Oxidative damage in tissues of juvenile crayfish (*Cherax quadricarinatus* von Martens, 1868) fed with different levels of proteins and lipid

Daño oxidativo en tejidos de acociles juveniles (*Cherax quadricarinatus* von Martens, 1868) alimentados con diferentes niveles de proteínas y lípidos.

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ABSTRACT

This experiment investigated the effect of dietary protein and lipid levels on superoxide radical production and lipid peroxidation in juvenile redclaw crayfish, *Cherax quadricarinatus*. Nine practical diets were formulated to contain a combination of three crude protein (CP) (26, 31, and 36%) and three crude lipid (CL) (4, 8, and 12%) levels. Four replicate groups of 15 crayfish (0.71 ± 0.13 g) per diet treatment were stocked in 40 L tanks, at 28 °C for 60 days. The control group was fed with a commercial shrimp diet. After the feeding period, superoxide radical (O_2^-) production and lipid peroxidation, measured as thiobarbituric acid reactive substances (TBARS) of muscle, digestive gland and gill were analyzed. In the group fed the control diet, O_2^- production and TBARS levels were significantly higher in the digestive gland than in muscles or gills. There was no effect of dietary protein or lipid level on O_2^- production in the digestive gland, muscle, and gill. However, dietary protein level significantly affected TBARS levels in crayfish gills ($p < 0.05$). The results suggest tissue-specific effects of dietary protein and lipid levels on indicators of oxidative stress in redclaw. Results indicate that a diet containing 31% CP and 8% CL provided adequate amounts of protein and lipid to satisfy nutritional requirements for optimal growth, while preventing diet-induced oxidative stress and protecting the integrity of the immune function.

Keywords: *Cherax quadricarinatus*; lipid peroxidation; oxidative stress; superoxide radical production.

RESUMEN

Se realizó un estudio para evaluar el efecto de diferentes niveles de proteínas y lípidos en dietas prácticas sobre la producción de radical superóxido y el daño oxidativo en acociles juveniles *Cherax quadricarinatus*. Se evaluaron nueve dietas prácticas que contenían tres niveles de proteínas crudas (PC) (28, 35 y 40%) y tres niveles de lípidos (LC) (4, 8 y 12%). Cuatro grupos de 15 acociles (0.71 ± 0.13 g) por tratamiento fueron sembrados en acuarios de 40 L a 28 °C durante 60 días. El grupo control fue alimentado con una dieta comercial para camarón. Transcurrido el periodo de alimentación, los organismos fueron sacrificados y se midió la producción endógena de radical superóxido (O_2^-) y la peroxidación de lípidos (sustancias reactivas al ácido tiobarbitúrico, TBARS) en extractos tisulares de músculo, glándula digestiva, y branquias. En los acociles alimentados con la dieta control, la producción de O_2^- y los niveles de TBARS fueron

significativamente mayores en la glándula digestiva que en el músculo o en las branquias. Los niveles de proteínas o lípidos en la dieta no tuvieron un efecto significativo sobre la producción de O_2^- en ninguno de los tejidos estudiados. Sin embargo, sí afectaron significativamente los niveles de TBARS en las branquias ($p < 0.05$). Los resultados indican que los niveles de proteínas y lípidos contenidos en la dieta del acocil, tienen un efecto sobre los indicadores de estrés oxidativo, y que este efecto es específico dependiendo del tejido; en este acocil una dieta con 31% PC y 8% LC proporciona el requerimiento adecuado de proteínas y lípidos para crecimiento óptimo, previniendo el estrés oxidativo y protegiendo la integridad de la función inmune.

Palabras clave: Estrés oxidativo; *Cherax quadricarinatus;* peroxidación lipidíca; producción de radical superóxido.

INTRODUCTION

Cherax quadricarinatus (von Martens, 1898), the redclaw crayfish, is an omnivorous species, native to northern Australia and recently introduced in Mexico for culture purposes (Cortés-Jacinto *et al*., 2003a,b). Under aquaculture conditions, an appropriate diet that meets the specific nutrient requirements for the species at each developmental stage must be provided to avoid nutritional imbalances, physiological changes, and disease (Harrison, 1990; Scott, 1999; Campaña-Torres *et al*., 2005). Dietary protein level is probably the most important element affecting growth of cultured species (Cortés-Jacinto *et al*., 2003a; Thompson *et al*., 2003; Campaña-Torres *et al*., 2005), while dietary lipids are an important source of calories (Hernández-Vergara *et al*., 2003; Cortés-Jacinto *et al*., 2005). The effects of dietary protein and lipid levels in aquaculture have been studied in terms of weight gain, and growth and survival rates (Hernández-Vergara *et al*., 2003; Thompson *et al*., 2003; Cortés-Jacinto *et al*., 2005).

