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Research Article

Superoxide dismutase activity in tissues of juvenile cauque river prawn (*Macrobrachium americanum* Bate, 1868) fed with different levels of protein and lipid

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ABSTRACT. The effect of different proteins and lipids levels on antioxidant response of superoxide dismutase (SOD) was tested in muscle, hepatopancreas and whole-body tissues of cauque river prawn *Macrobrachium americanum* juvenile. Six diets with two crude protein (35 and 40% diet) and three lipid levels (6, 10, and 14% diet) were tested for juveniles in a factorial manner (3×2), to provide six different dietary. Juvenile prawns (0.22 ± 0.03 g) were randomly placed in 18 plastic tanks (160 L), at a density of 15 juveniles per tank (3 tank replicates/treatment). The assay lasted 60 days. SOD activity was significantly different in muscle, hepatopancreas and whole body depending on proteins and lipids levels in the diet. Results indicate that the diet containing 35% protein and 10% lipid provided adequately to while preventing diet-induced oxidative stress and protecting the integrity of the antioxidant response of SOD.

Keywords: *Macrobrachium americanum*, diet, muscle, hepatopancreas, SOD, cauque river prawn.

INTRODUCTION

Macrobrachium americanum (Decapoda: Palaemonidae) is a freshwater prawn distributed from Mexico to Peru in watersheds and streams along the Pacific sector of America (Wicksten & Hendrickx, 2003; García-Guerrero *et al*., 2015). It has commercial value as cuisine product but there is still no management procedure for its culture (García-Guerrero *et al*., 2013; Méndez-Martínez *et al*., 2018a). Today, only some efforts have been carried out covering basic management aspects but optimal diets for every developmental stage have not been formulated yet. Because of this, previous research with this species have been executed using diets formulated for other species, but this might not produce the best possible results (Méndez-Martínez *et al*., 2018b). A specific protein/lipid balance is required to supply proper amounts of amino acids and energy to achieve best results (Goda, 2008). A balanced diet should include enough protein and lipids since those are essential for most metabolic processes that promote and regulate growth. Proteins are mainly required for growth (Kabir-Chowdhury *et al*., 2008; Davassi, 2011; Méndez-Martínez *et al*., 2017) while lipids are mainly required as fuel (Pezzato *et al*., 2008; Zhan *et al*., 2016). The effects of dietary proteins and lipids levels on tissue synthesis and bioenergetics have been previously studied in terms of growth rate, survival and physio-logical functions in some Decapoda (Cortés-Jacinto *et al*., 2005; Goda, 2008; Méndez-Martínez *et al*., 2018b).

Particularly for physiological functions, crustaceans have defense mechanisms that include antioxidant non-enzymatic and enzymatic processes whose functions depend on proteins and lipids levels and may require optimal amounts to express these kinds of responses against stressful conditions (Mohan *et al*., 2016). Superoxide dismutase (SOD) and catalase, have been recognized as good indicators of oxidative stress for aquatic organisms since the complex antioxidant system of aerobic organisms prevent the effect of reactive oxygen species (ROS) and plays a vital role in

protecting cells from oxidative stress (Zenteno-Savin *et al.*, 2008). Its activity is a potential indicator of oxidative stress in aquatic organisms (Chien *et al.*, 2003). Enzymatic antioxidant mechanisms include ascorbate peroxidase, glutathione reductase, catalase, and peroxidases. These enzymes efficiently remove hydrogen peroxide from cells, as does SOD, which degrades the superoxide anion (Muñoz *et al.*, 2000; Huang *et al*., 2018). SOD is acytosolic enzyme specific reacting with superoxide radical sand it executes protective mechanisms within the injured tissue, following oxidative processes and phagocytosis (Pan *et al.*, 2003). For example, Zenteno-Savin *et al.* (2008) reported that dietary proteins and lipids levels affect the oxidative stress response of juvenile crayfish *Cherax quadricarinatus.* Enzymatic antioxidant activity has been widely studied in cultured animals, mainly fishes (Li *et al.*, 2003). In crustaceans, recent discoveries concerning SOD, mention its role in immunity system (Chen *et al.*, 2005; Cortés-Jacinto *et al*., 2009; Huang *et al*., 2018) and modulation of oxidative responses that might increase or lower SOD activity (Campa-Córdova *et al.*, 2005; Huang *et al*., 2018).

This study pretends to evaluate the effects of three dietary lipids levels at two dietary proteins levels on the SOD activity in tissues of juvenile of cauque river prawn *M. americanum*.

