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ARTICLE

Effect of fasting on molting and survival rate in post-larvae of the shrimp *Litopenaeus vannamei*

Efecto del ayuno sobre la muda y la tasa de supervivencia en post-larvas del camarón *Litopenaeus vannamei*

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Resumen.- El objetivo del presente estudio fue evaluar el efecto de diferentes períodos de ayuno sobre la mortalidad de las post-larvas de *Litopenaeus vannamei* y el tiempo que les tomó mudar o morir. El estudio aplicó un diseño experimental utilizando post-larvas de 20 días de edad, con un grupo control sometido a alimentación continua (CF) y 9 tratamientos (S2, S4, S6, S8, S10, S12, S14, S16, S18) con períodos variables de ayuno, seguidos de alimentación continua. Se analizó el momento en el que la privación de alimentos provocó la muerte del 50% de los individuos, incluso cuando recibieron alimento después del ayuno. El análisis estadístico para el tiempo de muda identificó 3 grupos diferentes ($P < 0,05$). Grupo 1 con alimentación continua; Grupo 2 con periodos de ayuno de 2 y 4 días; y grupo 3 con periodos de ayuno entre 6-18 días. En el grupo control con alimentación continua se estimó un tiempo de muda de alrededor de 3,44 ± 0,68 días. En el tiempo de muerte hubo 2 grupos de tratamientos significativamente diferentes ($P < 0,05$). En el tratamiento con alimentación continua sobrevivieron todas las PL. El primer grupo consistió en pruebas de ayuno durante 2 y 4 días (S2 y S4). El segundo grupo correspondió desde los periodos de ayuno de S6 a S18 días, resultando el menor tiempo hasta la muerte con 71,43 y 100% de individuos muertos. El PNR50 se estimó alrededor de 3,97 ± 0,31 días, demostrando que los períodos de ayuno afectaron el desarrollo y potencialmente causan mortalidad en las post-larvas de *L. vannamei*.

Palabras clave: *Litopenaeus vannamei*, ayuno, muda, postlarva, índice de supervivencia

Abstract.- The objective of the present study was to evaluate the effect of different fasting periods on mortality of *Litopenaeus vannamei* post-larvae and the time it took to molt or die. The study applied an experimental design using 20-days-old post-larvae (PL), with a control group subjected to continuous feeding (CF) and 9 treatments (S2, S4, S6, S8, S10, S12, S14, S16, S18) with varying fasting periods, followed by continuous feeding. The time at which food deprivation resulted in death was analyzed for 50% of the post-larvae, even when they received food after fasting. Statistical analysis of time to molt identified 3 different groups ($P < 0.05$): group 1 with continuous feeding, group 2 with fasting periods of 2 and 4 days (S2, S4), and group 3 with fasting periods between 6-18 days (S6-S18). In control group with continuous feeding, the time to molt was estimated at about 3.44 ± 0.68 days. Time to death, showed 2 significantly different treatment groups ($P < 0.05$). In CF treatment, all PLs survived. The first group consisted of fasting tests for 2 and 4 days (S2, S4). The second group corresponded to fasting periods from S6 to S18 days, resulting in the shortest time to death with 71.43 and 100% of individuals dead. The PNR50 was estimated to be around 3.97 ± 0.31 days, demonstrating that fasting periods affected development and potentially caused death in PL of *L. vannamei.*

Key words: *Litopenaeus vannamei*, fasting, molting, post-larvae, survival rate

INTRODUCTION

The Pacific white shrimp *Litopenaeus vannamei* is the most important species representing over 70% of the global shrimp production (FAO 2018). It is vital to fisheries and farming activities in many Latin American, Asian, and African countries. The growth phases of shrimp comprise the larval, post-larval, juvenile, and adult stages; therefore, maintenance of the early stages is critical for further development. First, egg production occurs after maturation and breeding of mature *Litopenaeus vannamei* shrimp. Subsequently, the larval phases include nauplius, zoea, and mysis stages until the post-larval stage is reached. The zoea and mysis stages receive live food, which consists of one or several species of marine microalgae (Kiatmetha *et al.* 2011) and their concentration in the larval culture tank ranges around 1 x 10^5 cell mL^{-1} (Muller-Feuga *et al.* 2003). The microalgae are supplied in combination with the microcrustacean *Artemia salina* during the mysis and post-larval stages (Lavens & Sorgeloos 2000). While natural food is their primary nutritional resource, the aquaculture industry has developed a variety of artificial biotechnologically prepared foods currently used to feed

