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FECUNDITY AND BIOMASS OF *Artemia franciscana* FED DIFFERENT FEED TYPES

Fekunditas dan Biomassa *Artemia franciscana* pada Pemberian Jenis Pakan Berbeda

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ABSTRACT

Artemia franciscana is an essential live food in fish and shrimp hatchery operations, whose nutritional value is significantly influenced by the feed provided during culture. Providing high-quality, cost-effective feed remains a major challenge in the mass production of *A. franciscana*. This study aimed to evaluate the effect of different feed types on the fecundity and biomass of *Artemia franciscana*. The experiment was conducted at the Nutrition and Natural Feed Laboratory, Faculty of Fisheries and Marine Sciences, Mulawarman University, over 30 days (August–September 2024). A completely randomized design (CRD) with four treatments and four replications (n = 16 experimental units) was employed. Treatments consisted of P1 (spirulina flour), P2 (soybean meal), P3 (palm kernel cake), and P4 (a mixture of spirulina flour, soybean meal, and palm kernel cake at 1:1:1 w/w ratio). Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level. Results demonstrated that feed type had a highly significant effect ($P<0.01$) on the fecundity and biomass of *A. franciscana*. The highest fecundity was achieved in P4 (100.00 ± 4.40 nauplii/female), followed by P1 (86.00 ± 3.37 nauplii/female), P2 (71.25 ± 2.99 nauplii/female), and P3 (53.75 ± 3.50 nauplii/female). The highest final biomass was similarly recorded in P4 (1.75 ± 0.07 g/L), followed by P1 (1.49 ± 0.06 g/L), P2 (1.23 ± 0.05 g/L), and P3 (0.92 ± 0.05 g/L). All treatments differed significantly from each other based on DMRT ($P<0.05$). Water quality parameters remained within ranges supportive of *A. franciscana* survival and reproduction throughout the study. The mixed feed provided a more balanced nutrient profile, yielding the highest reproductive performance and biomass accumulation in *Artemia franciscana*.

Keywords: *Artemia franciscana*, Biomass, Fecundity, Mixed Feed, Spirulina Flour

ABSTRAK

Artemia franciscana merupakan pakan hidup penting dalam kegiatan pembenihan ikan dan udang yang nilai nutrisinya sangat dipengaruhi oleh jenis pakan yang diberikan selama kultur. Penyediaan pakan berkualitas dengan harga terjangkau masih menjadi tantangan utama dalam produksi massal *A. franciscana*. Penelitian ini bertujuan mengevaluasi pengaruh pemberian jenis pakan berbeda terhadap fekunditas dan biomassa *Artemia franciscana*. Penelitian dilaksanakan di Laboratorium Nutrisi dan Pakan Alami, Fakultas Perikanan dan Ilmu Kelautan, Universitas Mulawarman, selama 30 hari (Agustus–September 2024). Rancangan penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan empat perlakuan dan empat ulangan (n = 16 unit percobaan). Perlakuan terdiri atas P1 (tepung spirulina), P2 (tepung kedelai), P3 (tepung bungkil sawit), dan P4 (campuran spirulina, kedelai, dan bungkil sawit dengan rasio 1:1:1 b/b). Data dianalisis menggunakan analisis sidik ragam (ANOVA) satu arah dan uji lanjut Duncan's Multiple Range Test (DMRT) pada taraf kepercayaan 95%. Hasil penelitian menunjukkan bahwa jenis pakan berpengaruh sangat nyata ($P<0,01$) terhadap fekunditas dan biomassa *A. franciscana*. Fekunditas tertinggi diperoleh pada perlakuan P4 sebesar 100,00 ± 4,40 nauplii/induk, diikuti P1 (86,00 ± 3,37 nauplii/induk), P2 (71,25 ± 2,99 nauplii/induk), dan P3 (53,75 ± 3,50 nauplii/induk). Biomassa akhir tertinggi juga dicapai oleh P4 (1,75 ± 0,07 g/L), diikuti P1 (1,49 ± 0,06 g/L), P2 (1,23 ± 0,05 g/L), dan P3 (0,92 ± 0,05 g/L). Seluruh perlakuan berbeda nyata satu sama lain berdasarkan uji DMRT ($P<0,05$). Kualitas air selama pemeliharaan berada dalam kisaran yang mendukung kehidupan *A. franciscana*. Pakan campuran menghasilkan keseimbangan nutrisi yang lebih optimal sehingga memberikan performa reproduksi dan akumulasi biomassa tertinggi pada *Artemia franciscana*.

