

Animal performance and welfare of giant freshwater prawn (*Macrobrachium rosenbergii*) subjected to feed restriction

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Received: July 8, 2019

Accepted: June 9, 2020

How to cite: Pontes, C. S.; Arruda, M. F.; Santana, V. G. S. and Santos, D. B. 2020. Animal performance and welfare of giant freshwater prawn (*Macrobrachium rosenbergii*) subjected to feed restriction. Revista Brasileira de Zootecnia 49:e20190128. <https://doi.org/10.37496/rbz4920190128>

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ABSTRACT - Behavior activities of giant freshwater prawn *Macrobrachium rosenbergii* juvenile, their performance, and acquired lesions when under temporary feed restrictions were analyzed. Five animals/experimental units (10 prawns/m²) were employed for treatments DFL – daily feed supply in the light phase; DFD – daily feed supply in the dark phase; AFL – alternating feed supply in the light phase; and AFD – alternating feed supply in the dark phase, for a 24-hour period, with four replications each. Prawns were fed a pellet diet, twice a day, with 10% of their biomass/day. Specimens were monitored during 60 consecutive days, with behavior recording daily and weighed every 10 days, with assessment of lesions and/or amputated limbs at the end of the experimental period. Types of behavior comprised exploring, grooming, inactivity, crawling, swimming, excavation, burrowing, agonism, and feed ingestion by scan sampling, with instantaneous registration every 60 s, in 15-min windows, before and after feed, in light and dark phases. Feed restricted in alternate days during light and dark phases did not interfere in growth or survival of the specimens, with increase in apparent feed intake and less feed conversion ratio and specific growth rate. Agonistic behavior was higher in specimens with feed restrictions, with an increase in the number of lesions and amputations. The above demonstrates welfare decrease in farmed *M. rosenbergii* and depreciation in the quality of the final product.

Keywords: applied ethology, Malaysian giant prawn, prawn culture

1. Introduction

Different aquatic animal species are farmed to attend food demand worldwide. Crustaceans have been highlighted due to high market price and to their ability to adapt to several culture conditions (FAO, 2018). Freshwater prawns have stood out among crustaceans. In fact, they are farmed in several countries, with world production reaching 216,857 tons, worth 1.33 billion US dollars, in 2014 (FAO, 2016). Giant freshwater prawn, *Macrobrachium rosenbergii*, is the most important cultivable freshwater prawn species due to fairly high growth rates, wide temperature range (15-35 °C) and salinity tolerance (Mukhopadhyay et al., 2003).

Feed conversion ratio and cost-benefit in feeding management are, as a rule, the most significant fraction, with up to 50% of production costs, whilst feed management is a crucial factor to calculate the viability of a prawn farm (Akyiama and Polanco, 1997; Arnold et al., 2016). Feed-restriction strategy in

aquaculture is a stressful technique, even though it has been widely employed as a strategy to guarantee less feed expenditure and low labor costs (Maciel et al., 2018). Cyclic feed restriction may be a useful strategy to minimize negative environmental changes triggered by feed excess in ponds (Mohanty and Mohapatra, 2017).

In spite of the social and behavioral complexity in populations, studies in applied ethology to develop new management practices are scarce. Li et al. (2008) reported compensatory growth of *M. nipponense* during feed deprivation. Marques and Lombardi (2011) verified the occurrence of compensatory growth in *M. rosenbergii* stocked in cages, under high densities, during the nursery phase, and suggested partial compensatory growth in this phase. Additionally, Rahman et al. (2019) stated that time restriction could be applied in *M. rosenbergii* culture without detrimental effects on growth, feed efficiency, and nutrient utilization.

Research in applied ethology mainly aims at analyzing and enhancing the welfare of farmed animals. It is a concern of the contemporary society that founded the International Society for Applied Ethology (Huntingford et al., 2012). Since research on animal performance and its relationship with feeding and welfare of *M. rosenbergii* are scarce, it is relevant to assess the behavioral activities and lesions suffered by these animals when subjected to temporary feeding restrictions.

