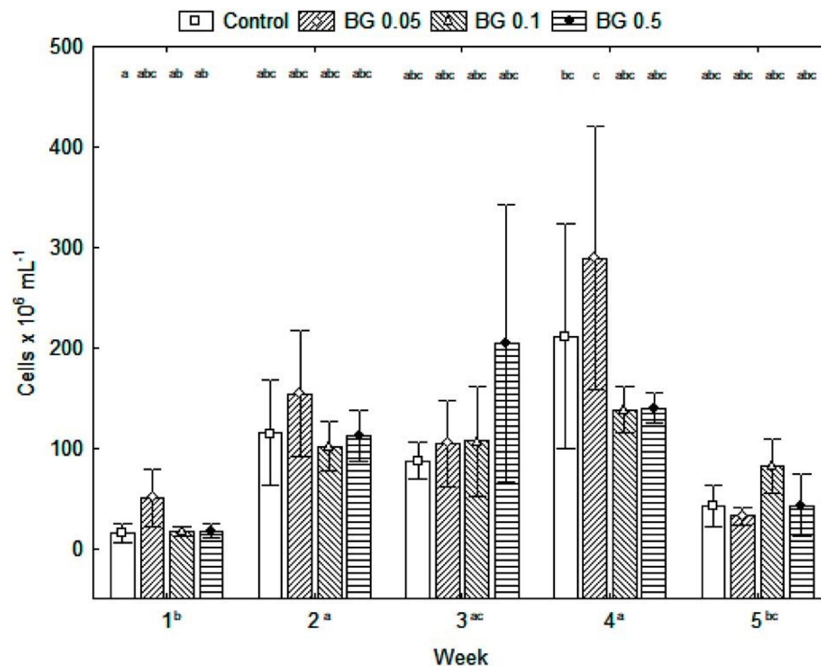

Figure 1. Leucocyte count of channel catfish feed with different levels (0, 0.05, 0.1, 0.5 %) of β-1,3/1,6 glucan using Natt-Herrick solution. Distinct letters indicates differences ($P < 0.05$) between diets and weeks.

values on week 5, especially for control and BG 0.05 diets (Fig. 2a).

DISCUSSION

The use of natural immunostimulants such as β-glucans has improved aquaculture production, positively impacting fish farming, modifying the biological response of some hematological and immunological parameters (Bohn & BeMiller, 1995), and enhancing the response of cultured organisms to infectious agents. These compounds are environmentally friendly, non-polluting and biodegradable, and they might indirectly help reduce environmental damage by aiding cultured organisms against diseases, reducing chemical treatments. Results from this research show a general improvement in some hematological and immunological parameters after β-1,3/1,6 glucan intake, especially during weeks 3 and 4. However, some hematological parameters (Ht and RBC) showed opposed results decreasing over experimental time, agreeing with other studies (Del Rio-Zaragoza *et al.*, 2011) which also reported high leucocytes values using same β-1,3/1,6 glucan concentration (0.05%) supplemented to red snapper *Lutjanus guttatus* commercial diet, and also a decrease in Ht and RBC values after the first treatment week.

No significant variation on total serum protein values during the experiment on any different diet groups were observed, which is similar to that reported in other species (*Dicentrarchus labrax*, *L. guttatus*) fed with β-1,3/1,6 glucan supplemented diets using similar concentrations (SitjÀ-Bobadilla & Pérez-Sánchez, 1999; Bagni *et al.*, 2005; Del Rio-Zaragoza *et al.*, 2011). No difference ($P > 0.05$) on glucose levels between treated and control fish were observed, suggesting a proper response from fish to biological modifiers like β-1,3/1,6 glucans. Glucose levels are used as an indirect stress indicator (Evans *et al.*, 2003), since high stress levels on organisms are reflected on higher cortisol amounts on circulating blood, which in turn elevates glucose levels, increasing disease susceptibility (Barandica & Tort, 2008).

