Effect of genotype and protein source on performance of Pacific white shrimp (*Litopenaeus vannamei*)

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**ABSTRACT**

Shrimp farming is supported by fast-growth and high-resistance genotypes. Protein requirement is important for shrimp performance. Fishmeal is the main source, but their limited production increase feed formulation costs for aquaculture. This study evaluated the effect of genotype and protein source on performance of two shrimp genotypes. Shrimps with fast-growth (FG) and high-resistance (HR) were randomly assigned to two dietary treatments (animal and vegetal protein) in a 2 x 2 factorial design. A 36-day bioassay was realised in clear-water hyper-intensive system to compare FG and HR performance using three replicate tanks for each genotype and dietary treatment. Each tank was seeded with 10 shrimp with average initial weight of 2.03 ± 0.10 g for FG genotype and 2.07 ± 0.02 g for HR genotype, at a density of 250 shrimp/m³. A weekly biometry was realised to monitor the shrimp performance. We observed a significant interaction (p < .05) between genotype and diet for shrimp growth performance. Compared with HR shrimp, FG shrimp showed higher growth and better feed efficiency. The maximum weight gain was recorded; FG shrimp was significantly higher than HR shrimp independently of protein source. Diet with different protein source significantly influence the performance of FG shrimp, while in HR shrimp there is no significant difference between dietary treatment. The comparison showed that genotype, diet and their interactions influenced the performance of *Litopenaeus vannamei*. This research may be useful for improving genetic selection programmes, reducing feed costs for shrimp industry and design-feeding strategies for shrimp genotypes.

**HIGHLIGHTS**

- *Litopenaeus vannamei* genotypes respond differently to diet.
- Fishmeal reduction on diet is posible in shrimps with high resistance.
- Genotype and diet interaction influence shrimp performance.

**INTRODUCTION**

Shrimp farming had a production of 7,862,000 tonnes in 2016 and the Pacific white shrimp (*Litopenaeus vannamei*) is the most important species with 53% of total production (FAO 2018). This growth demands better quality postlarvae (Gong et al. 2012). In northwest Mexico, approximately 20 laboratories produce 10 billion shrimp postlarvae annually through genetic improvement programmes (Mendoza-Cano et al. 2014). The production laboratories of shrimp postlarvae generate fast-growth genotypes based on two-stage selection process, considering body weight and growth between 28 and 130 days of age for to increase the genetic gain for growth at harvesting age (Castillo-Juárez et al. 2010); and high-resistance genotypes with shrimp broodstock of Ecuadorian origin survivors to white spot syndrome virus to reduce mortalities caused by this virus in farmed shrimp (Perez-Enriquez et al. 2018). However, genetic improvement programmes does not consider how the genotypic characterisation benefit or affect survival, growth, reproductive quality (Grijalva-Chon et al. 2013) and protein requirements of the *L. vannamei* genotypes.
The protein requirements of *L. vannamei* are mainly supplied in fishmeal, which usually constitutes between 5% and 40% of the manufactured feed (Tacon and Metian 2008). However, fishmeal production has decreased, and its cost has increased (FAO 2018); therefore, fishmeal has been replaced by soybean meal and soy protein concentrate as an alternative to supply the protein requirements for shrimp (Sookying and Davis 2011; Ye et al. 2011; Liu et al. 2012).

Several studies have evaluated the effect of diets containing soybean meal and/or soy protein concentrate on growth performance of *L. vannamei*, which have determined different levels of fishmeal replacement. Bauer et al. (2012) evaluated diets formulated with a mixture of soy protein concentrate and microbrial floc, suggesting that fishmeal can be completely replaced. Zhou et al. (2015) tested eight diets formulated mainly with soybean meal and only 6% of fish ingredients without significant difference with respect to shrimp performance. Soares et al. (2015) used diets with different level (0, 25, 50, 75, and 100%) of soybean protein concentrate, indicating that fishmeal can be replaced with 75% of soybean protein concentrate without negatively affecting the shrimp performance. Chen et al. (2017) suggested that a 25% of fishmeal can be replaced by soy protein concentrate; while Van Nguyen et al. (2017) suggested the same percentage but by fermented soybean meal without adverse effects on growth and feed utilisation of shrimp. Jatobá et al. (2017) demonstrated that fishmeal can be replaced with 33% of soy protein concentrate for to maintain adequate growth and lower feed intake in shrimp. Also, the effect of shrimp genotype and protein source has been evaluated (Gong et al. 2012); however, no significant differences were observed on growth performance due to the genetic relationship of shrimp genotypes.

Based on the above, the evaluation of diets formulated with alternative protein sources to fishmeal in different genotypes of cultured shrimp could help to determine the optimal dietary treatment for better growth performance and efficient feed use (Jatobá et al. 2014). Therefore, the aim of the present study was to determine the influence of genotype and protein source on performance of Pacific white shrimp (*L. vannamei*).

