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The Effect of Molasses in Cultivating Vanamei Shrimp (*Litopenaeus vannamei*) by a Shrimp Cultivation System without Water Changes on Conventional Feed Decrease

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Abstract

The primary challenge in developing the intensive hatchery of white shrimp (Litopenaeus vannamei) farming industry is the accumulation of nitrogen and organic toxins. The shrimp pond unit's feed serves as the source of inorganic nitrogen. This study investigated the effect of molasses in cultivating vaname shrimp (Litopenaeus vannamei) by a shrimp cultivation system without water changes on conventional feed decrease. This was done because molasses has the potential to stimulate the growth of heterotrophic bacteria in shrimp pond units without air exchange. In addition to their role in controlling inorganic nitrogen, heterotrophic bacteria also serve as a natural food source for the shrimp in the pond unit. This study employed an experimental method, testing five treatments using molasses and varying the concentration of conventional feed. This study found that the best treatment was a combination of molasses and 75% conventional feed, with a C:N ratio of feed = 20.0 : 1.0. This study found that the rate of decreasing natural feed provision had a significant effect on shrimp growth rate, feed conversion ratio, and quality parameters in the model without air exchange.

Keywords: *Feed reduction; Heterotrophic bacteria; Model without water exchange; Molasses; C:N feed*

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INTRODUCTION

One way to control inorganic nitrogen in aquaculture models is to change the C:N ratio of the feed. This can be done by watching the growth of heterotrophic bacteria (Burford et al., 2003). In fish experimental tanks, adding carbon materials can lower the amount of inorganic nitrogen (Avnimelech, 2019; Avnimelech, 2018) while producing single-cell protein for fish (Avnimelech et al., 2009; Avnimelech & Mokady, 2018). The addition of carbon materials can reduce the cost of pumping and feeding (Avnimelech et al., 2012; 2014; Kochva et al., 2014; Avnimelech, 2019; Decamp et al., 2003). Sources of carbon materials used in ponds include sorghum and wheat flour (Avnimelech et al., 2014). Stuart et al., (2009) maintained Penaeus monodon tiger prawns in a culture model without water changes, using daily carbon sources in the form of tapioca flour to increase the microbial community and improve the water quality in the culture unit.

Rosenberry (2001) stated that Shrimp metabolic products, such as urine and feces are consumed by bacteria cultivated in shrimp pond units using a model without water exchange; in turn, the shrimp consume them. Also, in shrimp ponds that don't exchange water, heterotrophic communities form bacterial cell flocs (Hoch & Kirchman, 2015; Johnsen et al., 2013; McIntosh, 2000; Chamberlain, 2001). Bacterial cell flocs can be found by itself or in combination with feed particles or clay (Harris and Mitchell, 2013; Avnimelech et al., 2019). Shrimp, especially white shrimp, can directly consume bacterial cell flocs, which are rich in protein, amino acids, and certain microelements (Ritvo et al., 2018; Lopez et al., 2002; McIntosh et al., 2000; Tacon et al., 2002). By consuming these bacterial cell flocs, shrimp are able to efficiently recycle pond nutrients into shrimp biomass and improve their nutrition (McIntosh, 2000; Hopkins et al., 2015; Thakur and Lin, 2003). Rosenberry (2001) stated that the non-water exchange shrimp culture model produced ten times more white shrimp than conventional semi-intensive pond units and forty times higher white shrimp production than conventional extensive ponds.

Avnimelech et al., (2014) observed that bacterial biomass consumption was significant because without lowering the fish development rate, the feeding rate could be lowered by 66% of the control. Therefore, the purpose of this study was to examine the impact of using molasses on the rate of feed reduction. Therefore, This study investigated the effect of molasses in cultivating vanamei shrimp (*Litopenaeus vannamei*) by a shrimp cultivation system without water changes on conventional feed decrease.

METHOD

Time and Place of Research

This research was conducted for two months, namely from April to May 2023 at the shrimp hatchery in Pantai Cermin sub-district, Serdang Bedagai district, North Sumatera province.

Tools and Materials

The tools used in this research included: a tank made of fiberglass with a capacity of 160 and oval in shape as a container for shrimp maintenance, equipped with aeration equipment (three aeration stones for each experimental unit), a water quality checker in the form of Horiba Model U-10, digital scales, equipment for bacterial culture, stationery and a laptop. The water was aerated with aeration stones and fertilized two weeks before the shrimp were spread to grow natural feed.

