Available online at
https://borneoakademika.uitm.edu.my

Borneo Akademika
Volume 9(1) June 2025, 1-8

e-ISSN: 2735-2250

Application of Coconut and Pineapple Probiotics on Copepod (*Acartia* sp.)

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Received Date: 20 April 2025
Accepted Date: 1 May 2025
Published Date: 30 June 2025

ABSTRACT

Copepod serves as a live food, supplementing essential amino acids and fatty acids into the aquatic larval organism. Nevertheless, the mass production of copepods remains unfeasible. A study was conducted at a fish hatchery in UiTM Mukah to investigate the effects of different probiotics on the proliferation rate of copepod *Acartia* sp. Three treatments were carried out during a 77-day feeding trial: a tank with no probiotic (T1), a tank with coconut-based probiotics (T2), and a tank with pineapple-based probiotics (T3). Yeast containing good bacteria (*Saccharomyces cerevisiae*) was used as feed to the copepods. The proliferation rate was measured by counting the number of copepods observed throughout the experimental period. Each tank was initially stocked with 10 copepods. T3 (pineapple probiotic) achieved the highest proliferation rate, with 222.67 ± 1.453 copepods, significantly outperforming the other treatments. T1 (no probiotic) exhibited the lowest proliferation rate, with only 43.33 ± 0.882 copepods, and was also significantly different from the other treatments. The results demonstrate that the use of pineapple juice as a probiotic significantly enhances copepod proliferation in a mass culture. Subsequently, this finding suggests a potential solution to the current challenges in the copepods production for aquatic hatcheries.

Keywords: Copepod; larval fish production; live food; probiotics; yeast

INTRODUCTION

The aquaculture industry is recognized as one of the fastest expanding sector in the global food production and supply. This expansion creates a key strategy in closing the gap of protein scarcity in human nutrition (Jian et al., 2021). The growing demand for safe and sustainable food has driven the application of natural growth promoters in fish feed. Among the most extensively studied supplements are probiotics, prebiotics, synbiotics, and phybiotics (Denev et al., 2009). Probiotics, a type of live microbial feed supplement, have been shown to create a beneficial environment for the host organisms. One of these hosts is live feed, such as copepods. Fish and other aquatic organisms rely on the superior nutritional quality of live feed during the early developmental stages for survival. A study by Yanes-Roca et al. (2014) demonstrated that the application of probiotics to live feed yields promising outcomes in disease prevention. The study also observed that approximately 10% of bacteria isolated from fish intestines exhibit antibacterial activity against fish pathogens. Similarly, Martínez Cruz et al. (2012) reported reduced in fish mortality during a lethal *Aeromonas* sp.

challenge when live feed was integrated with probiotics. During this fragile stage, larvae require high-quality live feed enriched with essential nutrients to support their optimal growth and immunity.
Copepods, a primary pelagic zooplankton, play a crucial role in transferring essential nutrients across trophic organisms (Samat et al., 2020). They are particularly important as live feed for fish larvae, with over 60 species successfully cultivated since the 1960s. *Acartia tonsa* is recognized as the first copepod species to be successfully cultured, carried out in Denmark in 1981 (Stottrup et al., 1986). Establishing a consistent and sustainable culture of copepods is vital to meet the growing demands of hatcheries, the aquarium trade, and research fields.

The mass production of copepods also offers a sustainable approach for natural fish stock depletion. Drillet et al. (2006) highlighted that nutritionally rich copepods are key strategies for achieving higher production of larval finfish. Species such as cod, halibut, flounder, and barramundi have all been successfully cultured with calanoid copepods from genera *Acartia*, *Eurytemora*, and *Gladioferens*. Precisely, some copepod species have the ability to elongate fatty acid chains, converting short-chain fatty acids into long-chain polyunsaturated fatty acids (Desvilettes et al., 1997). This metabolic ability allows copepods to be cultured using inexpensive ingredients such as yeast and flour.

Some copepod species, such as calanoids, are considered difficult to mass culture, whereas harpacticoids and cyclopoids are more adaptable. Differences in inherent traits and growth parameters within these taxa contribute to the cultivation challenges (Drillet et al., 2006). Nevertheless, maintaining optimal water quality is essential for achieving high copepod growth density.