Materials and methods

**Experimental organisms**

Approximately 2000 postlarvae (PL12 – PL14) were collected from each of two *L. vannamei* genotypes.

Shrimp with fast-growth (FG) came from a hatchery in Baja California Sur, Mexico, and shrimp with high-resistance (HR) came from a hatchery in Sonora, Mexico. Both shrimp genotypes were confirmed specific pathogen-free (SPF) by the Laboratorio de Análisis de Sanidad Acuícola del Instituto Tecnológico de Sonora according to World Organisation for Animal Health (OIE).

The postlarvae were maintained in the aquaculture laboratory in 1500 L vats with seawater under controlled conditions (temperature 30 ± 0.5 °C, dissolved oxygen ≥4 mg/L, salinity 37 g/L, pH ≥7 and a 12-light hour photoperiod) and fed 32% crude protein to allow acclimation and to gain weight for starting the performance bioassay.

**Experimental diet formulation**

Two experimental diets with different protein source (vegetal and animal) were formulated to contain medium protein level according to the algorithms described by previous reports (Calderón-Flores 2007; López-Vela et al. 2014). The animal protein-based diet (AD) contained fishmeal as the main protein source and a proportion of soybean meal, this diet was used as reference diet. While, the vegetal protein-based diet (VD) contained a mixture of soy protein and 6% fish raw materials (meal and oil) as an attractant. The proximate composition analysis was performed in the Laboratorio de Análisis Químico Proximal of CIBNOR, S.C., following the AOAC methods (Horwitz 2000; Table 1).

**Experimental design**

The experiment had a 2 × 2 factorial design. The factors were: Genotype (FG and HR) and protein source (animal and vegetal). The feeding trial lasted 36 d, using juvenile shrimp with an average initial weight of 2.03 ± 0.10 g for FG genotype and 2.07 ± 0.02 g for HR genotype. Shrimp were seeded in 12 rectangular tanks of 40 litres (50 × 30 × 30 cm) at a density of 10 shrimp per tank, equivalent to a density of 250 shrimp/m³. Three replicate tanks were randomly assigned for each of the two diets. Shrimp from all treatments were fed to satiation daily through trays with an initial rate of 12% biomass divided in three rations distributed at 8:00, 13:00 and 16:00. Uneaten feed was collected and dried for feed intake and feed conversion rate calculate.

Water was exchanged once daily to remove faeces, exuviae and dead shrimp; then, about 30% of the total...
volume of water was replaced. The water quality was monitored daily: salinity (36.9 to 37.0 g/L), temperature (27.8 to 28.3°C), dissolved oxygen concentration (4.0 to 5.1 mg/L), pH (7.6 to 7.8) and total ammonia (0.52 to 0.67 mg/L), remaining within the recommended limits for the shrimp farming. A weekly biometry was realised to monitor the shrimp performance according to the equations reported by Peña-Rodríguez et al. (2017) and Piétri et al. (2015): Final total weight (FWT) = Σ Final Individual Weight/Initial Number of Shrimp; Survival (%) = 100 × (Final Number of Shrimp/Initial Number Shrimp); Final biomass = FWT × Final Number of Shrimp; Weight Gain (WG) = 100 × (Final Weight – Initial Weight)/Initial Weight; Specific Growth Rate (SGR) = (ln average final weight – ln average initial weight)/number of days; Feed intake = Feed Input (g, dry weight) – Feed collected (g, dry weight); and Factor Conversion Rate (FCR) = Feed Intake/Final biomass.

Statistical analysis

The effects of the genotype, protein source and their interactions on performance were determined using a two-way ANOVA. A post-hoc Tukey’s test for multiple comparisons of means was realised in cases in which the results of ANOVA were significant. The statistical analysis was performed at a significance level of $p < .05$ using Statgraphics XVI software. $p$ values between .05 and .10 were considered as trends.

Results and discussion

Dietary protein is one of the main factors that influence the shrimp performance and the cost of formulated feed for cultured shrimp. On the other hand, protein requirements for shrimp growth are influenced by shrimp size, body weight, culture system, stocking density, environmental factors and biological value of protein sources (Shahkar et al. 2014). The ideal protein level for L. vannamei growth in the juvenile phase is approximately 35% to 40% in formulated feeds (NRC 2011), which implies using large amounts of protein resources, such as fishmeal. For this work, the animal and vegetal diets had medium level protein and similar proximate composition with small variations in lipid, fibre, ash and other ingredients according to protein source (Table 1). The protein percentages on experimental diets remained within the ranges established by previous studies (Ciro Calderón-Flores 2007; López-Vela et al. 2014). However, the small variations on protein level (35.6% in VD and 32.1% in AD) and the other components were due to the difference in moisture.