Research Procedure

Shrimp Rearing Experiment

The method used in this study was an experimental method with five treatments tested, including: treatment A (without using molasses with 100% conventional feed); treatment B (treatment using molasses with 25% conventional feed); treatment C (treatment using molasses with 50% conventional feed); treatment D (treatment using molasses with 75% conventional feed); and treatment E (treatment using molasses with 100% conventional feed). There were three replications for every treatment, and each treatment's tank allocation was chosen at random method. The tanks were aerated with three aerators suspended in the water column. In order to replenish water lost through evaporation, two liters of fresh water (tap water) were added each week to keep the 160 liter tank's capacity constant.

Each tank was stocked with 4 juvenile whiteleg shrimp (*Litopenaeus vannamei*) or equivalent to a shrimp density of 30 shrimp m2 (Csavas, 2014; Allan & Manguire, 2012). The average individual weight at the time of stocking was 5.014 ± 0.336 grams and The commercial shrimp feed, which was a product of a Taiwan company, was fed to the shrimp with a crude protein content of 38 %. In accordance with the feed company's instructions, the feeding rate used in the experiment was 5 % of body weight every day during the trial or known as conventional feed) and the amount of molasses given in each treatment.

Commercial feed (a product of the Taiwan Company) with a crude protein level of 38 % was utilized. For the duration, the standard feeding rate was 5 % of body weight daily (as advised by the feed provider). The amount of conventional feed was the amount of feed recommended by the feed company and was given three times a day (at 06.00 A.M, 12.00 A.M and 06.00 P.M). The amount of molasses (2.669 grams per gram of feed) was added daily to meet the C: N ratio of the feed to 20.0: 1 (McIntosh, 2000; Monroy et al., 1999). Molasses was used once a day, given every morning.

The main carbon sources in this experiment came from feed and molasses, whereas feed was the primary source of nitrogen. Furthermore, the nitrogen content of the feed was determined by assuming that 30 % of the feed protein contains 4.65 % nitrogen (Avnimelech, 2019). Therefore, While the carbon content of the feed and molasses utilized in this study were 38.5 % and 29.71 %, respectively, the nitrogen content of the feed with more than 30 % protein was estimated by conversion using this assumption. The amount of molasses required per 1 gram of feed in each treatment was 0.00 grams; 1.68 grams; 2.17 grams; 2.67 grams; and 3.16 grams for each treatment.

Water Quality Measurement and Parameters

A water quality meter was used to measure the water's salinity, temperature, pH, and dissolved oxygen content, namely Horiba Model U-10. Before measuring these parameters, the Horiba water quality meter was manually calibrated as described in its manual. According to Boyd (1998) The Palintest Photometer which is based on the Cadmium Reduction/Diazotization method, the Indophenol Method, and Diazotization Method for ammonia, nitrite, and nitrate, was used to photometrically quantify the concentration of these three elements in water.

By counting the colonies that developed on Tryptone Soya Agar (TSA) plates containing 10% NaCl, the quantity of heterotrophic bacteria was ascertained (Moriarty et al., (2006); Jorgensen et al., 2013). Before the bacteria were cultivated in agar media, dilution was carried out. The study was carried out for eight weeks. Every two weeks, the total weight of the shrimp, the number of living shrimp and the amount of feed used in each experimental unit were weighed. The data analysis displayed the shrimp's growth, survival rate, and feed conversion ratio.

Data Analysis

Survival Rate

Shrimp survival rate with reference formula based on Balazs (1993) was:

$$\text{Survival Rate (\%)} = \frac{(No - Nt}{No} \text{ x } 100\ \%$$

Information:
No = Number of shrimp at the beginning or t_0
Nt = Number of shrimp at time t

Shrimp Growth

Shrimp growth by referring to the formula based on Bages & Sloane (2001) was:

$$\text{Shrimp Growth (grams/day)} = \frac{Wt - Wo}{t}$$

Information:
W_0 = Shrimp body weight at the beginning or at the time t_0
Wt = Body weight of shrimp at time t

Shrimp Feed Conversion Ratio

Shrimp feed conversion ratio by referring to the formula based on Tseng et al., (2018) was:

$$\text{Feed Conversion Ratio (FCR)} = \sum \frac{\sum Wf}{\Delta W}$$

Information:
Wf = Amount of feed used
ΔW = Shrimp body weight gain

RESULTS AND DISCUSSION

We found that the treatment without molasses had the highest concentration of ammonia and nitrite. These results indicate that the addition of molasses has a significant effect on the ammonia, nitrite, and nitrate concentrations. These water quality metrics stayed constant and poor across all molasses treatments. Furthermore, in the experiment, the treatment with a 100% standard feeding rate and no molasses had the greatest levels of nitrite and ammonia.