2. Material and Methods

Juvenile specimens of giant freshwater prawn were retrieved from the municipality of Macaíba, state of Rio Grande do Norte, Brazil (5.88552 S; 35.365886 W), and conditioned in two 80-L plastic boxes, with constant aeration, at 27.2 °C and 0.5 g/L salinity. They were then transferred to Natal, RN, Brazil (5.841720 S; 35.202411 W).

Prawns were weighed on a scale (2.25 ± 0.53 g, $n = 20$) in the laboratory, conditioned, and acclimated in experimental units. During acclimatization, the feeding management of animals under experimental conditions was considered. Eight 250-L (100 × 50 × 50 cm) aquariums were employed for each treatment, with fine sandy substrate, and maintained within a closed water circulation system, with artificial light, constant aeration, and continuous filtration with biochemical and biological Canister filters. Five animals/aquarium were established at a stocking density of 10 prawns/m², following pattern used by producers (5-10 prawns/m² for mixed populations). Four artificial havens (PVC tubes) were placed in each aquarium. For animal behavior and performance under periodic feed restriction to be analyzed, *M. rosenbergii* underwent the following treatments, with four replications: DFL – daily feed supply in the light phase; DFD – daily feed supply in the dark phase; AFL – alternating feed supply in the light phase; and AFD – alternating feed supply in the dark phase, for a 24-h period.

The laboratory comprised two rooms with artificial lights. Photoperiod was controlled by a 12:12 h light/dark cycle timer. In one room, the light phase occurred from 06:00 to 18:00 h, whilst the dark phase occurred from 18:00 to 06:00 h (natural photoperiod); the photoperiod in the other rooms was inverted. Consequently, the four experimental units in the light phase and the four experimental units in the dark phase could be monitored at the same time. Aquariums were illuminated with 32-W white fluorescent lamps for the light phase and with 15-W red incandescent lamps for the dark phase. This type of lighting was used due to the prawn's lack of reaction to it (Pontes and Arruda, 2005).

Prawns were individually weighed on a digital balance every ten days (T_0 , T_{10} , T_{20} , and T_{30}) to monitor growth. Animals were observed in each treatment during 30 consecutive days to record the species' behavior. They were given pellet feed, throughout the experiment, at 10% of their biomass/day from troughs (transparent acrylic trays), with 35% crude protein, twice a day, at 08:00 and 16:00 h, complying with the treatment applied.

Reports on behavior of prawns started after 10 days of adaptation to the physical conditions of the aquaria (Sick et al., 1973; Pontes et al., 2006) and after the establishment of social hierarchy (Fero et al., 2007). Types of behavior registered every day in each treatment comprised exploration, grooming, inactivity, crawling, swimming, excavation, burrowing, and agonism (Santos and Pontes, 2016).

Registration method consisted of scan sampling (Martin and Bateson, 2007; Yamamoto and Volpato, 2011), applied in windows, 15 min before and 15 min after feed for each experimental unit twice a day (light and dark phases) per treatment, with instantaneous reports every 60 s, during 30 consecutive days for daily feed, with a further 30 days for alternating feed treatments, totaling 60 days and 320 h of observation. Timetables for windows lay between 08:00 and 09:00 h and between 20:00 and 21:00 h – when two aquariums were observed during the light phase and two during the dark phase, between 16:00 and 17:00 h, and between 04:00 and 05:00 h. Reports had to be undertaken by two observers at the same time. Observers underwent a trustworthy test with 95% confidence for data observation. At the end of the experiment, biometry was performed, and lesions or amputated body structures, an indication of fighting incidents characteristics of agonistic behavior, were detected to evaluate the relationship between feed restriction and animal welfare.

The water quality of the aquaria was monitored daily. Water salinity was kept at 0 g/L (portable refractometer); pH at 7.85 ± 0.2 (pH meter); water temperature at 27.5 ± 1 °C (electrode thermometer); ammonia at 0.02 ± 0.06 mg/L, and dissolved oxygen above 5 mg/L (Instrutherm digital oximeter). Water quality remained constant throughout the observation period, at the ideal standard for the culture of the species (Valenti, 1998).

