

# Compensatory growth and feed restriction in marine shrimp production, with emphasis on biofloc technology

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Abstract In Brazil, studies and production of penaeid shrimp in a biofloc technology (BFT) system are recent, but the results point to a promising future. Research with feed restriction inducing compensatory growth in shrimps has been shown to be a technique that allows a saving of around 25% in the use of feed for shrimp production. It also allows the reduction of costs with salaries and adapts shrimp farming to the world demand for environmentally friendly production, with the reduction of nitrogen and phosphorus levels in its effluents, as well as lower water use in shrimp farming. In crustaceans, it has been shown that after a period of feed restriction, the animals show a pronounced compensatory growth when they return to a sufficient food source. Studies with the penaeid shrimp Litopenaeus vannamei reported the ability of the species to obtain a complete compensatory growth after short feeding periods (1) to 3 days) followed by feeding; These short periods of fasting presented a greater efficiency in the feed conversion besides the decrease in the concentration of phosphorus present in the aquatic environment, coming from the excreta. The adoption of a restriction program in the feeding using BFT may contribute to a reduction in operating costs, reduction of metabolic nutrients dissolved in water, and, consequently, an increase in the number of cycles in which the same water can be reused for production reducing production costs and improving productivity indices in shrimp farming.

Keywords Aquaculture · Fasting · Litopenaeus vannamei · Penaeid shrimp

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## Introduction

Fasting is a practice experienced by various aquatic organisms throughout their life cycle. Wu and Dong (2002a) reported that this food discontinuity depends on factors such as water quality, environmental condition, and even the presence of pathogens in the aquatic environment.

In aquaculture activities, food restriction is a technique considered as stressful, but it has been used as a strategy to guarantee the economy in the use of ration, as well as in the reduction of costs with labor. It is believed that food restriction also contributes to the current worldwide demand for environmentally friendly production, reducing nutrient concentrations (e.g., nitrogen and phosphorus) and organic matter in its effluents (Zhu et al. 2016). Moreover, it is believed that compensatory growth (rapid growth after a period of feed restriction) can be fully achieved by the animals returning to the normal feeding condition (Zhu et al. 2016). According to Fóes et al. (2016), the economy of approximately 25% in feed use could be obtained with food restriction followed by compensatory growth in marine shrimps.

Shrimp farming is the sector within aquaculture that has the highest growth in production, with emphasis on the marine species *Litopenaeus vannamei*, commonly known as the Pacific white shrimp, a euryhaline species, which is able to tolerate salinities from 0 to 50% and is the most cultivated in the world (FAO 2014).

Despite the success of shrimp farming, the activity was considered an environmental villain due to the destruction of natural environments (e.g., marshes, swamps, estuaries, and other coastal environments), the construction of ponds, and the release of untreated effluents into recipient bodies (Crab et al. 2012). In addition, several episodes of considerable losses have been reported in recent years. These losses are mainly related to the pathogen action and depletion of water quality in farming environments (De Schryver et al. 2014).

The search for a sustainable technology and biosafety resulted in the development of shrimp farming in a closed superintensive system, with intense aeration, rich in suspended material, without water renewal, using smaller areas of breeding and utilizing the microbiota present in the environment (by manipulation of the carbon:nitrogen ratio) to control and decrease toxic inorganic nitrogen levels, and as a food source. Such a culture system was called biofloc technology (BFT). Other terms have been created to denote such a system as "zero-exchange aerobic heterotrophic system" (ZEAH) and cultivation in heterotrophic medium (Crab et al. 2012; Avnimelech 2014; Krummenauer et al. 2014). More specifically, the mentioned characteristics of the BFT system allow the formation of macroaggregates, consisting basically of microalgae, nitrifying and heterotrophic bacteria, protozoa, rotifers, metazoans, exoskeletons, feces, exudes, and remains of dead organisms, which are predominantly a heterotrophic and aerobic biota called biofloc (Schryver et al. 2008) that develop in an environment rich in carbon and nitrogen, which are characterized by microorganisms rich in protein and other nutrients (Asaduzzaman et al. 2008; Gao et al. 2012; Krummenauer et al. 2014). As mentioned, the socalled bioflocs are produced naturally, vary in different systems, and can be supplemented as a food source for cultivated organisms like penaeid shrimp (Kuhn et al. 2009; Emerenciano et al. 2012), tilapia (Avnimelech 2014), and mussels (Ekasari et al. 2014).