Table 1. Effect of Molasses Use in Vanamei Shrimp (*L. vannamei*) Cultivation with a Shrimp Cultivation System Without Water Changes on the Decrease in Conventional Feed

No	Water Quality Parameters	Treatment	Mean ± St. Deviation
1.	Ammonia (mg/liter)	1. Treatment A	0.3174 ± 0.0253^{a}
		2. Treatment B	0.0397 ± 0.0037^{b}
		3. Treatment C	0.0457 ± 0.0023^{b}
		4. Treatment D	0.0499 ± 0.0089^{b}
		5. Treatment E	0.0530 ± 0.0025^{b}
2.	Nitrite (mg/liter)	1. Treatment A	36.0768 ± 0.0005^{a}
		2. Treatment B	0.0000 ± 0.0000^{b}
		3. Treatment C	0.0000 ± 0.0000^{b}
		4. Treatment D	0.0000 ± 0.0000^{b}
		5. Treatment E	0.0000 ± 0.0000^{b}
3.	Dissolved Oxygen (mg/liter)	1. Treatment A	6.13 ± 0.06^{a}
		2. Treatment B	5.54 ± 0.17^{b}
		3. Treatment C	5.21 ± 0.07^{c}
		4. Treatment D	4.89 ± 0.07^{d}
		5. Treatment E	4.71 ± 0.03^{d}
4.	pH	1. Treatment A	7.56 ± 0.08^{a}
		2. Treatment B	8.16 ± 0.06^{b}
		3. Treatment C	8.00 ± 0.01^{c}
		4. Treatment D	7.82 ± 0.02^{e}
		5. Treatment E	7.74 ± 0.03^{e}
5.	Number of Heterotrophic Bacteria (CFU/ml)	1. Treatment A	$1.85\ X 10^{9} \pm 5.50\ \ 10^{7\ a}$
		2. Treatment B	$7.33\ X\ 10^{9} \pm 4.53\ X\ 10^{8\ b}$
		3. Treatment C	$8.84\ X\ 10^{9} \pm 3.28\ X\ 10^{8\ c}$
		4. Treatment D	$1.04\ X\ 10^{10} \pm 9.94\ X\ 10^{8\ d}$
		5. Treatment E	$1.21\ X\ 10^{10} \pm 2.65\ X\ 10^{8\ e}$
6.	Life sustainability (%)	1. Treatment A	100.00 ± 0.00^{a}
		2. Treatment B	100.00 ± 0.00^{a}
		3. Treatment C	100.00 ± 0.00^{a}
		4. Treatment D	100.00 ± 0.00^{a}
		5. Treatment E	100.00 ± 0.00^{a}

No	Water Quality Parameters	Treatment	Mean ± St. Deviation
7.	Shrimp Growth (grams/day)	1. Treatment A	0.030±0.005[a]
		2. Treatment B	0.014±0.004[b]
		3. Treatment C	0.037±0.006[c]
		4. Treatment D	0.052±0.001[d]
		5. Treatment E	0.040±0.001[e]
8.	Feed Conversion Ratio	1. Treatment A	3.521±0.178[a]
		2. Treatment B	0.770±0.194[b]
		3. Treatment C	1.140±0.085[c]
		4. Treatment D	1.238±0.074[d]
		5. Treatment E	1.998±0.0253[a]

Note: Values for each Water Quality Parameter and Biological Parameter of Shrimp Growth in the Same Column are Followed by a Superscript that is Significantly Different ($p<0.05$).

Regression analysis also revealed a negative correlation between the number of heterotrophic bacteria in the experiment and the ammonia concentration at its conclusion. Because molasses with a C:N feed ratio of 20.0:1 was used, heterotrophic bacteria were able to remove the inorganic nitrogen from the shrimp farming medium. This result is similar to that obtained in previous studies by several researchers (Tezuka, 2010; Avnimelech, 2019; Goldman et al., 2007; McIntosh, 2000), proving that a C:N feed ratio of 15.0:1 or more will effectively remove inorganic nitrogen from water.

The reduction in conventional feeding rate significantly affected the dissolved oxygen concentration in the experiment. In the molasses treatment, dissolved oxygen levels increased significantly in response to the reduction in experimental feeding rate. The experiment's treatment without molasses showed significantly higher levels of dissolved oxygen. Increased feeding and molasses could lead to an increase in the number of heterotrophic bacteria. A previous report indicated a decrease in dissolved oxygen as shrimp's feed amounts increase (Hopkins et al., 2013). Heterotrophic bacteria have a major contribution in decreasing dissolved oxygen concentration in shrimp culture media (Visscher and Duerr, 2001; Sun et al., 2001).