Kata Kunci: *Artemia franciscana*, Biomassa, Fekunditas, Pakan Campuran, Tepung Spirulina

INTRODUCTION

Artemia franciscana (Kellogg, 1906) is a primitive crustacean from the order Anostraca that has long been recognized as the most important live food in the fish and shrimp hatchery industry worldwide (Madkour et al., 2023; Van Stappen et al., 2024). This organism inhabits hypersaline lake ecosystems and salt production ponds, has a very wide salinity tolerance range, ranging from 5 to over 250 ppt, and can survive extreme environmental conditions (Madkour et al., 2023; Van Stappen et al., 2024). Among the various Artemia strains that have been identified, the *A. franciscana* strain from the Great Salt Lake, United States, is the most widely used commercially in aquaculture due to the consistent quality and availability of its cysts (Van Stappen et al., 2024).

Newly hatched Artemia nauplii from cysts range in size from 400 to 500 µm, fitting the mouth openings of most intensively cultured fish and shrimp larvae. In addition to their size, Artemia nauplii are slow-moving, easily captured by larvae, contain 40–60% protein by dry weight, and can be utilized immediately after hatching or further developed through adult broodstock culture to improve quantity and nutritional quality (Amin et al., 2023; Amin et al., 2025). Adult Artemia culture enables the continuous production of nauplii in large quantities, making it an essential component of modern hatchery management (Anand et al., 2024; Madkour et al., 2023). The contribution of Artemia to the successful hatchery production of marine fish, freshwater fish, and shrimp has been extensively documented in various international studies (Ashaari et al., 2024; Khan et al., 2025; Van Stappen et al., 2024).

The nutritional quality of Artemia is not fixed, but is strongly influenced by the composition of the feed provided during the culture phase, a phenomenon known as "you are what you eat" or nutrient bioencapsulation (Amin et al., 2022; Turcihan et al., 2021). The content of essential fatty acids such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in Artemia nauplii varies significantly depending on the feed source provided to the female parent (Amin et al., 2022; Pham et al., 2023; Turcihan et al., 2021). Adequate polyunsaturated fatty acids (PUFAs) are critical for nervous system development and optimal growth of fish and shrimp larvae that consume Artemia as live food (Amin et al., 2022; Pham et al., 2023). Fecundity and biomass of Artemia broodstock are also highly responsive to the quality and quantity of available feed, making feed optimization a key strategy for increasing culture productivity (Amin et al., 2023; Van Stappen et al., 2024).

One of the main challenges in mass production of *Artemia franciscana* is the reliance on high-quality, but often expensive, feed ingredients, such as commercial microalgae and fish meal (Macusi et al., 2023; Serra et al., 2024). Limited supply and fluctuating prices of premium feed ingredients encourage the exploration of more economical and readily available alternative feed ingredients, particularly from local sources (Hussain et al., 2024; Macusi et al., 2023). In East Kalimantan, agro-industrial feed ingredients such as palm kernel meal are abundant and relatively inexpensive, making them attractive for exploration as a component of Artemia feed. However, the use of single feed ingredients based on local sources risks producing certain nutritional deficiencies that can suppress the growth and reproduction of Artemia (Amin et al., 2023; Amin et al., 2025).

Spirulina flour (*Spirulina platensis*, synonym: *Arthrospira platensis*) is known as one of the most nutrient-rich natural feed supplements available for aquatic organisms. Spirulina protein content reaches 60–70% of dry weight with a complete and balanced essential amino acid profile (Alagawany et al., 2021; Ringø, 2025; Zhang et al., 2020). In addition to protein, spirulina is rich in functional pigments such as phycocyanin, β-carotene, and zeaxanthin, as well as γ-linolenic acid (GLA, 18:3n-6), which plays an important role in reproductive metabolism (Al Mamun et al., 2023; Alagawany et al., 2021; Soma et al., 2024). The carotenoids contained in spirulina function as biological antioxidants that protect gonad cells from oxidative damage and act as precursors for reproductive hormones in crustaceans (Ringø, 2025; Sun et al., 2024; Zahan et al., 2024). The cellulose-free cell walls of spirulina contribute to its high digestibility by filter feeders like Artemia, making nutrient utilization from spirulina more efficient than other plant-based feed sources (Alagawany et al., 2021; Zhang et al., 2020).

