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The Influence of Different Concentrations of Isolated Soy Protein (ISP) Powder in Rice Bran Suspension as Feed on the Production of *Artemia salina* Cysts

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ABSTRACT

Artemia salina is a natural food source known for its high nutritional quality. It is commonly used in larval rearing due to its ability to produce long-lasting cysts that can hatch at any time. These characteristics make *A. salina* very suitable for sustainable cultivation. In this study, the feed consisted of rice bran suspension enriched with Isolated Soy Protein (ISP) meal. The aim was to investigate the effect of different protein concentrations in the rice bran suspension feed on cyst production in *A. salina* broodstock. This study used a Completely Randomized Design (CRD) with five treatments and four replications. The treatments included P0 (without ISP meal, 25% protein concentration), P1 (ISP meal, 40% protein concentration), P2 (ISP meal, 45% protein concentration), P3 (ISP meal, 50% protein concentration), and P4 (ISP meal, 55% protein concentration). Data analysis was performed using ANOVA followed by Duncan's multiple range test. The results showed that variations in protein concentration with the addition of ISP flour had a significant effect ($P < 0.05$) on total cyst production and cyst production per broodstock of *A. salina*. The highest total cyst production (710.67 cysts/L) was achieved in P4 with a protein concentration of 55%, while the highest cyst production per broodstock (28.67 cysts/broodstock) occurred in P2 with a protein concentration of 45%.

Keywords: *Artemia salina*, enrichment, Isolate Soy Protein (ISP) flour

INTRODUCTION

The use of natural feed is one of the keys to success during aquaculture (Rasdi & Qin, 2014). This is because natural feed plays an important role as a source of initial nutrition for fish in the larval phase (Mohseni *et al.*, 2012). One type of natural feed that is widely used is *Artemia* (Wang *et al.*, 2022). *Artemia* is a natural feed with excellent nutritional qualities for larval rearing, containing approximately 51-55% protein, 14-15% carbohydrates, 13-19% fat, and 3-15% n-3 highly unsaturated fatty acids (HUFA) (Kumar & Babu, 2015). Based on research by Pham *et al.* (2022), giving *Artemia* enriched with organic selenium can increase growth in white snapper. Pratiwy *et al.* (2021) state *Artemia* can reduce mortality in fish larvae and increase larval immunity. *Artemia* as a natural feed has been widely used because *Artemia* cysts can be stored for extended periods and hatched as needed (Bengtson *et al.*, 2018). However, most *Artemia* cysts in Indonesia are imported at relatively high costs (Mubarak *et al.*, 2020). One effort in cultivating *Artemia* is using rice bran as feed (Madkour *et al.*, 2023). However, rice bran has a low protein and omega-3 content (Yin *et al.*, 2014; Sapwarobol *et al.*, 2021). This is a challenge in *Artemia* production because the need for Omega-3 is quite high (Pratama *et al.*, 2023). The need for Omega-3 can be met by adding fish oil rich in

Omega-3, namely EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) (Indelicato *et al*., 2023). The use of Omega-3 can accelerate the maturity and reproduction of *Artemia* (Pratama *et al*., 2023). In addition, the need for protein and the appropriate amino acid composition can increase the success of *Artemia* cultivation (Amin *et al*., 2023). One ingredient with a high protein content is Isolated Soy Protein (ISP). The protein content of ISP is >90%, with several essential amino acid contents that play a role in the growth and fecundity of *Artemia*, such as phenylalanine, histidine, and isoleucine, as well as non-essential amino acid contents such as cysteine, glutamine, and aspartic acids (Chamba *et al*., 2015). Research by Mubarak *et al*. (2023) shows that giving ISP with a protein concentration of 40% and 45% produces the highest *A. salina* cyst diameter results. Therefore, this study aims to determine the effect of rice bran suspension enriched with ISP flour and fish oil as feed on producing *A. salina* cysts.

MATERIALS AND METHODS

Research Design

This study employed a Completely Randomized Design (CRD) experimental method. The research consisted of five treatments: one control treatment (PO) and four Isolated Soy Protein (ISP) treatments with different protein levels (40%, 45%, 50%, and 55%).

