Apparent carbohydrate and lipid digestibility of feeds for whiteleg shrimp, *Litopenaeus vannamei* (Decapoda: Penaeidae), cultivated at different salinities

Milena Gucic[1], Edilmar Cortés-Jacinto[1]*, Roberto Civera-Cerecedo[1], Denis Ricque-Marie[2] & Luis Rafael Martínez-Córdova[3]

1. Programa de Acuicultura, Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo Santa Rita, La Paz, B.C.S. 23096, México; mgucic@gmail.com, ecortes@cibnor.mx*, rcivera04@cibnor.mx
2. Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Av. Pedro de Alba s/n, Cd. Universitaria, San Nicolás De Los Garza, Nuevo León, 66451, México; denis.ricquemr@uanl.edu.mx
3. Departamento de Investigación Científica y Tecnológica de la Universidad de Sonora, Luis Donaldo Colosio s/n, Col. Centro, Hermosillo, Sonora,83000, México; lmtz@guaymas.uson.mx

* Corresponding author

Received 06-XII-2011. Corrected 15-X-2012. Accepted 06-XI-2012.

Abstract: Digestibilidad aparente de carbohidratos y lípidos en alimentos para camarón blanco, *Litopenaeus vannamei* (Decapoda: Penaeidae), cultivados en diferentes salinidades. Whiteleg shrimp, *Litopenaeus vannamei* is one of the most commercially farmed species worldwide because of its fast growth, good survival rate at high farming densities, and osmoregulatory capacity, which makes it an excellent candidate for cultures at different salinities. The knowledge of shrimp nutritional requirements is critical in the formulation of diets to allow optimal growth at different environmental conditions and development stages. The effect of salinity on apparent digestibility of shrimp feed is not well known, and this information is required in shrimp diet formulation. For this purpose, the apparent digestibility coefficients of carbohydrates (ACD) and lipids (ALD) were determined for juvenile whiteleg shrimps under controlled culture conditions. We evaluated the apparent digestibility of six commercial (D1:37CP, D2:38CP, D3:39CP, D4:34CP, D5:35CP, and D6:37CP) and two experimental (E1:33CP and E2:33CP) diets for juvenile whiteleg shrimp cultivated at three salinities (5, 35 and 50psu) in 60L aquariums. ACD and ALD were determined *in vivo* using chromic oxide as an inert marker. Our results showed that ALD in most cases was over 80%, independent of salinity, except the E1:33CPdiet which had 74.0% at 50psu. Diet D3:39CP showed the highest ALD coefficient (90.1 and 90.6% at 5 and 35psu, respectively). For ACD, differences were detected between commercial and experimental diets at every salinity level, although salinity effect on ACD was not significant. Diet D4:34CP had the highest coefficient (92.4%) at 5psu, and E2:33CP at 35 and 50psu (97.3 and 94.7%). This study demonstrated that there is no significant effect of saline variations on carbohydrate and lipid digestibility by juvenile whiteleg shrimp, under the experimental conditions. Rev. Biol. Trop. 61 (3): 1201-1213. Epub 2013 September 01.

Key words: diets, *Litopenaeus vannamei*, different salinities, digestibility.

Feed is the main source of waste and responsible for most of the environmental impacts of aquaculture. The quantity and quality of the waste excreted by shrimp depend on ingestion, digestion, and metabolism of dietary compounds (Amirkolaie 2011). Valdez *et al.* (2008) reported that the highest energy from the food consumed by juvenile whiteleg shrimp *Litopenaeus vannamei* is obtained when they are maintained at 26 practical salinity units (psu). However, in areas with high evaporation rates, such as the coast of the States of Sonora and Sinaloa, Mexico (Martinez-Cordova *et al*. 2009), the levels of salinity are much higher

and it could be of great interest to examine if the diets' performance is affected by that condition. Diets that fulfill nutritional requirements at the lowest cost and lowest impact to the environment become imperative; in that context, digestibility of the ingredients needs to be accurately determined (Cruz-Suarez *et al*. 2001, Campaña-Torres *et al*. 2005, Guo *et al*. 2006). Studies of digestibility of feeds in commercial aquatic organisms have acquired great importance and interest as environmental regulations obligate farmers to use environmentally friendly sources, especially those having low nitrogen and phosphate output (Campaña-Torres *et al*. 2006). Feed is a very large part of operating expenses in crustacean aquaculture (Cortés-Jacinto *et al.* 2003), reaching a value as high as 50% (Shiau 1998).

Chemically well-defined diets provide greater confidence in the responses of animals to the feed offered (Glencross *et al*. 1999). Using highly digestible diets is environmentally beneficial under high-density cultivation, where accumulation of undigested feed contaminates the water, increases costs of water treatment, and promotes shrimp disease and mortality (Lin *et al*. 2006).