Historically, BFT shrimp farming is an innovative system of shrimp production developed in the USA in the 1990s, adjusting shrimp farming to increasing environmental pressure to reduce water use (Hopkins et al. 1993). The investigations were focused on the production of *L. vannamei* with minimal use of water changes and even reuse of water for several cycles, aiming also to reduce the introduction, infestation, and dissemination of viral and bacterial epidemics (Lara et al. 2012; Krummenauer et al. 2013). The minimum water exchange in the system is guaranteed by the recycling of the nitrogenous compounds carried out by the bacteria present in the aquatic environment (Avnimelech 2014).

In Brazil, studies and production in BFT are recent, but the results point to a promising future (Wasielesky et al. 2006; Poersch et al. 2006; Emerenciano et al. 2012). In cities that are not in a coastal area, the demand and value for marine food are high. This commercial factor compensates the installation of this system (using low salinity) in areas far from the valued coastal region (Maciel 2013).

Studies by The World Bank (2013) indicate that by the year 1980, only 10% of fishmeal and fish oil produced in the world were consumed by aquaculture, but in 2010, this percentage rose to 73%, and by 2030, it will be imperative to improve food efficiency in the consumption of fish meal for the production of animal feed.

Like all productive activities, scaling up has a detrimental effect on the environment. A productive company should aim at optimizing the use of natural resources and production, producing more and better qualities, reducing environmental impacts, and using the minimum natural resources necessary for its production (Américo et al. 2013)—aquaculture with less use of animal resources for its production, less use of water (non-renewable resource), and with a lower effluent level (Souza et al. 2003).

In this sense, the studies have been conducted to show a real efficiency of the biofloc in reducing the amount of fish meal and oil in the rations for shrimp farming (Scopel et al. 2011; Emerenciano et al. 2012).

# Biofloc as a food, compensatory growth, and productivity in marine shrimp farming

Studies have shown that the use of biofloc as a food contributed significantly to the growth of *L. vannamei*. Emerenciano et al. (2012), working with a nursery of *Farfantepenaeus brasiliensis*, reported the beneficial role of biofloc by improving water quality and shrimp growth when compared to clear-water treatment. These authors concluded that the rich microbiota, mainly protozoa and rotifers, contributed to better shrimp performance, corroborating Thompson et al. (2002) working with *Farfantepenaeus paulensis* juveniles ( $\sim 0.14$  g). Kuhn et al. (2009) described that diets containing biofloc (7.8 to 15.6%) can be used contributing to the growth and survival of *L. vannamei* juveniles ( $\sim 0.44$  g). These data were supported by Bauer et al. (2012) using 3.5 to 14% of biofloc in the diets of *L. vannamei* juveniles ( $\sim 2.5$  g). On the other hand, diets with biofloc inclusion above 25% resulted in lower growth of *L. vannamei* juveniles ( $\sim 0.59$  g) and the authors attributed the result to a restriction of the amino acids lysine and methionine and the high ash content in the dry biofloc. Probably, the trace elements present in the biofloc could generate some toxic effect to the animals (Gamboa-Delgado et al. 2017).

But what is the good thing about using biofloc as a food source? We can describe several positive points collected from successive researches. Chamberlain et al. (2001) and Kuhn et al. (2010) described the high crude protein (CP) content in the biofloc (35 to 51 and 38.8 to 40.5%, respectively), while Emerenciano et al. (2012) and Xu and Pan (2012), also working with *L. vannamei* in BFT, reported CP = 30.4 and 27.3 to 31.6%, respectively. Xu and Pan (2012) also highlight the biofloc lipid content from 3.7 to 4.2%, and in addition to supplemental microbial nutrition, these authors reported the production of extracellular enzymes by

the biofloc facilitating feed utilization and digestion in shrimps. In addition, Ferreira et al. (2015) stated that microbial biofloc can be a source of probiotic Gram-positive bacteria of the genus *Bacillus* spp., which is very effective in controlling the opportunistic bacteria of the *Vibrio* genus. Avnimelech (2014) also explains that the probiotic effect is due to a biopolymer (poly- $\beta$ -hydroxybutyrate) stored in microbial cells that can depolymerize, releasing butyric acid, an antimicrobial agent. Another interesting point is the biofloc size. According to Ekasari et al. (2014), biofloc > 100 µm presented the highest levels of protein (27.8%) and lipids (7.5%), while biofloc < 48 µm seemed to be the richest in essential amino acids.