Also, genotype affects feed tolerance and influences nutritional requirements, but little is known about how the genotype–diet interaction intervenes in nutrient use (Stover 2006, 2007). Mexican shrimp farming is supported mainly by two shrimp genotypes, fast-growth and high-resistance (Castillo-Juárez et al. 2015). Nevertheless, there is limited information on how protein source affects the performance of each shrimp genotype. For this reason, the present study focussed on evaluating the effects of genotype and protein source on shrimp performance. The L. vannamei performance results are described in Table 2.

### Table 1. Formulation and proximate composition of experimental diets (g/kg dry weight).

|                | AD     | VD     |
|----------------|--------|--------|
| Soy protein concentrate<sup>a</sup> | –      | 312.92 |
| Soybean meal<sup>b</sup> | 247.56 | 208.62 |
| Fishmeal<sup>c</sup> | 247.56 | 34.77  |
| Wheat meal<sup>d</sup> | 184.43 | 139.08 |
| Corn starch<sup>e</sup> | 166.96 | 190.08 |
| Fish oil<sup>f</sup> | 64.23  | 26.22  |
| Soybean lecithin<sup>g</sup> | 33.40  | 36.20  |
| Minerals<sup>h</sup> | 8.53   | 9.25   |
| Vitamins<sup>i</sup> | 8.53   | 9.25   |
| Alginic acid<sup>j</sup> | 17.06  | 18.49  |
| Ash<sup>k</sup> | 17.49  | 9.44   |
| Cellulose<sup>l</sup> | 2.23   | 5.67   |
| Antioxidant<sup>m</sup> | 0.01   | 0.01   |
| Proximate composition  |
| Moisture | 28.5 ± 0.5 | 12.2 ± 0.8 |
| Crude protein | 321.2 ± 1.2 | 356 ± 0.7 |
| Crude lipid | 58.4 ± 0.5 | 66.5 ± 0.0 |
| Crude fibre | 2.3 ± 0.6 | 11.7 ± 0.6 |
| Ash | 108.6 ± 0.3 | 55.9 ± 0.3 |
| Nitrogen free extract | 509.4 | 509.7 |
| Gross energy, Kcal/g | 4.33 ± 0.002 | 4.64 ± 0.004 |
| Nitrogen, g kg<sup>−1</sup> | 51.4 ± 0.2 | 57.0 ± 0.1 |

AD: animal protein-based diet and VD: vegetal protein-based diet. Both with medium protein level.
<sup>a</sup>Soy protein concentrate (SPC) (64.70% crude protein (CP), 1.13% lipid).
<sup>b</sup>Soybean meal (50.95% CP, 1.17% lipid). PIASA (La Paz, Baja California Sur, Mexico).
<sup>c</sup>Whole sardine meal (72.95% PC, 6.38% lipid). PIASA (La Paz, Baja California Sur, México).
<sup>d</sup>Wheat meal (13.38% PC, 0.4% lipid). PIASA (La Paz, Baja California Sur, México).
<sup>e</sup>Corn starch (0.3% PC, 0.1% lipid). Unilever de México, S. de R.L. de C.V. (Tultitlán-Estado de México, México).
<sup>f</sup>Sardine oil. PIASA (La Paz, Baja California Sur, México).
<sup>g</sup>Soybean lecithin. PIASA (La Paz, Baja California Sur, México).
<sup>h</sup>Minerals. PIASA (La Paz, Baja California Sur, México).
<sup>i</sup>Vitamins. PIASA (La Paz, Baja California Sur, México).
<sup>j</sup>Alginic acid. Sigma-Aldrich Corp. 180947 (St. Louis, MO).
<sup>k</sup>Ash. Sigma-Aldrich Corp. 180947 (St. Louis, MO).
<sup>l</sup>Cellulose. Sigma-Aldrich Corp. C6288 (St. Louis, MO).
<sup>m</sup>Butylated hydroxytoluene (BHT). Sigma-Aldrich Corp. 180947 (St. Louis, MO).
America evaluated two *L. vannamei* genotypes with feeds of different protein sources and protein levels and determined an genotype–diet interaction (*p* < .05) for survival but showed no significant differences in weight gain due to the genetic closeness between the genotypes (Gong et al. 2012). Our study found a significant genotype–diet interaction on growth performance parameters. These results suggest that protein requirement and consequently the growth performance of the *L. vannamei* shrimp was influenced by the genotype, which come from different laboratories located in different geographical regions from Mexico.