Carbohydrates and lipids components are important nutrients in shrimp diets (Gaxiola *et al*. 2005). From a practical point of view, understanding how carbohydrates are used will provide information to design better feeds for the different growth phases of shrimp. Increasing the proportion of carbohydrates instead of proteins to meet energy requirements reduces the quantity of fishmeal and the cost of feed. Adding vegetal protein to reduce costs has been a widely studied topic (Cuzon *et al*. 2000, Campaña-Torres *et al*. 2006, Radford *et al*. 2008, Olmos *et al.* 2011). Additionally, plant-based diets provide essential fatty acids, phospholipids, sterols and carotenoids for growth, survival, and normal metabolic function (Shiau 1998, Ouraji *et al*. 2010). Digestibility of feedstuffs for whiteleg shrimp was examined by Davis & Arnold (1993), Guo *et al*. (2006), Lin *et al*. (2006) and Terrazas *et al*. (2010).

Salinity and temperature are two of the most important environmental factors controlling shrimp growth and survival because the rate of physiological responses is directly affected (Mu *et al*. 2005, Bückle 2006, Kir & Kumlu 2008). For whiteleg shrimp, an optimal temperature and salinity leads to higher productivity (Perez-Velazquez *et al*. 2007). Nutrition of shrimp reared at low salinity improved growth and survival by adjusting levels of nutrients in the feeds (Gong *et al*. 2003, 2004a, Perez-Velazquez *et al*. 2007). When shrimp are exposed to low salinity, they have to counteract passive loss of Na^+ and Cl^- by active uptake of Na^+ from the water in exchange for H^+, which occurs in the apical membrane of the osmoregulatory cells to improve their osmoregulatory capacity (Palacios *et al*. 2004, Bückle 2006, Hurtado *et al*. 2006).

In arid and semi-arid climates, where high evaporation of pond water is common, salinity can increase to 50psu or higher, especially by the end of the growing season; despite that extreme variation, whiteleg shrimp survive and grow at high densities because of its capacity to regulate variations in osmotic and ionic conditions that permits it to inhabit waters ranging from 0.5-60psu (Roy *et al.* 2007, Jaime-Ceballos *et al.* 2008, Valdez *et al.* 2008). Additionally, the species is resistant to several diseases (Ponce-Palafox *et al*. 1997, Lin *et al*. 2006). For those reasons, *L. vannamei* is one of the most cultivated marine shrimp in Mexico and worldwide. The available scientific literature about the effect of salinity on digestibility of diets in shrimp is scarce; thus, the objective of this study was to determine the effect of salinity on apparent digestibility coefficients of lipids and carbohydrates of six commercial and two experimental diets for juvenile whiteleg shrimp, *L. vannamei*.

MATERIALS AND METHODS

Experimental specimens: Juvenile Whiteleg shrimp (3.6±0.3g average initial weight) were obtained from a hatchery firm in La Paz, B.C.S. Mexico and acclimated for seven

days in three 1 500L fiberglass tanks and fed commercial feed containing 35% crude protein (PIASA^{TM}, La Paz, Mexico) at 35psu.

Water quality: Seawater was pumped to the laboratory, filtered (5µm), and UV-sterilized. Salinity, water temperature, and dissolved oxygen were measured daily to control conditions in each trial. Temperature was set at ~27°C and dissolved oxygen at >5mg/L. Nitrates and nitrites were measured weekly by spectrophotometer analysis (Strickland & Parson 1972). Ammonium, orthophosphate, and total phosphorus were recorded weekly according to Murphy & Riley (1962) and Solórzano (1969). Alkalinity was determined volumetrically with phenolphthalein and bromocresol, using sulfuric acid in a 25mL digital burette with precision of ±30µL.

Diet preparation: Commercial diets were selected according to the recommended protein content of shrimp feed up to 30% (Molina-Poveda & Morales 2004). Diet E1:33CP was formulated by using MIXIT-WIN software (Agricultural Software Consultants, San Diego, CA, USA) and prepared in the laboratory using the ingredients specified in table 2. All ingredients were pulverized and sieved through a 250µm mesh. This diet was prepared by mixing macro-ingredients in a blender until a uniform mixture was obtained. The micro-ingredients (vitamin and mineral premixes, sodium alginate, chromic acid, and antioxidant BHT) were mixed in a plastic container and then added to the macro-ingredients. Fish oil and soy lecithin were homogenized into an emulsion and added to the mixture. Distilled hot water was added (~30% of the dry weight of the ingredients). The dough was passed through a meat grinder to form 2mm diameter pellets that were dried in an air flux oven at 45°C for 8hr. Pellets were packed in plastic bags and stored at -20°C. Diet E2:33CP was similar to a commercial, fishmeal-based diet, formulated according to Akiyama *et al.* (1991) (Table 1), which was first pelletized at an industrial shrimp feed factory (Cruz-Suárez *et al*. 2009) and then ground to obtain a maximum particle size of 500µm; this was mixed with 1% chromic oxide as an inert marker and 1% sodium alginate (A-7128, Sigma, St. Louis) as a high viscosity binder. The resulting mixture was pelleted in a meat grinder through a die with 2mm diameter holes. Using standard methods (AOAC 1995), the feed was analyzed for proximate chemical composition (Table 2).

