doi: 10.15578/iaj.17.1.2022.23-28 *Indonesian Aquaculture Journal, 17 (1), 2022, 23-28*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

EFFECT OF BIOACTIVE PROTEIN INGREDIENTS (MOTIV™) ON TOTAL HEMOCYTE AND SURVIVAL RATE OF VANNAMEI SHRIMP, *Litopenaeus vannamei*

Slamet Budi Prayitno[*)#], Bagus Rimbayu Ardie[*)], Romi Novriadi[**)], Vivi Endar Herawati[*)], and Seto Windarto[*)#]

[*)] Departement of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Diponegoro
Jl. Prof. H. Soedarto, S.H., Tembalang, Kecamatan Tembalang, Kota Semarang, Jawa Tengah 50275, Indonesia
[**)] Ministry of Marine Affairs and Fisheries, Jakarta, Indonesia

(**Received: February 21, 2022; Final revision: June 13, 2022; Accepted: June 14, 2022**)

ABSTRACT

One of the problematic factors in the cultivation of vannamei shrimp is the outbreak of bacteria and viruses. One way to prevent infection is by strengthening the shrimp's immune system. MOTIV™ is a fermented corn protein concentrate that has been reported to possess probiotic properties that can positively increase the disease resistance of shrimp. Therefore, this study aimed to determine the effect of bioactive protein (MOTIV™) on the total hemocyte count and shrimp survival rate. The study used an experimental method with a completely randomized design with five treatments and three replications: A (commercial feed), B (7.5% MOTIV add of 1.5% krill meal instead of 9% fish meal), C (7.5% MOTIV™ add of 1% krill meal to replaces of 2% poultry meal and 6.5% fish meal), D (7.5% MOTIV™ to replaces of 3.5% poultry meal and 4% fish meal), and E (7.5% MOTIV™ to replaces of 7.5% poultry flour). Feeding was done four times/day based on shrimp biomass and weekly feed counts based on daily shrimp survival. The vannamei shrimp used was one gram with a population density of 15 shrimps/aquarium with an aquarium volume of 100 liters.Total shrimp hemocytes, survival, and water quality were all measured. Treatment C had the highest total hemocyte, averaging 4.1 x 10^7 cells/mL, whereas treatment B had the lowest, averaging 1.4 x 10^7 cells/mL. During the trial, only treatment C supported 100% survival.

KEYWORDS: ***L. vannamei; THC;*** **survival rate; MOTIV™; corn**

INTRODUCTION

Vannamei shrimp, *Litopenaeus vannamei* is currently in great demand by the aquaculture industry for cultivation activities because this species has the advantage of higher survival than tiger shrimp. The cultivation of vannamei shrimp tends to require a short time, and market forces and the price of vannamei shrimp are also relatively high. The Indonesian Government has planned to increase vannamei shrimp production since 2015 by 12% per year so that in 2019 the target was to reach 842 thousand tons (KKP, 2017).

One of the problem factors in vannamei shrimp cultivation is the emergence of bacterial and virus attacks, which are the leading causes of crop failure to date. Vannamei shrimp culture that has been infected with the virus cannot be cured, so prevention before infection occurs is the best strategy. One of the efforts to prevent disease is to increase the defense system in shrimp. Increasing immunity in shrimp can be achieved chemically, with drugs or other ingredients to improve specific and non-specific response mechanisms in fish (Putri *et al*., 2013).

The provision of immunostimulants in feed is intended to activate the non-specific immune system of cells such as hemocytes in invertebrates. Hemocytes are part of the cellular defense system in vannamei shrimp, responsible for phagocytosis, nodulation, and encapsulation (Febriani *et al*., 2018). Hemocytes can be used as a quantitative parameter in measuring the stress response in shrimp. An increase in total hemocytes indicates an improvement in health status of the organism because it will form phagocytic cells that play a role in defending themselves from attack by microorganisms (Ismawati *et al*., 2019).