MATERIALS AND METHODS

Formulation and preparation of diets

Six experimental diets were formulated with two different proteins levels (30 and 40%) and three different lipids levels (6, 10 and 14%) and used in a factorial design (3×2) based on Méndez-Martínez (2017). All ingredients were sieved through a 250 µm mesh, and each diet was prepared by mixing all the macro-ingredients in an industrial blender until a uniform mixture was obtained. The micro-ingredients were mixed by hand in a plastic container before adding them to the macro-ingredients. Soy lecithin and fish oil were mixed until obtaining a homogeneous blend and then water was added. 2-mm pellets were extruded with a meat grinder and dried during 8 h at 45°C in an air flux oven. Thereafter, dried pellets were packed in plastic bags and kept refrigerated at -4°C until its use as suggested by Méndez-Martínez *et al*. (2017, 2018b). Formulation of the experimental diets is shown in Table 1.

Experimental design

Juvenile prawns (0.22 ± 0.03 g) were produced in the laboratory from three wild spawning females collected in the San Pedro de La Presa basin (17°03'36.14"N, 100°01'35.03"W) following the procedure by Méndez-Martínez *et al*. (2017, 2018a).

The specimens used in this study were previously separated and acclimated for one week in experimental tanks, which consisted of round fiber-reinforced with plastic (200 L). Plastic tanks (18) were filled with 160 L of water. A factorial experimental design (3×2) was applied to six different treatments with three replicates (tanks) each. Stocking density was 15 juveniles per tank, for a total of 45 juveniles for treatment. Specimens were fed with the experimental diets from day zero. The experiment lasted 60 days, and water temperature, dissolved oxygen, pH and ammonium content were measured daily with a multi-parameter probe (Aqua Troll 400, *in-situ*, Fort Collins, CO, USA). The photoperiod was 12 h light-12 h darkness, which is the norm for the season (Méndez-Martínez *et al*., 2018b).

All tanks were siphoned daily at 8:00 h before feeding. Daily cleaning implied water exchange of 20% and feces removal from each tank. Juvenile prawns were initially fed at a rate of 5% of its weight day^{-1} (Cortés-Jacinto *et al*., 2003). Each day 40% of food was supplied at 09:00 h and 60% at 17:00 h. Feed intake was determined by feeding to apparent satiation according to Méndez-Martínez *et al*. (2018b), and this ration was adjusted weekly.

Analysis of the proximate composition of diets

Proximate composition of diets, including dry matter, crude protein, ash, crude fiber, ether extract and nitrogen-free extract were performed three times by following the procedures of the Association of Official Analytical Chemists (AOAC, 2000). Moisture content was determined by drying the samples until constant weight at 105°C. Crude protein (N×6.25) was determined using the Dumas method or combustion nitrogen analysis (FP 2000 Leco-Corporation, Saint Joseph, MI, USA). The ether extract was determined using the Soxtec System (HT6, Tecator, Sweden, UK, USA). Ash content was determined using a muffle oven at 550°C for 8 h. The total energy of the diets was measured using automatic an isoperibol calorimeter (6400, Parr Instrument, Moline, IL, USA). It was assumed that burning of organic matter was done by oxidation (Méndez-Martínez *et al*., 2017, 2018b).

Antioxidant enzyme activity (SOD)

Prawns fasted during 24 h in agreement with Gu *et al*. (2014) and Méndez-Martínez *et al*. (2018b) at the beginning and at the end of the experiment.

Muscle and hepatopancreas portions of five specimens per tank were separated by dissection and four whole prawns per tank were sampled and stored at -20°C until its use for further analyses. For SOD determinations, the tissues were lysed in a mechanical

Table 1. Formulation and proximate composition of six experimental diets (% in dry matter) for juvenile *Macrobrachium americanum*.