Experimental design at different salinities: The experiment was initiated on 29th of June, 2007. A completely randomized 8×3 factorial experimental design with four replicates per treatment was used. Each tank was stocked with 10 juveniles (4.0±0.5g average initial weight) preconditioned at a salinity of 5, 35 or 50psu.Feeding treatments consisted of six commercial (D1:37CP, D2:38CP, D3:39CP, D4:34CP, D5:35CP and D6:37CP), and two experimental (E1:33CP and E2:33CP) diets for shrimp, each one tested at three salinities (5, 35 and 50psu). The salinity/feeding trials were set as follows:

Feeding trial 5psu: To reach 5psu for the low-salinity trials, fresh tap water was gradually added to the seawater at ~5psu per day, as described in Jaime-Ceballos *et al*. (2008).

Feeding trial 35psu: After the first trials, seawater was gradually added in the second set of trials to increase salinity at the rate of ~5psu per day until 35psu was reached. Salinity was maintained at 35±1psu.

Feeding trial 50psu: For the high-salinity trials, a 1 100L tank was filled with seawater and iodide-free salt was added. A refractometer (model RF20, Extech Instruments, Waltham, MA, USA) was used to record salinity every 15-20min until 50psu was reached. This water was pumped into the high-salinity experimental tanks.

The procedure was as follows: first organisms were acclimated to salinity of 5psu and experimental feed, feces collections were accumulated until 1.9g dry weight of fecal material (~1.9g wet feces) from each treatment had been collected; shrimps were then acclimated to salinity of 35psu and feces were collected

TABLE 1
Ingredient (g/100 dry wt) of experimental diets (E1:33CP and E2:33CP) used to measure apparent in vivo digestibility of lipids and carbohydrates in juvenile whiteleg shrimp *Litopenaeus vannamei*

Ingredients	Crude protein (%)		Diet	
	E1:33CP	E2:33CP	E1:33CP	E2:33CP
Wheat meal[1]	12	12	45.6	45.14
Sardine meal[1]	65	57	22.8	34.0
Soybean meal[1]	46	47	19.0	14.0
Fish oil[1]	-	-	4.0	2.8
Soy lecithin[1]	-	-	2.0	3.5
Vitamin premix[2,a]	-	-	1.8	0.3
Mineral premix[3,b]	-	-	1.7	0.15
Vitamin E (D-α-tocopherol 50%)	-	-		0.03
Vitamin C[4,c]	-	-	0.09	0.06
Antioxidant[5]	-	-	0.004	0.02
Sodium alginate[6]	-	-	2.0	-
Chromic oxide	-	_	1.0	-

1. ODONAJI, Distribuidora de Alimentos Naturales y Nutricionales, La Paz, B.C.S., Mexico.
2. Vitamin premix (g/kg): A acetate, 15; D3, 7.5; E, 4; K3, 2.0; cholinechloride, 400mg; thiamin, 150; riboflavin, 100; pyridoxine, 50; pantothenicacid, 100; niacin, 300; biotin, 1; inositol, 500; folicacid, 20; cyanocobalamin, 0.1. [a]Vitamin mixture composition: retinol, 4000 IU/g; thiamin, 24; riboflavin,16; DL Ca pantothenate, 30; pyridoxine, 30; cyanocobalamin, 80mg/kg; ascorbicacid, 60; menadione, 16; cholecalciferol, 3200IU/g; tocopherol, 60; biotin, 400mg/kg; niacin, 20mg/kg; folicacid, 4.
3. Mineral premix (g/kg of diet). BASF, D.F., Mexico; [b]Co, 2; Mn, 16; Zn, 40; Cu, 20; Fe, 1mg/kg; Se, 100 mg/kg; I, 2.
4. Butylated hydroxytoluen, Costa Mesa, CA, USA; [c]Vitamin C. BASF, Mexico.
5. Antioxidant: Dresquin 66, Dressen, D.F., Mexico.

