



Performance of *Litopenaeus vannamei* at different levels of daily protein intake and carbohydrate/protein ratios in low fishmeal content feeds

Rendimiento de *Litopenaeus vannamei* a diferentes niveles de ingesta diaria de proteína y proporciones carbohidrato/proteína en alimentos formulados con baja inclusión de harina de pescado

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ABSTRACT

The increase in shrimp aquaculture production relates to the development of increasingly efficient formulated feeds and feed management strategies. Protein, considered the most important ingredient in feeds, represent the highest economic and environmental cost. Therefore, determining the appropriate amount of protein is essential to maintain a balance between performance, cost, and environment in aquaculture systems. The objective of this study was to determine the mathematical functions of Daily Protein Intake (DPI), to evaluate feeds with different Carbohydrate/ Crude protein (CBH:CP) ratio and low inclusion of fishmeal for *L. vannamei* juveniles culture. The adjusted mathematical function that best represented aquaculture feeding practices was $DPI=44.7(BW)^{-0.714}$. In the first experiment, four variants of DPI (80, 100, 120 and 140 %) at a constant CBH:CP ratio was carried out, finding the best performance at 120 %. In the second experiment, diets with five levels of CBH:CP (g CBH: g crude protein; 2.1, 1.5, 1.1, 0.8 and 0.6) with constant Lip:CP were evaluated using the same DPI function. The CBH:CP levels variation had a significant effect on growth parameters, feed conversion rate, nitrogen retention and protein, determining that feed with 2.1 CBH:CP ratio presents the best results when using $DPI=53.64(BW)^{-0.714}$.

Keywords: Daily protein intake, nitrogen retention, *L. vannamei* performance, carbohydrates:protein rates.

RESUMEN

El aumento de la producción acuícola de camarón se ha logrado gracias al desarrollo de alimentos balanceados y estrategias de alimentación más eficientes. La proteína es considerada el insumo más importante para el desarrollo de los organismos, y representa el mayor costo económico y ambiental. El objetivo de este estudio fue determinar las funciones matemáticas de ingesta diaria de proteína (IDP), con objeto de utilizarlas para evaluar dietas con diferentes proporciones de carbohidratos/proteína cruda (CHO:CP) y baja inclusión de harina de pescado en juveniles de *L. vannamei*. El modelo matemático que representó de mejor manera las prácticas de alimentación acuícolas evaluadas fue $IDP=44.7(PC)^{-0.714}$. En el primer experimento se utilizaron

cuatro niveles de variación de IDP (80, 100, 120 y 140 %) a una proporción CHO:CP constante, encontrando el mejor rendimiento con IDP 120 %. En el segundo experimento; se evaluaron dietas con cinco niveles de CHO:CP (g CHO:g proteína cruda; 2.1, 1.5, 1.1, 0.8 y 0.6) usando la mejor función de IDP. La variación de los niveles de CHO:CP tuvieron un efecto significativo en crecimiento, el factor de conversión alimenticia, retención de nitrógeno y eficiencia proteica, determinando que la dieta con CHO:CP de 2.1 presentó mejores resultados con $IDP=53.64(PC)^{-0.714}$.

Palabras clave: Ingesta diaria de proteína, retención de nitrógeno, desempeño de *L. vannamei*, proporción carbohidrato: proteína.

INTRODUCTION

Shrimp aquaculture production has shown an accelerated increase in the last two decades, reaching 4.966 million tons in 2018 for *L. vannamei*, corresponding to 54 % of crustacean aquaculture (FAO, 2020). These production levels have been possible thanks to the culture intensification, where the use of formulated feeds and feed management has been a fundamental strategy (Wasielesky *et al.*, 2006). Shrimp feed quality include protein, fatty acids, carbohydrates, vitamins, minerals, and additives (Wang *et al.*, 2015). Protein is one of the most important nutrients required for growth (Hu *et al.*, 2008) and accounts the major economic and environmental cost in diets formulation, favoring the increase of nitrogen residuals to the environment (Tacon and Akiyama, 1997; Lee and Lee, 2018; Gil-Nuñez *et al.*, 2020). The proper use of protein favors rapid growth (Guroy *et al.*, 2012) and water quality in aquaculture systems (Limsuwan and Junratchakoo, 2004). A minimum dietary protein level is required to supply amino acids for normal maintenance of metabolism (Lee and Lee, 2018). Several studies have evaluated the adequate protein requirements in terms of protein levels in feeds, finding the best yields feeding with 36 % (Yaemssoksawat *et al.*, 2008) and 40 % (Liu *et al.*, 2005). Kureshy and Davis (2002) determined the daily protein intake (g DP kg⁻¹ BW d⁻¹) for maintenance and maximum growth for *L. vannamei* juveniles at different body weights (g) and protein retentions. In addition

to protein, the development of aquaculture systems requires a precise evaluation for the rest of the energy substrates.