In this way, BFT shrimp farming may allow lower feed utilization and also lower crude protein levels, maintaining the quality of the water medium by the absorption of nitrogenous compounds, allowing the same water to be reused for several consecutive production cycles. In addition, new studies with shrimp feed restriction indicate that productive management with periods of no feeding may be a trend in shrimp farming soon (Crab et al. 2007; Wu and Dong 2002a).

Due to spatial and temporal irregularities of food resources in the natural environment, aquatic animals may encounter periods of fasting or malnutrition during their life cycle (Wu and Dong 2002a). This adaptation to food deprivation induces metabolic responses that may vary according to factors such as life stage, seasons, environmental conditions, temperature, and nutritional status of the animals before the period of food deprivation, influencing biological adjustment by increasing or decreasing the effect of feed restriction on animals (Sheridan and Mommsen 1991).

Growth restriction during the period of food deprivation or fasting can be followed by a rapid growth phase when food is restored, known as compensatory growth (Farbridge et al. 1992). This compensatory growth is usually related to an increase in the rate and efficiency of weight gain during the recovery period (Dobson and Holmes 1984).

Compensatory growth may be an internal adjustment mechanism for animals to adapt in many situations of dramatic variations in the environment: It allows the animals to survive through a period of feed restriction and then to experience an accelerated growth trajectory under normal conditions (Ali et al. 2003; Wasielesky et al. 2013). Body energy reserves, such as lipids, have been considered as responsible for the induction of compensatory growth. Decrease of body lipids and proteins depends on different factors such as the species, size, development phase, duration of the food restriction, and nutritional quality of the food ingested, among others (Wang et al. 2000).

Among aquatic animals, compensatory growth has been most commonly studied in fish: hybrid sunfish (Hayward et al. 2000); several species of fish (Ali et al. 2003); Nile tilapia *Oreochromis niloticus* (Wang et al. 2009; Ali et al. 2016); gilthead sea bream *Sparus aurata* (Bavcevic et al. 2010; Peres et al. 2011), Asian catfish *Pangasius bocourti* (Jiwyam 2010); little research has been done with mollusks, such as scallop *Argopecten irradians* (Auster and Stewart 1984), and crustaceans, such as Chinese shrimp *Fenneropenaeus chinensis* (Wu et al. 2000, 2001a, b), Pacific white shrimp *L. vannamei* (Wasielesky et al. 2013; Zhu et al. 2016), and crayfish *Cherax quadricarinatus* (Stumpf and Greco 2015).

Studies have shown that during the absence or reduction of food, fish use hormonal and metabolic strategies to survive, causing a decrease in the size of the gastrointestinal tract and liver as a consequence (Souza et al. 2003). When the feed is normalized, the physiological processes are reestablished. Fish use food primarily to meet energy needs in maintaining vital processes by restoring tissue catabolism, and only after that, the rest is used for growth (Souza et al. 2003).

Studies on feed restriction in fish demonstrate that a feedback strategy with hyperphagia can restore normal fish growth after a period of decreased weight gain and length, which may be more pronounced the longer the feed restriction time (Arauco and Costa 2012). This survival strategy can be used to obtain better zootechnical indices for fish farming, because studies indicate that the adoption of joint strategies of feeding practices that meet the nutritional requirements of fish can maximize the effect of compensatory growth.

In crustaceans, it has been shown that after a period of feed restriction, they exhibit a pronounced compensatory growth when they return to a sufficient food source (Wu et al. 2000, 2001a, b). But what is the limit to this compensation? Wu et al. (2001a), working with juveniles of *F. chinensis* ( $\sim 2.189$  g) during 10 days of feed restriction (fed 4 to 12% of body weight per day) and then feeding them ad libitum under a 30-day recovery period, reported that 12% of the group was able to regain body weight in comparison with the control group. Cycles of starvation and refeeding, such as 1:4, 2:8, 4:16, and 8:32, were tested by Wu and Dong (2002a), and the authors reported that the shrimp subjected to the different cycles had greater weight gain than the control group (fed ad libitum continously). However, after 32 days of experiment, none of the cycles reached the same body weight as the control group.