larval and post-larval shrimp (Meyer-Willerer 2005). After the hatchery cycle, shrimp farmers purchase the post-larvae, and their early development and mortality are still major concerns (De Schryver *et al*. 2017). Shrimp post-larva complete different developmental stages by a molting process, whereby they shed their exoskeleton to allow growth. The molt cycle has 4 stages: post-molt (metecdysis), inter-molt (anecdysis), premoult (proecdysis), and shedding the old cuticle (ecdysis) (Corteel *et al.* 2012). Molting delay and survival rate are associated with physiological states that could be caused by low energy intake, lack of or limited food consumption (Urzúa & Anger 2013). In the advanced larvae of decapod crustaceans, there is a physiological state in which well-fed crustaceans can move from one larval stage to the next without the need for subsequent feeding (Anger & Balkema 2001). The development of one stage to another depends on the quantity and quality of food intake. The development of organisms in commercial culture systems may be subject to nutritional strategies that strengthen their immune system (Chen *et al.* 2015).

There is enormous scientific interest in the larval development, physiology, and behavior of crustaceans, as well as the factors that affect their development and survival rates (Bardena *et al.* 2019). When shrimp are exposed to fasting conditions or lack of food, a recovery period occurs that alters their physiological condition. Hence, in the early stages of development, the effect of fasting is more severe, causing internal biochemical changes (Pascual *et al.* 2006, Sánchez-Paz *et al.* 2007). Molting occurrence would also be a physiological consequence of the number of nutritional reserves contained in the organism (Urzúa & Anger 2013). Consequently, internal damage and poor recovery can occur when the post-larvae do not have enough energy to capture and manipulate food (Sacristán *et al.* 2016), causing growth retardation and mortality (Calvo *et al.* 2012).

At the level of culture of aquatic organisms, the origin of physiological disorders is still incomprehensible, and high mortalities can be due to the lack or poor quality of food (Piccinetti *et al*. 2015). Studies about the effects of food deprivation on the development of the white shrimp post-larvae are limited, particularly in the context of the rearing conditions applied in Latin America (Dastidar *et al*. 2013). The research was conducted with post-larvae cultured in commercial hatcheries and applying a practical experimental design based on the estimation of the point of no return (PNR50), a critical physiological point at which the animal no longer recovers from the effects of food deprivation (Paschke *et al*. 2004, Calvo *et al*. 2012). Therefore, the objective of this study was to evaluate the effect of different fasting periods on the mortality of post-larval *Litopenaeus vannamei* and the time it takes for them to molt or die. The results of this research will be beneficial for understanding the effect of food deprivation on the development and survival of white shrimp and its implications for feeding management and the quality larvae produced as a requirement for sustainable production.

Materials and methods

Post-larvae selection and maintenance conditions

The nauplii stage larvae were provided by a spawner supplier located in Ecuador. The nauplii were obtained from the spawning of 6 female shrimps. All phases of larval culture were carried out in a commercial shrimp laboratory in Puerto Bolivar, El Oro, Ecuador, completing the larval stages nauplii, zoea, and mysis, till reaching the 4-days-old post-larvae stage. During culture conditions in the commercial hatchery, the larvae in the zoeae stages received marine microalgae *Chaetoceros gracilis* in combination with artificial feed ZM zoea containing 58% protein. The concentration of *Chaetoceros gracilis* in the culture tank was measured twice a day using a Neubauer hemocytometer and oscillated around 1×10^5 cell mL^{-1}. At the stages of mysis, the larvae completed a formulated diet (ZM-mysis) containing 58% protein in combination with frozen *Artemia salina* (Fig. 1). During early post-larval stages, the diet consisted of Zeigler brine shrimp flakes (Zeigler Bros, Inc), 50% of Crude Protein, 13% of Crude Fat, 3% of Crude Fiber, 10% of Moisture and 10% of Ash. The quantity of artificial food provided during culture conditions in the commercial hatchery was according to the instructions of the feed manufacturer.