Soybean meal is the most widely used plant-based protein source in aquaculture feed formulations, with a protein content ranging from 44–48% dry weight (Abdul Kari et al., 2023; Ma et al., 2022). Soybean meal's widespread availability, relatively affordable price, and relatively complete amino acid profile make it an attractive candidate feed ingredient for Artemia culture. However, soybean meal contains several antinutritional factors, including trypsin inhibitors, saponins, phytates, and lectins, which can inhibit digestive enzyme activity, reduce protein and mineral bioavailability, and potentially damage the integrity of the gastrointestinal epithelium (Ma et al., 2022; Peng et al., 2022). In aquatic organisms, the presence of these antinutrients has been reported to suppress growth rates, reduce feed utilization efficiency, and at high concentrations can affect reproductive success (Ma et al., 2022; Peng et al., 2022). Therefore, although soybean meal quantitatively contains adequate protein, its effectiveness as a sole feed for Artemia requires further study (Ma et al., 2022; Yang et al., 2024).

Palm kernel cake/meal is a byproduct of the palm oil processing industry and is abundantly available in East Kalimantan, one of the largest palm oil plantation centers in Indonesia. This ingredient contains protein ranging from 15–18% with a relatively high crude fiber content of 13–18%, as well as significant proportions of mannan and galactomannan (Enyidi, 2023; Huang et al., 2024; Mamat et al., 2021). High crude fiber content can limit nutrient digestibility and bioavailability, especially in organisms with limited enzymatic capacity such as suspension-feeding Artemia (Van Stappen et al., 2024). However, palm kernel meal contains essential minerals such as phosphorus, calcium, and magnesium, as well as saturated fatty acids that can contribute energy to Artemia growth when combined with other high-quality feed ingredients (Huang et al., 2024; Mamat et al., 2021).

To the author's knowledge, comprehensive studies comparing the effects of spirulina flour, soybean flour, and palm kernel meal, either singly or in a proportional mixture, on the fecundity and biomass of *Artemia franciscana* are still very limited in the scientific literature. Most previous studies have focused on fatty acid bioencapsulation or enrichment of Artemia nauplii with commercial lipid emulsions (Amin et al., 2022; Ashaari et al., 2024; Balachandar & Rajaram, 2019; Van Stappen et al., 2024), while studies on locally sourced, readily available, and economically available feeds for adult Artemia culture are still very rare. Furthermore, the potential of palm kernel meal—a dominant agro-industrial commodity in East Kalimantan—as a component of Artemia feed has never been specifically reported in the context of reproductive parameters and biomass. Based on these research gaps, this study was designed to evaluate the effects of spirulina flour, soybean flour, palm kernel meal, and a mixture of the three on the fecundity and biomass of *Artemia franciscana*. This study is expected to provide scientific contributions related to the optimization of alternative feed based on local ingredients for more efficient and sustainable *A. franciscana* production, as well as serve as a scientific reference for aquaculture development in East Kalimantan.

RESEARCH METHODS

Research Time and Location

The research was conducted for 30 days from August to September 2024 at the Nutrition and Natural Feed Laboratory, Faculty of Fisheries and Marine Sciences, Mulawarman University, Samarinda, East Kalimantan.

Tools and Materials

The equipment used included a 2 L transparent glass jar as a culture container, an aerator and its accessories (aeration hose, airstone, and air pump), a digital scale (accuracy 0.01 g), a stereo microscope and magnifying glass, a dropper, a 100 mL graduated cylinder, a refractometer (accuracy 0.5 ppt), a digital thermometer, a pH meter, a DO meter, an ammonia test kit, and a 100 µm plankton sieve. The materials used included *Artemia franciscana* cysts from the Great Salt Lake strain, seawater with a salinity of 30–32 ppt, spirulina flour (*Spirulina platensis*, protein content ≥ 60% dry weight), soybean flour (protein content ≥ 44% dry weight), palm kernel meal flour (protein content ≥ 16% dry weight), and distilled water for diluting the test solution.