Feed intake, or rather, the difference between the quantity provided (dry weight) and the surplus in the trough (removed 2 h after placed in the water) was evaluated daily during the experiment. At the end of the experiment, surplus of each treatment was added so that the apparent intake of prawns could be calculated. Feed efficiency (FE) was determined by intake rates (Kureshy and Davis, 2002):

$$\text{Ingestion (apparent consumption)} = \text{feed provided} - \text{surplus}$$

$$\text{Feed efficiency} = \frac{\text{final weight} - \text{initial weight}}{\text{feed consumption}}$$

Weight gain was calculated at the end of the experiment with a two decimal digit precision scale. Weight gain, retrieved from rates obtained (Kureshy and Davis, 2002), determined specific growth rate (SGR) (Wu and Dong, 2002):

$$\text{Weight gain (g)} = \text{final weight} - \text{initial weight}$$

$$\text{SGR (\%} \cdot \text{day}^{-1}\text{)} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{number of days}} \times 100$$

Dead specimens were counted every day, in the morning, to calculate the final survival rate (SR), defined by Bautista-Teruel et al. (2003):

$$\text{SR (\%)} = \frac{\text{final number of prawns}}{\text{initial number of prawns}} \times 100$$

Statistical Analyses were performed with SIGMAPLOT 10.0 (2006). Depending on premises required for data parametricity (Normality – Kolmogorov-Smirnov; Homoscedasticity – Shapiro-Wilks) (Zar, 1999), results were analyzed with ANOVA or Kruskal-Wallis test. For significant differences, we applied the *post hoc* Tukey's test. A significance level of 5% was used to assess the results. Lesions or amputated body structures were represented through descriptive statistics, and SR was reported in the text.

Behavioral variables (exploring, grooming, inactivity, crawling, swimming, excavation, burrowing, agonism, and feed intake) were analyzed according to the model below, in which N is the total sample size, n_i is the size in each treatment, and R_i is the sum of the ranks of each treatment of dependent variable:

$$H = \frac{12}{N(N+1)} \times \sum_{i=1}^k \frac{R_i^2}{n_i} - 3(N+1)$$

Animal performance (weight, apparent intake, FE, and FCR) were analyzed with one-way ANOVA, according to the following model, in which K is the treatment number, N is the sample size, n_k is the amount of individuals per treatment, \bar{x}_k is the mean of dependent variable in a treatment, \bar{x}_{total} is the mean of dependent variable in all treatments, x_{ik} is the value of na individual of a treatment, $K - 1$ is the degree of freedom of the model, and $N - K$ is the degree of freedom of the residue:

$$F = \frac{\left(\frac{\sum n_k (\bar{x}_k - \bar{x}_{total})^2}{K - 1} \right)}{\left(\frac{\sum (x_{ik} - \bar{x}_k)^2}{N - K} \right)}$$

Then, in the *post hoc* Tukey's test, we followed the model:

$$HSD = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(\frac{\sum (x_{ik} - \bar{x}_k)^2}{N - K} \right) \times \left(\frac{1}{n} \right)}}$$