For the fasting experiment, approximately 1,000 4-days-old post-larvae were obtained from the commercial hatchery. They were acclimatized and maintained at a temperature of 27 ± 1 °C and a salinity of 26 in a 100 L capacity tank with sterilized seawater. The 4-days-old post-larvae were maintained for 15 days until they reached the age of 19-days-old and fed *ad libitum* with Zeigler brine shrimp flakes, proximal composition previously described.

Experimental conditions

At 19-days-old, a group of PLs was placed on absorbent paper to remove any remaining water from the organisms and to evaluate their length and weight. The size of each PL was measured from the rostrum to telson using graph paper and an AmScope© model SE303R-P compact binocular stereo microscope. The length of PLs was expressed as mean ± standard deviation. The weight of each PL was determined using a Denver analytical balance model X-100 (Denver Instrument Company, Germany), weighing approximately 1 g of organisms and dividing the resulting weight by number of organisms.

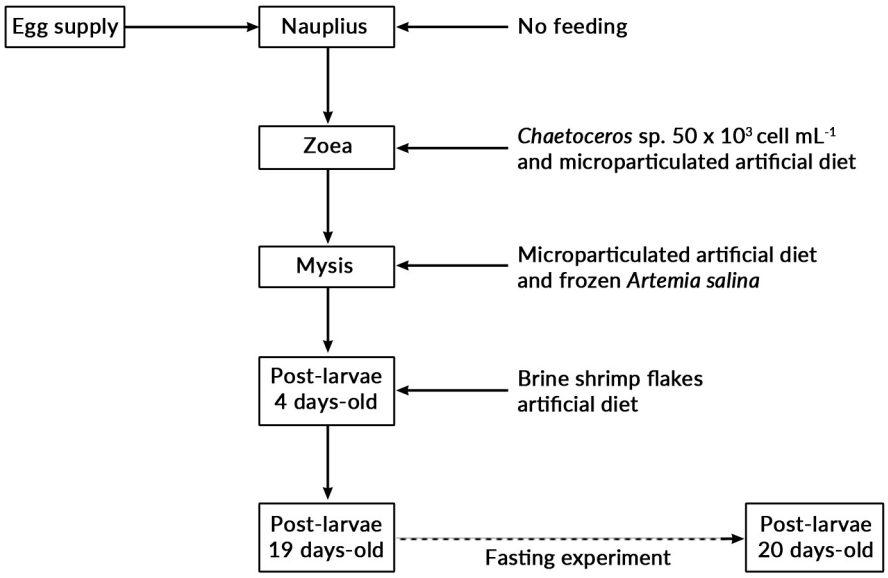

Figure 1. Flow chart of shrimp larval culture applied in the commercial production hatchery that supplied the post-larvae used for point-of-no-return (PNR50) experiments / Diagrama de flujo del cultivo de larvas de camarón aplicado en el criadero de producción comercial que suministró las post-larvas utilizadas para experimentos de punto de no retorno (PNR50)

At 19-days-old, 500 post-larvae (PL) were taken randomly and placed individually in small glass flasks containing 200 mL of sterilized seawater. The PLs were accurately observed every hour to detect the molt passage or ecdysis (freshly molted when shedding old cuticle). From here, a group of PL was obtained exactly after the molting passage for the fasting experiment.

Freshly molted 20-days-old PL were individually placed in small glass flasks with 200 mL of sterilized seawater. From the 20-days-old PL to the next stage, the organisms were exposed to PNR50 experiments, a method to examine time to molt and time to death of post-larvae exposed under different fasting periods. Each treatment consisted of 16 replicates (PL) exposed to a control group subjected to continuous feeding (CF) and 9 fasting treatments (S2, S4, S6, S8, S10, S12, S14, S16, S18) with variable periods of fasting, followed by continuous feeding. Figure 2 describes the design of the experiment, which comprises a control group subjected to continuous feeding and 9 treatments of varying fasting periods (2-18 days), followed by continuous feeding. The continuous feeding consisted of delivered brine shrimp flakes after each fasting period. For example, in the S2 treatment, the PL did not receive food for 2 days; after completing their treatment, they received food till they died or molt. When corresponded, the formulated larval feed was diluted in water and provided to the organisms three times a day after each period of fasting.