Research Design

The study used a Completely Randomized Design (CRD) with four treatments and four replications, resulting in 16 experimental units. The four treatments tested were P1 (spirulina flour as the sole microalgae-based feed), P2 (soybean flour as the sole vegetable protein-based feed), P3 (palm kernel meal as the sole agro-industrial waste-based feed), and P4 (a mixture of spirulina flour, soybean flour, and palm kernel meal in a 1:1:1 ratio on a weight/weight basis as a combined feed).

Table 1. Feed Treatment Composition in the Study

Treatment	Types of Feed	Categories
P1	Spirulina flour	Microalgae single feed
P2	Soybean flour	Plant-based single feed
P3	Palm kernel meal flour	Agro-industrial single feed

P4	Mix (1:1:1 b/b)	Combination feed

Media Preparation and Cyst Hatching

The maintenance medium used was filtered and sterilized seawater with a salinity of 30–32 ppt. Each culture vessel was filled with 1 L of seawater. Continuous aeration was provided using an aerator to maintain dissolved oxygen levels above 5 mg/L and prevent feed from settling at the bottom of the vessel (Van Stappen et al., 2024).

A. franciscana cysts were hydrated and hatched in separate containers filled with water with a salinity of 30–32 ppt with intensive aeration and 40-watt lighting for 24–36 hours at room temperature (28–30°C). Hatched nauplii were separated from the cyst shell using a positive phototaxis response using the light gradient method, then collected using a 100-µm plankton sieve and stocked into rearing tanks at an initial density of 100 individuals per liter (Van Stappen et al., 2024).

Feed Preparation and Feeding

Each feed ingredient was ground using a dry blender and sieved through a 100-µm plankton sieve to adjust the particle size to the filtering capacity of Artemia (Van Stappen et al., 2024). In the P4 treatment, the three ingredients were mixed homogeneously in proportions of 33.3% spirulina flour, 33.3% soybean flour, and 33.3% palm kernel meal flour (weight/weight). Feed was provided twice daily at 7:00 AM and 5:00 PM WITA with a total dose of 0.2 g/L/day (0.1 g/L per feeding) following the recommendations of Van Stappen et al. (2024). Before feeding, each portion of feed was diluted in 10 mL of seawater and stirred until homogeneous to facilitate the distribution of feed particles in the water column.

Maintenance

Maintenance was carried out for 30 days until the Artemia reached adulthood and the female broodstock began to reproduce (around the 20th to 30th day). Aeration was maintained continuously. Leftover feed and debris that settled on the bottom of the container were removed daily using a dropper. A 20–30% water change was performed every 2–3 days to maintain the quality of the culture environment. Water quality parameters (temperature, salinity, pH, dissolved oxygen/DO, and ammonia) were measured every three days using a calibrated instrument.

Research Parameters

Fecundity

Fecundity was observed after the Artemia reached adulthood and the female broodstock began producing nauplii. Observations were conducted by randomly selecting 10 female broodstock per replication using a dropper, counting the number of nauplii produced per reproductive cycle using a magnifying glass, and then returning the broodstock to the rearing container. Fecundity was calculated using the following formula (Van Stappen et al., 2024):

$$F = N / I$$

Where:
F = fecundity (nauplii/broodstock)
N = total number of nauplii produced
I = number of female broodstock observed.

Biomass

Artemia biomass was measured at the end of rearing (day 30). All Artemia in each container were harvested using a 100 µm plankton sieve, drained on filter paper for 30 seconds to remove surface water, and then weighed using a digital scale. Biomass was calculated using the formula:

$$B = W / V$$

Where:
B = biomass (g/L)
W = total weight of harvested wet Artemia (g)
V = volume of rearing medium (L).

Data Analysis

Fecundity and biomass data are presented as mean ± standard deviation (SD). Prior to ANOVA analysis, data normality was tested using the Shapiro-Wilk test, and homogeneity of variance was tested using Levene's test. After the assumptions were met, data were analyzed using one-way analysis of variance (ANOVA) at the 95% confidence level ($\alpha = 0.05$). If the ANOVA showed a significant difference between treatments ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was performed to identify differences between treatment pairs. Water quality data were analyzed descriptively and compared with optimal reference values for *A. franciscana* survival according to Van Stappen et al. (2024). All statistical analyses were performed using SPSS version 26 software.