In studies with *L. vannamei*, Lin et al. (2008) reported that this species was able to obtain a complete compensatory growth (3 to 9 days) from the reestablishment of feeding after short periods of fasting (1 to 3 days). Likewise, studies with *L. vannamei* in China found that feed conversion efficiency and mean protein efficiency for groups of shrimp subjected to short periods of feed restriction (1 to 3 days) were significantly better than those of the control groups, suggesting that after short periods of feed deprivation, with return to normal feed, there is potential to increase digestion and nutrient uptake by shrimp (Zhu et al. 2016). Comoglio et al. (2004) reported that *L. vannamei* (~ 0.998 g) was able to survive 16 days without food and after 9 days of fasting, the survival decreased to 65%. In another study, *L. vannamei* (~ 8.18 g) had been starved for 7 to 28 days with survival from 100 to 59% and lost of body weight of 3.2 to 10.4%, respectively. Shrimps starved for 14 days had three stages of modulation of gene expression related to immunity (Lin et al. 2012).

Some specific genetic aspects and physiological responses related to low feeding have been described. Gene expression induced by starvation and refeeding also changes muscle metabolism and growth rate and sometimes can impair muscle growth (Hornick et al. 2000; Hagen et al. 2009). Thus, methods for maximizing growth have been tested for many years in aquaculture, i.e., the use of fasting conditions that result in low growth rate followed by refeeding, when many organisms attempt to accelerate the growth rate (Hornick et al. 2000). This accelerated growth is identified by being significantly faster than the growth rate of those individuals that have not experienced growth depression and have been kept under the same conditions (Nikki et al. 2004).

Muscle growth is also controlled by the expression of myostatin (Mstn), known as growth and differentiation factor-8 (GDF-8), member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily of proteins. However, Mstn is known to inhibit muscle differentiation and growth during myogenesis of vertebrates and functions as a negative regulator of skeletal muscle growth (McPherron et al. 1997). In invertebrates, Mstn has also been shown to restrict muscle growth in the fly *Drosophila melanogaster*, scallop *A. irradians* (Kim et al. 2004), and sea cucumber *Apostichopus japonicus* (Li et al. 2016). In crustaceans, Mstn has been cloned from the lobster *Homarus americanus* (Kim et al. 2009), the crab *Gecarcinus lateralis* (Covi et al. 2008), and the shrimp *Penaeus monodon* and *L. vannamei* (De Santis et al. 2011; Qian et al. 2013, Zhuo et al. 2017). In the shrimp *P. monodon*, the Mstn gene was widely expressed across all tissues under investigation including the muscle, hepatopancreas, eyestalk, heart, gill, and stomach and presented a high concentration in the muscle after the molt cycle of shrimp (De Santis et al. 2011).

According to Wu and Dong (2002a), the activity of molting and growth pattern in crustaceans may be related to feeding. Apparently, compensatory growth to some extent contributes to increased frequency of ecdyses by hyperphagia after periods of food restriction. These same authors, studying compensatory growth responses in Chinese shrimp juveniles, *F. chinensis*, under different temperatures and different diets, found that the period between ecdyses was strongly influenced by food restriction (Wu and Dong 2002b). Also, the consequence of the natural discontinuous growth of crustaceans can be separated into two moments: the first would be the increase in the frequency of ecdyses, that is, in each molt, the old exoskeleton is expelled, and a rapid and extensive growth occurs during a short period before the new exuviae become hardened; the other is the period between ecdyses, during which the exuviae are hard and growth is limited.

In the case of food restriction in a biofloc system, Lara (2016) found high productivity indices in the establishment of *L. vannamei* in a biofloc system during 60 days of cultivation. The study was carried out with six different treatments with a density of 400 individuals/m<sup>2</sup>, varying the feed restriction regimes using fixed feed conversion rates. The period of feed restriction and feedback did not affect shrimp growth in the different treatments. There were no statistically significant differences in survival among treatments (means above 95%). Likewise, no significant differences were found regarding the final weight, weekly growth, and final yield among treatments with higher feed intake. This indicates that a savings of 25% in the production of shrimp in the biofloc system can be obtained by reducing the use of artificial feed.

In another study, Lara (2016) demonstrated that juvenile shrimps  $(1.14 \pm 0.38 \text{ g})$  produced in a biofloc system with artificial feed restriction techniques, with eight different feeding rates, using low feeding rates and divided into two periods, with 21 days of artificial food restriction, and with 29 days with artificial feedback in biofloc showed a partial compensatory growth in the second period and high survival (averages over 95%), resulting in a savings of 24.79% in artificial feed.