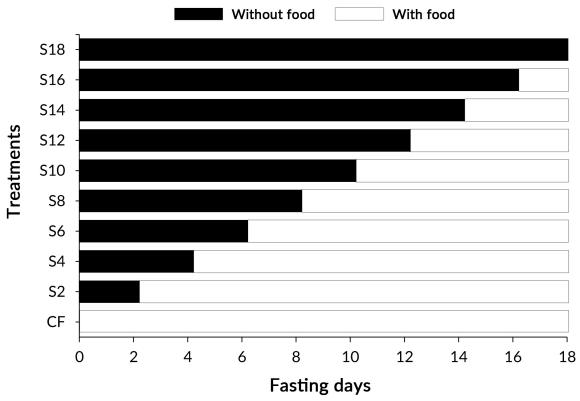

Figure 2. Experimental design used to determine the effect of fasting on 20-days-old *Litopenaeus vannamei* shrimp post-larvae. Nine treatments (S2, S4, S6, S8, S10, S12, S14, S16, S18) of different fasting periods (2-18 days) followed by continuous feeding are shown. The control group was subjected to continuous feeding (CF) / Diseño experimental utilizado para determinar el efecto del ayuno en post-larvas de camarón *Litopenaeus vannamei* de 20 días de edad. Se muestran 9 tratamientos (S2, S4, S6, S8, S10, S12, S14, S16, S18) de diferentes períodos de ayuno (2-18 días), seguido de alimentación continua. El grupo de control fue sometido a alimentación continua (CF)

The time to molt was measured based on the molting response of each post-larvae. The experiment consisted of observing the organism till molting to the following life stage. Each PL was taken out from the experimental flask when it either molted or died, registering, and quantifying the number of molts, deaths, time to molt, time to death and percentage of mortality.

Statistical analysis

Data were processed using SPSS statistical software Version 25.0 (SPSS, Chicago, IL). Descriptive statistical analysis for time to molt or death followed standard methods. According to the experimental design, the time to molt and the time to death were taken as dependent variables and the fasting period as the independent variable with a control group. The normal distribution and homogeneity of the time to molt and time to death were tested with the Shapiro-Wilk median tests. Parametric tests were applied when the data met the assumptions of the model (one-way ANOVA); otherwise, the Kruskal-Wallis H tests were used. Subsequently, paired comparisons for the time to molt and percentage of mortality were made using Duncan's (parametric) test and Mann-Whitney U test (non-parametric). When the probability of error to reject the null hypothesis was greater than 0.05, the differences were considered not significant (Sokal & Rohlf 1995).

A nonlinear sigmoid Boltzmann regression was performed on the percentage of mortality. The type of curve used to estimate the lethal dose of a toxic substance was used to obtain the PNR50 where the lethal dose in the present study was the fasting time. The PNR50 was defined as the point at which 50% of PL died after a period of fasting, even if they received feed later (Paschke *et al.* 2004).

Results

In total, 150 PL were analyzed to investigate the time for molting and dying after fasting in the post-larvae of the white shrimp *Litopenaeus vannamei*. At 20 days old, before the PNR50 experiments, the post-larvae achieved a dry weight of 1.64 mg, and their length ranged from 6-15 mm. The average length average was 11.2 ± 1.88 mm, with 16.8% of the coefficient of variation. Table 1 shows the summary results, mean and standard deviation for time to molt and time to death, with minimum and maximum values for molts in each fasting treatment.

Normality tests performed with Shapiro Wilks test for the time to molt showed that the data are normally distributed (P value > 0.05), but for time to death, the data are not normally distributed (P value < 0.05). The analysis of time to molt showed that in the control treatment, with continuous feeding, PL molted in a mean of 3.44 days. In the treatment with 2 days of fasting, molting occurred at the beginning of the fourth day and culminated on the ninth day, with a molt delay of approximately 2.5 days with respect to the control treatment. Successively, in the treatment with four days of fasting, molting occurred on the fifth day and culminated on the ninth day, with a molting delay of 3.0 days with respect to the control treatment. Treatment S6 recorded 4 molts that occurred on the ninth day. In treatments S10 and S12, there was only one molt on the seventh and eighth day, respectively. In treatments S14 to S18, no molt was recorded. ANOVA test for time to molt determined statistical differences between fasting treatments [$F(9) = 9.421$; $P < 0.05$]. Duncan's multiple range test identified 3 different groups ($P < 0.05$) illustrated in Figure 3A: control group 1, with continuous feeding; group 2, with S2 and S4, fasting periods of 2 and 4 days, respectively; and group 3, corresponding to S6.