The diet supplemented with 0.05% of β-1,3/1,6 glucans induced a significant ($P < 0.05$) increase on WBC numbers on week 4. Higher WBC values are associated with health, as they are involved on immunological response; leucocyte receptors are promoted by β-glucan, enhancing cell activity resulting on an improvement of phagocytic activity, and cytokine secretion which stimulate new WBC formation (Raa, 1996). In other species, *S. cerevisiae* yeast β-glucan intake also increases WBC in *Catla catla* juveniles fed at 0.8% (Jha *et al.*, 2007) and *Labeo rohita* fingerlings using 1% (Andrews *et al.*, 2009). Whereas, these reports did not measure the time needed for leucocyte proliferation, Del Rio-Zaragoza *et al.* (2011) established that higher WBC values are observed du-

Table 1. Blood, spleen and HK parameters of channel catfish (*Ictalurus punctatus*) feed with supplemented diets with β-1,3/1,6 glucan. Mean ± SE n: 3 in each group. Ht: hematocrit; TP: total protein; RBC: red blood cells; WBC: white blood cells; NbT: nitroblue tetrazolium; HK: head kidney; IgM: immunoglobulin M. Ht (%); TP (g 100 mL^{-1}); RBC (cells x 10^5 mL^{-1}); WBC (cells x 10^6 mL^{-1}); glucose (mg dL^{-1}); blood NbT, NbT spleen, NbT HK (cells $field^{-1}$); IgM (g L^{-1}). *Differences ($P < 0.05$) with at least another group on different weeks. **Values are means of 3 reps $aquaria^{-1}$