One major bottleneck in using copepods as the primary feed source for fish larvae is the difficulty in mass culture. Limited studies on culture improvement further hinder progress in the aquaculture industry. These factors have contributed to the limited commercial-scale cultivation of this unique species. According to Holste & Peck (2006), immediate research on cost-effective methods for copepod culture could address issues related to low fish larval productivity. Host organisms such as copepods benefit from the application of probiotic bacteria, which promote health by improving water quality, balancing beneficial microbial populations, and reducing pathogenic bacteria (Gomez-Gil et al., 2000).

At present, major producers commercial probiotics are led by China. Manufacture over 500,000 tons annually, they focus primarily on photosynthetic bacteria, antagonistic bacteria, lactic acid bacteria, nitrifying bacteria, and denitrifying bacteria (Qi et al., 2009). According to Denev et al. (2009), there is a fundamental difference in the factors influencing aquatic and terrestrial-based probiotics. In aquatic environments, there is no clear distinction between the microbial communities within inside and outside the host, due to the constant interaction between the aquatic environment and the host.

After fish hatch, microbes begin colonizing their gastrointestinal tract and regulate gene expression, forming a conducive environment while inhibiting the invasion of other bacteria (Carvalho et al., 2012). The benefits of integrating and administering probiotics include synthesis of inhibitory compounds, competition for nutrients, energy, and adhesion sites, improvement of water quality, enhancement of immune responses, and support for the host's digestion (Andrejčáková et al., 2016; de la Fuente et al., 2015; Sopková et al., 2017).

Ensuring the effectiveness of probiotics in aquaculture requires careful consideration of dosage and application methods. The efficacy of probiotics heavily depends on their ability to survive and proliferate within the host's intestinal tract. In feed, a concentration of 10^6 to 10^7 live probiotic organisms per gram or milliliter is necessary to maintain effectiveness (Yadav et al., 2004). Probiotics can be administered either through feed or by directly adding them to the fish tank. Adequate aeration is essential to support probiotic development, with a minimum dissolved oxygen level of 3% (Denev et al., 2009). One successful application of probiotics is the bioencapsulation of live organisms, as demonstrated by a significant increase in rotifer populations when cultivated with *Lactobacillus plantarum* (Yanes-Roca et al., 2014). *Lactobacillus* sp. and *Bacillus* sp. are among the beneficial bacteria commonly incorporated into commercial probiotic products under strict regulation (Martínez et al., 2012).

Organism health can be enhanced through the application of probiotics via competitive elimination and the production of antimicrobial compounds that combat pathogenic bacteria (Pérez-Sánchez et al., 2014). According to Lin et al. (2019), the effects of probiotics vary depending on the host and probiotic species. The response of copepods (*Acartia* sp.) may differ from other genera such as *Eurytemora*, *Gladioferens*, *Parvocalanus*, and *Centropages*. However, there are limited studies on the application of probiotics in copepod cultures (Drillet et al., 2006; Drillet et al., 2011). Therefore, this study was conducted to evaluate the effectiveness of coconut and pineapple probiotics in the mass culture of copepods (*Acartia* sp.).

The utilization of fruit juices as substrates for probiotic fermentation has garnered significant attention among researchers. Studies on blueberry juice (Li et al., 2023), watermelon juice (Shi et al., 2023), passion fruit juice (Fonseca et al., 2022), and jackfruit juice (Muhialdin et al., 2021) have highlighted the unique composition of these juices, which are rich in essential nutrients, sugars, and bioactive compounds. These nutritional profiles provide an ideal environment for the cultivation of beneficial microorganisms. Previous investigations consistently emphasize the effectiveness of fruit juices as potent media for probiotic fermentation. This study investigates the potential for mass culturing copepods (*Acartia* sp.) using probiotics that are enriched with pineapple and coconut juices, which are recognized to support the growth of probiotic strains such as *Lactobacillus casei*.

METHODOLOGY

Sampling of copepod

Copepods were collected from ten aquatic ponds located behind a fish hatchery at UiTM Sarawak Branch, Mukah Campus. Samples were obtained using a 140 μm mesh sieve. The organisms from the samples were observed under a light microscope. Copepods (*Acartia* sp.) were isolated and accumulated in a beaker.

Preparation of coconut and pineapple probiotics

A 10 L basin was prepared, containing 5 L of water mixed with 1 L fresh coconut water (for the coconut probiotic), 1 L of fresh pineapple juice, 1 L of molasses, 195 mL of Yakult containing *Lactobacillus casei* strain Shirota, and 100 g of dry yeast. The mixture was stirred well to combine the ingredients. It was then distributed evenly into several empty bottles, with tightly sealed lids. The lids were opened occasionally every two days to release gas buildup. The probiotic solution was considered ready for use once a sour smell was detected.