[#] Correspondence: Departement of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Indonesia.
Jl. Prof. H. Soedarto, S.H., Tembalang, Kecamatan Tembalang, Kota Semarang, Jawa Tengah 50275
E-mail: *sbudiprayitno@gmail.com*

MOTIV™ is a fermented corn protein concentrate (CPC) made by enzymatically removing non-protein components from corn. CPC is rich in lysine, contains almost no ash and starch, and has high levels and concentrations of lysine, methionine and other essential amino acids. It includes crude protein, crude fat, lysine, methionine, and has a much higher protein than the commonly used corn gluten meal (Khalifa *et al*., 2016). The use of foods containing beneficialfood ingredients can enhance the innate defense mechanisms based on the non-specific immune system required in response to invading pathogens. Fermented corn protein concentrate is reported to contain probiotic properties that can proactively increase disease resistance in shrimp (Galkanda *et al*., 2021). Therefore, this study aimed to determine the effect of bioactive protein (MOTIV™) on total hemocytes and vannamei shrimp survival.

MATERIALS AND METHODS

The test organism used as research material was vannamei shrimp, *Litopenaeus vannamei* with a weight of one gram. Vannamei shrimp were kept in aquaria at a density of 15 shrimp/aquarium.

The aquaria used in the study were 50 cm x 50 cm x 40 cm with a volume of 100 liters equipped with aeration to supply oxygen. Each aquarium was washed with soap until clean, dried, and filled with seawater to about 80% of its volume. The research site was at *Marine Science Techno Park* (MSTP), Jepara, Central Java.

The study used a completely randomized design (CRD) consisting of five treatments and three replications. A completely randomized design is a type of experimental design where the experimental units are randomly assigned to the different treatments. The various treatments in this study were:

A : control using a commercial feed

B : 7.5% MOTIV™ add 1.5% krill meal to replaces of 9% fish meal

C : 7.5% MOTIV™ add 1% krill meal to replaces of 2% poultry meal and 6.5% fish meal

D : 7.5% MOTIV™ to replaces 3.5% poultry meal and 4% fish meal

E : 7.5% MOTIV™ to replaces of 7.5% poultry flour

Feeding was done four times/day based on shrimp biomass and weekly feed counts based on daily shrimp survival.

The hemolymph collection method for the *L. vannamei* shrimp was based on the research of Darwantin *et al*. (2016). The data displayed includes total hemocyte count, survival rate, and water qua-lity data.

Total Hemocyte Count

According to Arifin *et al*. (2014), total hemocyte count (THC) is calculated by the formula:

$$\text{THC} - \text{average of total cells} \times \frac{1}{\text{volume}} \times \text{diluent factor}$$

Survival Rate

According to Ihsanudin *et al.* (2014), the survival rate is calculated by the following formula:

$$SR = \frac{Nt}{No} \times 100\%$$

where:

SR = survival rate (%)

Nt = number of shrimp at the end of rearing

No = number of shrimp at the initial stocking

Water Quality

Water quality measurements include temperature, salinity, pH, and dissolved oxygen (DO) were conducted every day and measured using the water quality checker (WQC). Ammonia levels were monitored once a week.

RESULTS AND DISCUSSION

Total Hemocyte Count

Based on the research, total hemocyte count for white shrimp (*Litopenaeus vannamei*) was determined for 30 and 60 days of rearing. The results of the total hemocyte count of vannamei shrimp are presented in Figure 1.

The highest total hemocyte count occurred in treatment C of 4.1×10^7 cells/mL, and the lowest value was found in treatment B of 1.4×10^7 cells/mL. Analysis of variance (ANOVA) showed that the MOTIV™ feed significantly affected the total hemocytes of white shrimp with rearing period of 60 days.

Hemocyte cells consist of a granular (hyaline) hemocytes, semi-granular and granular cells. The purpose of seeing THC is to find out indicators of stress levels and shrimp health. In the first hemocyte test, it was found that the MOTIV™ feed had no significant effect on hemocytes with ANOVA results ($P > 0.05$). The results of the ANOVA test showed that MOTIV™ feeding had a significant effect on shrimp hemocytes during 60 days of rearing ($P < 0.05$).