Diet	Week	Ht	TP	RBC	WBC	Glucose	Blood NbT**	NbT spleen**	NbT HK**	I_S	I_N	IgM
Control	1	36.67 ± 2.40	4.47 ± 0.10	21.50 ± 2.55	15.9 ± 9.6*	54.67 ± 9.17	0.07 ± 0.05*	1.11 ± 0.22	0.78 ± 0.22	0.063 ± 0.003	0.16 ± 0.04	21.39 ± 4.58
	2	31.67 ± 1.45	4.00 ± 0.06	16.17 ± 1.44	114.7 ± 52.5	42.67 ± 2.33	0.08 ± 0.05*	0.56 ± 0.22	0.56 ± 0.56	0.066 ± 0.007	0.25 ± 0.03	8.02 ± 4.59
	3	27.33 ± 2.73	3.53 ± 0.17	13.53 ± 5.11	87.5 ± 18.4	53.67 ± 3.18	0.45 ± 0.03*	0.78 ± 0.22	2.22 ± 0.99	0.054 ± 0.002	0.29 ± 0.03	15.75 ± 4.44
	4	25.67 ± 2.05	3.07 ± 0.10	17.20 ± 3.79	211.3 ± 112.2*	33.33 ± 4.66	0.02 ± 0.02*	0.44 ± 0.29	0.56 ± 0.40	0.093 ± 0.034	0.28 ± 0.05	16.30 ± 3.99
	5	26.33 ± 0.73	3.60 ± 0.12	16.10 ± 2.70	42.1 ± 20.9	47.00 ± 14.57	0*	0.22 ± 0.11	0.56 ± 0.56	0.049 ± 0.007	0.22 ± 0.04	8.74 ± 3.80
BG 0.05	1	35.67 ± 4.09	3.80 ± 0.23	21.80 ± 3.78	50.5 ± 28.1	51.00 ± 4.93	0.22 ± 0.19	0.33 ± 0.19	1.67 ± 1.02	0.060 ± 0.009	0.18 ± 0.04	13.72 ± 4.57
	2	31.50 ± 2.18	3.50 ± 0.23	15.23 ± 2.97	153.7 ± 62.8	49.67 ± 7.26	0.10 ± 0.03	0.22 ± 0.22	0.78 ± 0.29	0.073 ± 0.010	0.27 ± 0.02	15.12 ± 1.80
	3	28.00 ± 1.53	3.27 ± 0.10	13.07 ± 1.51	104.4 ± 42.7	60.67 ± 3.76	0.47 ± 0.14*	1.44 ± 0.78	1.33 ± 0.19	0.051 ± 0.005	0.22 ± 0.02	22.98 ± 1.31
	4	25.00 ± 2.64	3.63 ± 0.21	14.63 ± 2.03	289.3 ± 131.1*	46.00 ± 5.51	0*	1.33 ± 1.00	0.22 ± 0.11	0.090 ± 0.026	0.26 ± 0.02	18.45 ± 5.32
	5	22.83 ± 1.36	2.97 ± 0.16	12.30 ± 0.76	32.0 ± 8.0	58.67 ± 8.19	0.02 ± 0.02*	0.11 ± 0.11	0	0.046 ± 0.008	0.20 ± 0.02	13.83 ± 1.81
BG 0.1	1	28.33 ± 3.29	3.20 ± 0.47	19.97 ± 1.62	16.9 ± 5.1*	48.67 ± 11.47	0.25 ± 0.08*	0.44 ± 0.29	1.44 ± 1.44	0.058 ± 0.002	0.19 ± 0.03	18.42 ± 5.20
	2	30.33 ± 2.19	3.93 ± 0.23	18.87 ± 1.55	101.4 ± 24.4	51.33 ± 1.20	0.08 ± 0.06*	0.22 ± 0.22	0.33 ± 0.19	0.066 ± 0.010	0.18 ± 0.05	19.27 ± 3.62
	3	27.00 ± 4.36	3.47 ± 0.27	13.87 ± 5.13	106.7 ± 54.9	58.67 ± 10.17	0.42 ± 0.02*	0.67 ± 0.19	1.22 ± 0.62	0.054 ± 0.005	0.27 ± 0.01	16.77 ± 3.86
	4	29.00 ± 3.60	3.73 ± 0.32	19.50 ± 1.94	138.2 ± 22.5	58.67 ± 8.37	0*	0.33 ± 0.19	0.11 ± 0.11	0.060 ± 0.008	0.18 ± 0.03	13.21 ± 3.92
	5	27.00 ± 2.52	3.90 ± 0.19	19.17 ± 0.84	81.9 ± 26.3	55.33 ± 4.34	0.02 ± 0.02*	0.22 ± 0.22	0.22 ± 0.11	0.054 ± 0.009	0.23 ± 0.01	17.19 ± 3.15
BG 0.5	1	33.00 ± 4.04	4.07 ± 0.25	22.13 ± 1.76	17.16 ± 6.6*	51.33 ± 8.74	0.15 ± 0.06	0.56 ± 0.56	0.33 ± 0.19	0.064 ± 0.002	0.20 ± 0.05	11.83 ± 5.20
	2	31.00 ± 3.22	3.73 ± 0.19	12.80 ± 3.60	112.4 ± 26.0	50.33 ± 2.60	0.07 ± 0.02	0.44 ± 0.11	0.78 ± 0.22	0.063 ± 0.003	0.27 ± 0.03	7.91 ± 1.17
	3	29.00 ± 2.52	3.80 ± 0.42	11.60 ± 4.27	204.7 ± 137.9	60.33 ± 7.53	0.57 ± 0.20*	1.11 ± 0.59	2.56 ± 1.79	0.066 ± 0.007	0.23 ± 0.03	18.16 ± 2.21
	4	29.33 ± 1.85	3.33 ± 0.08	16.80 ± 1.30	140.0 ± 15.5	49.33 ± 6.36	0.10 ± 0.05	0.89 ± 0.73	0.56 ± 0.11	0.054 ± 0.002	0.14 ± 0.05	17.06 ± 0.24
	5	26.33 ± 1.33	3.53 ± 0.04	15.63 ± 1.09	43.0 ± 30.7	56.33 ± 5.36	0*	0.11 ± 0.11	0.11 ± 0.11	0.093 ± 0.034	0.22 ± 0.02	19.41 ± 2.15
Factorial ANOVA												
Diet		$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Week		$P < 0.01$	$P > 0.05$	$P < 0.01$	$P < 0.001$	$P > 0.05$	$P < 0.001$	$P < 0.05$	$P < 0.01$	$P < 0.05$	$P < 0.05$	$P > 0.05$
Diet *x* week		$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Table 2. Channel catfish (*I. punctatus*) phagocytic activity against *S. aureus* (ATCC 25923) using diets supplemented with of β-1,3/1,6 glucan (0, 0.05, 0.1 and 0.5%). Mean ± SE, n: 3 in each group, phagocytic rate (R_P), phagocytic index (I_P).