Material and method

The equipment used includes a 25-litre volume gallon, 20 plastic cups sized 300 ml each, and an aeration device consisting of an LP-100 water pump aerator, aerator tubing, and aerator stones. Water quality measurement tools include a pH meter, thermometer, dissolved oxygen (DO) kit, and ammonia test kit. For the observation of *A. salina* or cysts, a Charge Coupled Device (CCD) microscope and object glass were utilized. Additionally, equipment for sampling and culture needs included sieves, plankton nets, 40 µm mesh sieves, digital scales, blender, spoon, bucket, plastic bottles, measuring cups, Erlenmeyer flask, Petri dishes, pipettes, syringes, 2 ml Eppendorf tubes, hand counter, aluminum foil, label paper, and Olympus Studio 2 application. On the other hand, the materials used consisted of *A. salina* broodstock cultivated by the Faculty of Fisheries and Marine Sciences, Universitas Airlangga, freshwater, seawater, rice bran from rice milling, lemuru fish oil, Isolated Soy Protein (ISP) flour extracted from soy protein, and rock salt.

Culture Media Preparation and Broodstock Provisioning

The culture medium used seawater with an initial salinity of 35 ppt, and was gradually increased to 100 ppt, 125 ppt, and 140 ppt by adding rock salt. *A. salina* broodstock, which was cultured for one month in a filter tank, was selected for this study. The adult gonad broodstock were randomly distributed into gallon containers, each filled with 7 liters of seawater and containing 50 individuals (male and female) each.

The frequency of feeding was twice a day (morning and evening) at 2 ml of natural food/gallon. For cyst production, 20 plastic cups (volume 300 ml) were filled with 250 ml of seawater with a salinity of 140 ppt. Each cup was filled with three *A. salina* broodstock from the gallon, and fed 0.2 ml of food every day, divided into morning and evening portions.

Preparation of rice bran suspension feed

The rice bran suspension was prepared by mixing rice bran, Isolated Soy Protein (ISP) flour, fish oil, and seawater. Fish oil was added uniformly at 5% across all treatments to isolate the effect of ISP flour's protein content. The manufacturing process commenced with precise weighing of ingredients according to treatment specifications. The prepared suspension was then bottled and refrigerated. Prior to utilization in the feeding study, proximate analysis verified the protein content in each treatment.

Cyst Harvesting

Cyst harvesting is conducted through total cyst harvesting or per volume and broodstock harvesting. Total cyst harvesting is done weekly, and in this study, harvesting was performed five times. Meanwhile, per broodstock cyst harvesting was conducted four times.

Cyst Production

The calculation of total cyst production of *A. salina* involves collecting cysts in a petri dish divided into four sections marked with a marker. Cysts are manually counted in one section of the petri dish using a hand counter, multiplied by four, and stored back in the refrigerator. The calculation of cysts per broodstock is determined using the formula for *Ephippia* production per broodstock (Sellami *et al.*, 2021).

$$\text{Cyst production} = \frac{\Sigma\, cyst}{\Sigma\, parent}$$

Survival Rate

The calculation of survival rate can be conducted using the survival rate formula according to Minabi *et al.* (2020):

$$SR\ (\%) = (N_t/N_o) \times 100\%$$

SR = Survival Rate (%)
N_t = final number of *A. salina* broodstock
N_o= Initial number of *A. salina* broodstock

Water Quality

Water temperature, pH, and dissolved oxygen (DO) levels were monitored using a thermometer, digital pH meter, and digital DO meter. Additionally, ammonia (NH_3) levels were measured throughout the study.

Data Analysis

The data in this study will be analyzed using Analysis of Variance (ANOVA) with a significance level of α = 5% (0.05), followed by Duncan's Multiple Range Test (DMRT) to determine the best treatment among all treatments (Kusriningrum, 2010). The results of the analysis will be presented and discussed using descriptive methods.