RESULT

***Artemia franciscana* Fecundity**

The average fecundity of *Artemia franciscana* in each treatment is presented in Figure 1. Measurement results indicate significant variation in fecundity between treatments. The highest fecundity was achieved in treatment P4 (feed mixture) at 100.00 ± 4.40 nauplii/parent, followed sequentially by P1 (spirulina flour) at 86.00 ± 3.37 nauplii/parent, P2 (soybean flour) at 71.25 ± 2.99 nauplii/parent, and P3 (palm kernel meal) at 53.75 ± 3.50 nauplii/parent.

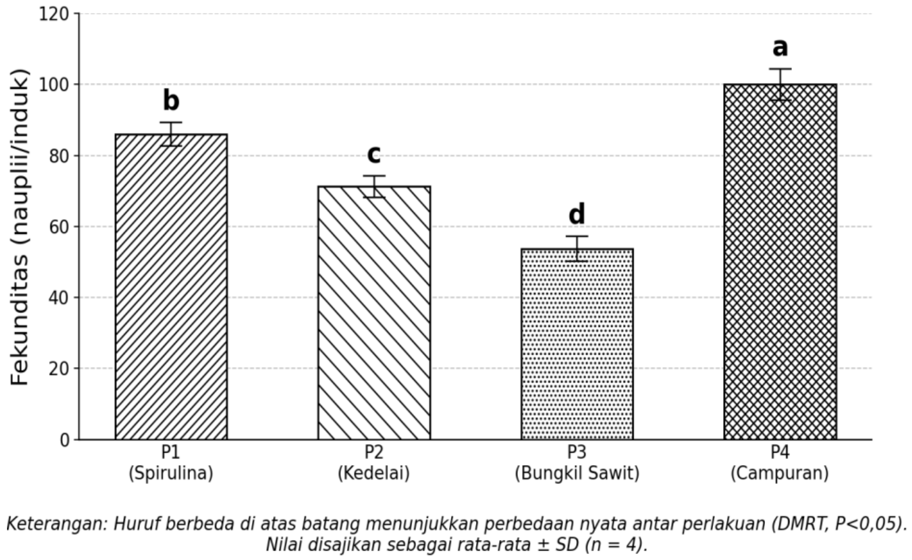

Figure 1. Average Fecundity of *Artemia franciscana* (nauplii/parent ± SD) in Each Treatment. Different Letters Above the Bars Indicate Significant Differences Based on the DMRT Test ($P<0.05$).

The results of the analysis of variance (ANOVA) on the fecundity of *Artemia franciscana* are presented in Table 2. Feed type had a highly significant effect on fecundity (F-count = 121.56 > F-table 1% = 5.95; P<0.01). The coefficient of variation (CC) of 4.63% indicates that the data variability within the treatments is low, making the research results reliable.

Table 2. Analysis of Variance (ANOVA) of *Artemia franciscana* Fecundity

Sources of Diversity	JK	db	KT	F-count	F-table (1%)
Treatment	4,725.50	3	1,575.17	121.56**	5.95
Error	155.50	12	12.96	-	-
Total	4,881.00	15	-	-	-

Note: ** = highly significant difference (P<0.01); CC = 4.63%; JK = sum of squares; db = degrees of freedom; KT = mean square.

The results of the further DMRT test (Table 3) show that all treatments are significantly different from each other (P<0.05), with all letter notations being different. This confirms that each feed type produced statistically different fecundity responses, with no treatments producing equivalent responses.

Table 3. Results of the DMRT Fecundity Follow-Up Test for *Artemia franciscana*

Treatment	Types of Feed	Mean ± SD (nauplii/broodstock)	DMRT Notation	Ranking
P3	Palm kernel meal flour	53.75 ± 3.50	d	4
P2	Soybean flour	71.25 ± 2.99	c	3
P1	Spirulina flour	86.00 ± 3.37	b	2
P4	Mix (1:1:1 b/b)	100.00 ± 4.40	a	1

Note: Different letter notations in the same column indicate significant differences between treatments based on the DMRT test (P<0.05). SE = 1.7999; LSR(p=2) = 5.547; LSR(p=3) = 5.805; LSR(p=4) = 5.963.

Artemia franciscana Biomass

The final biomass measurements of *Artemia franciscana* for each treatment are presented in Figure 2. The highest biomass was obtained in treatment P4 at 1.75 ± 0.07 g/L, followed by P1 at 1.49 ± 0.06 g/L, P2 at 1.23 ± 0.05 g/L, and P3 at 0.92 ± 0.05 g/L. The biomass ranking pattern is consistent with the fecundity pattern across all treatments.