Additionally, the amount of heterotrophic bacteria in the experiment was inversely correlated with the dissolved oxygen content. The dissolved oxygen level decreased with the time of the experiment. Previous studies of Landesman, (2014); Sun et al., (2001); Montoya et al., (2002) support this result by demonstrating a substantial inverse relationship between the quantity of heterotrophic bacteria and the dissolved oxygen content.

In the experiment, the pH value significantly decreased as the feeding rate increased, and the treatment without molasses had the lowest pH level. This trend could be attributed to the highest nitrifying bacterial activity, as describe by the greatest nitrite and nitrate values. According to Hargreaves (2018); Tacon (2001) The potential hydrogen (pH) is eventually lowered by oxidizing each mole of ammonia, which produces two hydrogen ions. Also, Tacon et al., (2002) revealed that raising the nitrite in shrimp culture lowers the pH. Allan et al., (2015) discovered that the breakdown of surplus feed caused the pH to drop as the feeding rate increased. The study's findings also demonstrated that raising the feeding rate of molasses-cultured shrimp significantly reduced the pH value.

As the conventional feeding rate when molasses was added to the experiment, the quantity of heterotrophic bacteria rose. Conversely, the treatment without molasses, but with a feeding rate of 100%, had the lowest number of bacteria in the experiment, as shown in Table 1. This is clear from the fact that in shrimp aquaculture, more molasses results in more heterotrophic bacteria, and treatments with a greater feeding rate consistently produce more molasses. Several researchers, including Parson et al., (2001); Azam et al., (2003); Avnimelech et al., (2012); Kochva et al., (2014); Middelboe et al., (2015); and Avnimelech (2019), have investigated the same results, reporting that the addition of carbon in water leads to an increase in the number of heterotrophic bacteria.

The experiment's shrimp survival rate remained 100% across all treatments, unaffected by the reduction in the conventional feeding rate. The growth rate, percentage of weight gain, and feed conversion ratio in the experiment were all greatly impacted by the decrease in the traditional feeding rate, as indicated in Table 1. In the experiment, the treatment with a conventional feeding rate of 25% had the lowest growth rate and the lowest percentage of weight gain among all the treatments. Similarly, in the molasses experiment, the treatment with a conventional feeding rate of 50% had a lower weight gain percentage and growth rate compared to the treatments with feeding rates of 75% and 100%. Despite applying molasses with a C:N ratio of 20:1, the results demonstrate that conventional feeding rates of 25% and 50% are insufficient for shrimp growth.

These results indicate that the treatment is not providing enough feed quantity for the shrimp. Daily observations support this opinion, revealing that the shrimp cultivation treatments, specifically treatments 2 and 3, had no remaining feed, even with additional molasses at 25% and 50% of the conventional feeding rate. Additionally, these results imply that natural feed, which includes heterotrophic bacteria is insufficient to compensate for a feeding rate reduction of 50 or more, which is crucial for maintaining shrimp growth.

Interestingly, in the experiment, there was a decrease in the feed conversion ratio in response to the decrease in feeding rate. Therefore, because we used the same feed, Feed consumption, not feed quality, is reflected in the variation in feed conversion ratio values between test treatments. Tacon et al., (2002) stated that the feed conversion ratio decreased with decreasing feed amount and increased with increasing feed amount given. Similarly, Abdelghany & Ahmad (2002) showed that treatments with decreased feeding rates had a lower fish feed conversion ratio. These results demonstrate that we evaluate the efficiency of shrimp farming not only by feed conversion, but also by percentage weight gain and growth rate.

Although the results of this study clearly imply that shrimp can use bacterial flocculation as feed, even if it did not directly prove it. and can partially replace feed in treatments using molasses at a rate of 75% of conventional feed. Several researchers (Harris & Mitchell, 2013; Schroeder, 2018; Avnimelech et al., 2002) have reported that fish consume heterotrophic flocculation, which significantly contributes to fish production. Additionally, Tacon et al., (2002) reported that whiteleg shrimp grew rapidly in a model without water exchange due to their ability to produce their own food and the abundance of microbes available for consumption, a process known as bacterial flocculation. Shrimp need amino acids like threonine, valine, isoleucine, and phenylalanine, which are abundant in bacterial flocculation.

CONCLUSION

This study discovered that the rate of decreasing natural feed provision had a significant effect on shrimp growth rate, feed conversion ratio, and quality parameters in the model without air exchange. Moreover, this study identified the most effective treatment as a combination of molasses and 75% conventional feed, with a C:N ratio of feed = 20.0 : 1.0.

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