RESULT AND DISCUSSION

***A. salina* total cyst production**

Based on the research results, adding isolated soy protein flour to rice bran suspension feed over one month significantly affected cyst production in each treatment. The differing protein concentrations significantly influenced cyst production in each treatment in the feed. This study found that the highest average total cyst production of *A. salina* was in treatment P4 (55% protein concentration) with 710.67 cysts/L, followed by treatments P1, P2, and P3 with total cyst productions of 562.33, 671, and 473 cysts/L, respectively (Figure 1).

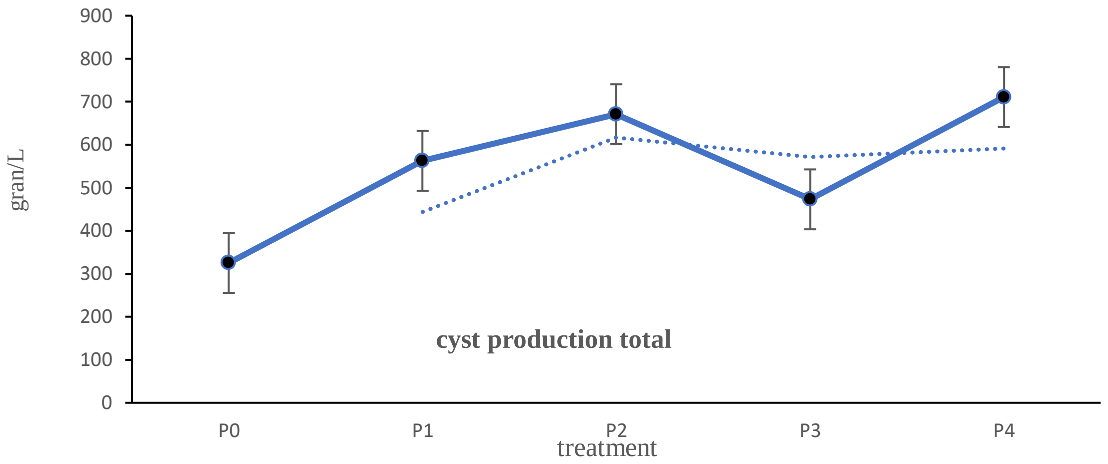

Figure 1. Total cyst production (grains/L) in each treatment during the maintenance period.

Meanwhile, the lowest cyst production were found in the treatment without ISP flour (P0), which was 325.33 cysts/L. This is because the protein content in the rice bran suspension without ISP flour is relatively low. The protein content in the feed affects the ability of *A. salina* to produce cysts because protein plays an important role in the formation and reproduction of eggs (Amin et al., 2023). Higher protein content in the feed increases the ability of *Artemia* to produce cysts in terms of quality and quantity. The optimal protein requirement for *Artemia* is between 40-60% (Hariansyah *et al.*, 2013). In addition, soy protein isolate contains amino acids such as phenylalanine, histidine, isoleucine, cysteine, glutamine, and aspartic acid, which can increase fecundity in *Artemia* (Chamba *et al.*, 2015). An increase in total cyst production correlates with increased protein concentration in the feed across all treatments.

Based on the total cyst production per week, the first week had the highest average total production compared to the second and third weeks (Figure 2a). The highest total cyst production in the first week was in treatment P2 (45% protein concentration), with an average production ranging from 147.33 to 518.33 cysts/L. Furthermore, the highest total production in the second week was found in the P4 treatment (protein concentration 55%), which was 69-251.67 cysts/L. This condition continued until the third week still in the P4 treatment, with an average production ranging from 55 to 108 cysts/L. However, the total cyst production in the second week decreased compared to the first week due to the maturation of the *Artemia* broodstock, resulting in cysts hatching and becoming nauplii (Baxevanis et al., 2004). Likewise, in the third week there was a decrease in total cyst production because the broodstock reached peak maturity on the 26th day of maintenance with a maturity level reaching 95.03% (Baxevanis et al., 2004).