3. Results

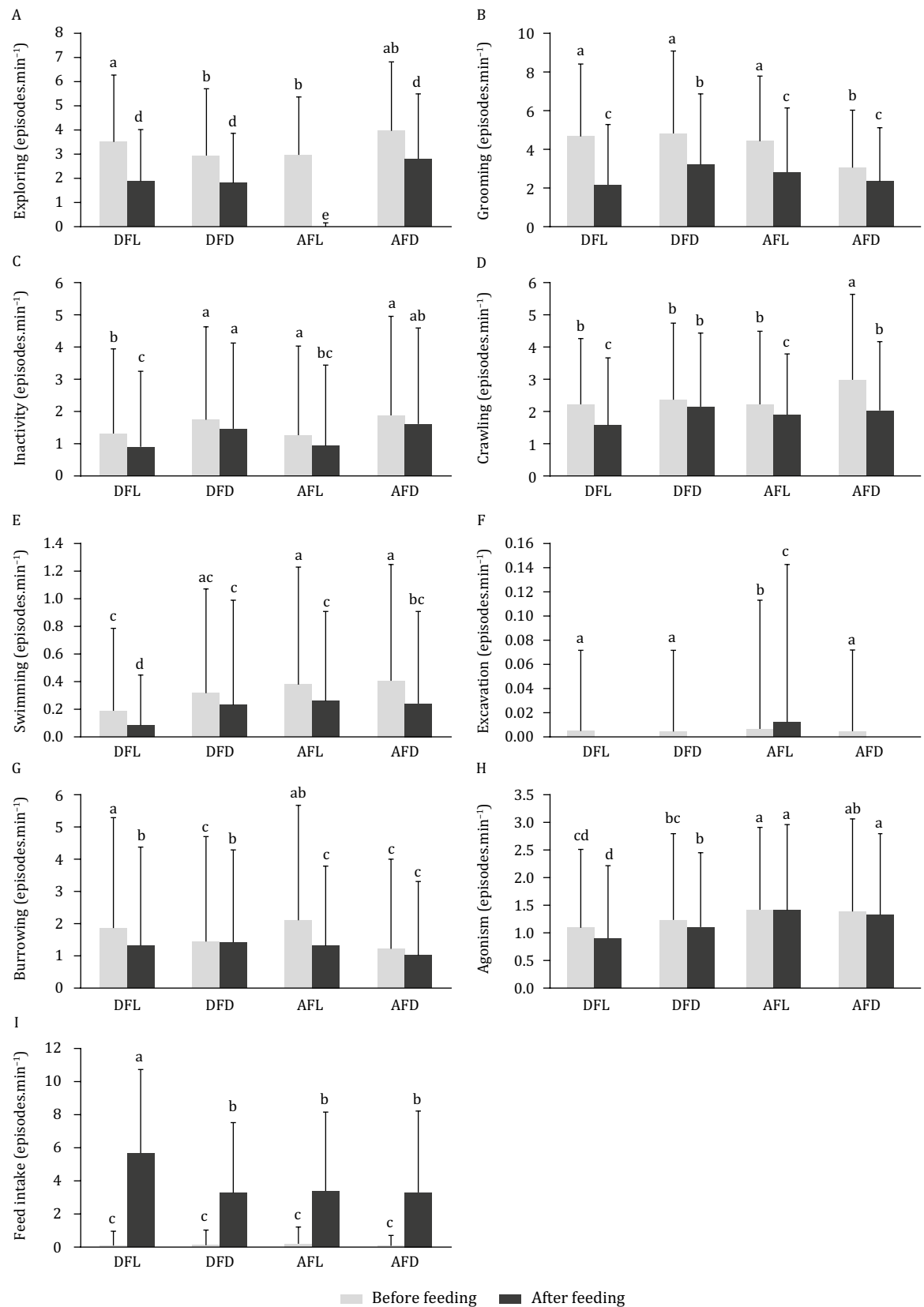
As a rule, after feed provision, we observed decreased exploration, grooming, inactivity, crawling, swimming, and burrowing ($P < 0.05$) (Figures 1A, 1B, 1C, 1D, 1E, and 1G, respectively) in all treatments. Exploration was more conspicuous prior to feeding ($H = 1813.054$; $gl = 7$; $P \leq 0.05$) with greater frequency rates when feed was provided at alternate days during the dark phase (3.95 ± 2.86) and daily during the light phase (3.50 ± 2.77) (Tukey's test at $P < 0.05$) (Figure 1A). Grooming behavior mainly occurred prior to feeding ($H = 633.533$; $gl = 7$; $P < 0.05$), especially in daily feed in light (4.72 ± 3.73), daily feed in dark (4.87 ± 4.30), and alternate feed in light (4.48 ± 3.36) ($P < 0.05$) (Figure 1B). Inactivity, crawling, and swimming had greater frequency rates prior to feeding for all treatments in the two phases (light/dark), with a decrease in frequency of such behaviors after feeding ($P < 0.05$) (Figure 1C, 1D and 1E). Crawling occurred more frequently prior to feeding ($H = 214.176$; $gl = 7$; $P \leq 0.001$), with greater scores for alternate feeding in dark phase (AFD = 3.02 ± 2.65) (Figure 1D). The above also occurred for swimming ($H = 163.135$; $gl = 7$; $P \leq 0.001$), mainly in treatments with feed restrictions (AFL and AFD) (Tukey's test at $P < 0.05$) (Figure 1E). Results evidence behavior changes triggered by feed during the light and dark phases for the 24-h period.