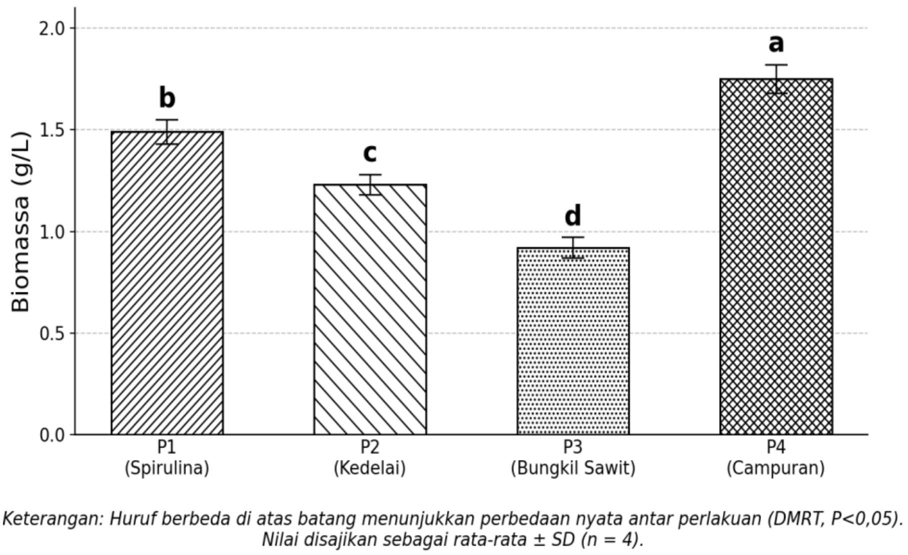

Figure 2. Average Final Biomass of *Artemia franciscana* (g/L ± SD) for Each Treatment.

Different Letters Above the Bars Indicate Significant Differences Based on the DMRT Test (P<0.05).

The ANOVA results for *A. franciscana* biomass (Table 4) showed that the type of feed had a highly significant effect (F-count = 151.61 >> F-table 1% = 5.95; P <0.01). The coefficient of variation of 4.29% indicated good data uniformity within the treatments. The DMRT test (Table 5) confirmed that all treatments were significantly different from each other (P <0.05), with all letter notations differing from P3 (d) to P4 (a).

Table 4. Analysis of Variance (ANOVA) of *Artemia franciscana* Biomass

Sources of Diversity	JK	db	KT	F-count	F-table (1%)
Treatment	1.5246	3	0.5082	151.61**	5.95
Error	0.0402	12	0.0034	-	-
Total	1.5648	15	-	-	-

Note: ** = highly significant (P <0.01); CC = 4.29%.

Table 5. Results of the DMRT Follow-Up Test for *Artemia franciscana* Biomass

Treatment	Types of Feed	Mean ± SD (nauplii/broodstock)	DMRT Notation	Ranking
P3	Palm kernel meal flour	0.92 ± 0.05	d	4
P2	Soybean flour	1.23 ± 0.05	c	3
P1	Spirulina flour	1.49 ± 0.06	b	2
P4	Mix (1:1:1 b/b)	1.75 ± 0.07	a	1

Note: Different letter notations indicate significant differences based on the DMRT test (P <0.05). SE = 0.02895; LSR(p=2) = 0.0892; LSR(p=3) = 0.0934; LSR(p=4) = 0.0959.

Water Quality During Maintenance

Water quality parameters during *Artemia franciscana* maintenance are presented in Table 6. All water quality parameters in all treatments were within the range that supports normal *A. franciscana* life, based on reference values from Van Stappen et al. (2024).

Table 6. Water Quality Range During *Artemia franciscana* Maintenance

Treatment	Temperature (°C)	Salinity (ppt)	pH	DO (mg/L)	NH_3 (mg/L)	Optimal Reference*
P1	28.3–29.1	30–32	7.8–8.1	5.8–6.4	0.03–0.07	Van Stappen et al. (2024)
P2	28.2–29.0	30–32	7.7–8.0	5.5–6.2	0.05–0.09	
P3	28.4–29.3	30–33	7.6–7.9	5.1–5.8	0.08–0.13	
P4	28.1–29.0	30–32	7.8–8.2	5.9–6.6	0.03–0.06	
Optimal*	25–30	25–40	7.5–8.5	≥ 4.0	< 0.20	

*Optimal values based on Van Stappen et al. (2024). NH_3 = dissolved free ammonia.