Dietary protein and lipid levels affect the production of reactive oxygen species (ROS) and the oxidative stress response in mammals (Rana *et al*., 1996; Mataix *et al*., 1998; Schwerin *et al*., 2002; Luna-Moreno *et al*., 2007). In fish, *in vivo* lipid peroxidation from ROS is a cause of several diseases (Sakai *et al*., 1998). The oxidative stress response is an important component of the defence mechanism in crustaceans (Winston *et al*., 1996; Holmblad & Söderhäll, 1999; Campa-Córdova *et al*., 2002; Kovacevic *et al*., 2006, Mercier *et al*., 2006a). Changes in dietary protein and lipid levels can potentially compromise the immune function via an altered oxidative stress response in crustacean species in aquaculture (Campa-Córdova *et al*., 2002). The effect of dietary protein or lipid levels on the oxidative stress response in crustaceans has only recently been addressed (Dutra *et al*., 2007). This study was undertaken to investigate the effect of different levels of dietary protein and lipids on free radical production and oxidative damage in juvenile redclaw crayfish.

MATERIALS AND METHODS

Experimental diets. Experimental diets were formulated to contain three levels of crude protein (26, 31, and 36%) and three levels of lipids (4, 8, and 12%) and were prepared as described by Cortés-Jacinto *et al.* (2003a). Proximate analyses of diets (Table 1) were determined according to AOAC (1995). Gross energy of the diet was measured in an adiabatic bomb calorimeter (Model 1261, Parr, Moline, IL, USA). Juvenile redclaw crayfish were initially fed a ration of 5% of biomass per day. Each day, 30% was feed at 8:00, 30% feed at 14:00 and 40% feed at 20:00 according to Cortés-Jacinto *et al.* (2003b) during a 60-day trial. The following morning leftover feed, which could be readily identified by its swollen pellet shape, was removed and quantified by estimating the amount in its original dry form, and the rations were adjusted to minimize the amount of uneaten feed.

Diet water stability. The amount of dry matter leached from the pellets was determined as previously described (Obaldo *et al*., 2002).

Experimental redclaw crayfish. Juvenile redclaw crayfish (n = 525, 0.71 ± 0.13 g initial wet weight) were obtained from a stock at CIBNOR, La Paz, B.C.S., Mexico. The animals were maintained according to recommendations by Cortés-Jacinto *et al.* (2003b). Redclaw crayfish were held in 40 L fiberglass aquaria at a stocking density of 15 redclaw per tank. Juvenile crayfish that died during the first three days of experiment were replaced with those held under identical conditions. Temperature was maintained at 28.01 ± 0.33 °C with 100-W heaters (Aquarium Pharmaceuticals, Paris, France), and a 14L/10D-photoperiod during the 60-day experiment. An air stone in each tank provided constant aeration. Uneaten feed and feces were siphoned from each tank daily. After siphoning, 30% of the tank water was replaced daily with fresh tap water. Water quality was monitored and maintained well within recommended limits for redclaw crayfish (Villarreal, 2000). Each diet was fed to only one of the crayfish groups. The control group was fed a commercial shrimp diet of pellets with 36.7% protein, and 12.6% lipids (PIASA®, La Paz, B.C.S., Mexico). Five specimens with a mean weight of 6.3 ± 0.27 g were randomly selected from each group for analyses of superoxide radical (O_2^-) production and lipid peroxidation.

Biochemical analysis of superoxide radical (O_2^-) production and lipid peroxidation. At the end of the experiment, five crayfish from each treatment were sacrificed by immersion in

Table 1. Proximate composition of the experimental diets (g/100 g dry matter).

	Diets (% protein/% lipids)								
	26/4	26/8	26/12	31/4	31/8	31/12	36/4	36/8	36/12
Protein[1]	26.7±0.01	26.7±0.07	26.7±0.12	31.7±0.06	31.5±0.03	31.5±0.12	36.4±0.09	36.3±0.16	36.6±0.09
Ether extract[1]	4.3±0.06	8.8±0.03	12.3±0.02	4. 9±0.09	8.3±0.07	12.2±0.08	4.9±0.03	8.7±0.09	12.1±0.08
Ash[1]	7.0±0.02	6.9±0.03	8.2±0.03	8.3±0.06	8.3±0.05	6.9±0.02	9.5±0.04	9.8±0.03	9.6±0.08
Fiber[1]	0.39±0.04	0.69±0.07	0.25±0.01	0.27±0.02	0.39±0.01	0.69±0.01	1.53±0.18	1.04±0.23	1.23±0.03
NFE[2]	54.8	50.0	45.1	48.7	44.4	41.5	41.1	35.3	33.6
Gross energy (kJ^{-1})	17.5	18.0	19.2	17.5	18.2	19.1	17.9	18.7	19.4
P:E ($mg\ kJ^{-1}$)[3]	15.2	14.8	13.9	18.1	17.3	16.5	20.3	19.4	18.8
Water stability (%)[4]	92.3±1.2	94.9±0.2	96.0±0.4	92.8±0.6	93.2±1.5	95.3±0.9	93.7±0.7	94.8±1.1	96.8±0.7