TABLE 2
Proximate analysis[1] (g/100 dry wt) of experimental and commercial diets used

	E1:33CP	E2:33CP	D1:37CP	D2:38CP	D3:39CP	D4:34CP	D5:35CP	D6:37CP
Dry matter	93.0±0.23^{a}	93.6±0.17^{c}	92.4±0.09^{d}	91.3±0.09^{e}	92.3±0.07^{d}	91.7±0.23^{e}	93.3±0.04^{e}	93.0±0.22^{a}
Crude protein(N×6.25)	33.5±0.27^{g}	33.4±0.45^{g}	37.9±0.15^{c}	38.3±0.25^{b}	39.0±0.08^{a}	34.5±0.08^{f}	35.6±0.30^{e}	37.4±0.0.5^{d}
Ether extract	7.0±0.1^{b,c}	7.0±2.0^{b,c}	8.2±0.02^{a,b}	9.4±1.3^{a}	9.3±0.02^{a}	5.9±0.78^{c}	9.5±0.03^{a}	7.2±2.0^{b,c}
Ash	8.7±0.03^{h}	11.5±0.04^{d}	11.2±0.07^{e}	14.4±0.04^{a}	12.0±0.10^{c}	9.7±0.04^{g}	12.2±0.03^{b}	10.3±0.03^{f}
Crude fiber	3.0±0.26a	1.5±0.00^{b}	1.3±0.00^{b,c}	1.4±0.00^{b}	0.6±0.61 ^{d}	1.0±0.06^{c,d}	0.9±0.03^{d}	1.5±0.22^{b}
Nitrogen-free extract	49.2±0.26^{a}	45.0±2.26^{b}	41.3±0.24^{cd}	36.4±1.58^{f}	39.2±0.79^{ef}	49.0±0.72^{a}	41.8±0.39^{c,d}	43.6±0.75^{b,c}
Gross energy (kJ/g)	18.3±0.2^{b}	18.2±0.1^{c}	18.2±0.1^{c}	17.9±0.0^{d}	18.2±0.0^{cd}	17.8±0.1^{e}	18.2±0.1^{c}	18.4±0.0^{a}

E1:33CP=Experimental diet 1, **E2:33CP**=Experimental diet 2, **D1:37CP**=Commercial diet 1, **D2:38CP**=Commercial diet 2, **D3:39CP**=Commercial diet 3, **D4:34CP**=Commercial diet 4, **D5:35CP**=Commercial diet 5, **D6:37CP**=Commercial diet 6

1. Values are means of three determinations ±SD. Values within the same row with different superscripts are significantly different ($p<0.05$).

again. Finally, the shrimp were acclimated to 50ups for the final collection of fecal material in the same quantity. Shrimp were fed to apparent satiation twice daily (09:00 and 17:00hr). Total daily feed was initially set at 5% total shrimp biomass in each experimental tank. Molted cuticles, dead shrimp, and unconsumed feed were removed daily. Shrimp that died during each bioassay were replaced with specimens from the same batch and weight, which was kept in a reserve 500L fiberglass tanks. Replacement specimens had undergone the

same treatment of salinity and corresponding feed. At the end of the 70-day experimental period, shrimp were weighed on a digital balance (Ohaus Scale. Florham Park, NJ, USA).

***In vivo* digestibility:** Shrimp were fed *ad libitum* three times daily for seven days to acclimate to the experimental diets containing chromic oxide before starting collection of feces in each feeding trial (5, 35 and 50psu). Unconsumed feed, molts, overnight feces and dead shrimp were removed daily. After the seven-day acclimation, as described in Velurtas *et al*. (2011), feces were collected twice daily at 90min after each feeding by siphoning fecal strands with a Pasteur pipette. Feces were gently rinsed with distilled water, transferred to 30mL conic tubes, and frozen at -20°C. When feces from all tanks were collected after the first feeding, a second round of collecting feces was done. One hour after the second feeding, feces were collected again. Pooled samples of frozen fecal material from each day and each tank were freeze-dried, then ground and thoroughly mixed, and kept frozen at -80°C until analysis. To maintain the same density, dead shrimp were replaced by similar-sized shrimp. Apparent digestibility coefficients (ADC) for carbohydrates and lipids were determined by methods described by Cho & Slinger (1979), using the equation: ADC of nutrients (%)=100-100 [(%Cr_2O_3 in feed)/(%Cr_2O_3 in feces)×(%nutrient in feces)/(%nutrient in feed)].