Excavation of substrate mainly occurred prior to feeding, during the day light phase, with no statistical differences ($H = 21.480$; $gl = 7$; $P = 0.003$; *post hoc* Tukey's test, $P < 0.05$), except for treatment with feeding on alternate days, during the day light phase (Figure 1F). Behavior burrowing in havens was observed in all treatments, with greatest frequency prior to feeding ($H = 101.938$; $gl = 7$; $P \leq 0.001$) and in the light phase (Tukey's test at $P < 0.05$) (Figure 1G).

Agonistic behavior (Figure 1H) was reported in all treatments, regardless of photoperiod (light/dark), with significantly higher scores ($H = 116.800$; $gl = 7$; $P \leq 0.001$) for animals with feed restriction (AFL and AFD) (Tukey's test at $P < 0.05$). Intake behavior prior to feeding was attributed to feed surplus on the substrate and seedlings in the experimental units. Higher intake rates were observed after feeding ($H = 2414.529$; $gl = 7$; $P \leq 0.001$), with higher means for treatment DFL, followed by AFL, DFD, and AFD (5.68 ± 5.08 , 3.38 ± 4.77 , 3.30 ± 0.23 , and 3.29 ± 4.94 , respectively) (Tukey's test at $P < 0.05$) (Figure 1I).

Lesions and amputations in all body parts of farmed prawns were reported in all treatments. Pereiopods, pleopods, antennae, and uropods were the body limbs most affected by agonistic events. Highest rates of occurrences of lesions and amputations were reported for animals fed on alternate days, during the dark phase (Figure 2).

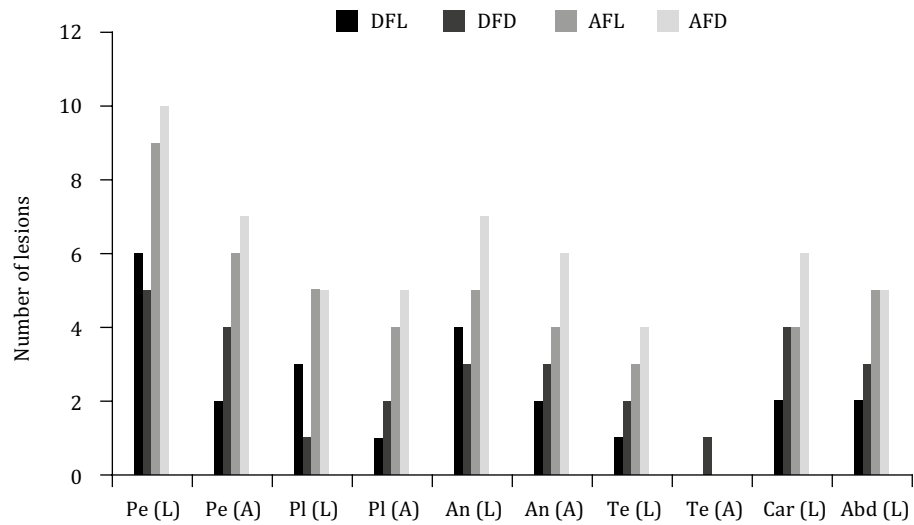
The above result is corroborated by a great number of agonistic events by animals with feed restrictions (Figure 1H), coupled with the common occurrence of autotomy (self-amputation as an animal defense



DFL - daily feed supply in light phase; DFD - daily feed supply in dark phase; AFL - alternating feed supply in light phase; AFD - alternating feed supply in dark phase.