[1]Mean ± SD, n = 3.
[2]NFE = nitrogen free extract, calculated by difference.
[3]P:E = protein to energy ratio
[4](%) = Percent dry matter retention

liquid nitrogen. After dissection over ice, the muscle, digestive gland, and gills were removed, placed in cryovials, and immediately immersed in liquid nitrogen and stored at –80 °C until analyzed.

Superoxide radical production. Endogenous O_2^- production was assessed as an index of the tissue capacity for production of ROS by spectrophotometry during the reduction of ferricytochrome *c* (Drossos *et al.*, 1995; Zenteno-Savín *et al.*, 2006). Each sample was placed in a test tube containing Krebs-Henseleit buffer (0.11 M NaCl, 4.7 mM KCl, 12 mM $MgSO_4$, 12 mM $NaH2PO_4$, 25 mM $NaHCO_3$, 1 mM glucose). Then 15 µM cytochrome *c* (Type VI from horse heart, SIGMA) was added to the sample and was incubated for 15 min in a shaking water bath at 37 °C; then 3 mM N-ethylmaleimide was added to inhibit further reduction of cytochrome *c*. The tubes were then centrifuged at 4000 × *g* at 4 °C for 10 min. Supernatants were removed and the absorbance was read at 550 nm in a spectrophotometer (Model 6305, Jenway, Princeton, NJ, USA). A mixture containing the same reagents was added to the pellet and used as a blank for each sample, after incubation and centrifugation at the same conditions. The amount of O_2^- produced was calculated by dividing the absorbance by the extinction coefficient for the change between ferricytochrome *c* and ferrocytochrome *c*, E550 = 21 nM cm^{-1}. Results were expressed in nanomoles of O_2^- per minute g^{-1} wet tissue.

Lipid Peroxidation. Lipid peroxidation was assessed as an index of the damage induced by ROS by measuring the tissue content of TBARS (Ohkawa *et al.*, 1979; Olsen & Henderson, 1997; Zenteno-Savín *et al.*, 2006). Each sample was homogenized in two volumes of isotonic crustacean solution (450 mM NaCl, 10 mM KCl, 1 mM PMSF). The homogenized sample was incubated for 15