Additionally, higher protein concentrations in the feed lead to lower cyst production because the proteins in the feed are utilized by *Artemia* broodstock for nauplii release and the formation of new cysts, thus using more protein as an energy source for sustaining these processes (Jubaedah *et al.*, 2006). The decline in cyst production is also attributed to the possibility that the cysts obtained may be from subsequent generations, whereas the initial generation cysts have already hatched into nauplii. This aligns with findings by D'Agustino (1980), where a complete reproductive cycle of *Artemia* typically spans 4 to 5 days. Subsequent cyst groups immediately enter the oviduct after the release of the first group (Jubaedah *et al.*, 2006).

Production of *A. salina* brood cysts

The results showed that variations in protein concentration in the feed had a significant impact on cyst production per broodstock of *A. salina*. Treatment P2 showed the highest cyst production per broodstock at 28 eggs, followed by treatments P3, P4, P0, and P1 with 27, 22, 19, and 18 eggs per broodstock, respectively. In addition, cyst production per broodstock varied between weeks: the first week produced between 1.67 and 6.33 eggs per broodstock, the second week produced 5 to 10 eggs per broodstock, and the third week produced 4.33 to 8.67 eggs per broodstock (Figure 2b). This indicates that protein content in the feed plays an important role in optimizing the reproductive results of *A. salina*.

The variation of cyst production per week shows the lowest value of cysts per broodstock in the first week compared to other weeks. The low production is thought to be because the broodstock is still trying to adapt to the maintenance environment. In the second week, cyst production increases when parthenogenesis begins, resulting in rapid cyst production and reaching peak production (Mubarak *et al.*, 2009). The factor that influences this is feed composition. Amin *et al.* (2024) highlighted that protein content can affect growth, survival rate, and fertility. *Artemia* can grow optimally with a protein content in the feed of around 40% and 45% (Mubarak *et al.*, 2023). Fulfillment of optimal protein content can increase the endurance and survival of *Artemia* (Hariansyah *et al.*, 2013).

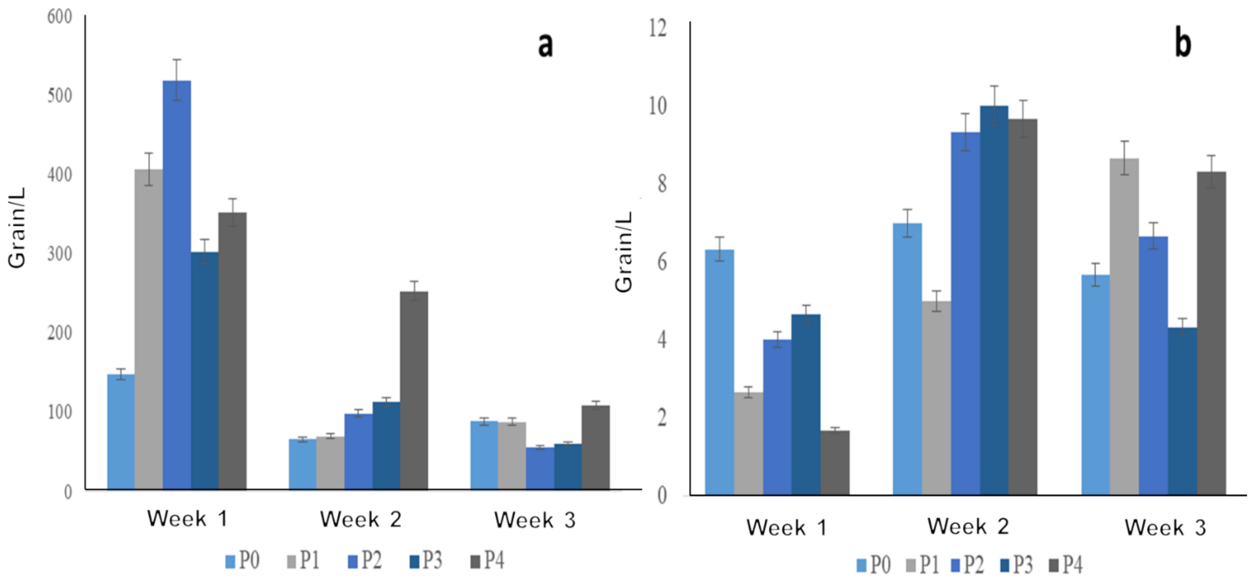

Figure 2. a; Total cyst production (grains/L) and b; cyst production (grains/L) per brood per week during rearing.