Diet	Week	Phagocityc rate (R_P)	Phagocityc index (I_P)
Control	2	36.1 ± 13.0	0.19 ± 0.03
	3	105.6 ± 15.1	0.32 ± 0.08
	4	52.8 ± 17.4	0.25 ± 0.08
	5	171.9 ± 63.0	0.26 ± 0.08
BG 0.05	2	49.9 ± 31.7	0.22 ± 0.04
	3	99.0 ± 33.4	0.51 ± 0.10
	4	34.1 ± 12.7	0.45 ± 0.10
	5	151.4 ± 23.8	0.33 ± 0.20
BG 0.1	2	53.6 ± 15.8	0.31 ± 0.08
	3	135.9 ± 58.2	0.29 ± 0.04
	4	56.9 ± 19.9	0.26 ± 0.07
	5	82.2 ± 30.8	0.19 ± 0.07
BG 0.5	2	28.4 ± 3.4	0.40 ± 0.11
	3	76.0 ± 31.5	0.37 ± 0.08
	4	49.5 ± 11.1	0.32 ± 0.04
	5	77.0 ± 3.0	0.16 ± 0.05
Factorial ANOVA			
Diet		$P > 0.05$	$P > 0.05$
Week		$P < 0.05$	$P < 0.05$
Diet *x* week		$P > 0.05$	$P > 0.05$

ring the second week of β-1,3/1,6 glucan supplementation on *L. guttatus*.

Significant differences ($P < 0.05$) in phagocytic cell counts reactive to NbT were observed on the third week; these cells are capable of engulfing and destroying bacteria through a process called respiratory burst, generating superoxide anion (O_2^-) which transforms in hydrogen peroxide (H_2O_2) and some hydroxyl free radicals (OH). However, other studies (Yildirim *et al.*, 2003) do not report significant differences ($P > 0.05$) on channel catfish *I. punctatus* immune response fed control and treated diets, when different concentrations of gosypol, a polysaccharide obtained from cotton was used, probably due to widely dispersed results in data analysis, which can cause the absence of statistical significance. This experiment showed elevated values of phagocytic cell counts reactive to NbT in fish fed the diets supplemented with both doses of β-1,3/1,6 glucans, but with no difference ($P > 0.05$) in between, indicating that there is probably no need to increase the concentration of β-1,3/1,6 glucans to obtain a biological response. Jaafar *et al.* (2011) report that β-glucan immunostimulation activity can vary according to its source, intake period and treated species; in this study a higher I_P was observed on fish given the 0.05% β-1,3/1,6 glucan supplemented diet, keeping above the rest of the treatments during weeks 3, 4 and 5; nonetheless, the wide dispersion of data observed did not allow to highlight any statistical differences ($P > 0.05$) among treatment groups. On the other hand, there are other reports (Zhu *et al.*, 2012) that determined that channel catfish *I. punctatus* immune system may be improved through the phagocytic activity increment due to the intake of feed supplemented (0.3%) with compounds containing at least of 25% of β-1,3/1,6 glucans.

Immune response modifier biological agents like β-1,3/1,6 glucans possess the ability to widely stimulate organisms in both the specific and nonspecific immune systems (Barandica & Tort, 2008). The spleen and head kidney are lymphoid organs involved on the fish immune system responsible of antibody production (Manning, 1994) and measurable indirectly through the relationship between the organ weight relative to the body weight, thus obtaining the spleen and nephro somatic indices. Comparing results from different variables, this study showed marked differences on I_S during the weeks 2 and 4 with respect to the rest of the weeks; particularly in week 2 the BG 0.05 diet showed higher values than the rest of diets. On the other hand, spleen NbT reactive phagocytic cells exhibited higher values in fish treated with 0.05% of β-1,3/1,6 glucans during weeks 3 and 4 with respect to the others. The above findings are consistent with those reported by Rohlenová *et al.* (2011) who have reported an association between the phagocytic activity measured with NbT stain reactive test and the increment of I_S studied on carps *Cyprinus carpio*; this may be due to proliferation of B lymphocytes in the spleen; this organ when it is immunologically stimulated, increases its activity and size, and vice versa, when it is immunosuppressed it reduces its size (Harford *et al.*, 2006). Considering weekly times that show an increment on immunological parameters mentioned before, it denotes that there is a similitude to what National Research Council (1992) reported categorizing the I_S as a relevant immune parameter. Nowadays this assertion is inconsistent over fish immune literature due to other research that finds no relationship between spleen size and immune status in an organism (Poisot *et al.*, 2009).