Chemical analysis and determination of leaching: Experimental diets were finely ground and sieved, then analyzed in triplicate for dry matter (AOAC 934.01), crude protein (AOAC 984.13, %N×6.25) Kjeldahl nitrogen equipment (Foss, Hillerød, Denmark). Crude lipids (AOAC 920.39) were determined by the ether-extraction method (Soxtec; Foss) and crude fiber (AOAC 962.09, Fibertec; Foss). Ash (AOAC 942.05) and nitrogen-free extract were determined according to standard methods (AOAC 1995). Gross energy was determined with an adiabatic calorimeter (Parr Instrument, Moline, IL, USA). A leaching trial was conducted for experimental and commercial diets to determine the amount of retained dry matter, using the method of Obaldo *et al*. (2002). Approximately 2g feed was placed in 250mL Erlenmeyer flasks with 200mL water for one hour at 5, 35 and 50psu. The flasks were gently swirled to disperse and submerge the feed pellets on a platform shaker at 100rpm at room temperature (~27°C) for one hour. The residual contents were collected on Whatman No. 3 filter paper and dried in an oven at 105°C for 24hr. Dry matter retention (DMR) was calculated as: DMR(%)=(dw residual feed after immersion/dw initial feed)×100. Diets and feces samples were lyophilized and analyzed for total lipids (Bligh & Dyer 1959) and carbohydrates with the Anthrone method (Dreywood 1946). Concentration of chromic oxide (Cr_2O_3) in diets and feces was determined by digesting the organic matter with nitric acid, oxidizing Cr_2O_3 to Cr_2O_7 with perchloric acid, followed by colorimetric analysis of the dichromate ion with diphenylcarbazide (Furukawa & Tsukahara 1966).

A two-way ANOVA was used to determine the effect of salinity (at 5, 35 and 50psu) and treatments (diets) on the digestibility coefficients. Significance was set at $p<0.05$. In the presence of significant interactions, one-way ANOVA of single factors was used. Tukey's multiple-range test was used to identify significant differences among digestibility coefficients ($p<0.05$) (Zar 1999). Data were analyzed with software (Statistica7.0, STATSOFT, Tulsa, OK, USA).

RESULTS

Water quality: There were no significant differences among treatments for water temperature (27.5±0.9°C) and dissolved oxygen (5.4±0.4mg/L). Nitrites and nitrate showed higher concentrations at 35psu, compared to 5psu (0.26 and 400µm/L, respectively) and 50psu (<100 and 11.5µm/L, respectively) (Table 3).

Diet stability: Stability of dry matter in water for all diets was >86% (Table 4).

TABLE 3
Physicochemical parameters of water quality on *Litopenaeus vannamei* cultivated at salinities of 5, 35 and 50psu

Sample	Temperature	Oxygen	Nitrites	Nitrates	Ammonium	Ortophos-phates	Total phosphorus	Total hardness	Alkalinity
	(°C)	(mg/L)	(µM/L)	(µM/L)	(µM/L)	(µM/L)	(µM/L)	(mg/L $CaCO_3$)	(mg/L $CaCO_3$)
5psu	27.5±0.9	5.0±0.78	0.26±0.2	400.0±9.0	5.21±0.3	0.24±0.0	0.23±0.0	1 029±129.5	241.0±9.5
35psu	27.5±0.9	5.0±0.78	0.14±0.2	12.8±0.9	0.50±0.0	0.58±0.0	0.68±0.1	6 893±117.5	137.9±6.9
50psu	27.5±0.9	5.0±0.78	0.10±0.0	11.5±1.5	1.66±0.3	0.32±0.0	0.60±0.1	6 993±107.5	138.8±5.4

µM=Micromoles.

TABLE 4
Hydrostability of dry matter (%)[1] in two experimental (E1:33CP and E2:33CP) and six commercial (D1:37CP, D2:38CP, D3:39CP, D4:34CP, D5:35CP and D6:37CP) diets evaluated in *Litopenaeus vannamei* cultivated at different salinities

Diet	Salinity (psu)					
	5		35		50	
E1:33CP	94.8	$\pm0.3^{a}$	99.6	$\pm0.6^{a}$	99.9	$\pm0.1^{a}$
E2:33CP	91.5	$\pm0.3^{b}$	94.7	$\pm2.7^{b}$	99.0	$\pm1.5^{ab}$
D1:37CP	89.5	$\pm0.4^{bc}$	97.5	$\pm2.6^{ab}$	97.7	$\pm0.6^{abc}$
D2:38CP	86.8	$\pm1.5^{d}$	96.7	$\pm2.3^{ab}$	96.8	$\pm0.5^{bc}$
D3:39CP	88.4	$\pm1.0^{cd}$	96.5	$\pm1.7^{ab}$	97.7	$\pm1.1^{abc}$
D4:34CP	93.5	$\pm0.8^{a}$	99.0	$\pm1.5^{ab}$	99.1	$\pm0.8^{ab}$
D5:35CP	88.0	$\pm0.8^{cd}$	92.0	$\pm2.8^{b}$	93.2	$\pm2.1^{c}$
D6:37CP	91.4	$\pm0.7^{b}$	96.4	$\pm3.4^{ab}$	99.0	$\pm1.8^{ab}$
Mean	90		97		98	

1. Values are means of three determinations ± SD. Values within the same column with different superscripts are significantly different ($p<0.05$).