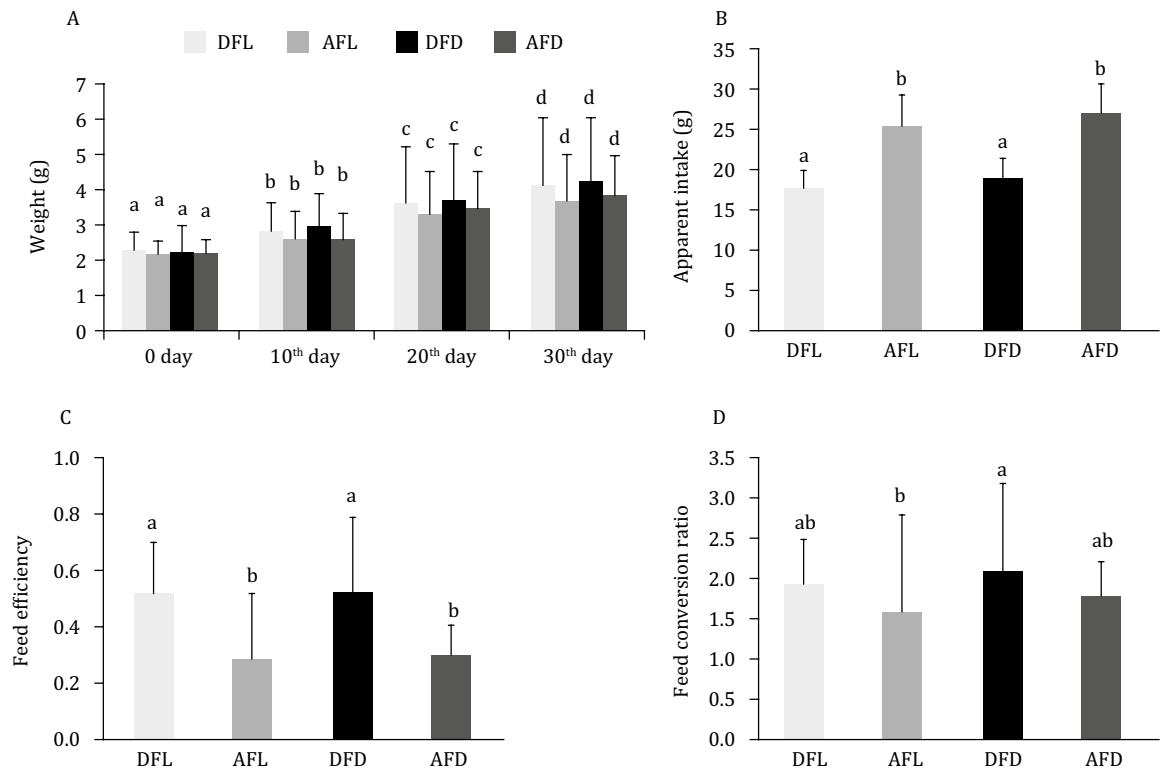
Different uppercase letters in the box plots denote significant differences (Mann-Whitney test at $P < 0.05$).

Figure 1 - Frequency (episodes.min⁻¹) of behavior of *Macrobrachium rosenbergii* prawns (mean \pm standard deviation) prior and after feeding in light and dark phases during a 24-h period.



DFL - daily feed supply in light phase; DFD - daily feed supply in dark phase; AFL - alternating feed supply in light phase; AFD - alternating feed supply in dark phase; Pe (L) - lesions on pereopods; Pe (A) - amputated pereopods; PI (L) - lesions on pleopods; PI (A) - amputated pleopods; An (L) - antennae/antennules with lesions; An (A) - amputated antennae/antennules; Te (L) - telson/uropods with lesions; Te (A) - amputated telson/uropods; Ca (L) - cephalothorax with lesions; Abd (L) - abdomen with lesions.

Figure 2 - Number of lesions and amputations in body structures of *M. rosenbergii* prawns subjected to different feeding treatments.



DFL - daily feed supply in light phase; DFD - daily feed supply in dark phase; AFL - alternating feed supply in light phase; AFD - alternating feed supply in dark phase. Different uppercase letters in the box plots denote significant differences (Tukey's test $P < 0.05$).

Figure 3 - Weight (g), apparent intake (g) (mean \pm standard deviation), feed efficiency, and feed conversion ratio of *M. rosenbergii* specimens subjected to different feeding treatments.

mechanism in conflict episodes). Although damages did not kill the animal, mutilated animals and animals with lesions depreciated the final product.