As much as the β-1,3/1,6 glucan intake increase do not mean that immune response would improve in the same way to organism, it should takes in consideration that for every specie it should establish the appropriate dosage or concentration of β-1,3/1,6 glucans that leads to an improvement of immune system, otherwise, instead of enhance immunity, initial fight against pathogens may be compromised. In this regard, Robertsen *et al.* (1990) report that high β-glucan admi-

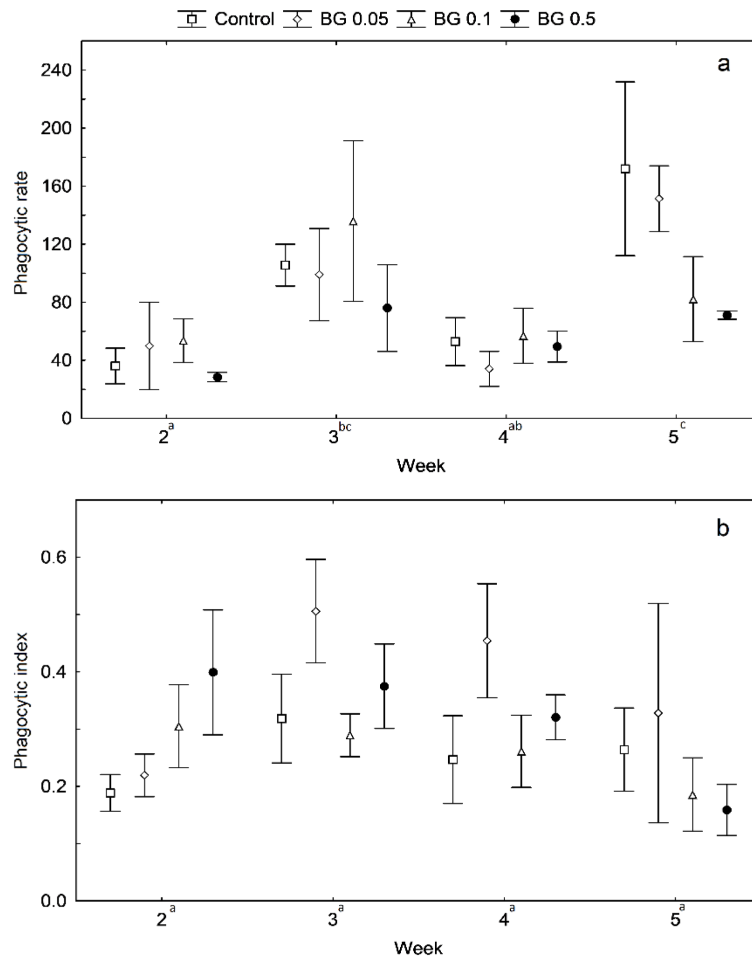

Figure 2. Channel catfish phagocytic activity against *Staphylococcus aureus* (ATCC 25923) using diets supplemented with of β-1,3/1,6 glucan (0, 0.05, 0.1 and 0.5%). a) Phagocytic rate (R_P), b) Phagocytic index (I_P). Distinct letters indicates differences ($P < 0.05$) between treatment weeks.

nistration (1800 µg fish^{-1}) from *S. cerevisiae,* increase the mortality on Atlantic salmon *Salmo salar* once it challenged with *Vibrio anguillarum* a week after product intake.