Table 1. Summary of results and descriptive statistics for time of molting and death in different fasting treatments in 20-days-old *Litopenaeus vannamei* shrimp post-larvae / Resumen de los resultados y estadística descriptiva para el tiempo de mudas y muertes en diferentes tratamientos de ayuno en post-larvas de camarón *Litopenaeus vannamei* de 20 días de edad

Treatment*	Time of fasting	N	Deaths	Time to death (days)**	Time to molt (days)**	Minimum time to molt (days)	Maximum time to molt (days)
CF	0	9	0	0.00 ± 0.00	3.44 ± 0.68	3	5
S2	2	16	6	4.50 ± 1.80	5.90 ± 1.81	4	9
S4	4	15	7	3.71 ± 3.10	6.50 ± 1.32	5	9
S6	6	14	10	8.90 ± 2.66	9.00 ± 0.00	9	9
S8	8	16	16	8.69 ± 2.64	0.00 ± 0.00	0	0
S10	10	16	15	8.13 ± 2.33	7.00 ± 0.00	7	7
S12	12	16	15	7.87 ± 3.00	8.00 ± 0.00	8	8
S14	14	16	16	8.67 ± 3.05	0.00 ± 0.00	0	0
S16	16	16	16	9.38 ± 2.34	0.00 ± 0.00	0	0
S18	18	16	16	9.69 ± 0.92	0.00 ± 0.00	0	0

*continuous feeding (CF) and 9 treatments (S2, S4, S6, S8, S10, S12, S14, S16, S18)