Component	Experimental diets (%) Protein/lipid					
Protein/lipid	35/14	35/10	35/6	40/14	40/10	40/6
Sardine meal[1]	25.0	25.0	25.0	25.0	25.0	25.0
Soybean meal[1]	20.0	20.0	20.0	20.0	20.0	20.0
Wheat flour Int[1]	17.3	17.7	16.6	5.3	4.4	3.2
Cellulose	3.8	9.9	16.4	6.5	12.5	19.0
Cod liveroil[1]	5.8	2.4	3.7	6.0	2.3	2.7
Corn gluten[1]	5.1	5.0	5.3	15.5	15.8	16.1
Cornstarch[1]	2.0	1.0	1.0	1.0	1.0	1.0
Alginic acid[2]	2.0	2.0	2.0	2.0	2.0	2.0
Soy lecithin[4]	10.0	8.0	1.0	9.7	8.0	2.0
Vitamin premix crustaceans[3]	0.3	0.3	0.3	0.3	0.3	0.3
Calcium chloride[5]	6.0	6.0	6.0	6.0	6.0	6.0
Premez mineral crustaceans[6]	2.5	2.5	2.5	2.5	2.5	2.5
Choline chloride[7]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin C[8]	0.1	0.1	0.1	0.1	0.1	0.1
Proximate composition (% in dry matter)						
Moisture	93.0	92.1	94.0	93.3	96.2	95.4
Crude protein	35.0	35.4	35.6	40.9	40.8	40.7
Ether extract	14.9	10.3	6.1	14.8	10.3	6.7
Crude fiber	4.2	7.3	10.6	5.8	6.5	9.9
Ash	10.3	10.4	10.4	10.3	10.4	10.4
NFE[10]	35.6	36.6	37.3	28.1	32.0	32.3
Gross energy (MJ g^{-1})	20	19	18	20	19	18

[1]PIASA feed (Productora Industrial Acuasistemas, La Paz, B.C.S., MX). [2]Sigma-Aldrich, St. Louis, MO. [3](g 900 g^{-1}) Vitamin premix: Vitamin A acetate, 100, 000 IU; Vitamin D3, 850 UI; α-tocopherolacetate 2000 IU; menadione (2); thiamine HCl (0.5); riboflavin (3); pyridoxine HCl (1); dl-Capantothenicacid (5); nicotinicacid (5); biotin (0.05); inositol (5); Vitamin B12 (0.02); folicacid (0.18). All from Sigma-Aldrich. [4]ODONAJI, Mexico City, MX.[5]Reactive ACS, Sigma-Aldrich. [6](g 200 g^{-1} Mineral premix: KCl (28.57); $MgSO_4 \cdot 7H_2O$ (28.57); $ZnSO_4 \cdot 7H_2O$ (5.14); $MnCl_2 \cdot 4H_2O$, 1.34; $CuSO_4 \cdot 5H_2O$ (0.29); Kl (0.29); $CoCl_2.2H_2O$ (0.14); Na_2HPO_4 (135.43). [7]Sigma-Aldrich, 62% active agent, [8]35% stable active agent, Roche Diagnostics, Risch-Rotkreuz, Switzerland. [9]Data are expressed as mean ± SD of three replicates.[10]Nitrogen free extract = 100 - (% crude protein + % ether extract + % crude fiber + % ash).

homogenizer containing 0.5 mL phosphate buffer (50 mM, pH 7.8). The homogenate was centrifuged (5,724 g for 5 min at 4°C). Then, the supernatant was heated during 5 min at 65°C. Another supernatant sample was obtained from the second centrifugation of crude extract and stored separately at -20°C. SOD activity was determined according to Beauchamp & Fridovich (1971), using nitro blue tetrazolium (NBT) in the presence of riboflavin. Briefly, a 2 mL reaction mixture (0.1 mM EDTA, 13 µM methionine, 0.75 mM NBT, and 20 µM riboflavin in 50 mM phosphate buffer at pH 7.8) and 100 µL of the crude extract were placed under fluorescent light for 2 min or until A560 in control tubes reached 0.2 to 0.25 OD. The specific activity (units per mg protein) was calculated using a computer program (Vázquez-Juárez *et al*., 1993).

Statistical analysis

The Kolmogorov-Smirnov ($P < 0.05$) and Bartlett ($P < 0.05$) tests were applied to the data first in order to determine normality and homogeneity of variance, respectively. A two-way analysis of variance (ANOVA) was executed using Statistica 10.0 software (StatSoft, Tulsa, OK, USA). Duncan multiple range test was executed to compare differences between treatment means when significant F values were observed, at $P < 0.05$ level (Zar, 1999).