MOTIV™ feed affected the THC of vannamei shrimp with increasing yield at day 60. Treatment C indicated the highest THC yield than A, B, D, and E treatments with a feed composition of 7.5% MOTIV™ plus 1%

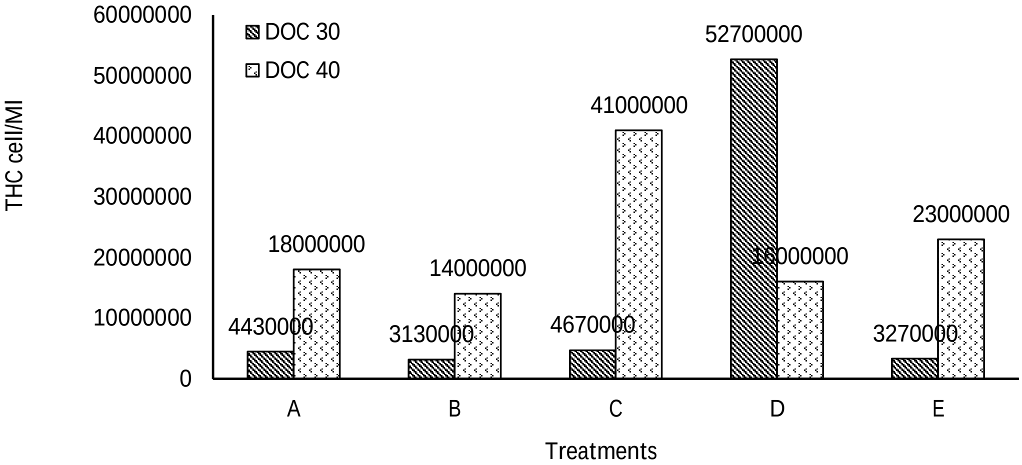

Figure 1. The total hemocyte count of *L. vannamei* shrimp fed five different diets for 30 and 60 days reared.

krill flour replace 2% poultry meal and 6.5% fish meal. Treatment C increased on day 60, with the average number of 4.67 x 10^6 cells/mL to 4.10 x 10^7 cells/mL. Likewise, in treatments A, B, D, and E, in treatment A, there was an increase from the average number of 4.43 x 10^6 cells/mL to 1.80 x 10^7 cells/mL; in treatment B, the average number was 3.13 x 10^6 cells/mL to 1.4 x 10^7. cells/mL, in treatment D with an average number of 5.27 x 10^6 cells/mL to 1.60 x 10^7 cells/mL, and in treatment E with an average number of 3.27 x 10^6 to 2.3 x 10^7 cells/mL.

The increase in the total number of hemocytes is closely related to foreign materials: fermented corn protein concentrate containing active ingredients. One of the active ingredients that play a role in increasing the total number of hemocytes is phenol. According to Prasiddha *et al*. (2016), phenolic compounds are secondary metabolites produced by plants involved in various special physiological functions such as growth, development, and standard defense mechanisms. Examples of phenolic compounds found in corn are flavonoids such as quercetin, alcohol, simple phenols such as p-coumaric acid, saponins, tannins, anthocyanins, and protocatechins. Meanwhile, according to Utomo *et al*. (2015), the active ingredients can directly stimulate the formation of hemocyte cells. One of the active ingredients that play a role in increasing the total number of hemocytes is saponins. Saponins contain a molecule of glysitinic acid, anti-inflammatory, and anti-tumor activity. This study did not examine whether the shrimp were infected with pathogens during rearing.

THC results in this study said to be higher than the research of Oktaviana & Febriani (2019), which produced THC of 9.18 x 10^6 cells/mL while the results of this study resulted in an increase of 4.1 x 10^7 cells/mL in treatment C. Hemocytes are one of the defense systems in white shrimp that are responsible for against phagocytosis, nodulation, and encapsulation. A high number of hemocytes indicates a good level of shrimp health. According to Suleman *et al*. (2019), changes in the number of hemocytes mean stress and health status in shrimp. This increase in total hemocytes indicates an increase in the body's defense reaction due to foreign particles entering the shrimp's body. The hemocyte cell receptors will recognize foreign particles that enter the shrimp body to produce cellular responses such as phagocytosis. Phagocytosis is a defense mechanism carried out by phagocytic cells by digesting foreign particles. The number of shrimp hemocytes can decrease if environmental conditions deteriorate, such as low dissolved oxygen content, temperature, and salinity or the presence of pathogen attack. The number of hemocytes obtained during the study ranged from 1.4 x 10^7 to 4.1 x 10^7 cells/mL. According to Febriani *et al*. (2018), the number of hemocytes of healthy shrimp weighing 11-12 g/head is 1.80 ± 9.28 x 10^7 cells/mL.