Significant differences occurred among salinities, with greater leaching at 5psu for all diets, and lower at 50psu. In general, the most stable pellets were diets E1:33CP and D4:34CP, and the least stable were D2:38CP and D5:35CP.

***In vivo* digestibility of lipids and carbohydrates:** Apparent digestibility coefficients (ADC) of lipids and carbohydrates for all diets and at all salinities are presented in tables 5 and 6. Lipid coefficients were >80%, except for Diet E1:33CP (74%) at 50psu. Significant differences among diets were observed. The highest values of lipid ADC were in Diet D3:39CP (90.1% at 5psu salinity and 90.6% at 35psu).The lowest value obtained was for Diet E1:33CP (74% at 50psu salinity). No differences were detected among salinities.

For carbohydrate ADC, some significant differences were found among diets at three salinities. The highest coefficient at 5psu was for Diet D4:34CP (92.4%), while E2:33CP had 97.3% at 35psu and D1: 37CP had 94.9% at 50psu. From statistical analysis, salinity was not responsible for different carbohydrate ADC ($F=0.3$, $p>0.05$, d.f.=2, $p=0.733$) or lipids ($F=1.6$, $p>0.05$, d.f.=2, $p=0.209$). No effects of salinity or an interaction between lipids and carbohydrates were detected at the end of the three trials (5, 35 and 50psu).

Shrimp growth: Mean weight gain of juveniles in treatments E1:33CP and E2:33CP, were 11.9 and 9.6g, respectively; in treatments D1:37CP, D2:38CP, D3:39CP, D4:34CP, D5:35CP and D6:37CP, weight gain

TABLE 5
Apparent lipid digestibility coefficients (%)[1] in two experimental (E1:33CP and E2:33CP) and six commercial (D1:37CP, D2:38CP, D3:39CP, D4:34CP, D5:35CP and D6:37CP) diets for juvenile Whiteleg shrimp *Litopenaeus vannamei* cultivated at three different salinities

Diet	Salinity (psu)					
	5		35		50	
E1:33CP	83.5	±2.7^{a}	80.5	±4.4^{b}	74.0	±3.2^{b}
E2:33CP	86.8	±1.8^{ab}	87.2	±2.5^{ab}	88.8	±2.9^{a}
D1:37CP	81.0	±4.4^{b}	82.6	±4.7^{b}	85.2	±1.6^{ab}
D2:38CP	87.3	±1.4^{ab}	86.9	±1.0^{ab}	87.8	±1.2^{a}
D3:39CP	90.1	±0.9^{a}	90.6	±0.7^{a}	86.1	±3.1^{a}
D4:34CP	85.2	±3.7^{ab}	86.5	±3.4^{ab}	86.2	±0.8^{a}
D5:35CP	83.1	±1.2^{b}	85.7	±0.8^{ab}	86.2	±2.3^{a}
D6:37CP	86.6	±1.8^{ab}	86.0	±1.4^{ab}	80.7	±1.8^{ab}
Mean	85.5		85.8		84.4	
Two-way ANOVA						
	DF		F		p	
Diet	7		11.4		0.000	
Salinity	2		1.6		0.209	
Interaction	14		2.5		0.006	

1. Values are means ± SD of three tanks of shrimp per dietary treatment. Values within the same column with different superscripts are significantly different ($p<0.05$).

TABLE 6
Apparent carbohydrate digestibility coefficients (%)[1] of two experimental (E1:33CP and E2:33CP) and six commercial (D1:37CP, D2:38CP, D3:39CP, D4:34CP, D5:35CP and D6:37CP) diets for juvenile *Litopenaeus vannamei* cultivated at three salinities

Diet	Salinity (psu)					
	5		35		50	
E1:33CP	87.9	±0.7^{bcd}	91.0	±2.2^{bc}	83.3	±1.9^{bc}
E2:33CP	86.4	±2.1^{cd}	97.3	±1.6^{a}	94.7	±0.7^{a}
D1:37CP	89.6	±0.9^{abc}	84.6	±2.1^{cd}	94.9	±0.4^{a}
D2:38CP	87.2	±2.5^{bcd}	75.9	±3.5^{d}	89.0	±0.7^{ab}
D3:39CP	90.5	±0.8^{ab}	86.3	±5.1^{bcd}	85.9	±5.2^{bc}
D4:34CP	92.4	±1.6^{a}	93.7	±1.0^{ab}	90.5	±4.2^{ab}
D5:35CP	86.3	±2.8^{cd}	89.6	±1.0^{bc}	85.0	±2.0^{bc}
D6:37CP	84.9	±0.6^{d}	87.3	±3.1^{bc}	77.4	±2.8^{c}
Mean	88.2		88.2		87.6	
Two-way ANOVA						
	DF		F		P	
Diet	7		12.5		0.000	
Salinity	2		0.3		0.733	
Interaction	14		8.4		0.000	

1. Values are means ± SD of three tanks of shrimp per dietary treatment. Values within the same column with different superscripts are significantly different ($p<0.05$).

obtained was 8.4, 10.9, 11.8, 8.9, 11.4 and 10.2g, respectively.