RESULTS

Proximate composition (protein, fat, fiber and ash), nitrogen-free extract, and gross energy content of six practical diets are shown in Table 1. Along the study, water quality parameters were as following: tempera-

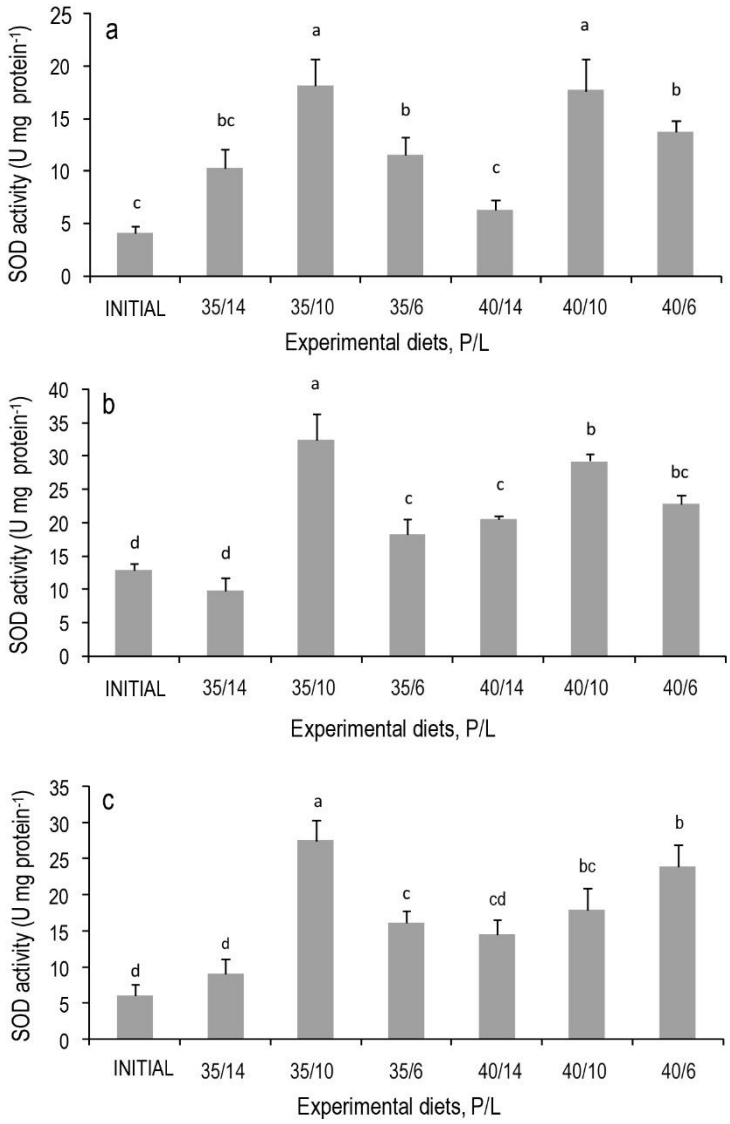

Figure 1. Superoxide dismutase (SOD) activity in a) muscle, b) hepatopancreas and c) whole-body of juvenile prawn *Macrobrachium americanum* fed two crude protein levels and three lipid levels during cultivation for 60 days. P: protein, L: lipid. The data are expressed as the mean ± SD of three replicates. The values in the same line with different superscripts are significantly different ($P < 0.05$).

ture varied from 28.0 to 29.5ºC, pH from 6.7 to 7.6, dissolved oxygen from 4.7 to 6.2 mg L^{-1}, ammonia from 0.05 to 0.08 mg L^{-1} and alkalinity from 167 to 191 mg L^{-1}. These values were within regular limits for indoor production of prawns so it is assumed that variations in results were due to treatments.

The SOD enzyme activity in whole-body, muscle and hepatopancreas are presented in Figure 1. Results show that feeding with two different levels of proteins and three levels of lipids induced significant differences in SOD activity in prawn tissues along the trial.

The highest SOD (U mg^{-1} protein) content in the muscle was observed in prawns fed with the diet at proportion of P/L of 35/10 and 40/10 whereas the highest SOD content in hepatopancreas was observed in prawns fed diets with a proportion of P/L = 35/10. Whole body SOD showed the highest activity when fed with diets containing a proportion of P/L = 35/10. The highest SOD values of in whole-body, muscle and hepatopancreatic tissue were observed for prawns fed the diet containing 35% protein, irrespective of dietary lipids levels. Prawns fed the diet with 10% lipid had a higher content regardless of dietary proteins levels.

DISCUSSION

Among the few previous studies dealing with dietary protein and lipids amounts on aspects of physiology, hematology or histology of *Macrobrachium americanum* are those published by Méndez-Martínez (2017) and Méndez-Martínez *et al*. (2017, 2018b) and those studies stated that improper protein and lipid amounts may affect most physiological functions. However, nutritional requirements of *Macrobrachium americanum* has been poorly studied and this is the first attempt to understand the oxidative stress in tissues of this species. In the present study, the SOD activity was significantly affected in muscles, digestive gland or whole-body by the amount of protein and lipid in the diet. SOD is one of the main antioxidant enzymes but the antioxidant defense systems consist of various enzymes involved in oxidative reactions that produces a reduction in oxidative stress (Lee *et al*., 2009; Huang *et al*., 2018). Aquatic animals with nutritional deficiencies are prone to oxidative stress caused by extreme temperatures, hypoxia, pollution or exposure to xenobiotics (Hwang & Lin, 2002; Huang *et al*., 2018).