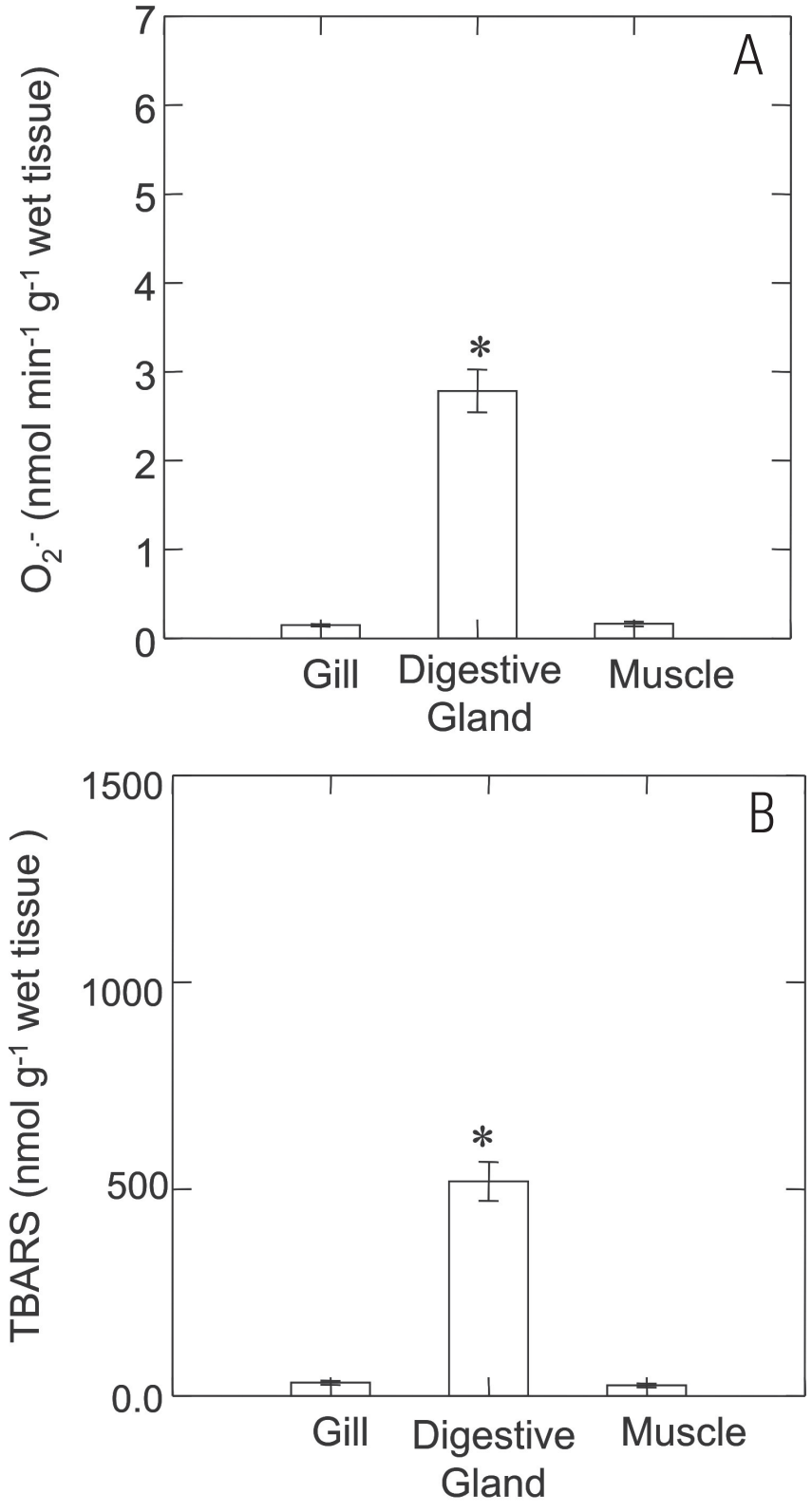

Figure 1. (A) Superoxide radical production (O_2^-, nmol min^{-1} g^{-1} wet tissue); (B) lipid peroxidation levels (TBARS, nmol g^{-1} wet tissue) in tissues of juvenile redclaw crayfish *Cherax quadricarinatus* fed the control diet. N = 5. * = $p < 0.05$ differences among tissues. Endogenous O_2^- production was assessed following the method of Drossos *et al.* (1995); lipid peroxidation was quantified as the concentration of thiobarbituric acid reactive substances (TBARS, Ohkawa *et al.*, 1979).

Table 2. Superoxide radical production (O_2^-, nmol min^{-1} g^{-1} wet tissue) and lipid peroxidation levels (TBARS, nmol g^{-1} wet tissue) in tissues of juvenile redclaw crayfish fed different levels of protein and lipid.

Diet (protein/lipids)	Tissue	O_2^- (nmol min^{-1} g^{-1}) Mean ± s.e. (n)	TBARS (nmol g^{-1}) Mean ± s.e. (n)
26/4	Gills	0.204 ± 0.033 (5)	19.7 ± 4.7 (5) a
	Digestive gland	0.913 ± 0.130 (4) *	340.7 ± 16.5 (4)*
	Muscle	0.008 ± 0.001 (5)	10.5 ± 3.0 (5)
26/8	Gills	0.188 ± 0.048 (5)	17.2 ± 3.0 (5) b
	Digestive gland	1.226 ± 0.155 (4)*	182.2 ± 127.4 (4)*
	Muscle	0.008 ± 0.002 (5)	10.4 ± 3.2 (5)
26/12	Gills	0.174 ± 0.042 (5)	34.9 ± 9.0 (5)
	Digestive gland	2.540 ± 0.162 (4)*	281.7 ± 185.9 (4)*
	Muscle	0.005 ± 0.002 (4)	3.1 ± 0.3 (5)
31/4	Gills	0.178 ± 0.042 (5)	73.5 ± 3.7 (5) a,b,c
	Digestive gland	1.990 ± 0.874 (4)*	2041 ± 47.3 (4)*
	Muscle	0.010 ± 0.005 (5)	2.9 ± 0.9 (4)
31/8	Gills	0.189 ± 0.050 (5)	80.0 ± 4.7 (5) a,b,d
	Digestive gland	1.275 ± 0.368 (4)*	6553 ± 148.0 (4)*
	Muscle	0.010 ± 0.004 (5)	113 ± 2.5 (5)
31/12	Gills	0.166 ± 0.006 (5)	68.8 ± 2.7 (5) b,e
	Digestive gland	1.310 ± 0.275 (5)*	344.4 ± 158.8 (5) *
	Muscle	0.008 ± 0.003 (5)	7.4 ± 2.7 (5)
36/4	Gills	0.302 ± 0.072 (5) a	7.0 ± 1.4 (5) c,d,e
	Digestive gland	1.540 ± 0.024 (5)*	649.7 ± 335.5 (5)*
	Muscle	0.012 ± 0.005 (5)	8.1 ± 1.8 (5)
36/8	Gills	0.275 ± 0.080 (5)	17.0 ± 6.1 (5) c,d,e
	Digestive gland	1.090 ± 0.327 (5)*	610.9 ± 188.3 (5)*
	Muscle	0.014 ± 0.003 (5)	13.2 ± 1.6 (5)
36/12	Gills	0.166 ± 0.059 (5)	10.5 ± 4.9 (4) c,d,e
	Digestive gland	2.541 ± 0.933 (4)*	596.6 ± 386.7 (4)*
	Muscle	0.004 ± 0.001 (5)	6.3 ± 1.4 (5)
Control	Gills	0.147 ± 0.137 (45) a	31.3 ± 4.7 (43) c,d
	Digestive gland	2.784 ± 0.238 (44)*	518.8 ± 46.5 (44)*
	Muscle	0.163 ± 0.026 (45)†	25.1 ± 5.0 (45)