The protein in the diet can be reduced if the energy required comes from another source such as carbohydrates or lipids, which can reduce the emission of residual nutrients and feed costs (Zainuddin *et al.*, 2014). Shrimp require lipids in their diet to perform a series of metabolic functions, where the recommended lipid levels in the diet range between 6 and 10 %, with negative effects on growth with values greater than 10 % (González-Félix *et al.*, 2002; Xu *et al.*, 2017; Jannalhulla *et al.*, 2019). Zhang *et al.* (2013) determined that the effect of lipids could vary depending on the protein, reporting highest growth rates at high inclusion percentages in diets with 34 % protein. Despite the low digestibility of carbohydrates by the shrimp (Shiau, 1997), carbohydrates are often used in formulated feeds as an energy source through the protein-sparing mechanism (Wang *et al.*, 2015), where feeding with frequency of four times per day, diets carbohydrate-rich presents a good performance (Zainuddin *et al.*, 2014).

The importance of protein amount provided is clear in aquaculture, also related to other different nutrients in the feeding of shrimp. On the other hand, there is a lack of studies related to the growth of *L. vannamei* with fixed amounts of protein anchored to different ratios of other nutrients. Thus, the aim of this study was to determine the daily protein intake (DPI) mathematical functions related to body weight, in order to use in experiments to evaluate carbohydrate:crude protein ratios in feeds with low inclusion of fishmeal that present the best performance in terms of growth, protein efficiency and nitrogen retention. The use of mathematical functions for the supply of protein will favor the use of the appropriate amount of this, reducing economic and environmental costs while maintaining good performance, allowing evaluate feed formulas and variation of other energetic substrates such as carbohydrates.

MATERIAL AND METHODS

Shrimp feeding tables

Different shrimp feeding tables reported for *L. vannamei* aquaculture in several countries or recommended by relevant feed companies were reviewed. Feeding tables used in this study were based on biomass percentages (%Biomass) for different shrimp Body Weights (g).

DPI mathematical model

Daily protein intake (g protein/kg biomass/day) mathematical function related to *L. vannamei* Body Weight (BW) in grams, was adjusted in this study from experimental data reported by Kureshy and Davis (2002), using selected DPI data for different BWs where Protein Retention (%) was similar (37.2 % in average).

Data from different feeding tables reviewed in this study were transformed to Daily Protein Intake DPI (g protein /Kg Biomass/day) related to individual BW and were analyzed to determine the best fit for mathematical functions between

both variables, using the same mathematical model mentioned previously. The best fit for DPI mathematical function for all the data reported for *L. vannamei* in an explicit or implicit manner (through feeding tables) was $DPI=a(BW)^b$, where DPI is expressed in g protein/Kg biomass/day and BW in grams.

The mathematical function adjusted from data reported by Kureshy and Davis (1992) was considered as 100 % DPI, and four functions were developed as a variation in 20 % between them (80, 100, 120 and 140 %) to cover the explicit or implicit DPI data reviewed for *L. vannamei* aquaculture at different body weights. These mathematical functions were evaluated experimentally at different levels of DPI and same BW respect to growth, protein efficiency and nitrogen retention using feeds with fixed carbohydrate: crude protein (CBH:CP) and lipid:crude protein (Lip:CP) ratio.

First trial: DPI mathematical functions related to 80, 100, 120, 140 % DPI levels

The experiment was carried out in the experimental nutrition laboratory at the Centro de Investigaciones Biológicas del Noroeste S.C. (La Paz, Baja California Sur, México). *L. vannamei* juveniles (1 ± 0.5 g) from Aquacultura Mahr local company, kept under laboratory conditions for one week, were randomly distributed in three experimental units (60 L fish tanks) (n=12) for each DPI level. Shrimp were reared under controlled conditions of salinity 37 ± 0.5 psu (practical salinity units), dissolved oxygen (6 ± 1.0 mg O₂ L⁻¹), temperature (28 ± 1 °C) and photoperiod (12:12h light:dark).

Shrimp were fed with a commercial diet (40 % protein and fixed CBH:CP ratio) in four daily rations (9:00, 12:00, 15:00 and 18:00 h) to decrease the amount of feed not eaten according to four DPI levels (80, 100, 120 and 140 %) for 35 days. The DPI levels were adjusted weekly according to the shrimp body weight and biomass. The shrimp tanks were siphoned every day to remove uneaten food, with a weekly water exchange (80 %), to remove organic carbon, nitrogen and phosphorous residuals accumulated during the week. Also, the experimentally selected DPI mathematical function was used to evaluate five experimental low fishmeal feeds with different CBH:CP ratios using the same Lipid:Crude protein level and DPI mathematical function.