Survival Rate

The results of the measurement of the survival rate of vannamei shrimp are presented in Figure 2.

The Figure 2 shows that there is no significant difference in survival rate between of five treatments. The results of the calculation of the vannamei shrimp survival rate in Figure 2 show that the highest vannamei shrimp survival rate was 100% in the shrimp group with treatment C, the shrimp group that was fed corn fermented feed added with 1% krill to replace 2% poultry flour and 6.5% flour fish. While treatment A, B, and D results have a survival rate of 95.56%, respectively. According to Saloko *et al*. (2015), the high level of survival can be influenced by several fac-

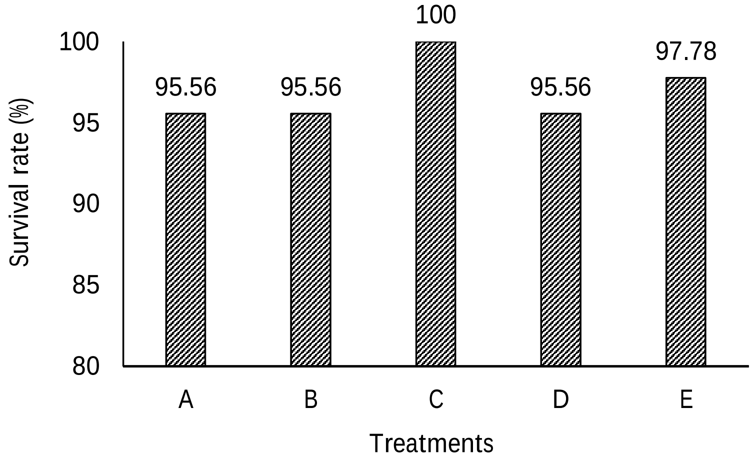

Figure 2. The survival rate of *L. vannamei* shrimp fed five different diets for 60 days of reared.

tors, including the temperature, which is always stable, the water quality is always good.

Based on vannamei shrimp survival calculation results during the study, each treatment did not significantly differ. This indicated that the fermented corn protein concentrate feed had almost the same survival rate as the control. This suggests that feed with fermented corn protein concentrate does not cause many deaths, or it can be said that white shrimp can accept the feed. According to Ni'mah *et al*. (2021), a good survival rate indicates that immunostimulators that enter the shrimp body can protect or be proactive against external factors such as pathogens that enter the shrimp body.

This increase in the survival rate indicates that the immune system in the shrimp body can protect (protective) white vannamei shrimp against external factors that enter the shrimp body, for example, infection by pathogens which is one of the leading causes shrimp death. According to Utomo *et al*. (2015), the survival of the tested shrimp is also suspected to be closely related to the increase in THC because the shrimp's immune system is non-specific. Rohmin *et al*. (2017) the immune system in shrimp is still primitive, and unlike fish and mammals, which have immunoglobulins, the immunoglobulins in shrimp are replaced by prophenoloxidase activating enzyme (PPA). The PPA is a protein located in the hemocyte granular cells.

Water Quality

Based on the research, the values of water quality as supporting data include dissolved oxygen (DO), hydrogen power (pH), temperature, salinity, and ammonia, and the results are presented in Table 1.

The water quality results show that the water quality during rearing was optimal for cultivation activities and could support the growth of vannamei shrimp (*L. vannamei*).

Water quality observations were carried out two times a day in the morning and evening. The variables observed were temperature, DO, salinity, pH, and ammonia. Ammonia observations were carried out once a week. The value of water quality have salinity levels ranging from 28 to 31 ppt. Salinity with a 28-31 ppt value indicates that the salinity is quite good. Nababan *et al*. (2015) stated that good salinity for growth ranged from 10-30 ppt with optimal salinity ranging from 15-25 ppt. This is reinforced by WWF-Indonesia (2014), the ideal salinity for shrimp growth is between 10-35 ppt.