In our study, shrimp genotypes fed diets containing different protein sources had differentiated growth responses. Growth performance parameters of FG genotype as final weight, survival, final biomass, weight gain and specific growth rate decreased when shrimp were fed vegetal protein-based diets. While, HR genotype had the same growth performance with the two dietary treatments. These results indicate that FG genotype assimilated the animal-based diet and HR genotype can be fed diets containing animal or vegetal protein. In general, FG genotype gained 68.5% more weight than HR genotype when fed vegetal-based diet. The relationship between feed intake and final biomass per dietary treatment was measured with the FCR indicator (Piérri et al. 2015) and values within the acceptable ranges were observed. According to FCR values, FG shrimp genotype had a higher feed efficiency than HR shrimp genotype, but FG shrimp genotype requires animal protein for higher final biomass. Both FG and HR shrimp genotypes showed no difference in feed efficiency when fed with animal-based diet or vegetal-based diet. The shrimp growth performance and feed efficiency results are preliminary but show typical values of a hyper-intensive shrimp culture system in clear water and also agree with previous works where the shrimp was fed with formulated feed containing total or partial fishmeal replacement with soybean meal and/or soy protein concentrate (Bauer et al. 2012; Soares et al. 2015; Zhou et al. 2015; Chen et al. 2017; Jatobá et al. 2017; Van Nguyen et al. 2017).

In summary, the differences of shrimp growth performance, feed intake and feed efficiency may be due to many factors, but mainly to shrimp genotypic conditions. Shrimp from FG genotype were selected according to the body weight and growth for increase genetic gain for growth (Castillo-Juarex et al. 2010), which could imply a higher metabolism and therefore a greater demand of feed containing essential nutrients. While the shrimp from HR genotype only are selected from broodstock shrimp survivors to white spot syndorme virus (Perez-Enriquez et al. 2018) without any genetic condition on growth or body weight. Nevertheless, these results are preliminary, so it would be interesting to carry out these types of studies challenging shrimp with frequent pathogens and determine how performance and disease-resistance are affected by the protein source. Also, more dietary treatments with at least five levels of vegetal-based protein supplemented with essential amino acids would help to determine the optimal level of vegetal protein for each shrimp genotype and long-term bioassays to assess whether growth performance, feed intake and feed efficiency could be improved.

Despite this evidence, nutrigenetics and nutrigenomics could be applied to identify genetic variations that provide information on the nutritional responses of the different shrimp genotypes (Benítez and Nuñez 2017). This would allow understanding how genetics and nutrition interact to impact *L. vannamei* growth performance and to support the postlarvae production industry in developing tools for choosing shrimp broodstocks and/or shrimp genotypes with desirable characteristics such as greater growth and better use of formulated feed.

### Conclusions

The genotypes influence performance parameters while protein source tends to affect feed efficiency.

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**Table 2. Effect of genotype, diet and their interactions on performance of *L. vannamei*.**

| ITEM               | FG         | HR         | Pooled SE | Genotype | Diet | Genotype-diet |
|--------------------|------------|------------|-----------|----------|------|----------------|
| Mean              | AD         | VD         | AD        | VD       |      |                |
| FWT, g            | 8.1a       | 7.0b       | 4.3a      | 4.4a     | 0.17 | <.01           |
| Survival, %       | 89.3a      | 77.3b      | 96.0a     | 97.3a    | 2.08 | <.01           |<.01 |<.01 |
| Final biomass, g  | 72.8a      | 54.2b      | 41.4a     | 42.4a    | 2.01 | <.01           |<.01 |<.01 |
| WG, %             | 304.6a     | 243.1b     | 106.8a    | 111.9a   | 11.97| <.01           |.05  |<.02 |
| SGR, g day⁻¹      | 3.9a       | 3.4a       | 2.0a      | 2.1a     | 0.11 | <.01           |<.01 |<.05 |
| Feed intake, g    | 130.2a     | 95.1b      | 82.9a     | 88.0a    | 3.43 | <.01           |<.01 |<.01 |
| FCR               | 1.8a       | 1.8a       | 2.0a      | 2.1a     | 0.05 | <.01           |<.01 |<.38 |

FG: fast-growth genotype; HR: high-resistance genotype; AD: animal protein-based diet; VD: vegetal protein-based diet; Pooled SE: pooled standard error; FWT: final total weight; WG: weight gain; FCR: factor conversion rate. Mean with different superscript letters indicate significant differences (*p* < .05).
Likewise, the FG genotype needs animal-based feed for higher performance, while HR genotype can be fed with vegetal-based diets without affecting shrimp performance. These may be useful for improving genetic selection programmes, reducing feed costs for shrimp industry and design-feeding strategies for shrimp genotypes.

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Author contributions

J.R.G.G. and R.C.H. designed the study and wrote the manuscript. M.B.F.P. and J.C.G.N. carried out the feeding bioassay, collected the data and reviewed the manuscript. F.L.V. and R.A.B.L. helped with the statistical analysis. All authors read, revised and approved the manuscript.

Disclosure statement

The authors declare that they have no competing interests.

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Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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