Experimental setting

Three treatments were prepared: no probiotic (T1), coconut probiotic (T2), and pineapple probiotic (T3). Each treatment included three replicates, prepared in 1 L glass bottles containing 800 mL of water and 10 copepods. The treatments were arranged randomly using a Completely Randomized Design in the fish hatchery. To prepare the feed, 10 g of dry yeast was dissolved in 50 mL of dechlorinated water. Each treatment was fed with 1 mL of the prepared liquid yeast solution. For T2 and T3, 5 mL of coconut and pineapple probiotic, respectively, were also added. Once the water in the treatments changed from cloudy to clear, each treatment was supplemented with an additional 1 mL of the yeast solution. For T2 and T3, additional 5 mL of the respective probiotic was added.

Data collection

The number of copepods in each treatment was counted and recorded weekly.

Data analysis

Data was analysed using SPSS version 16 software. A one-way analysis of variance (ANOVA) was applied to determine statistically significant differences ($p<0.05$) among the treatment means. Tukey's test was used to identify significant differences between means at an alpha value of 0.05. All results were reported as Mean ± Standard Error (S.E).

RESULTS AND DISCUSSION

Table 1 indicates that copepods treated with pineapple probiotics (T3) displayed significantly improved growth performance, with the highest final number (222.67 ± 1.453a), compared to those treated with no probiotic (T1) and coconut probiotic (T2). The lowest growth performance was observed in T1 (no probiotic), yielding the lowest final count (43.33 ± 0.882c), which was significantly lower than other treatments. The differences in the final number of copepods across treatments could be attributed to the varying responses of copepods to the probiotics in enhancing growth rate, disease tolerance, and immunity (Hoseinifar et al., 2018; Tan et al., 2019; Zhang et al., 2019).

Table 1. Number of copepods after 77 days exposure to different probiotics.

Treatment	Initial Number	Final Number	Number Gain
T1: No Probiotic (Control)	10.00 ± 0.000	43.33 ± 0.882 ^{c}	33.33 ± 0.882 ^{c}
T2: Coconut Probiotic	10.00 ± 0.000	61.67 ± 0.882 ^{b}	51.67 ± 0.882 ^{b}
T3: Pineapple Probiotic	10.00 ± 0.000	222.67 ± 1.453 ^{a}	212.67 ± 1.453 ^{a}

Values are mean ± standard error of mean of three replicates. Means in the same column with different superscripts are significantly different at alpha value 0.05. Means in the same column with similar superscripts are not significantly different at alpha value 0.05.

The addition of probiotics has been shown to be successful in numerous studies involving aquatic species, such as white shrimp (*Litopenaeus vannamei*) (Amjad et al., 2022), shrimp (*Litopenaeus stylirostris*) (Singh et al., 2011), and *Macrobrachium rosenbergii* postlarvae (Khumsrisuk et al., 2022). Amjad et al. (2022) reported that *Bacillus amyloliquefaciens* probiotics improved biofloc systems in shrimp, resulting in better survival rates, total weight gain, and food conversion ratios. Similarly, *Pediococcus acidilactici* probiotics enhanced shrimp growth and survival. These findings align with the present study. Higher survival rates were observed in treatments containing probiotics compared to the control group. Meanwhile, the control group exhibited the lowest number of copepods in the final feeding trial. The improved survival rates are likely attributed to the beneficial effects of probiotics on the host microbiota, which aids suppress pathogenic bacteria (Amjad et al., 2022).

Several studies have also highlighted the benefits of pineapple-derived properties in aquatic animals. Singh et al. (2011) found that common carp fingerlings exhibited optimal growth and the lowest Feed Conversion Ratio (FCR), when their diet was supplemented with 1% pineapple waste extract. Similarly, Khumsrisuk et al. (2022) discovered that Nile tilapia fed with 1% pineapple peel extract exhibited significant improvements in weight gain, average daily weight gain, specific growth rate, FCR, protein efficiency ratio, and feed utilization efficiency. Sharma et al. (2019) also observed superior growth performances in common carp fingerlings when their diet included pineapple peel extract at a 1:2 ratio. Furthermore, Silabam and Fuad (2021) reported a significant increase in the weight and length of catfish (*Clarias batrachus*) fed a commercial diet enriched with 1.5% pineapple extract.