This trial demonstrated that β-1,3/1,6 glucan diet supplementation to channel catfish *I. punctatus* up to 0.5% is safe for this species, likewise, it is perceptible that just 0.05% of β-1,3/1,6 glucan supplementation results enough to stimulate nonspecific catfish immune system on parameters like WBC count and phagocytic cells activity. Further research of β-1,3/1,6 glucans activity challenged to infectious pathogens is necessary for the benefit to industry and environmental care.

ACKNOWLEDGMENTS

Thanks are extended to the National Council of Science and Technology of Mexico (CONACYT) and to the Professional Development Program for Teachers from Mexico (PRODEP), for the Ph.D. scholarship to F. Benavides-González. Thanks also to farm El Tirón Parejo, Xicotencatl, Tamaulipas, for donating the fish for this study, and to the laboratory technician María Dora López Aguilar who helped with the hematological tests.

REFERENCES

Akramienė, D., A. Kondrotas, J. Didžiapetrienė & E. Kėvelaitis. 2007. Effects of β-glucans on the immune system. Medicina (Kaunas, Lithuania), 43(8): 597-606.

Andrews, S., N. Sahu, A. Pal & S. Kumar. 2009. Haematological modulation and growth of *Labeo rohita* fingerlings: effect of dietary mannan oligosaccharide, yeast extract, protein hydrolysate chlorella. Aquacult. Res., 41(1): 61-69.

Bagni, M., N. Romano, M. Finoia, L. Abelli, G. Scapigliati, P. Tiscar, M. Sarti & G. Marino. 2005.

Short- and long-term effects of a dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*). Fish Shellfish Immunol., 18(4): 311-325.

Barandica, C.L.M. & B.L. Tort. 2008. Neuroendocrinología e inmunología de la respuesta al estrés en peces. Rev. Acad. Colomb. Cienc., 32(123): 267-284.

Benavides-González, F., R. Gomez-Flores, J. Rábago-Castro, J. Sánchez-Martínez & I. Montelongo-Alfaro. 2015. Effects of hydrogen peroxide and metrifonate on monogenean *Ligictaluridus floridanus* on catfish (*Ictalurus punctatus*, Rafinesque) gills. J. Parasitol., 101(6): 707-710.

Bohn, J. & J. BeMiller. 1995. (1→3)-β-d-Glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohyd. Polym., 28(1): 3-14.

Boltaña, S., F. Reyes-Lopez, D. Morera, F. Goetz & S.A. MacKenzie. 2011. Divergent responses to peptidoglycans derived from different *E. coli* serotypes influence inflammatory outcome in trout, *Oncorhynchus mykiss*, macrophages. B.M.C. Genomics, 12(1): 1-34.

Bonaldo, A., K.D. Thompson, A. Manfrin, A. Adams, E. Murano, A.L. Mordenti & P. Gatta. 2009. The influence of dietary β-glucans on the adaptive and innate immune responses of European sea bass (*Dicentrarchus labrax*) vaccinated against vibriosis. Ital. J. Anim. Sci., 6(2): 151-164.

Bridle, A.R., C.G. Carter, R.N. Morrison & B.F. Nowak. 2005. The effect of beta-glucan administration on macrophage respiratory burst activity and Atlantic salmon, *Salmo salar* L., challenged with amoebic gill disease-evidence of inherent resistance. J. Fish Dis., 28(6): 347-356.

Cain, K.D., L. Grabowski, J. Reilly & M. Lytwyn. 2003. Immunomodulatory effects of a bacterial-derived β-1, 3 glucan administered to tilapia (*Oreochromis niloticus* L) in a spirulina-based diet. Aquacult. Res., 34(13): 1241-1244.

De, B.C., D.K. Meena, B.K. Behera, P. Das, P.D. Mohapatra & A.P. Sharma. 2014. Probiotics in fish and shellfish culture: immunomodulatory and ecophysiological responses. Fish Physiol. Biochem., 40(3): 921-971.

Del Rio-Zaragoza, O.B., E.J. Fajer-Ávila & P. Almazán-Rueda. 2011. Influence of β-glucan on innate immunity and resistance of *Lutjanus guttatus* to an experimental infection of dactylogyrid monogeneans. Parasite Immunol., 33(9): 483-494.