DISCUSSION

The effect of water quality on feed digestibility and ingestion had not been previously determined for crustaceans, but it does not appear to have a significant effect, unless the quality of the feed is nutritionally limiting (Lee & Lawrence 1997). Lawrence *et al.* (2001) reported that working with limited water exchange, Pacific whiteleg shrimp could tolerate high level of ammonia nitrogen (>25mg/L) and nitrite (>26mg/L) without compromising survival or health. The values of physicochemical parameters of water quality were within acceptable limits for indoor production of shrimp (Martínez-Córdova *et al*. 2009, Campaña-Torres *et al*. 2010).

Commercial shrimp feeds are commonly reported to include fish meal at levels between 25% and 50% (Dersjant-Li 2002). Significant decrease in apparent digestibility of crude lipid (E1: 74% at 50psu salinity) may be caused by the high amount (3%) of crude fiber in this diet, significantly greater than other diets. However, recent studies have shown that commercial shrimp feeds containing 30–35% crude protein can include as little as 7.5-12.5% fish meal, without compromising shrimp performance (Fox *et al*. 2004). Feed type and salinity can significantly affect dry matter retention of shrimp feeds (Obaldo *et al*. 2002, Cruz-Suárez *et al*. 2006). Our analyses confirmed significant differences in hydrostability among diets and salinities. The best results occurred at higher salinities; Diet E1was more hydrostable than the others. High hydrostability does not necessarily indicate high performance. On the contrary, it may indicate greater hardness and difficulty to digest (Samocha *et al.* 2001, Cerecer-Cota *et al*. 2005), and leaching of crude protein and lipid in the feed exhibited a reduced trend with length of time immersed in water (Carvalho & Nunes 2006). In our study, Diet E1 had the highest hydrostability and the lowest digestibility of lipids and carbohydrates.

Apparent digestibility coefficients (ADC) of feeds depend on their chemical composition and digestive characteristics of the target species, as well as environmental conditions (Brunson et al. 1997). Salinity has a significant effect on metabolism of penaeid shrimp (Rosas *et al*. 2001, Zhu *et al*. 2006, Valdez *et al*. 2008). As salinity deviates from the iso-osmotic point, the osmotic balance in shrimp is modified and it expends more energy on osmoregulation and less on growth (Hurtado *et al*. 2006). Shrimp are excellent osmoregulators if ionic ratios in the water are adequate (Gong *et al*. 2004b, Roy *et al*. 2007). Juvenile *L. vannamei* show hyper-osmotic regulation at low salinity and exhibit hypo-osmotic regulation at high salinity with an isomotic point of 718mOsm, which is equivalent to 25psu (Do *et al*. 2010). These results indicate that *L. vannamei* possesses the ability to osmoregulate in seawater and brackish water (low salinities).

High dry matter retention indicates that ingredients used for formulation and methods of preparing diets were satisfactory. Our results show that salinity has a significant effect on retention of dry matter. Leaching was significantly higher at low salinity. At the highest salinity, concentrations of ions Cl^-, HCO_3^- and SO_4^{2} (APHA 1980) are higher, which retards rapid leaching of feed.

The ability to use carbohydrates as an energy source varies widely among fish, shrimp, and terrestrial animals. Fish and shrimp use carbohydrates less efficiently than other animals (Guo *et al*. 2006). A negative physiological effect results from glucose saturation and high digestive system saturation (Shiau 1998). We found some differences in the apparent digestibility coefficient of carbohydrates in commercial and experimental diets at the three levels of salinity. The effect of salinity on carbohydrate ADC was not significant. In penaeids, several digestive carbohydrases have been identified (*vgr*. α-amylase, α-glucosidase, α-maltase, α-saccharase, galactosidase, chitinase and chitobiase), which suggests an ability to digest a wide range of carbohydrate sources (Ceccaldi 1997).

In general, carbohydrate ADC coefficients were similar or higher than other reports, which is probably related to the preparation of the diets. When hot water is used for pellet production, as in our study, carbohydrates are gelatinized, which improves their digestibility (Mohapatra *et al*. 2003, Campaña-Torres *et al*. 2006). Results from our study suggest that an increase in gelatinization of starch during manufacture of commercial diets is expected to have a positive effect on digestibility (Thomas *et al*. 1998).