Full survival of animals was reported during the treatments under analysis, perhaps due to good water quality, feed amount, and the ability of prawns to acquire feed. There was a significant difference in the weight of animals every ten days of culture within the specific treatment. However, no difference between treatments was reported when they were compared on weighing days (T_0 , T_{10} , T_{20} , and T_{30}) (Figure 3A). An apparent higher intake (g) was detected after feeding provision in all treatments when animals were subjected to feed restrictions (ANOVA, $P \leq 0.001$) (Figure 3B), resulting in lower feed efficiency (ANOVA, $P = 0.859$) (Figure 3C) and FCR (ANOVA, $P = 0.222$) (Figure 3D). Although no statistical difference was detected, a trend for greater feed efficiency and SGR was observed for daily treatments (DFL and DFD).

4. Discussion

Results showed that a short temporary restriction (24 h) in feed during light or dark phases did not affect survival, weight, feed efficiency, and SGR of *M. rosenbergii*. Feed intake had a higher rate in treatments to which animals were subjected with feed restriction during the light and dark phases when compared with those without any restrictions. Prawns reached similar weight and specific growth and survival rates. Since there was no difference between those subjected or not to temporary feed restriction, *M. rosenbergii* demonstrated good feed benefits within all the experimental conditions. Compensatory growth may be defined as past growth achieved after a period of fasting or reduced feed rates. In the case of aquatic animals, the strategy has been under observation over time (Weatherley and Gill, 1987; Broekhuizen et al., 1994; Bull and Metcalfe, 1997; Rhaman et al., 2019) due to its implication on fish farming and investigation on the biological phenomenon. However, the literature on prawns and on invertebrates subjected to feed restrictions is rather scarce.

According to Maclean and Metcalfe (2001), animals subjected to feed restriction periods lose weight and growth is impaired. However, when feed is reestablished, the animals overeat and may achieve weight gain similar to those fed every day (control groups). In the case of shrimps, Maciel et al. (2018) reported that short fasting periods produced a greater efficiency in FCR for *Litopenaeus vannamei*, with decrease in phosphorus concentration in the water, due to excreta. Wu et al. (2001) reported that groups of *Fenneropenaeus chinensis* subjected to fasting periods and re-feeding had compensatory growth, appetite, and overeating. The authors reported a compensatory growth in all groups subjected to restrictions and that, due to their previous nutritional record, prawns may regulate their appetite and growth rate. Pontes et al. (2010) analyzed temporary replacement of feed to shrimp by feed for broilers in the farming of *L. vannamei* to decrease costs, at short time intervals, and did not detect any difference in performance. In other words, parameters were not affected by the feed regime.

Few papers deal with feed restriction in fresh water prawns. Malecha et al. (1989) suggested the occurrence of compensatory growth in *M. rosenbergii* when small prawns, subjected to space restrictions, were separated from the original population or transferred to low stocking densities. Li et al. (2008) tested feed restriction in *Macrobrachium nipponense* for 18 days in a laboratory and reported that animals with two- and four-day feed restriction had similar weight as those fed daily (control). Further, the authors observed that animals with feed restrictions decreased oxygen consumption and increased feed conversion efficiency as a compensatory effect.

Marques and Lombardi (2011) evaluated compensatory growth in post-larvae *M. rosenbergii* specimens cultivated at high densities (50, 400, 800, and 1200 prawns/m²) in net tanks during 244 days and reported that prawns farmed within the highest density showed a visible compensatory growth when compared with prawns in a density of 800 animals/m². Ghosh et al. (2018) reported that *M. rosenbergii*'s growth rate increased significantly when the animals were subjected to a 50% feed restriction, albeit with significant effects on survival rate. More recently, Rahman et al. (2019) found that feed restriction two days a week would not hamper the growth of *M. rosenbergii*, and followed by proper refeeding,

feed could be restricted three days a week in improved traditional prawn culture. In addition, feed restriction does not hamper final production and showed a negligible reduction in protein content in body composition as well as better feed efficiency.

The current analysis demonstrated that prawns did not show any difference in final weight, feed efficiency, and SGR. Or rather, animals subjected to temporary feed restriction (fed on alternate days) had the same growth rate to those fed daily either in the light or in the dark phase of the 24-h period. El Ghazali et al. (2009) insisted that to obtain a compensatory growth, two phases are needed: a first stress phase during which animal growth is reduced, followed immediately by the second phase during which the growth depression disappears, allowing the resumption of growth, but at a rate below the animal's physiological capacity (compensatory growth). However, total or partial compensation depends on several factors, such as severity and duration of restriction, development stage of the organism, and re-feeding pattern (Wilson and Osbourn, 1960; Ryan, 1990).