Formulated diets

For the second experiment, five diets were formulated with Nutrion® software, using a low inclusion of fish meal (6.5 %; table 1), yeast and soybean meal as protein, sardine oil for lipids and wheat flour as carbohydrate source, to obtain five CBH:CP (2.1, 1.5, 1.1, 0.8 and 0.6) ratios with the same Lip:CP ratio (table 1). The ingredients were ground in an ultrafine sprayer set at 250 µm and mixed with water and a fermented mix of marine yeasts and lactobacilli selection. Once extruded and dried, the diets were subjected to two steps of organic acid and fish oil baths. Table 2 shows the proximal composition of the diets.

Table 1. Diets composition formulated with five different carbohydrate:protein (crude protein) (CBH:CP) ratios with same lipid:protein (Lip:CP) ratio for *L. vannamei*. Ingredients are expressed in dry weight (%).

Tabla 1. Composición de dietas formuladas con cinco proporciones diferentes carbohidrato:proteína (proteína cruda) (CHO:CP) con la misma proporción lipido:proteína (Lip:CP) para *L. vannamei*. Los ingredientes se expresan en base seca.

Diet	1	2	3	4	5
CBH:CP	2.1	1.5	1.1	0.8	0.6
Lip:CP	0.2	0.2	0.2	0.2	0.2
Wheat meal	51.4	37.4	23.6	16.6	8.4
SPC ¹	0	0	10	32.4	49.1
Soybean meal	21.9	36	36.8	20.4	11.5
Fishmeal	6.5	6.5	6.5	6.5	6.5
Fish oil	0.7	1	1.5	1.8	2.2
Yeast ²	4	4	4.5	4.5	4.5
PM vit ³	2.6	2.6	2.6	2.6	2.6
PM min ⁴	6.3	6.1	5.8	5.9	5.7
Soybean lecithin	2.4	3.1	3.8	4.4	5
Aminoacids mix ⁵	0.4	0.14	0.3	0.3	0.5
Alginate acid	2	2	2	2	2
Probiotic Mix ⁶	1	1	1	1	1
Cellulose	0.8	0.16	1.6	1.6	1
	100	100	100	100	100

1.- Soybean protein concentrate, 2.- *Saccharomyces cerevisiae*, 3.- Choline, DL-alpha-tocopherol, retinol, butylated hydroxytoluene, astaxanthin, ascorbic acid, biotin, inositol, folic acid, cholecalciferol, menadione, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, cobalamin, carnitine. 4.- NaH₂PO₄, CaCO₃, KH₂PO₄, MgSO₄-7H₂O, ZnSO₄-7H₂O, CuSO₄-7H₂O, CoCl₂, Na₂SeO₃, KI. 5.- Methionine, arginine, tryptophane. 6.- Formic, propionic, acetic and citric acid.

Second trial: Different levels of CBH:CP ratio using same DPI model

L. vannamei juveniles (2.5 ± 0.5 g) were randomly distributed in 15 experimental tanks with 60 L (12 organisms per aquarium) by triplicate. The feeding was based on the DPI mathematical function selected in the first trial. The shrimp tanks were daily siphoned to remove uneaten feed, with a weakly water exchange (40 %) and environmental conditions like the experiment mentioned above.

Statistical analysis

The following zootechnical parameters were obtained in both experiments: Weight gain (%) (WG) = ((final weight (g) – initial weight (g))*100)/ initial weight (g); Specific growth rate (%) (SGR) = 100 * (ln final weight (g) – ln initial weight (g))/days of experiment; Survival rate (%) = ((shrimp initial number – dead shrimp number)/shrimp initial number) × 100; Feed conversion ratio (FCR) = feed intake (dry matter) (g)/weight gain (g); Protein efficiency ratio (PER) = wet weight gain (g) / dry protein intake (g).

Homoscedasticity of variances and normality of data were verified by Bartlett and Shapiro Wilk's test. The effect of DPI in the first experiment and dietary CBH:CP in the second one on zootechnical parameters was analyzed separately using one way ANOVA. Differences were considered significant at $p < 0.05$ and were indicated to the end of the tables. Differences between means for each group (individual means) were determined using a post hoc Tukey's HSD test.

RESULTS

Feeding tables review

In all the shrimp feeding tables reviewed the daily ration based on biomass (%) diminish as the Body Weight increase. A greater variability in DPI was found in the feeding ratio based on Biomass percentage for shrimps with BW lower than 5 grams in all analyzed cases; from BW 5 to 12 grams most cases show similar DPI levels. Feeding tables reported for México by Quintero and Roy (2010) and for Honduras by Teichert-Coddington and Rodriguez (1995) show greater feeding ratios from 2 to 18 grams compared to other feeding tables (figure 1).