**mean and standard deviation

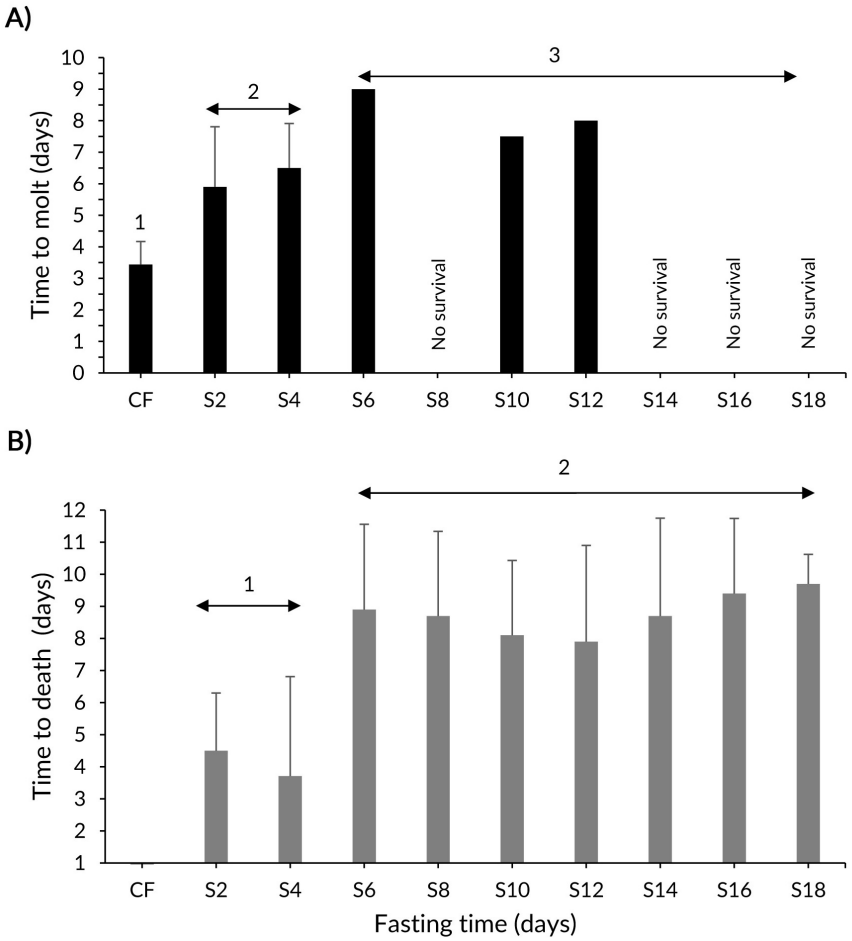

Figure 3. Time to molt (A) and time to death (B) in 20-days-old *Litopenaeus vannamei* post-larvae in response to different fasting periods. X-axis shows treatments, and Y-axis shows time to molt and time to death in each treatment. A) Time to molt shows three distinct and significantly different groups ($P < 0.05$): Group 1, control with continuous feeding; Group 2 with 2 and 4 days of fasting, respectively; and Group 3, from 6 to 18 days of fasting. B) Time to death shows two distinct and significantly different groups ($P < 0.05$). Group 1 with 2 and 4 fasting days and Group 2 with fasting periods from 6 to 18 days / Tiempo de muda (A) y tiempo de muerte (B) en post-larvas de *Litopenaeus vannamei* de 20 días de edad en respuesta a diferentes períodos de ayuno. El eje X muestra los tratamientos y el eje Y muestra el tiempo hasta la muda y tiempo hasta la muerte en cada tratamiento. A) El tiempo de muda muestra tres grupos distintos y significativamente diferentes ($P < 0,05$): Grupo 1, control con alimentación continua; Grupo 2 con 2 y 4 días de ayuno, respectivamente; y el Grupo 3, desde 6 a 18 días de ayuno. B) El tiempo de muerte muestra dos grupos distintos y significativamente diferentes ($P < 0,05$). El Grupo 1 con 2 y 4 días de ayuno; y el Grupo 2 con periodos de ayuno de 6 a 18 días

The analysis of deaths showed that in the control treatment all individuals survived. In the 2-day fasting group, deaths begin on 3-day until 7-day, with a mean of 4.50 and standard deviation of 1.80 days. In the S4 treatment group, deaths began on the 1-day and occurred until 10-day, with a mean of 3.71 and standard deviation of 3.10 days. In the rest of the treatments from S6 to S16, deaths begin on 1-day and culminate on 11-day, and in the S18 treatment deaths began on the 8-day of fasting and end on 11-day. Analysis of all fasting experiments shows that deaths culminated on the 11-day. The time to death decreases with increasing fasting period. The Kruskal-Wallis's test H= 28.44 ($P < 0.05$) with 8 degrees of freedom for time-to-death data determined differences between treatment levels. For this test, the control treatment was not considered because all PLs survived. For time to death, there were 2 significantly different treatment groups (Mann-Whitney U test; $P < 0.05$), shown in Figure 3B. The first group consisted of food deprivation for 2 and 4 days (S2-S4) with percentage mortalities between 37.5 and 46.67%, respectively. The second group corresponded to fasting days S6 to S18, resulting in the highest percentage of mortality, between 71.43% and 100%. Based on the percentage of mortality, the estimated dose-dependent nonlinear Boltzmann-sigmoidal regression for *L. vannamei* shows that 50% of the mortality occurs in 4 fasting days (Fig. 4), and approximately the other 50% of PL resisted between 9-11 fasting days.