*$P < 0.05$ differences among tissues; †$P < 0.1$ effect of diet; values within columns with the same alphabetical superscript are significantly different at $p < 0.05$.

min at 37 °C on a shaking water bath; the reaction was stopped by addition of ice cold 0.8 M HCl in 12.5% trichloroacetic acid (SIGMA). After adding 1% thiobarbituric acid (SIGMA), samples were incubated for 10 min in a boiling water bath, cooled to room temperature, and centrifuged at 4000 × *g* for 10 min at 4 °C. The supernatant was read at 532 nm in a spectrophotometer (Model 6305, Jenway, Princeton, NJ, USA). A standard curve of malondialdehyde bis (diethyl acetal) (SIGMA) was run in parallel with the samples and the concentration of TBARS in the samples was calculated from this standard curve. Results were expressed in nM of TBARS g^{-1} wet tissue.

Statistics. The SYSTAT software (SPSS, Richmond, CA, USA) was used for data analysis. Results are presented as mean ± SE for at least five redclaw crayfish in each treatment group. Normality and homogeneity of variances of the data were tested with the Kolmogorov–Smirnoff and Cochran's C tests, respectively. Differences between means with respect to diet were tested with ANOVA followed by Bonferroni post-hoc tests for multiple comparisons. Statistical analyses were performed independently for O_2^- production and TBARS. To determine if there were differences among tissues, an ANOVA with the Bonferroni post hoc test was performed for each diet group. Significance was set at $p < 0.05$.

RESULTS

Experimental diets. Reasonably good pellet stability in water was achieved in all experimental diets. Between 92.3 ± 1.2% and 96.8 ± 0.7% of the dry matter was retained after 1 h for all pellet types. Proximate composition (protein, fat, fibre, and ash), nitrogen-free extract, and gross energy content of nine practical diets are shown in Table 1.

Superoxide radical production. Superoxide radical production in muscle, digestive gland, and gills of *C. quadricarinatus* fed different levels of proteins and lipids are presented in Table 2. Production of O_2^- was different among tissues, even in crayfish fed the control diet (Fig. 1A). In all treatment groups, O_2^- production was higher in the digestive gland than in muscle or gills. There was no effect of dietary protein/lipid levels on O_2^- production in the crayfish digestive gland. However, O_2^- production was higher in muscle in crayfish fed the control diet than those fed experimental diets ($p < 0.1$, Table 2). O_2^- production in the gills of crayfish fed the 31/4 diet was significantly higher than in the gills of animals fed the control diet (Table 2).

Lipid peroxidation. Lipid peroxidation (TBARS) levels in juvenile redclaw tissues are presented in Table 2. TBARS was different among tissues, even in crayfish fed the control diet (Fig. 1B). In all treatment groups, TBARS levels were higher in the digestive gland than in muscle or gills. There was no differential effect of levels of dietary protein/lipids in TBARS levels in crayfish muscle or digestive gland. However, significant differences were found among diets in crayfish gills (Table 2). TBARS levels in gills of crayfish fed diets with 31% protein, regardless of dietary lipid content, were significantly higher than in crayfish fed the control diet (31.3 ± 4.7 nmol g^{-1}). TBARS levels in gills from crayfish fed the 26/4, and 26/8 (19.7 ± 4.7, and 17.2 ± 3.0 nmol g^{-1}, respectively) diets were significantly lower than those fed the 31/4, and 31/8 diets (73.5 ± 3.7, and 80.0 ± 4.7 nmol g^{-1}, respectively), while TBARS levels in gills from crayfish fed 31% protein were significantly higher than those fed 36% protein, regardless of dietary lipid content ($p < 0.05$).