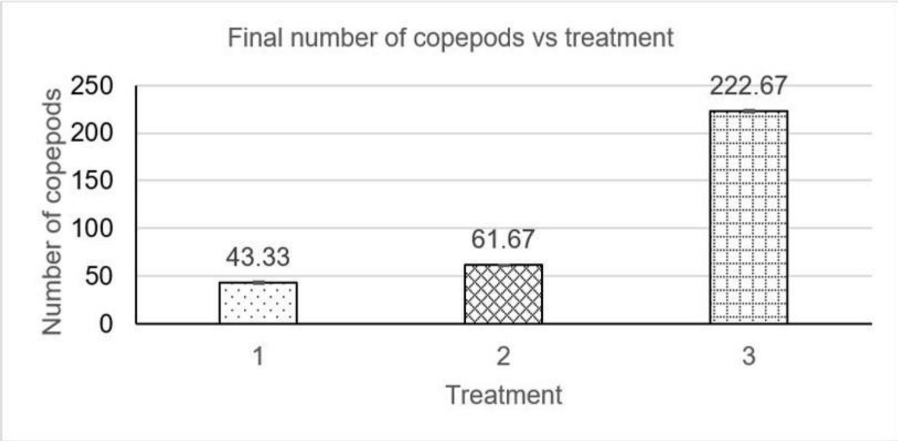

Figure 1. Bar graph on the final number of copepods versus different treatments of probiotics.

The observed increase in the number of copepods in the current study may be attributed to the beneficial effects of probiotics in enhancing digestion and nutrient absorption. Similar findings were reported by Amjad et al. (2022) in *L. stylirostris* shrimp, where probiotic treatments improved hepatic storage in the digestive gland. The unique biological traits of copepods, such as their small size, sexual dimorphism, and only 12 larval stages, contribute to their rapid population growth.

In addition to adequate nutrition, key environmental parameters such as temperature, salinity, dissolved oxygen, and light must be carefully controlled to ensure successful copepod culture. Maintaining optimal water quality can be achieved through automatic control systems equipped with specialized probes to monitor these parameters effectively.

The low final number of copepods observed in T1 (without probiotics) is consistent with the findings by Raj et al. (2008). They reported poor survival rates in fry of Indian major carp (*Catla catla*) when not supplemented with probiotics (Efinol® L). The absence of probiotics likely resulted in low immune defence and poor digestive enzyme activity, hence contributing to poor growth performance.

Another contributing factor is the increased likelihood of cannibalism as copepod populations grow. When copepods frequently encounter one another in the same medium, larger individuals often prey on smaller ones, ultimately reducing the final population. This phenomenon has been reported in various copepod species, including *Sinocalanus tenellus*, *Centropages abdominalis*, *Tigriopus fulvus*, and *Acartia sinjiensis* (Drillet et al., 2011). Effective management strategies, such as segregating copepods by size or grading, are necessary to minimize cannibalism (Brandl, 1972). Additionally, regularly harvesting eggs that sinking to the bottom can help optimize egg production. The sex ratio of male to female copepods also requires careful management. For example, in 2.5 months, only one spermatophore is needed to fertilize eggs (Ohtsuka and Huys, 2001). A single male *Temora longicornis* can mate with multiple females in a day, suggesting that reducing the number of males may improve productivity and profitability.

In the future, the growth performance of copepods could be enhanced by applying a combination of probiotics. Dash (2018) demonstrated the effectiveness of using *Bacillus pumilus* and *Lactobacillus delbrueckii* on *Cyprinus carpio*, which exhibited strong gut colonization ability and improved growth. Multispecies probiotic applications have been shown to provide synergistic benefits, improving overall efficacy (Ajiboye et al., 2011; Hauville et al., 2016; Ibrahem, 2013).

CONCLUSIONS

Copepods treated with pineapple probiotics exhibited the highest growth rate population, suggesting that pineapple probiotics are the best candidate to address the challenges of mass culturing copepods (*Acartia sp.*).

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of Universiti Teknologi Mara (UiTM), Sarawak Branch, Mukah Campus, for providing the apparatus and materials for this research.

CONFLICT OF INTERESTS

The authors declare that this research was conducted in the absence of any personal, commercial or financial conflicts of interest related to the funders.

AUTHORS' CONTRIBUTIONS

Ros Anizah carried out the research, wrote and revised the article. Ros Anizah also conceptualised the central research idea and developed the theoretical framework. Norliza and Nurain Johar designed the research and supervised research progress Ros Anizah led the review process, managed revisions and approved the final article submission.

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