Survival Rate and Water Quality

The highest survival rate was observed during the first week of maintenance, ranging from 85% to 88%. Treatment P2 exhibited the highest survival during this period, followed by P1, P4, P3, and P0. In the second week, treatment P4 (55% protein) showed the highest survival, followed by P2, P3, P1, and P0. By the third week, treatment P4 (55% protein) again recorded the highest survival, followed by P1, P0, P2, and P3. Detailed SR values are summarized in Table 1.

The survival rate of *A. salina* is influenced by several factors, primarily the quality of the rearing water and feed. Water quality parameters are crucial and must be carefully monitored during the study. Water quality can affect an organism's growth and survival rate (Huang *et al.*, 2022). Measurements showed DO levels ranging from 3.20 to 4.61 mg/L, temperatures between 28.4 to 31.8 °C, pH values ranging from 7.0 to 8.3, and ammonia concentrations from 0.25 to 0.75 mg/L (Table 2). These results still show that the water quality during maintenance is still within the optimal range for broodstock maintenance and cyst production.

The optimal dissolved oxygen and pH levels for *Artemia* are oxygen above 2 mg/L, and pH between 6.5-8.0 (Islam *et al.*, 2019). Oxygen levels themselves need to be maintained because they can affect changes in the reproductive system and cyst formation (Lee *et al.*, 2025). Temperature affects growth, metabolism, osmoregulation, and respiration. *Artemia* can survive at temperatures ranging from 15 °C to 55 °C but grows optimally at temperatures ranging from 20 °C to 40 °C (Kumar & Babu, 2015). Ammonia is produced from the decomposition of organic matter, especially protein, from leftover feed and metabolic waste. According to Schumann (2000), the tolerance limit for ammonia is below 10 mg/L, which indicates that the ammonia levels in this study are still within the tolerance limit.

Table 1. Mean of Survival Rate (%)

Treatment	Diet protein concentration (%)	SR		
		1	2	3
P0	25%	85,67±3,05	74,33±4,16	67,00±1,00
P1	40%	88,33±0,57	74,67±3,51	68,33±2,51
P2	45%	88,67±1,52	75,33±5,68	64,67±5,03
P3	50%	86,00±1,00	75,33±3,51	64,00±4,58
P4	55%	86,67±1,15	76,00±2,00	69,00±1,00

Table 2. Water quality measurement during the study

Treatment	Parameters			
	DO (mg/L)	Temp (ºC)	pH	Ammonia (mg/L)
P0	3.43	29.98	7.4	0.25 – 0.50
P1	3.56	30.30	7.3	0.25 – 0.75
P2	3.62	30.22	7.4	0.25 – 0.75
P3	3.40	30.12	7.3	0.50 – 0.75
P4	3.52	29.75	7.4	0.50 – 0.75

In this study, salinity significantly affected cyst formation. Yang and Sun (2023) stated that by adjusting salinity appropriately, it can induce *Artemia* sp. broodstock to produce nauplii. *A. salina* itself reproduces sexually by forming cysts and asexually by forming nauplii. According to Lee *et al.* (2025), in environmental conditions with high salinity and low dissolved oxygen, female *Artemia* reproduce sexually. *Artemia* can produce cysts optimally at a salinity range of 60-80 ppt, and cyst production will decrease if it is carried out above 150 ppt (Far *et al.*, 2009; Toi *et al.*, 2021).

CONCLUSION

Culturing *A. salina* using rice bran suspension feed with different protein concentrations significantly affects total cyst production. A protein concentration of 55% in rice bran suspension feed resulted in a total cyst production of 710.67 grains/L. Cultivating *A. salina* using rice bran suspension feed with varying protein concentrations also influences cyst production per broodstock. Providing a protein concentration of 45% in rice bran suspension feed resulted in a cyst production per broodstock of 28.67 grains.