Table 2. Proximate chemical analysis for formulated diets with five different carbohydrate:protein (crude protein) (CBH:CP) and same lipid:protein (Lip:CP) ratio for *L. vannamei*.

Tabla 2. Análisis químico proximal de dietas formuladas con cinco proporciones diferentes de carbohidrato:proteína (proteína cruda) (CHO:CP) con la misma proporción lipido:proteína (Lip:CP) para *L. vannamei*.

Diet	CBH:CP (g/g)	Lip:CP (g/g)	Moisture (%)	Crude protein (%)	Crude lipids (%)	Crude fiber (%)	Ash (%)	NFE (%)	Energy (cal/g)
0	1.07	0.2	6.4±0.1	40.4±0.2	8.1±0.1	1.0±0.1	7.2±0.04	43.3	4809.6±3.1
1	2.1	0.2	8.8±0.10	26.5±0.24	5.1±0.03	0.4±0.10	10.0±0.01	57.9	4091±0.61
2	1.5	0.2	11.5±0.1	32.7±0.1	6.3±0.1	0.6±0.00	10.8±0.1	49.6	4276.8±3.9
3	1.1	0.2	9.1±0.1	37.9±0.1	7.5±0.1	1.0±0.1	10.6±0.1	42.9	4398.1±6.1
4	0.8	0.2	11.2±0.1	42.2±0.1	8.5±0.03	1.8±0.0	10.34±0.1	37.1	4488.0±4.8
5	0.6	0.2	9.7±0.1	46.1±0.1	9.1±0.1	0.7±0.1	10.2±0.01	33.8	4608.1±1.2

Diet 0: Commercial Feed for First trial, diets 1-5: Experimental formulated feeds for second trial.

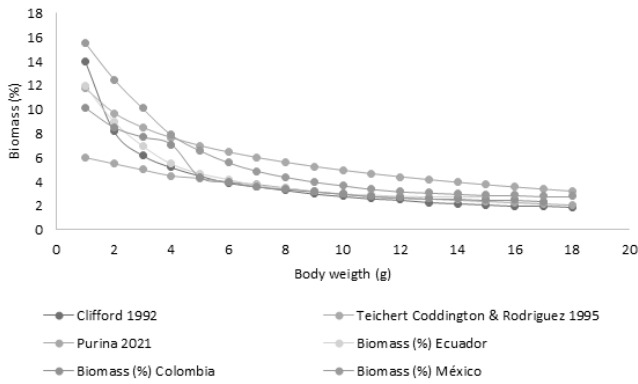


Figure 1. Feeding rates as % Biomass related to shrimp *L. vannamei* body weight (g) for different cases reported by Clifford (1992) and Teichert Coddington and Rodríguez (1995) for Honduras, Davis et al. (2006) para Nicovita, Quintero and Roy (2010) for Ecuador, Colombia and México, Purina 2021 https://www.nutrimentospurina.com/archivos_aplicaciones/files_fkceditor/file/Acuacultura/Guia_Alimentacion.pdf
Figura 1. Tasas de alimentación en porcentaje de biomasa en función del peso corporal (g) del camarón *L. vannamei* para diferentes casos analizados que incluyen datos reportados por; Clifford (1992) y Teichert Coddington and Rodríguez (1995) para Honduras, Davis et al (2006) para Nicovita, Quintero y Roy (2010) para Ecuador, Colombia y México, Purina 2021 https://www.nutrimentospurina.com/archivos_aplicaciones/files_fkceditor/file/Acuacultura/Guia_Alimentacion.pdf

DPI mathematical functions related to body weight (BW)

The DPI mathematical function developed in this study from experimental data reported by Kureshy and Davis (2002) with similar protein efficiency levels, was $DPI = 44.7 BW^{0.714}$ (Figure 2).