DISCUSSION

Significant differences were found in TBARS levels among diets in gills of crayfish (Table 2). Crayfish fed 26% protein had lower TBARS levels in gills than those fed 31% protein, while the latter had higher TBARS levels than those fed 36% protein, regardless of dietary lipid content ($p < 0.05$). Increased production of TBARS could be the result of increased levels of other ROS, such as hydrogen peroxide or hydroxyl radical, or a direct effect of the dietary protein/lipid composition. In crayfish, gills also have an excretory function (Vogt, 2002); it is possible that the oxidative damage in crayfish gills is a consequence of the excretion of an increased protein load.

Production of O_2^- and TBARS levels were different among tissues, even in crayfish fed the control diet (Fig. 1). In all treatment groups, O_2^- production and TBARS levels were higher ($p < 0.05$) in the digestive gland than in the muscle or gills (Fig. 1). Similar differences in O_2^- production and TBARS levels between digestive gland, muscle, and gills in whiteleg shrimp *Litopenaeus vannamei* were found by Zenteno-Savín *et al.* (2006).

Dietary nutrient supply affects health and performance of terrestrial and aquatic organisms. Other reports indicate that dietary lipid and protein levels increase free radical production and oxidative damage indicators. Ingestion of specific fatty acids, such as polyunsaturated fatty acids, play an important role in O_2^- production (Mercier *et al.*, 2006b) and free radical-mediated lipid peroxidation (Tocher *et al.*, 2002). Ingestion of dietary protein in excess of metabolic amino acid requirements increases production of ROS in mitochondria, leading to oxidative stress and resulting in lipid peroxidation (Harper, 1994; Benzie, 1996). Decreased antioxidant defences and increased lipid peroxidation were found in liver of rats fed a protein-deficient diet compared to rats fed an isocaloric normal protein diet; severe protein energy malnutrition resulted in hepatic injury (Rana *et al.*, 1996). Schwerin et al. (2002) found increased expression of genes involved in the oxidative stress response, along with upregulation of gene expression and neuronal signaling in pigs fed soy (versus casein) as dietary protein. Dietary lipids have a differential effect on specific tissue membrane composition in rats, and it was suggested that lipid peroxidation levels are dependent on both tissue type and diet (Mataix *et al.*, 1998).

The effects of dietary protein or lipid levels on free radical response have only recently been studied in crustaceans. While Dutra *et al.* (2007) found decreased lipoperoxidation levels in *Hyalella* fed a restricted caloric diet, the results from our study suggests that isocaloric changes in dietary protein or lipid content do not significantly increase oxidative damage to lipids in muscle or digestive gland of juvenile crayfish. That $O_2^{\bullet-}$ production and lipid peroxidation levels were not significantly changed in the digestive gland or muscle of crayfish and that the diets were not supplemented with antioxidants suggest that crayfish have sufficient antioxidant defences to counteract the oxidative stress potentially induced by changes in dietary protein or lipid levels. Still, further detailed studies are needed to corroborate this.

Increased lipid peroxidation in gills of crayfish on diets with 31% protein was not expected and suggests that protein and lipid metabolism, absorption, and deposition are adjusted to maintain structural and functional properties in active tissues, such as muscles and the digestive gland. This result agrees with findings in mammals of tissue-specific effects of dietary protein and lipids on indicators of oxidative stress (Mataix *et al.*, 1998).

Alternatively, the differences among tissues may reflect their regenerative capacity (Ochoa *et al*., 2003), suggesting that gills of juvenile crayfish have a lower regenerative capacity than muscles or the digestive gland. It is possible that dietary protein and lipid levels directly affect the membrane lipid composition in crayfish gills by increasing either availability or oxidation rates of fatty acids.

O_2^- production did not change with diet in tissues, specifically the gills of juvenile redclaw crayfish. This does not rule out increased formation of other ROS, which were not measured in this study. It would be interesting to find other ROS produced in crayfish gills and if this production is dependent on protein or lipid contents in the diet. Our results suggest that crayfish have enhanced antioxidant defences and warrant a detailed study of the main antioxidant enzymes in this species. ROS production is an important component of the immune response in crustaceans (Winston *et al*., 1996; Holmblad & Söderhäll, 1999; Campa-Córdova *et al*., 2002; Kovacevic *et al*., 2006; Mercier *et al*., 2006a); the increased lipid peroxidation found in gills suggests a closer look at the immune response in tissues of crayfish fed diets with different protein and lipid levels. Details on growth, survival, and feed conversion are presented in Cortés-Jacinto *et al.* (2005).