REFERENCES

Amin, M., Hasanah, M. D., Alamsjah, M. A., Wisudyawati, D., & Musdalifah, L. (2024). The Effects of Formulated Diet Inclusion on Growth and Reproductive Performances of *Artemia franciscana*. Aquaculture Studies, 25(1), 40-46.

Amin, M., Intan, B., Putri, M. A. R., Mukti, A. T., & Alamsjah, M. A. (2023). Effect of protein sources in formulated diets on growth performance, feed utilization, survival rate, and reproductive performance of *Artemia franciscana*. Aquaculture International, 31(4), 1893-1910.

Baxevanis, A. D., El-Bermawi, N., Abatzopoulos, T. J., & Sorgeloos, P. (2004). Salinity effects on maturation, reproductive and life span characteristics of four Egyptian *Artemia* populations (International Study on *Artemia*. LXVIII). Hydrobiologia, 513, 87-100.

Bengtson, D. A., Léger, P., & Sorgeloos, P. (2018). Use of *Artemia* as a food source for aquaculture. In *Artemia* biology (pp. 255-286). CRC Press.

Chamba, M. V. M., Hua, Y., Murekatete, N., & Chen, Y. (2015). Effects of synthetic and natural extraction chemicals on yield, composition and protein quality of soy protein isolates extracted from full-fat and defatted flours. Journal of food science and technology, 52, 1016-1023.

D'agostino, A. (1980). The vital requirements of *Artemia*: physiology and nutrition. The Brine Shrimp. Physiology, Biochemistry, Molecular Biology, 2, 474.

Far, M. R., Gorttappeh, A. H., Mousanejhad, V., Zardoshti, M. R., & Asl, N. H. H. (2009). Evaluating the energy balance sheet *Artemia* of Uremia Lake and the methods of optimizing its. Nase More, 56.

Hariansyah, S., Amir, & Setyono, B. D. H. (2013). The effect of different feeds on the growth and nutrient content of *Artemia salina*. Jurnal Perikanan Unram, 3, 68-74.

Huang, H. H., Li, C. Y., Song, Y., Lei, Y. J., & Yang, P. H. (2022). Growth performance of shrimp and water quality in a freshwater biofloc system with a salinity of 5.0‰: effects on inputs, costs and wastes discharge during grow-out culture of *Litopenaeus vannamei*. Aquacultural Engineering, 98, 102265.

Indelicato, S., Di Stefano, V., Avellone, G., Piazzese, D., Vazzana, M., Mauro, M., ... & Bongiorno, D. (2023). HPLC/HRMS and GC/MS for Triacylglycerols Characterization of Tuna Fish Oils Obtained from Green Extraction. Foods, 12(6), 1193.

Islam, M. S., Kibria, M. M., & Bhuyan, S. (2019). Production of *Artemia* biomass in indoor culture tank in Bangladesh. Journal of scientific research, 11(1), 101-110.

Jubaedah, D., Djokosetiyanto, D., & Soni, A. F. M. (2009). Quantity and quality of *Artemia* cysts at various levels of salinity changes. Jurnal Perikanan, 8(2), 194-200.

Kumar, G. R., & Babu, D. E. (2015). Effect of light, temperature, and salinity on the growth of *Artemia*. International Journal of Engineering Science Invention, 4(12), 7-14

Kusriningrum, R. S. (2012). Experimental design. Airlangga University Press. Surabaya.

Lee, W. Y., Liu, C. H., Yu, B. H., Lee, Y. K., Kuo, C. H., Huang, C. Y., Teng, C.Y., Xu, Z.Y., & Chiu, K. (2025). 1H NMR-based metabolomic analysis of hypersalinity-induced oviparity in brine shrimp. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 101409.

Madkour, K., Dawood, M. A., Sorgelos, P., & Sewilam, H. (2023). Effects of desalination brine on the fecundity of brine shrimp fed on rice bran. Annals of Animal Science, 23(3), 869-875.

Minabi, K., Sourinejad, I., Alizadeh, M., Ghatrami, E. R., & Khanjani, M. H. (2020) Effects of different carbon to nitrogen ratios in the biofloc system on water quality, growth, and body composition of common carp (*Cyprinus carpio* L.) fingerlings. Aquac Int., 28,1883–1898.