Unbalanced diets provide no adequate levels of nutrients and that may cause low growth rates and feed conversion ratios, low resistance to stress and low capability for healing (Sánchez *et al*., 2005; Huang *et al*., 2018). Previously, the effect of diet on SOD activity has been reported for *Penaeus monodon* (Sivagnanavelmurugan *et al*., 2014), *M. malcolmsonii* (Annamalai *et al*., 2016) and *M. rosembergii* (Mohana *et al*., 2016), fed with different diets showing that malnutrition affects the capability of crustacean species to cope with stress because this cause malfunctions on the immune defense mechanisms. Under such malfunctions, the disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses are more difficult to cope with (Matozzo *et al*., 2005; Villa-Cruz *et al*., 2009; Huang *et al*., 2018). Previous reports indicate that dietary lipid and protein amounts have also an effect on free radical production and are oxidative damaging indicators (Chen *et al*., 2005; Zenteno-Savín *et al*., 2008). Fridovich (1995) linked increased SOD activity with longevity and increased tolerance to ischemic or reperfusion events as well as to factors that induce oxidative stress. Ingestion of dietary protein in excess (more amino than required) increases production of ROS in mitochondria, leading to oxidative stress and resulting in lipid peroxidation (Harper, 1994; Benzie, 1996). Thus, the increase in SOD activity in this experiment can be considered as a response to changes in lipid composition of hemocyte cell membranes, probably because of nutritional deficiencies, which interfere with the production of cell-activating factors (cytokines or chaperonins) that improve or decrease phagocytic capability (Itami *et al*., 1998).

Dietary proteins and lipids levels affect the production of reactive oxygen species (ROS) and the oxidative stress response in fish and crustaceans, where the oxidative stress response is an important component of the defense mechanisms (Winston *et al*., 1996; Holmblad & Söderhäll, 1999; Kovacevic *et al*., 2006). These results suggest a common trend in a variety of animals such as mammals, in which different dietary proteins and lipids levels in diets might produce differences in the oxidative stress antioxidant enzymatic system functions (Mataix *et al*., 1998; Zenteno-Savín *et al*., 2008). Several previous studies have shown how antioxidant enzymes activities might increase or decrease causing affectations in the activity of immune functions. For example, Li *et al*. (2003) stated that during starvation, the activities of SOD and CAT decreases, but might gradually increase after refeeding.

Lipids peroxidation activity is dependent on both tissue kind and diet (Mataix *et al*., 1998) and in agreement with previous findings, this seems to be in all animals. In the present study, the P:L = 35:10 treatment induced the highest SOD activity in juvenile prawns showing the direct consequence of nutrients over oxidative stress. Arun & Subramanian (1998), studied SOD activity in different tissues of the freshwater prawn *M. malcolmsonii* suggesting that higher antioxidant values are a consequence of multiple oxidative reactions. Hence, this may be the site of greatest free-radical production. In fact, values of SOD activity found by Arun & Subramanian (1998) were similar to those observed in present study in which the dietary protein-and-lipid amount was the only difference between treatments. Digestibility of animal-derived and plant-derived ingredients vary depending in protein and lipid amounts and type as reported by Mohan *et al*. (2016) and those differences may produce differences in antioxidant abilities. For example, Chien *et al*. (2003) enhanced antioxidant capacity of the giant tiger prawn *Penaus monodon* by including astaxanthin in the diet. Mercier *et al*. (2009) reported high tolerance to handling stress in the white leg shrimp *P. vannamei* when fed with highly unsaturated fatty acids, so this ingredient increased its immune response.

Our results suggest that, in comparison with previous studies, levels of dietary proteins and lipids have an effect on specific tissue membrane composition, affecting SOD levels in different tissues and different circumstances. All these findings suggest that a balanced diet in terms of protein and lipid, might improve SOD activity and consequently, resistance to

stress. Finally, in terms of oxidative stress prevention, a proper diet for juvenile *M. americanum* prawns is suggested to have a proportion of P/L = 35/10.

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