Our results suggest that, in a fashion similar to what has been observed in mammals (Mataix *et al*., 1998; Ochoa *et al*., 2003), levels of dietary protein and lipid have a differential effect on specific tissue membrane composition, affecting lipid peroxidation levels in different ways in gills, muscle, and the digestive gland in juvenile redclaw crayfish. Similarly, these results suggest that a diet for juvenile redclaw crayfish that provides adequate protein and lipids is 31/8. This appears to satisfy nutritional requirements for optimal growth, prevent diet-induced oxidative stress, and protect the integrity of the immune function.

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REFERENCES

AOAC, 1995. Association of Official Analytical Chemists. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 16th Ed. Arlington, VA, USA. 1422 p.

Benzie, I. F. 1996. Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. *International Journal of Food Sciences and Nutrition* 47: 233-261.

Campa-Córdova, A. I., N. Y. Hernández-Saavedra & F. Ascencio. 2002. Superoxide dismutase as modulator of immune function in American white shrimp (*Litopenaeus vannamei*). *Comparative Biochemistry Physiology C* 133: 557-565.

Campaña-Torres, A., L. R. Martínez Córdova, H. Villarreal Colmenares & R. Civera Cerecedo. 2005. Estudio de los parámetros de producción del acocil australiano *Cherax quadricarinatus* (von Martens, 1858), variando el nivel de proteína en su dieta. *Hidrobiológica* 15: 255-260.

Cortés-Jacinto, E., H. Villareal-Colmenares, R. Civera-Cerecedo & L. R. Martínez-Córdova. 2003a. Effect of dietary protein level on growth and survival of juvenile freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquaculture Nutrition* 9: 207-213.

Cortés-Jacinto, E., H. Villarreal-Colmenares & M. Rendón-Rumualdo. 2003b. Efecto de la frecuencia alimenticia en el crecimiento y sobrevivencia de juveniles de langosta de agua dulce *Cherax quadricarinatus* (Decapoda: Parastacidae). *Hidrobiológica* 13: 151-158.

Cortés-Jacinto, E., H. Villarreal-Colmenares, L. E. Cruz-Suárez, R. Civera-Cerecedo, H. Nolasco-Soria & A. Hernández-Llamas. 2005. Effect of different dietary protein and lipid levels on growth and survival of juvenile Australian redclaw crayfish *Cherax quadricarinatus* (von Martens). *Aquaculture Nutrition* 11: 283-291.

Drossos, G., A. Lazou, P. Panagopoulos & S. Westaby. 1995. Deferoxamine cardioplegia reduces superoxide radical production in human myocardium. *The Annals of Thoracic Surgery* 59: 169-172.

Dutra, B. K., R. B. Santos, A. A. P. Bueno & G. T. Oliveira. 2007. Variations in the biochemical composition, lipoperoxidation and reproductive success *Hyalella pleoacuta* and *Hyalella curvispina* maintained on different diets. *Comparative Biochemistry and Physiology A* 148: S86-S90, Abstract.

Harper, A. E. 1994. Sine concluding comments on emerging aspects of amino acid metabolism. *The Journal of Nutrition* 124: 1529S-1532S.

Harrison, K. E. 1990. The role of nutrition in maturation, reproduction, and embryonic development of Decapod crustacean: A review. *Journal of Shellfish Research* 9: 1-28.

Hernández-Vergara, M. P., D. B. Rouse, M. A. Olvera-Novoa & A. D. Davis. 2003. Effects of dietary lipid level and source on growth and proximate composition of juvenile redclaw (*Cherax quadricarinatus*) reared under semi-intensive culture conditions. *Aquaculture* 223: 107-115.

Holmblad, T. & K. Söderhäll. 1999. Cell adhesion molecules and antioxidative enzymes in a crustacean, posible role in immunity. *Aquaculture* 172: 111- 123.

Kovacevic, T. B., S. S. Borkovic, S. Z. Pavlovic, R. M. Radojicic, Z. S. Saicic & S. Zorica. 2006. The concentrations of antioxidant compounds in the hepatopancreas, the gills, and muscle of some freshwater crayfish species. *Acta Biologica Hungarica* 57: 449-458

Luna-Moreno, D., O. Vázquez-Martínez, A. Báez-Ruiz, J. Ramírez & M. Díaz-Muñoz. 2007. Food restricted schedules promote differential lipoperoxidative activity in rat hepatic subcellular fractions. *Comparative Biochemistry and Physiology Part A* 146: 632-643.