The degree of acidity (pH) of water-based on measurements ranges from 6.5 to 8.0. This pH range can support the continuation of the shrimp farming business. According to Sahrijanna & Septiningsih (2017), a suitable pH value for shrimp culture is between 7.4 and 8.9, with the optimum standard value of 8.0. pH.

Dissolving oxygen for cultivation will have a good effect on shrimp. Cultured organisms use oxygen for metabolic processes. The DO results ranged from 5.3-6.5 mg/L. According to Anas *et al*. (2015), a dissolved oxygen content of fewer than 2 mg/L can cause shrimp death, and the optimum limit is 4-7 mg/L.

Water temperature based on measurements ranging from 28.2°C-29.5°C. According to Sahrijanna & Septiningsih (2017), a suitable temperature value for shrimp cultivation is 27°C-31°C. While the levels of ammonia during the study ranged from 0.02-0.2 mg/L. According to Chrisnawati *et al*. (2018), the opti-

 Copyright @ 2022, Indonesian Aquaculture Journal, p-ISSN 0215-0883; e-ISSN 2502-6577

Table 1. The average of water quality values on water reared of *L. vannamei* shrimp

Variables	Morning	Afternoon
Temperature (°C)	28.890 ± 0.209	28.953 ± 0.200
DO (mg/L)	5.715 ± 0.375	5.727 ± 0.323
Salinity (ppt)	30.847 ± 0.481	30.817 ± 0.645
pH	7.405 ± 0.285	7.414 ± 0.308
Ammonia (mg/L)	0.02 ± 0.004401	

mum ammonia level in vannamei shrimp rearing water is 0.05-0.1 mg/L. Ammonia levels began to affect growth by 50% at 0.45 mg/L levels and caused death at 1.29 mg/L levels.

CONCLUSIONS

The use of feed with bioactive protein ingredient (MOTIV™) can increase the number of vannamei shrimp hemocytes this can be used to indicate of strengthening the shrimp's immune system an.d health status in shrimp. However, the use of bioactives in feed from different fermented corn protein concentrate did not significantly differ in survival rate. It is suggested to use a feed composition with 7.5% MOTIV™ add of 1% krill flour to replace 2% poultry meal and 6.5% fish meal, which is the best result of this study and can increase the total number of hemocytes of *L. vannamei* shrimp.

REFERENCES

Anas, P., Sudinno, D., & Jubaedah, I. (2015). Carrying capacity of water for vannamei shrimp cultivation semi-intensive system in utilization of coastal areas in Pemalang Regency. *Jurnal Penyuluhan Perikanan dan Kelautan*, 9(2), 29-46.

Arifin, M.Y., Supriyono, E., & Widanarni. (2014). Total hemocyte, glucose and survival rate of mantis shrimp (*Harpiosquilla raphidea*) post-transportation with two different systems. *Jurnal Kelautan Nasional*, 9(2), 111-119.

Chrisnawati, V., Rahardja, B.S., & Satyantini, W.H. (2018). The effect of giving probiotics with different times on reduction of ammonia and total organic matter in the maintenance media of vannamei shrimp (*Litopenaeus vannamei*). *Journal of Marine and Coastal Science*, 7(2), 68-77.

Darwantin, K., Sidik, R., & Mahasri, G. (2016). Efficiency of the use of immunostimulants in feed on growth rate, immune response and survival of vannamei shrimp (*Litopenaeus vannamei*). *Jurnal Biosains*, 18(2), 1-18.

Febriani, D., Marlina, E., & Oktaviana, A. (2018). Total hemocytes of vannamei shrimp (*Litopenaeus vannamei*) maintained at 10 ppt salinity with different stocking density. *Journal of Aquaculture Science*, 3(1), 100-107.

Galkanda-Arachchige, H.S., Hussain, A.S., & Davis, D.A. (2021). Fermented corn protein concentrate to replace ûshmeal in practical diets for Paciûc white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*. https://doi.org/10.1111/anu.13303.

Ihsanudin, I., Rejeki, S., & Yuniarti, T. (2014). Effect of administration of recombinant growth hormone (rGH) through oral methods with different time intervals on growth and survival of Larasati tilapia (*Oreochromis niloticus*) seeds. *Journal of Aquaculture Management and Technology*, 3(2), 94-102.