Mohseni, M., Pourkazemi, M., Hassani, S. H., Okorie, O. E., Min, T. S., & Bai, S. C. (2012). Effects of different three live foods on growth performance and survival rates in Beluga

(*Huso huso*) larvae. Iranian Journal of Fisheries Sciences, 11(1), 118-131.

Mubarak, A. S., Jusadi, D., Junior, M. Z., & Suprayudi, M. A. (2017). Evaluation of the rice bran and cassava suspension use in the production of male *Moina* offspring and *Ephippia*. AACL Bioflux, 10(3), 512.

Mubarak, A. S., Pursetyo, K. T., & Monica, A. (2020). Quality and quantity of *Ephipia moina* macrocopa resulting from mating using tilapia fish feces suspension feed. The Iraqi Journal of Agricultural Science, 51, 157-167.

Mubarak, A. S., Sulmartiwi, L., & Nurhayati, M. D. A. R. (2023). The Effect of Different Protein Concentration in Rice Bran Suspension as Feed on Cyst Diameter of *Artemia salina*. Journal of Aquaculture Science, 8(1), 57-66.

Mubarak, A. S., Tias, D. T. R., & Sulmartiwi, L. (2009). The addition of dolomite in the culture of *Daphnia* spp. Daily Feeding System in *Daphnia* spp. populations and stability of water quality. Jurnal Ilmiah Perikanan dan Kelautan, 1(1), 67-72.

Pham, H. D., Le, M. H., Dinh, K. V., Siddik, M. A., Hoang, D. H., & Van Ngo, M. (2022). Effects of enrichment *Artemia* with organic selenium and essential fatty acids on growth performance and fatty acid composition of barramundi (*Lates calcarifer*) larvae. Regional Studies in Marine Science, 55, 102595.

Pratama, R. W., Sulmartiwi, L., & Mubarak, A. S. (2023). Different omega-3 concentrations in bran suspension toward the first reproduction time of *Artemia* sp. In IOP Conference Series: Earth and Environmental Science, 1273(1), 012037.

Pratiwy, F. M., Grandiosa, R., & Arifah, F. N., (2021). The enrichment of live feeds: an inquiry for feeding at early stages of fish. Int. J. Fish. Aquat. Stud., 9, 131–134.

Rasdi, N. W., & Qin, J. G. (2014). Improvement of copepod nutritional quality as live food for aquaculture: a review. Aquaculture Research, 47(1), 1-20.

Sapwarobol, S., Saphyakhajorn, W., & Astina, J. (2021). Biological functions and activities of rice bran as a functional ingredient: A review. Nutrition and metabolic insights, 14, 11786388211058559.

Sellami, I., Naceur, H. B., & Kacem, A. (2021). Reproductive performance in successive generations of the brine shrimp *Artemia salina* (Crustacea: Anostraca) from the Sebkha of Sidi El Hani (Tunisia). Animal Reproduction Science, 225, 106692.

Toi, H. T., Quynh, H. T., & Van, N. T. (2021). Study on *Artemia* culture in low salinity water in earthen ponds: how to avoid poor cysts production due to erratic rains and prolonged high temperatures resulting from the climate change. Aquaculture, Aquarium, Conservation & Legislation, 14(6), 3189-3196.

Wang, Y. Y., Liang, X. F., He, S., Tang, S. L., & Peng, D. (2022). The potential use of *Artemia* for larval rearing of mandarin fish (*Siniperca chuatsi*). Aquaculture Reports, 25, 101216.

Yang, J. Y., & Sun, S. C. (2023). Combined effects of temperature, photoperiod, and salinity on reproduction of the brine shrimp *Artemia sinica* (Crustacea: Anostraca). PeerJ, 11, e15945.

Yin, Z. J., Liu, H. L., Dong, X., Tian, L., Xiao, L., Xu, Y. N., & Qu, L. Q. (2014). Increasing α-linolenic acid content in rice bran by embryo-specific expression of ω3/Δ15-desaturase gene. Molecular breeding, 33, 987-996.