Studies on the effects of fasting and re-feeding on the growth of prawns have revealed that hyperphagia is a mechanism that triggers compensatory growth (Wu et al., 2001). Current experiment shows that prawns increased feed intake after diet provision. Intake is greater when animals fed daily during the light phase and may indicate that it is the preferential phase for diet provision. Since there is no difference in animal indexes among restriction and non-treatments, labor costs may be decreased through diet provision in alternate days. Barki et al. (1991) observed that *M. rosenbergii* prawns compete for resources (feed, haven, and access to partners for mating) and that size and morphotype of males affect dominance hierarchy. More recently, Rahman et al. (2019) found that when feed is provided, mainly to groups subjected to temporary feed restriction, competition among the animals ensued.

Agonistic behavior mainly occurs in treatments when feed is given at alternate days, prior to and after feed provision, during the light and dark phases. The above indicates that feed restriction escalates the aggressiveness of *M. rosenbergii* and may interfere in the quality of the final product. These data are corroborated by Short (2004) and Balasundaram et al. (2004) who stated that *Macrobrachium* prawns are nocturnal, aggressive, and territorial, preferring havens during the day. As a rule, animals compete to achieve resources (havens, feed, and mates), whilst one of the most obvious mechanisms to this end is aggressiveness (Martin and Moore, 2007).

Specimens in all treatments seek havens during the light day period. Havens are relevant resources to increase access to mates and to minimize aggressive encounters and predation. They provide protection to subdued animals in the presence of dominant animals (Englund and Krupa, 2000; Martin and Moore, 2007; Alcock, 2011). Current authors reported that, prior to feed provision, the animals showed higher behavior rates, such as grooming, excavation, and crawling. According to Bauer (1989), several decapod crustacean species spend much time and energy in grooming. High exploration and crawling rates prior to feed provision are natural seeking for food. These types of behavior have been detected for the prawn species *L. vannamei* (Pontes et al., 2006). Feeding occurred in the light and dark phases and corroborated current results. Nunes et al. (1996) and Pontes and Arruda (2005) reported that prawns of the species *Farfantepenaeus subtilis* and *L. vannamei*, respectively, fed during the day and during the night.

Feed restriction in species with territorial behavior may trigger serious conflicts, with loss of limbs and even death. The current research showed that, although no death occurred in any of the treatments, lesions and amputations were common, especially in animals subjected to temporary feed restriction. Karplus et al. (1989) reported that the lack of chelae (second pair of pereopods) suppressed aggressiveness in adult *M. rosenbergii* and *M. amazonicum*, respectively. The authors detected that agonistic behavior in almost all reports was performed by the second pair of pereopods. Karplus et al. (1992) reported a significant increase in the survival of male *M. rosenbergii* featuring orange-colored and transparent chelae when dominant males (blue chelae) did not have any chelae. Brugiolo et al. (2007) suggested that the removal of chelae in *M. rosenbergii* is viable in commercial prawn farms, even though it entails more technical labor.

The data above show that repeated cycles of short fasting and feeding may be a useful protocol to improve the use of feed and to decrease labor costs in feed management. Field studies with *M. rosenbergii* populations in monosex farming may elucidate other aspects on the physiology, behavior, and growth of the animals.

5. Conclusions

Short feed restriction with feed provision on alternate days may be employed without any impairment to growth and survival of the animals. Apparent intake of feed increases after provision on alternate days, coupled to low feed conversion ratio and specific growth rate during the light and dark phases. It should be highlighted that increase in aggressive behavior and, consequently, in the number of lesions and amputations indicates low welfare conditions of *M. rosenbergii* and depreciation in the quality of the final product.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: C.S. Pontes, M.F. Arruda and D.B. Santos. Data curation: D.B. Santos. Formal analysis: C.S. Pontes, M.F. Arruda and V.G.S. Santana. Investigation: M.F. Arruda, V.G.S. Santana and D.B. Santos. Methodology: C.S. Pontes and D.B. Santos. Project administration: C.S. Pontes

Acknowledgments

The present work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES) – financing code 001.

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