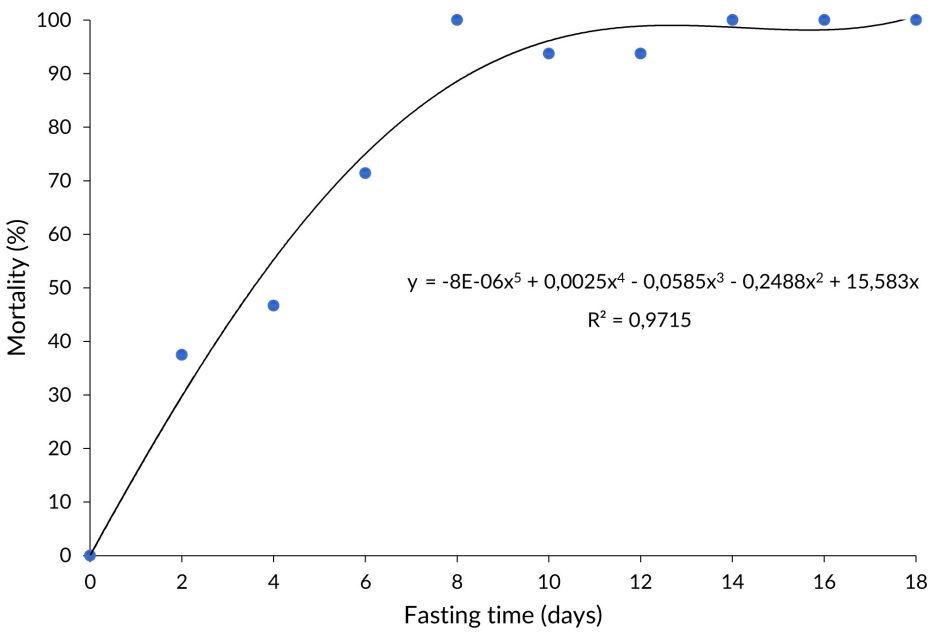

Figure 4. PNR50 (point of no return) in 20-days-old *Litopenaeus vannamei* shrimp post-larvae in relation to fasting time. X-axis shows the treatments, and Y-axis shows the percentage of mortality related to each treatment / PNR50 (punto de no retorno) en post-larvas de camarón *Litopenaeus vannamei* de 20 días de edad en relación con el tiempo de ayuno. El eje X muestra los tratamientos y el eje Y muestra el porcentaje de mortalidad relacionado con cada tratamiento

The analysis of molts and death in each treatment shows that in the control group 100% of the individuals molted completely and no deaths were recorded. The treatment level with two days of fasting shows that 62.5% of the PL molted, and 37.5% died. The group with four days of fasting (S4) presented 53.33% of molts and 46.7% of mortality, showing a decrease in molts compared to the group with two days of fasting. For PL group with six days of fasting, the percentage of mortality increased to 71.4% and only 28.57% molted. Practically no further changes for the rest of the treatment levels, and only deaths were recorded. As fasting days increase, molting percentage decreases and mortality increases at each treatment level.

Discussion

Response to molting or death in white shrimp *Litopenaeus vannamei* varied according to fasting time. Experiments in the present study showed that molting began on 3-day in the control group and ended on 9-day in the S6 group of 6 fasting days. Molting was affected after 2 fasting days, as evidenced by the time interval to molt from one stage to the next, with molting ability decreasing with increasing fasting time. When the fasting period increased, post-larvae showed signs of muscle tissue loss, stress, and pallor, possibly due to autolysis. Previous research has reported a significant decrease in dry weight of post-larvae, resulting in weakness and stress after fasting (Dall 1974, Cuzon *et al.* 1980, Regnault 1981, Barclay *et al.* 1983, Stuck *et al.* 1996). Other studies reported that feed deprivation and molt status are critical factors in aquaculture (Bardenas *et al.* 2019). It has been reported that during the fasting period or low quality of food intake, energy for sustainment comes only from endogenous material, and tissue deterioration may occur due to catabolic activity (Calvo *et al.* 2012). On the other hand, fasting affects digestive enzymes activity in *Litopenaeus vannamei* (Muhlia-Almazan & Garcia-Carreño 2002) and *Cherax quadricarinatus* (Sacristán *et al.* 2014), causing internal biochemical changes and elevated stress (Barclay *et al.* 1983, Pascual *et al.* 2006, Sánchez-Paz *et al.* 2007). Diet management during early life greatly affects the physiological response of organisms to molting as it constitutes the animals' reserves for normal development (Sánchez-Paz *et al.* 2007).

After exposure to fasting periods due to low or no food availability, shrimp go through a recovery period that alters their physiological state and survival rate (Calvo *et al.* 2012). In the present study, 20-days-old PLs showed that in the continuous feeding control group, all PLs survived, and at 4 days, a 50% survival rate was detected. The survival rate at