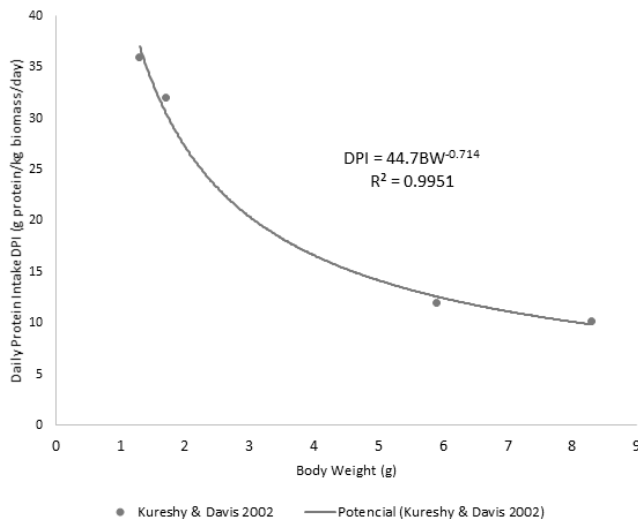


Figure 2. Daily protein intake (g prot/kg biomass/day) mathematical function ($DPI=44.7(BW)^{0.714}$) related to *L. vannamei* Body weight (BW) in grams, obtained in this study from experimental data reported by Kureshy and Davis (2002), using selected DPI data for different shrimp sizes only where protein retention (%) was similar (37.2 % in average).
Figura 2. Función matemática de la Ingesta Diaria de Proteína (g prot/ kg biomasa / día), $IDP=44.7PC^{0.714}$, en función del peso corporal (PC) en gramos, obtenida en este estudio a partir de datos experimentales reportados por Kureshy and Davis (2002), utilizando datos de IDP seleccionados para diferentes pesos corporales donde la retención de proteína (%) fue similar (37.2 % en promedio).

Figure 3 show the DPI mathematical functions adjusted from transformed data obtained from the different feeding tables, compared with the DPI mathematical function reported in figure 2.

DPI mathematical functions: $DPI = 65.58 BW^{0.714}$, $DPI = 53.64 BW^{0.714}$, $DPI = 44.7 BW^{0.714}$ and $DPI = 37.76 BW^{0.714}$, corresponding to 80, 100, 120 and 140 % DPI respectively, were $DPI = 44.7 BW^{0.714}$ for *L. vannamei* is considered 100 %, are presented in figure 4 and compared to the transformed DPI data obtained from feeding tables reviewed in this study.

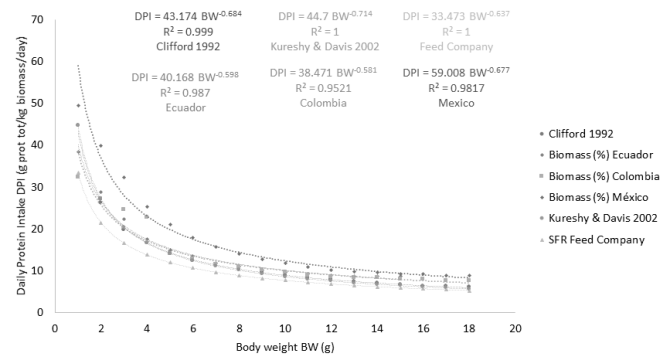


Figure 3. Daily protein intake (g prot/kg biomass/day) mathematical functions related to *L. vannamei* Body weight (g) adjusted from transformed data contained in different feeding tables based on %Biomass related to Body Weight (Clifford 1992, Davis et al., 2006 and Quintero and Roy 2010, for Ecuador, Colombia and México), compared with $DPI=44.7(BW)^{0.74}$.
Figure 3. Funciones matemáticas de la ingesta diaria de proteína (g prot/ kg biomasa/día) en función del peso corporal (g) de *L. vannamei* ajustadas a partir de datos transformados provenientes de diferentes tablas de alimentación de %Biomasa en relación con el Peso Corporal (Clifford 1992, Davis et al 2006 y Quintero and Roy 2010 para Ecuador, Colombia y México) comparadas con $DPI=44.7BW^{0.74}$.

First trial: Daily protein intake (DPI)

Significant differences were found in weight gain with different DPI functions (Table 3), with organisms fed 80 and 100 % DPI showing significantly less growth than those fed 120 and 140 %. At the end of the experiment, no significant differences were found between 120 and 140 %. We found significant differences in specific growth rate, average weekly growth, feed conversion rate, protein efficiency and nitrogen retention (Table 4). The organisms fed with 80 and 140 % DPI rates showed the lowest and highest values respectively in all cases. Three homogeneous groups were found in the

Table 3. *L. vannamei* Weight gain (g) under laboratory conditions fed with 80, 100, 120 and 140 % (crude protein) Daily Protein Intake (DPI).

Tabla 3. Ganancia en peso (g) de *L. vannamei* bajo condiciones de laboratorio alimentados con 80, 100, 120 y 140 % (proteína cruda) de ingesta diaria de proteína (IDP).