DISCUSSION

Artemia franciscana Fecundity

The highest fecundity in the P4 (mixed feed) treatment, at 100.00 ± 4.40 nauplii/parent, is thought to be closely related to a more optimal balance of nutrient composition compared to the single-feed treatment. Mixed feeds that proportionally combine spirulina, soybeans, and palm kernel meal have the potential to provide a more complete nutrient spectrum, including high-quality protein with a balanced essential amino acid profile, carotenoid and phycocyanin pigments, essential fatty acids, B-complex vitamins and vitamin E, and complementary macro- and micro-minerals (Alagawany et al., 2021; Amin et al., 2022; Zhang et al., 2020). The availability of complete essential amino acids in mixed feed is crucial for supporting vitellogenesis—yolk protein synthesis—which is a key prerequisite for successful female crustacean reproduction (Amin et al., 2023; Ma et al., 2022). The simultaneous provision of all nutrient precursors in a single mixed feed is thought to result in higher reproductive efficiency compared to providing single protein sources, each of which has a limited nutritional profile (Amin et al., 2023; Van Stappen et al., 2024).

The high fecundity of P1 (single spirulina meal) of 86.00 ± 3.37 nauplii/broodstock is consistent with spirulina's superior nutritional characteristics for aquatic organisms. Spirulina contains protein with a very high digestibility, reaching 80–90% in fish, which far exceeds the digestibility of soybeans and palm kernel meal (Alagawany et al., 2021; Zhang et al., 2020). The carotenoid content of spirulina, especially β-carotene and zeaxanthin, acts as an antioxidant that protects gonadal cell membranes from oxidative damage, as well as being a precursor of vitamin A which is important for the process of reproductive cell differentiation (Al Mamun et al., 2023; Sun et al., 2024; Zahan et al., 2024). The γ-linolenic acid (GLA) contained in spirulina can be metabolically converted to arachidonic acid (ARA) and EPA which act as reproductive signals and components of oocyte cell membranes (Amin et al., 2022; Ringø, 2025). Alagawany et al. (2021) and Zhang et al. (2020) reported that Spirulina has the potential to improve the growth performance and physiological status of aquatic organisms through its contribution of protein, pigments, and bioactive compounds. The absence of antinutritional factors in spirulina and its high digestibility due to the absence of a cellulose cell wall make its nutrients more accessible to Artemia, which act as filter feeders (Alagawany et al., 2021; Ringø, 2025; Zhang et al., 2020).

The fecundity of the P2 (soybean meal) treatment, at 71.25 ± 2.99 nauplii/parent, was in the middle, despite its quantitatively high protein content (44–48%). The decrease in fecundity compared to P1 is likely due to the presence of antinutritional factors in soybean flour, particularly protease inhibitors (trypsin and chymotrypsin inhibitors), saponins, and phytate, which can inhibit digestive enzyme activity and reduce protein and mineral bioavailability (Ma et al., 2022; Peng et al., 2022). In aquatic organisms, chronic exposure to soybean antinutrients has been reported to cause gastrointestinal mucosal disorders and reduced nutrient absorption efficiency (Ma et al., 2022; Peng et al., 2022). Furthermore, the relatively low methionine deficiency in soybeans and imbalances in certain amino acid ratios can limit protein utilization for optimal vitellogenin synthesis (Abdul Kari et al., 2023; Ma et al., 2022). Nevertheless, the fecundity of P2, which is still higher than that of P3, indicates that adequate soybean protein content can be partially utilized by *A. franciscana*, although not as efficiently as spirulina.