The lipid ALD was not significantly affected by salinity. Moreover, significant differences were present between feeds at every salinity level. In the bioassays, lipid digestibility coefficients were higher than those reported by Merican & Shim (1995), Ramos *et al*. (2001) and Lin *et al*. (2006) concluded that the method of preparing feed affects lipids and certain fatty acids ADCs due to autooxidation. Digestibility of feed can be caused by numerous factors, including the presence of enzyme inhibitors in the diet, inappropriate diet formulation, and proteins that are chemically or physically undigestable (Oujifard *et al*. 2012).

Mean weight gain of juveniles in treatments E2:33CP, D3:39CP, and D5:35CP was superior to other treatments. They contain a low level of crude fiber with a lipid level between 7and 9%. It is possible that the ingredients of these treatmentshave excellent nutritional value with high palatability.

Acclimation to changes in salinity induces modifications in the activity of processes directly related to ion transport mechanisms, but also of processes related to lipid metabolism (Pequeux 1995, Gong *et al*. 2004b). Li *et al*. (2008) conclude that greater activity of digestive enzymes in shrimp occurs at high salinities and an increase of B cells facilitates synthesis and excretion of digestive enzymes; this enables *L. vannamei* to derive more energy from its food. Additional studies of digestive enzymes would help our understanding of digestive physiology and nutritional requirements of whiteleg shrimp. Under our experimental conditions, no significant effect of salinity on digestibility of lipids and carbohydrates was found, nor an interaction between salinity and shrimp diet. The lack of differences observed between treatments suggests effective carbohydrate and lipid digestibility in *L. vannamei* that are fed commercial and experimental feeds under a range of saline conditions. Further studies should focus on the digestibility of fatty acids and amino acids in the diets.

ACKNOWLEDGMENTS

We thank Sonia Rocha, Dolores Rondero, Sandra de La Paz, Ernesto Goytortúa and Carlos Ceseña for their assistance and dedication to complete this study. Ira Fogel of CIBNOR provided editing services. Shrimp were obtained from Acuicultores de la Península de La Paz, B.C.S., México. Funding was provided by SAGARPA-CONACYT project 2003-C02-149 and CIBNOR grant AC0.8. The Secretaría de Relaciones Exteriores provided a student fellowship (811.5(6193)/137) to M.G.

RESUMEN

El camarón blanco, *Litopenaeus vannamei*, es una de las especies más cultivadas comercialmente en el mundo, debido a su velocidad de crecimiento y tasa de supervivencia en altas densidades de cultivo, y su capacidad de osmoregulación, que lo hacen un excelente candidato para cultivo en diferente salinidades. El conocimiento de los requerimientos nutricionales del camarón es fundamental en la formulación de dietas que permita el crecimiento óptimo en diferentes condiciones ambientales y fases de desarrollo del animal. El efecto de la salinidad sobre la digestibilidad aparente de alimentos comerciales para camarones no está documentado. Esta información es necesaria en el cultivo de camarón para la formulación de los alimentos comerciales. Se determinó la digestibilidad aparente de los carbohidratos (ACD) y lípidos (ALD), en juveniles del camarón blanco *L. vannamei* cultivado en condiciones controladas. Los tratamientos fueron seis dietas comerciales (D1:37CP, D2:38CP, D3:39CP, D4:34CP, D5:35CP y D6:37CP) y dos dietas experimentales (E1:33CP y E2:33CP) para juveniles de camarón blanco en cultivo, a tres salinidades (5, 35 y 50ups) en acuarios de 60L. Los coeficientes de digestibilidad aparente de los carbohidratos (ACD) y los lípidos (ALD) fueron determinados *in vivo* utilizando óxido crómico como marcador inerte en la dieta. ALD en la mayoría de los tratamientos fue superior al 80%, independientemente de la salinidad, con excepción

de la dieta E1:33CP que presentó 74.0% a salinidad de 50ups. La dieta D3:39CP presentó el mayor coeficiente ALD (90.1 y 90.6 en 5 y 35ups, respectivamente). En ACD, se presentaron diferencias entre dietas comerciales y experimentales en cada nivel de salinidad, aunque la salinidad no presentó un efecto significativo. La dieta D4:34CP presentó el coeficiente de digestibilidad más alto (92.4%) en la salinidad de 5ups, mientras que E2:33CP en salinidades de 35 y 50ups fue de 97.3 y 94.7%, respectivamente. En general, el presente estudio demuestra que en juveniles de camarón blanco la variación de salinidad no afecta significativamente la digestibilidad de lípidos y carbohidratos en las condiciones experimentales de este estudio.

Palabras clave: nutrición, alimento comercial, salinidad, camarón blanco.

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