Mataix, J., J. L. Quiles, J. R. Huertas, M. Battino & M. Mañas. 1998. Tissue specific interactions of exercise, dietary fatty acids, and vitamin E in lipid peroxidation. *Free Radical Biology and Medicine* 24: 511-521.

Mercier, L., E. Palacios, A. I. Campa-Córdova, D. Tovar-Ramírez, R. Hernández-Herrera, & I. S. Racotta. 2006a. Metabolic and immune responses in Pacific whiteleg shrimp *Litopenaeus vannamei* exposed to a repeated handling stress. *Aquaculture* 258: 633-640.

Mercier, L., E. Palacios & I. S. Racotta. 2006b. Efectos de los ácidos grasos altamente insaturados sobre la susceptibilidad al estrés y el sistema de defensa del camarón blanco del pacífico (*Litopenaeus vannamei). XLIX Congreso Nacional de Ciencias Fisiológicas*; Querétaro, Qro. Septiembre 4-8, 2006. C007.

Obaldo, L. G., S. Divakaran & A. G. Tacon. 2002. Method for the determining the physical stability of shrimp feeds in water. *Aquaculture Research* 33: 369-377.

Ochoa, J. J., J. L. Quiles, S. Ibañez, E. Martínez, M. López-Farías, J. R. Huertas & J. Mataix. 2003. Aging-related oxidative stress depends on dietary lipid source in rat postmitotic tissues. *Journal of Bioenergetics and Biomembranes* 35: 267-275.

Ohkawa, H., N. Ohishi & K. Yagi. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95: 331-358.

Olsen, R. E. & R. J. Henderson. 1997. Muscle fatty acid composition and oxidative stress indices of Artic charr, *Salvelinus alpinus* (L.), in relation to dietary polyunsaturated fatty acid levels and temperature. *Aquaculture Nutrition* 3: 227-238.

Rana, S., C. P. Sodhi, S. Mehta, K. Vaiphei, R. Katyal, S. Thakur, & S. K. Mehta. 1996. Protein-energy malnutrition and oxidative injury in growing rats. *Human and Experimental Toxicology* 15: 810-814.

Sakai, T., H. Murata, M. Endo, T. Shimomura, K. Yamauchi, T. Ito, T. Yamaguchi, H. Nakajima & M. Fukudome. 1998. Severe oxidative stress is thought to be a principal cause of jaundice of yellowtail *Seriola quinqueradiata*. *Aquaculture* 160: 205-214.

Schwerin, M., U. Dorroch, M. Beyer, H. Swalve, C. C. Metges & P. Junghans. 2002. Dietary protein modifies hepatic gene expression associated with oxidative stress responsiveness in growing pigs. *FASEB Journal* 16: 1322-1324.

Scott, R. W. 1999. Marketing bioactive ingredients in food products. *Food Technology* 53: 69-53.

Tocher, D. R., G. Mourente, A. Van Der Eecken, J. L. Evjemo, E. Diaz, J. G. Bell, P. Geurden, P. Lavens, & Y. Olsen. 2002. Effects of dietary Vitamin E on antioxidant defense mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). Aquaculture Nutrition 8: 195-207.

Thompson, K. R., L. A. Muzinic, T. D. Christian, C. D. Webster, L. Manomaitis & D. B. Rouse. 2003. Lecithin requirements of juvenile Australian red claw crayfish *Cherax quadricarinatus. Aquaculture Nutrition* 9: 223-230.

Villarreal, H. 2000. El cultivo de la langosta de agua dulce. Una oportunidad para la diversificación acuícola. *In*: Bonilla, Z. & I. Burciaga (Eds.). *Memorias III Simposium Internacional de Acuacultura*. 5-7 de Octubre, 2000. Culiacán, Sinaloa, pp. 110-135.

Vogt, G. 2002. Functional Anatomy. *In*: Holdich, D. M. (Ed.). *Biology of Freshwater Crayfish. Blackwell* Science, Oxford. p. 53-151.

Winston, G. W., M. N. Moore, M. A. Kirchin & C. Soverchia. 1996. Production of reactive oxygen species by hemocytes from the marine mussel, *Mytilus edulis*: Lysosomal localization and effect of xenobiotics. C*omparative Biochemistry and Physiology C* 113: 221-229.

Zenteno-Savín, T., R. Saldierna & M. Ahuejote-Sandoval. 2006. Superoxide radical production in response to environmental hypoxia in cultured shrimp. *Comparative Biochemistry and Physiology C* 142: 301-308.

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