6 fasting days was 30%, and at 8 fasting days, 100% of PLs died due from starvation. Studies published by Stuck & Overstreet (1994) with 13-14 days-old *Litopenaeus vannamei* post-larvae reported that 98% of individuals died after 10 fasting days. In their experiment they used larvae infected with *Baculovirus penaeid*, and the larvae were reared under controlled experimental conditions and fed *ad libitum* with live food such as *Chaetoceros gracilis* during zoeae and *Artemia salina* during all mysis and post-larvae stages. The 14-days-old post-larvae showed comparable resistance to the 20-days-old PLs tested in the present study. The nauplii acquired to produce the larvae were not found to be free of pathogen, and during the production cycle, larval and post-larvae stages received formulated feeds. The results of this study are based on classic feeding procedures achieved in a commercial laboratory, rather than other fully controlled culture conditions or feeding strategies, such as the use of 100% of live feed. Feed quality and feeding conditions are important factors for the development and survival rate of shrimp (Lavens & Sorgeloos 2000). Recovery after food deprivation in some individuals is known to result in the accumulation of energy reserves from previously received nutrition (Rosas *et al*. 2002, Sánchez-Paz *et al.* 2003, Pascual *et al*. 2006). Hence, feed quality and management applied during all stages of the larval cycle or just prior to exposure to fasting periods could affect molt response and survival. In commercial hatcheries, post-larvae may show poor recovery and mortality when fed after a period of feed deprivation, either because their energy reserves have become too depleted, limiting feed capture and handling, or because internal damage (autolysis) occurs, meaning they are unable to digest or assimilate feed (Sacristán *et al*. 2016). Vogt *et al.* (1985), in their study of *Penaeus monodon* post-larvae, observed that the organisms were able to resist fasting after the fifth day without damage. In another study, juveniles *L. vannamei* weighing about 0.99 g did not survive when subjected to food deprivation for more than 15 days (Comoglio *et al*. 2004). Other crustaceans such as freshwater lobster (Speck & Urich 1969) and marine lobster (Dall 1974) survive up to 28 and 41 days, respectively, without food in their adult stages. Juvenile blue crabs can survive for long periods without food (Wang & Stickle 1986). Although some crustaceans can survive long periods of fasting in post-larval and adult stages, once the organism has endured an extreme period of fasting close to the point of no return, they are unable to recover despite receiving abundant food (Anger 1987). Observations of early post-larvae and juvenile stages under culture conditions indicate that animals that survive periods of food deprivation are more susceptible to disease and potential death.

High variability in post-larval size can affect shrimp survival and development (Luan *et al.* 2020). Furthermore, it has been suggested that size variability is a good indicator of larval quality and stress resistance (Hernández *et al*. 2001). In this study, the PL group tested corresponded to a random sample of the batch without size grading, resulting in 16.8% variability, which could explain the resistance found in one PL at 10 days and in another at 12 days of fasting. In commercial hatcheries, many factors affect the size uniformity of PLs and, consequently, their response to molt time and survival rate. For further studies, it is suggested to consider PLs uniformity to reduce the effect of this factor. Abnormal feeding rate or unadjusted feed quantity in larval culture can lead to high variability in post-larval size, observed in many commercial hatcheries (Brito *et al*. 2004). In cases of unsuitable mixing conditions or inaccurate estimates of the number of organisms in the culture medium, inadequate feeding, and competitive behavior unintentionally occur, affecting PL size uniformity due to lack of feed and poor intake in part of the shrimp population. Besides, when feeding regimes are not under the requirements of the post-larvae population, organisms that do not receive sufficient food for maintenance will not be able to recover enough to develop healthily (Naegel & Rodriguez-Astudillo 2004). Shrimp farmers believe that abrupt changes in feed type during early rearing conditions affect the efficiency of feed assimilation, behavior and development of post-larvae (Alday-Sanz 2010). The effect of PL size and uniformity on their resistance to complete the molt cycle and survival after fasting requires further investigation. The effect of PL size and uniformity on their resistance to complete the molt cycle and survival after fasting requires further investigation. However, this study will be a basis for future research taking into account various factors such as chronological age and size of post-larvae. It should be considered that genetic variability affects the PLs size as suggested by Racota *et al.* (2003).

This study showed that fasting affected the shrimp post-larva molting process substantially, and more than 8 days of fasting caused up to 100% of mortality in PLs of 11.2 ± 1.88 mm of length, 1.64 dry weights and a chronological age of PL-20-days-old. A decrease in the energy of the post-larvae was also evident as the days of fasting increased, without being able to reach the next stage. After the fourth day of fasting, the return diminished significantly, exhibiting an increase in deaths. PNR50 point of no return, where the animal is no longer able to take advantage of the food due to prolonged fasting periods, was located around four days, so it can be concluded that the critical point was located at the fourth day of fasting. However, the study also showed that PL 20-days-old can resist up to 8 days of fasting. Although there is a prospect of expansion to the body of knowledge in this field, the outcomes of the present study provide the opportunity to reflect on the physiological behavior of the post-larvae rearing conditions from the perspective of development and survival when faced with food deprivation.

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