Time (Week)	80	100	120	140
1	0.62±0.11a	0.62±0.01a	0.71±0.04a	0.79±0.17a
2	1.24±0.07a	1.31±0.06ab	1.53±0.07b	1.73±0.18c
3	3.03±0.10a	3.08±0.06ab	3.39±0.15b	3.79±0.21c
4	3.77±0.19a	4.02±0.08a	4.58±0.21b	5.00±0.30b
5	3.84±0.16a	4.3±0.18a	5.01±0.28b	5.60±0.37b

Table 4. Performance of *L. vannamei* under laboratory conditions fed with 80, 100, 120 and 140 % (crude protein) Daily Protein Intake (DPI).**Tabla 4.** Desempeño de *L. vannamei* en condiciones de laboratorio alimentados con 80, 100, 120 y 140 % (proteína cruda) de Ingesta Diaria de Proteína (IDP).

	Daily Protein Intake (%DPI)			
	80	100	120	140
WG (%)	481.14±13.98a	526.34±6.87ab	602.55±34.10bc	636.81±61c
SGR (%)	3.14±0.06ab	3.32±0.03ab	3.59±0.11bc	3.7±0.20c
AWG (g)	0.76±0.03a	0.85±0.36a	1.00±0.5b	1.12±0.07b
S (%)	95.83±4.81a	88.8±4.81a	97.2±4.81a	94.4±4.81a
FCR	1.12±0.02a	1.26±0.03ab	1.32±0.07b	1.41±0.09b
PER	2.37±0.12a	2.09±0.1b	2.01±0.05b	1.89±0.05b
NR	40.45±0.90a	35.76±0.03b	34.38±1.92b	32.26±2.09b

WG=Weight gain, SGR=Specific growth rate, AWG=Average weekly growth, S=Survival rate, FCR=Feeding conversion ratio, PER=Protein efficiency ratio. C:N ratio of diet=7.98.

specific growth rate, showing significantly differences at 140 %. A higher feed conversion rate was found in treatments with 120 and 140 %. The protein efficiency ratio and nitrogen retention showed two homogeneous groups, where (120 %) DPI= 53.64 BW^{-0.714} was better in growth, PER and NR. No differences were found in survival rate due to DPI at any level.

Second trial: Bioassay with five CBH:CP ratios using same DPI level and Lip:CP ratio

We found a significant effect by CBH:CP ratio on weight gain, specific growth rate, feed conversion rate, protein efficiency ratio and nitrogen retention (Table 5). No significant differences were found in the survival rate. Significant differences were observed between treatments with 2.1 and 1.5 compared to the rest CBH:CP ratios. The specific growth rate also presented significant differences between treatments, with higher values in 2.1 and 1.5 CBH:CP. The feed conversion rate and protein efficiency ratio showed the highest values at the highest levels of CBH:CP (2.1 and 1.5). The weekly average growth showed an effect by Carbohydrate:Crude protein rate, with higher values in 2.1, 1.5 and 1.1 CBH:CP. Nitrogen retention was statistically higher in 2.1 and lower

in 0.6 CBH:CP respectively. Survival rate was not affected by CBH:CP ratios.

DISCUSSION

The review of the feeding tables shows great variability in %Biomass for Shrimp BW lower than 5 grams (Figure 1), mainly due to the variability in the protein content (level or percentage) in feeds recommended for small shrimps. In this sense, feeding tables not necessarily represent the real amount of protein provided to the aquaculture system, since they are not related with the protein content in feeds. When different protein levels in feeds are tested (25-45 % CP), the use of feeding tables as a protocol to feed the shrimps, with same feeding ratio based on % Biomass and BW, implicitly provide different amounts of protein according to the feed protein level (%), generating differences not necessarily due to the protein level, but the real amount of protein provided for shrimps. Kureshy and Davis (2002), using the same approach, tested different amounts of protein (g protein/kg shrimp biomass/day) as DPI for different shrimp BW and found a growth rate increase related to DPI increase, but also found an increase in Protein Efficiency (PE) from lower to medium DPI, but a decrease in PE at greater amount of DPI. Therefore, the maximum growth at high DPI have consequences in lowering PE and Nitrogen retention (NR), with side effects on nitrogen residuals in the aquaculture environment.