Evans, J.J., C.A. Shomaker & P.H. Klesius. 2003. Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to *Streptococcus agalactiae* in Nile tilapia *Oreochromis niloticus*. J. Aquat. Anim. Health, 15(3): 202-208.

Guzmán-Villanueva, L.T., F. Ascencio-Valle, M.E. Macías-Rodríguez & D. Tovar-Ramírez. 2014. Effects of dietary β-1, 3/1, 6-glucan on the antioxidant and digestive enzyme activities of Pacific red snapper (*Lutjanus peru*) after exposure to lipopolysaccharides. Fish Physiol. Biochem., 40(3): 827-837.

Harford, A., K. O'Halloran & P. Wright. 2006. Flow cytometric analysis and optimisation for measuring phagocytosis in three australian freshwater fish. Fish Shellfish Immunol., 20(4): 562-573.

Jaafar, R.M., J. Skov, P.W. Kania & K. Buchmann. 2011. Dose dependent effects of dietary immunostimulants on rainbow trout immune parameters and susceptibility to the parasite *Ichthyophthirius multifiliis*. J. Aquacult. Res. Development, (S3): 001. doi: 10.4172/2155-9546.S3-001.

Jha, A., A. Pal, N. Sahu, S. Kumar & S. Mukherjee. 2007. Haemato-immunological responses to dietary yeast RNA, ω-3 fatty acid and b-carotene in *Catla catla* juveniles. Fish Shellfish Immunol., 23(5): 917-927.

Jiménez-Guzmán, F. 2007. Técnicas de diagnóstico presuntivo para enfermedades de peces. Memoria Técnica, Universidad Autónoma de Tamaulipas. Cd. Victoria, Tamaulipas, 129 pp.

Kim, Y.S., F. Ke & Q.Y. Zhang. 2009. Effect of β-glucan on activity of antioxidant enzymes and Mx gene expression in virus infected grass carp. Fish Shellfish Immunol., 27(2): 336-340.

Lara-Rivera, A.L., G.M. Parra-Bracamonte, A.M., Sifuentes-Rincón, H.H. Gojón-Báez, H. Rodríguez-González & I.O. Montelongo-Alfaro. 2015. El bagre de canal (*Ictalurus punctatus* Rafinesque, 1818): estado actual y problemática en México. Lat. Am. J. Aquat. Res., 43(3): 424-434.

Manning, M. 1994. Fishes. In: R.J. Turner (ed.). Immunology: a comparative approach. John Wiley & Sons, New York, pp. 69-100.

Meena, D.K., P. Das, S. Kumar, S.C. Mandal, A.K. Prusty, S.K. Singh., M.S. Akhtar, B.K. Behera, K. Kumar, A.K. Pal & S.C. Mukherjee. 2013. Beta-glucan: an ideal immunostimulant in aquaculture (a review). Fish Physiol. Biochem., 39(3): 431-457.

Meshram, S.J., H.S. Murthy, H. Ali, H.S. Swain & A. Ballyaya. 2015. Effect of dietary β-glucan on immune response and disease resistance against *Aeromonas hydrophila* in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). Aquacult. Int., 23(2): 439-447.

National Research Council. 1992. Use of biologic markers in controversial areas of environmental health. In: Biologic Markers in Immunotoxicology. National Academy Press, Washington DC, pp. 127-139.

Natt, M. & C. Herrick. 1952. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Sci., 31(4): 735-738.

Parmar, P.V., H.S. Murthy, C.S. Tejpal & B.N. Kumar. 2012. Effect of brewer's yeast on immune response of giant freshwater prawn, *Macrobrachium rosenbergii*, and its resistance to white muscle disease. Aquacult. Int., 20(5): 951-964.

Poisot, T., A. Šimková, P. Hyršl & S. Morand. 2009. Interactions between immunocompetence, somatic condition and parasitism in the chub *Leuciscus cephalus* in early spring. J. Fish Biol., 75(7): 1667-1682.

Raa, J. 1996. The use of immunostimulatory substances in fish and shellfish farming. Rev. Fish Sci., 4(3): 229-288.