Ismawati, R.A., Destryana, & Huzaimah, N. (2019). The immunity of vanname shrimp (*Litopenaeus vannamei*) given supplementary feed with kasembukan leaves (*Paederia foetida* Linn.). *Jurnal Kelautan*, 12(2), 201-206.

Khalifa, N.S.A., Belal, I.E.H., El-Tarabily, K.A., Tariq, S., & Kassab, A.A. (2016). Evaluation of replacing fish meal with corn protein concentrate in nile tilapia *Oreochromis niloticus* fingerlings commercial diet. *Aquaculture Nutrition*, 24(1), 143-152.

KKP. (2019). Cultivation of vaname shrimp (*Litopenaeus vannamei*) in millenial fish farming. Direktorat Jenderal Perikanan Budidaya: Balai Perikanan Budidaya Air Payau Situbondo.

Nababan, E., Putra, I., & Rusliadi. (2015). Maintenance of vannamei shrimp (*Litopenaeus vannamei*) with different feeding percentages. *Jurnal Ilmiah Perikanan dan Kelautan*, 3(2), 1-9.

Ni'mah, U., Pringgenies, D., & Santosa, G.W. (2021). Effect of *Stichopus hermanii* semper extract, 1868 (Stichopodidae, Holothuroidea) on total hemocyte count of *Litopenaeus vannamei* Boone, 1931 (Penaeidae, Crustacea). *Journal of Marine Research*, 10(3), 387-394.

Oktaviana, A. & Febriani, D. (2019). Total hemocyte count in vannamei shrimp (*Litopenaeus vannamei*) given additional banana stem flour in feed. *Jurnal Perikanan*, 9(2), 188-193.

Prasiddha, I.J., Laeliocattleya, R.A., Estiasih, T., & Maligan, J.M. (2016). Potential of corn hair bioactive compounds (*Zea mays* L.) for natural sunscreen: Literature review. *Jurnal Pangan dan Agroindustri*, 4(1), 40-45.

Purnamasari, I., Purnama, D., & Utami, M.A.F. (2017). Vannamei shrimp (*Litopenaeus vannamei*) growth in intensive pond. *Jurnal Enggano*, 2(1), 58-67.

Putri, F.M., Sarjito, & Suminto.(2013). Effect of addition of *Spirulina* sp. in artificial feed on total hemocyte count and phagocytic activity of vannamei shrimp (*Litopenaeus vannamei*). *Journal of Aquaculture Management and Technology*, 2(1), 102-112.

Rohmin, M.F.T., Mahasri, G., & Rantam, F.A. (2017). Response analysis of urban vannamei (*Litopenaeus Vannamei*) which is exposed to crude protein zoothamniumpenaei oral and maintained in ponds. *Jurnal Biosains Pascasarjana*, 19(2), 143-157.

Sahrijanna, A. & Septiningsih, E. (2017). Time variations of water quality in shrimp cultivation ponds using integrated multitrophic aquaculture (IMTA) technology in Mamuju, West Sulawesi. *Jurnal Ilmu Alam dan Lingkungan*, 8(16), 52-57.

Saloko, Y.T., Rachimi, E.I., Raharjo, & Yanto, H. (2015). Effect of different fermented yellow corn in feed on growth rate and survival rate of jelawat fish (*Leptobarbus hoevenii* Blkr). *Jurnal Ruaya*, 5(1), 6-10.

Suleman, Andayani, S., & Yuniarti, A. (2019). Potency of *Ulva lactuta* crude extract in increasing total hemocyte count (THC) and phagocytic activity in vannamei shrimp (*Litopenaeus vannamei*). *Jurnal Ilmu Perikanan*, 10(1), 1-7.

Utomo, A.S., Prayitno, S.B., & Sarjito. (2015). Addition of binahong leaf powder (*Anredera cardivolia*) to feed against immune response, survival and health status of windu shrimp (*Penaeus monodon*) infected with *Vibrio harveyi*. *Journal of Aquaculture Management and Technology*, 4(3), 61-68.

WWF Indonesia. (2014). Vannamei shrimp cultivation: Semi-intensive pond with wastewater treatment plant. Jakarta: WWF Indonesia.