For this reason, in this study, we select the DPI data provided by Kureshy and Davis (2002), for different BWs with the better Protein retention (37.2 % in average) to adjust the mathematical model $DPI=44.7(BW)^{-0.714}$ (Figure 2). Using this mathematical model, we adjust the transformed data provided by the feeding tables from different sources and found not only high correlation (>0.9501), but surprisingly a similar trend between the adjusted DPI model obtained in this study from data reported by Kureshy and Davis (2002), compared to the DPI adjusted from Clifford (1992) data, recommended by relevant shrimp feed companies like Nicovita and Purina (Figure 3). The four different variants in DPI mathematical functions ($DPI=37.76(BW)^{-0.714}$, $DPI=44.7(BW)^{-0.714}$, $DPI=53.64(BW)^{-0.714}$, and $DPI=65.58(BW)^{-0.714}$), respectively corresponding to 80, 100, 120 and 140 % $DPI=44.7(BW)^{-0.714}$

Table 5. Performance of *L. vannamei* cultured in laboratory with five experimental diets with different CBH:CP ratios and same Lip:CP ratio (crude protein).**Tabla 5.** Desempeño de *L. vannamei* cultivado en laboratorio con cinco dietas experimentales con cinco proporciones diferentes CHO:CP y una proporción Lip:CP (proteína cruda)

CBH:P	Lip:P	C:N	WG	SGR	AWG	S	FCR	PER	NR
(g/g)	(g/g)		(%)	(%.day ⁻¹)	(g)	(%)			
2.1	0.2	12.15	323.3±15.7a	2.3±0.10 a	1.27±0.12 a	86.5±5.8a	2.25±0.08 a	1.84±0.07 a	30.65±1.93 a
1.5	0.2	9.87	297.4±7.6ab	2.2±0.10ab	1.14±0.06ab	94.4±2.8a	2.16±0.04ab	1.60±0.03 b	25.87±0.78 b
1.1	0.2	8.51	267.0±1.5 b	2.0±0.10 b	1.14±0.19 b	89.5±6.7a	2.07±0.01ab	1.40±0.001bc	23.33±0.13bc
0.8	0.2	7.64	269.0±9.7 b	2.0±0.10 b	1.22±0.12ab	92.3±7.7a	1.95±0.08 b	1.37±0.06 c	22.28±1.67 c
0.6	0.2	7.00	285.3±5.2ab	2.1±0.03ab	1.04±0.05 b	89.3±2.3a	1.66±0.02 c	1.45±0.02bc	23.91±0.58bc

WG=Weight gain, SGR=Specific growth rate, AWG=Average weekly growth, S=Survival rate, FCR=Feeding conversion ratio, PER=Protein efficiency ratio. C:N ratio of diet=7.98

for *L. vannamei*, cover and represent the different DPI data calculated from the feeding tables reviewed in this study (Clifford, 1992; Kureshy and Davis, 2002; Davis *et al.*, 2006; Quintero and Roy, 2010). For this reason were used to select the DPI mathematical model to evaluate the different Carbohydrate:Crude Protein ratios with same DPI and shrimp BW (Figure 4).

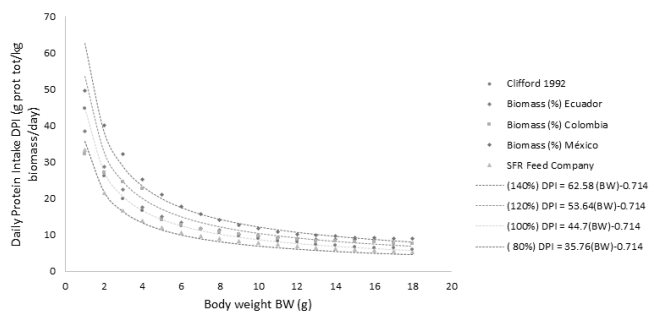


Figure 4. Daily Protein Intake (g prot/kg biomass/day) mathematical functions: $DPI=37.76(BW)^{-0.714}$, $DPI=44.7(BW)^{-0.714}$, $DPI=53.64(BW)^{-0.714}$ and $DPI=65.58(BW)^{-0.714}$, respectively corresponding to 80, 100, 120 and 140 % DPI= $44.7(BW)^{-0.714}$ for *L. vannamei*, where BW is Body weight (g), compared with DPI data calculated from different feeding tables (%Biomass related to Body Weight).

Figura 4. Funciones matemáticas de Ingesta Diaria de Proteína (g prot/kg biomasa/día): $IDP=37.76(PC)^{-0.714}$, $IDP=44.7(PC)^{-0.714}$, $IDP=53.64(PC)^{-0.714}$ e $IDP=65.58(PC)^{-0.714}$, corresponden respectivamente a 80, 100, 120 y 140 % $IDP=44.7(PC)^{-0.714}$ para *L. vannamei* Donde PC es el peso corporal (g), comparadas con datos de IDP calculados a partir de datos provenientes de diferentes tablas de alimentación (%Biomasa con relación al Peso Corporal).