Foés et al. (2016), working with the post-larvae of 20 days (PL20) of *L. vannamei* in the nursery stage, using high storage density (2000 PL/m<sup>2</sup>), tested two different treatments, one long-term of 144 days and one short-term (traditional nursery) of 18 days. Many advantages can be described by using nurseries, such as storage of larger and healthier juveniles in grow-out ponds, shorter periods of shrimp culture with higher growth rates (Cavalli et al. 2008), and lesser risk of disease introduction and mortality (Samocha et al. 2000). Thus, Foés et al. (2016) sought to study the zootechnical indices for *L. vannamei* and their compensatory growth after being released in the definitive culture tanks. In this study, it was demonstrated by the values of specific growth rates that after the transfer to the final grow-out ponds with lower storage density, the shrimp presented compensatory growth after a long period of being subjected to stressful storage in the nursery phase. The growth rates were similar to those obtained in traditional cultures without subjecting the PL to induced stress. This study demonstrated that *L. vannamei* can be maintained in nurseries for long periods of storage at high densities without production losses, since its compensatory growth can be activated when later maintained in a less stressful environment.

As for the environmental issue, Zhu et al. (2016) reported that short periods of fasting caused a decrease in the amount of feces and decrease of phosphorus in the water by leaching of unconsumed feed. After feeding, these short periods of fasting also showed a higher feed

conversion efficiency, which resulted in a decrease in the phosphorus discharge in the water through the feces when compared to the control group.

These same authors concluded that the establishment of a protocol of feeding cycles with short periods of food restriction has a positive potential in the environmental issue for shrimp farming, since the adoption of a feeding protocol with short periods of fasting can reduce the contribution of nitrogen and phosphorus in the water without apparently affecting the growth of *L. vannamei*. These results suggest that the use of feeding and fasting cycles, triggering compensatory growth in the shrimp, has a positive effect both from the point of view of production, improving feed conversion, and from the environmental point of view.

#### Final considerations

Marine shrimp farming in a biofloc system is a viable reality, technically and economically proven. However, more studies are needed to achieve greater productivity and greater knowledge, so that producers can be assured of an economic activity capable of generating jobs and income with the lowest risks.

The adoption of short periods of feed restriction on shrimp farming in biofloc, such as on Saturdays and Sundays, can save 25% on feed costs, apart from the savings with staff payroll, since there is no need to maintain a full staffing schedule for feeding activities and other daily activities in a large-scale shrimp culture, and it may be possible to have a simple system of on-call staff with a reduced status, which in Brazil can mean a reduction of 10% in overtime payments and payroll charges on Sundays, besides reducing costs.

Thus, the adoption of a restriction program on feeding of marine shrimp in a biofloc system may contribute to a reduction in operating costs and reduction of metabolic nutrients dissolved in the water and can lead to a reduction in the number of cycles in which the same water can be reused for consecutive productions.

According to Kuhn et al. (2010) bioflocs can be a suitable ingredient in shrimp feed. This option may offer the shrimp industry a means of mitigating the impacts of aquacultural wastes while producing a substitute for traditional proteins. Furthermore, produced bioflocs could potentially be used as a feed-grade ingredient for a different class of aquatic species, so the added benefit of identifying a suitable replacement for fish meal could ease the pressures on wild fisheries.

Studies on food restriction and compensatory growth in shrimp culture in biofloc could allow an economic increase and become a great ally of socio-environmental advocates, with reduction in operating costs, less use of inputs and pressure on hired labor, and, above all, it may enable future studies in relation to the food and nutritional capacities of the organisms forming the bioflocs and their use as feed for the target species.

According to Avnimelech (2014) and Emerenciano et al. (2012), differences in microorganism profiles or microbial assembly, their benefits for nutrition, microbial ecology of bioflocs, and nitrogen assimilation rates using different carbon source (e.g., glycerol) are knowledge gaps and certainly deserve more investigation. Xu and Pan (2012) believe that further research is needed to better understand pathways and mechanisms of biofloc effects on the nutrition physiology of shrimp and how the bioflocs can be manipulated to maximize shrimp production performance. Last but not the least, Avnimelech (2014) emphasizes the importance of research on biofloc composition under different conditions and the possibility of biofloc manipulation to achieve the desired nutritional content as a supplement.

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