Rábago-Castro, J., J.G. Sánchez-Martínez, J. Loredo-Osti, R. Gomez-Flores, P. Tamez-Guerra & C. Ramírez-Pfeiffer. 2011. Temporal and spatial variations of ectoparasites on cage-reared channel catfish, *Ictalurus punctatus*, in Tamaulipas, Mexico. J. World Aquacult. Soc., 42(3): 406-411.

Ringø, E., R.E. Olsen, J.L. Vecino, S. Wadsworth & S.K. Song. 2011. Use of immunostimulants and nucleotides in aquaculture: a review. J. Mar. Sci. Res. Develop., 2: 1-22.

Robertsen, B., G. Rørstad, R. Engstad & J. Raa. 1990. Enhancement of non-specific disease resistance in Atlantic salmon, *Salmo salar* L., by a glucan from *Saccharomyces cerevisiae* cell walls. J. Fish Dis., 13(5): 391-400.

Rohlenová, K., S. Morand, P. Hyršl, S. Tolarová, M. Flajšhans & A. Šimková. 2011. Are fish immune systems really affected by parasites? an immunoecological study of common carp (*Cyprinus carpio*). Parasites Vectors 4(1): 1 pp.

SitjÀ-Bobadilla, A. & J. Pérez-Sánchez. 1999. Diet related changes in non-specific immune response of European sea bass (*Dicentrarchus labrax* L.). Fish Shellfish Immunol., 9(8): 637-640.

Vaseeharan, B. & R. Thaya. 2014. Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture. Aquacult. Int., 22(3): 1079-1091.

Received: 8 August 2016; Accepted: 10 January 2017

Villamil, L. , A. Figueras & B. Novoa. 2003. Immunomodulatory effects of nisin in turbot (*Scophthalmus maximus* L.). Fish Shellfish Immunol., 14(2): 157-169.

Welker, T., C. Lim, M. Yildirim-Aksoy, R. Shelby & P.H. Klesius. 2007. Immune response and resistance to stress and *Edwardsiella ictaluri* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell yeast or yeast subcomponents. J. World Aquacult. Soc., 38(1): 24-35.

Yang, G., X. Tian, S. Dong, M. Peng, D. Wang & K. Zhang. 2016. Effects of dietary rhubarb, *Bacillus cereus*, yeast polysaccharide, and florfenicol supplementation on growth, intestinal morphology, and immune responses of sea cucumber (*Apostichopus japonicus*). Aquacult. Int., 24(2): 675-690.

Yildirim, M., C. Lim, P. Wan & P. Klesius. 2003. Growth performance and immune response of channel catfish (*Ictalurus puctatus*) fed diets containing graded levels of gossypol-acetic acid. Aquaculture, 219(1): 751-768.

Zanuzzo, F.S., E.C. Urbinati, G.W. Nash & A.K. Gamperl. 2015. Steelhead trout *Oncorhynchus mykiss* metabolic rate is affected by dietary *Aloe vera* inclusion but not by mounting an immune response against formalin-killed *Aeromonas salmonicida*. J. Fish Biol., 87(1): 43-53.

Zar, J.H. 1999. Biostatistical analysis. Pearson Education, New Delhi, 662 pp.

Zeng, L., Y.H. Wang, C.X. Ai, J.L. Zheng, C.W. Wu & R. Cai. 2016. Effects of β-glucan on ROS production and energy metabolism in yellow croaker (*Pseudosciaena crocea*) under acute hypoxic stress. Fish Physiol. Biochem., 42: 1395-1405.

Zhao, H.X., J.M. Cao, A.L. Wang, Z.Y. Du, C.X. Ye, Y.H. Huang, H.B. Lan, T.T. Zhou & G.L. Li. 2012. Effect of long-term administration of dietary β-1, 3-glucan on growth, physiological, and immune responses in *Litopenaeus vannamei* (Boone, 1931). Aquacult. Int., 20(1): 145-158.

Zhu, H., H. Liu, J. Yan, R. Wang & L. Liu. 2012. Effect of yeast polysaccharide on some hematologic parameter and gut morphology in channel catfish (*Ictalurus punctatus*). Fish Physiol. Biochem., 38(5): 1441-1447.