We observed that variations in DPI levels using the same protein and CBH:CP ratios have a direct effect on growth, PER and NR, showing a linear increase in all growth parameters with DPI. In survival, this trend was not observed. A variation of the optimal protein requirements of *L. vannamei* from 20 to 45 % has been reported (Kureshy and Davis, 2002, Hu *et al.*, 2008; Yaemssoksawat *et al.*, 2008; Sui *et al.*, 2015, Lee and Lee, 2018), where these variations are influenced by the stocking density and aquaculture system (Brito *et al.*, 2001). Xia *et al.* (2010) reported that adequate protein values in diet under high-density conditions, in the absence of natural productivity, must be at least 43 %, results in a linear growth as protein increases with a slight decrease at higher percentages. Similarly, Jang *et al.* (2014) found a greater weight gain and specific growth rate feeding with 40 and 45 % protein, not having significant differences between them, and suggesting that values higher than 40 % are not necessary.

In the experiments with variants of the DPI mathematical model, we find an increase in growth parameters and feed conversion rates, but a decrease in protein efficiency and nitrogen retention with the increase in DPI level. Although no significant differences were found between the highest DPI (120 % and 140 %), in terms of growth parameters and nitrogen retention, these results suggest that the change in the feed protein levels must be adjusted by DPI mathematical functions to compare feeds with different formulas or

CBH:CP ratios. In this study, a decrease in protein efficiency and nitrogen retention was found with the increase in DPI at similar levels of protein content and CBH:CP ratio, suggesting that the rest of the energy substrates in the diet provided favor the adequate use of this.

Regarding DPI, the levels used agree with the daily protein level required by *L. vannamei* determined by Kureshy and Davies (2002), Clifford (1992) and Tacon (2002), as well as the recommendations by relevant shrimp feed companies such as Nicovita (Clifford, 1992) and Purina (2021). In addition, the flexibility in the mathematical model may favor their adjustment to obtain better performance of the different aquaculture systems in economic and environmental terms, also testing different genetic linages and proportionality between different ingredients and protein.

Modern aquaculture requires maximum growth with a reduction in protein intake and nitrogen residuals. The protein in the diet can be reduced if the energy required comes from another source such as carbohydrates, which can reduce the emission of residual nutrients and feed costs (Zainuddin *et al.*, 2014). The results obtained in this study shows that increasing the proportion of carbohydrates/protein in feeds, with the same DPI mathematical model that meets the requirements of the organisms, will significantly favor the performance of the system without cost in survival. Carbohydrates can provide important energy content and can be used by shrimp for the synthesis of chitin, however, their ability to use them is limited, due to their low capacity to digest and regulate plasma glucose concentrations (Guo *et al.*, 2006).

It has been observed that the best performance is at levels around 40 %. Zainuddin *et al.* (2014) determined the best growth for *L. vannamei* with 38 % carbohydrates compared to lower levels, suggesting that high carbohydrates levels can be used if it is fed with a high frequency in small amounts, which favors the use of carbohydrates and increases fat reserves with a more efficient lipogenesis process. Pascual *et al.* (2004) found better immunological performance with high carbohydrates levels, determining that these can favor the functioning of the organisms feeding appropriately. In this sense, we observed that by supplying the same DPI and Lip:CP ratio, the differential contribution of carbohydrates increasing CBH:CP ratio to 2:1 has a significant effect on growth, protein efficiency and nitrogen retention without cost in survival.

CONCLUSION

Feeding based on $DPI=53.64(BW)^{-0.714}$ corresponding to 120 % $DPI=44.7(BW)^{-0.714}$, is sufficient to achieve optimal growth of *L. vannamei* juveniles, at least within a range of 2 to 8 g in clear water systems without natural productivity. DPI mathematical model show elasticity to adapt to different shrimp feed management scenarios and can be used to evaluate feed formulations in the context of genetic linages. A significant effect on weight gain, specific growth rate, feed conversion rate, protein efficiency and nitrogen retention

were found due to DPI levels and CBH:CP ratios. Growth parameters and feed conversion rates increase in both cases, but protein efficiency and nitrogen retention diminish with DPI levels using feeds with similar protein level and CBH:CP ratio and increases with CBH:CP ratios at same DPI level using feeds with low fishmeal content, with potential benefits to decrease risks of contamination and eutrophication. Satisfying the protein needs of the organisms, it allows the use of CBH:CP ratios to maximize the use of protein in feeds and performance of the aquaculture system without complications, such as decreases growth and mortality or environmental impacts like pond or ecosystem eutrophication.

ETHICS STATEMENT

Mexican law excludes fish and crustacean taxa with regard to (a) humane care during the transport of animals (NOM-051-ZOO-1995), (b) the technical specifications for the production, care and use of animals in laboratory conditions (NOM-062-ZOO-1999) and (c) the humane sacrifice of domestic and wild animals (NOM-033-ZOO-1995). Nonetheless, we voluntarily followed special procedures for shrimp care, at the end of the experiments were sacrificed in ice water.

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