The lowest fecundity in P3 (palm kernel meal) at 53.75 ± 3.50 nauplii/broodstock is consistent with the relatively lowest nutritional content of palm kernel meal among the three feed ingredients tested. The protein content of palm kernel meal (15–18%) is much lower than that of spirulina and soybeans, so that the adequacy of essential amino acids to support the reproductive process cannot be met when used as a sole feed source (Huang et al., 2024; Mamat et al., 2021). The high crude fiber content in palm kernel meal (13–18%), which consists mainly of mannan and galactomannan, can make it difficult for Artemia to filter feed particles and reduce the efficiency of soluble nutrient absorption (Huang et al., 2024; Van Stappen et al., 2024). Artemia, as a non-selective suspension feeder, uses an appendicular filtration mechanism to filter particles measuring 1–50 µm. Therefore, coarse, fibrous particles that are difficult to break down in its short digestive tract provide very limited nutritional value (Amin et al., 2023; Van Stappen et al., 2024). This protein deficiency and limited digestibility of palm kernel meal result in insufficient energy and amino acid intake to support optimal gametogenesis and nauplii production.

Artemia franciscana Biomass

The consistent biomass pattern with fecundity across all treatments indicates a close relationship between feed quality, somatic growth, and reproductive capacity of *A. franciscana*. Biomass is the cumulative result of individual growth and reproductive success of the population over the culture period, so a feed that supports both processes simultaneously will produce the highest biomass (Amin et al., 2023; Amin et al., 2025). The highest biomass in P4 (1.75 ± 0.07 g/L) can be explained by the nutritional complementarity mechanism in mixed feeds, where the limitations of the amino acid profile of one feed ingredient are compensated for by the other, resulting in a more comprehensive nutrient intake and supporting more efficient population growth (Amin et al., 2023; Ma et al., 2022).

The higher biomass in P1 (spirulina flour, 1.49 ± 0.06 g/L) compared to P2 and P3 is related to the superior biological value of spirulina protein and its better digestibility. The lack of a cellulose layer in spirulina cell walls allows nutrients to be more easily released and absorbed by Artemia during filtration and digestion (Alagawany et al., 2021; Zhang et al., 2020). The vitamin B12 content in spirulina, which is rarely found in other plant-based feed ingredients, is also thought to contribute to energy metabolism and protein synthesis, supporting somatic growth (Ringø, 2025; Soma et al., 2024). The GLA fatty acid in spirulina, after being converted to ARA and EPA through the elongation and desaturation pathways, supports cell membrane integrity and stimulates somatic tissue growth (Amin et al., 2022; Pham et al., 2023).

The lowest biomass in P3 (palm kernel meal, 0.92 ± 0.05 g/L) is consistent with the limited nutritional value of palm kernel meal as a sole feed. The low protein content limits body protein synthesis, while the high crude fiber content reduces the efficiency of available energy utilization (Huang et al., 2024; Mamat et al., 2021). It should be noted that although palm kernel meal as a sole feed yielded the lowest yield, its presence in the P4 feed mixture still provided a positive contribution through its mineral content, mannan oligosaccharides, which can function as a moderate prebiotic, and as an additional energy source (Mamat et al., 2021). This is demonstrated by the fact that the biomass of P4 was higher than that of P1, indicating that the combination of the three ingredients is more synergistic than spirulina alone.

Water Quality and Its Influence on Artemia Performance

Water quality during maintenance was generally within the optimal range for *A. franciscana*. Water temperatures between 28.1–29.3°C are within the optimal range of 25–30°C, which supports optimal Artemia metabolism, growth, and reproduction (Van Stappen et al., 2024). Salinity of 30–32 ppt (P3: up to 33 ppt) is still well below the tolerance threshold of *A. franciscana*, which can withstand over 250 ppt, with optimal growth and reproduction at 30–40 ppt (Madkour et al., 2023; Van Stappen et al., 2024). A pH of 7.6–8.2 corresponds to the mild alkalinity required for Artemia digestive enzyme activity (Van Stappen et al., 2024). DO levels in all treatments (5.1–6.6 mg/L) were well above the minimum requirement of Artemia, which is approximately 2–3 mg/L, so oxygen was not a limiting factor (Amin et al., 2023; Van Stappen et al., 2024).

The relatively higher ammonia levels in treatment P3 (0.08–0.13 mg/L) compared to the other treatments indicated a greater accumulation of organic matter due to inefficiently digested palm kernel meal residue. The high crude fiber content in palm kernel meal results in more organic matter not being decomposed quickly, thus increasing the organic load in the culture vessel (Huang et al., 2024; Mamat et al., 2021). Although P3 ammonia levels (0.08–0.13 mg/L) are still well below the toxic threshold for Artemia (0.3–0.5 mg/L dissolved NH_3), these conditions can cause chronic sublethal stress that contributes to decreased reproductive performance and growth in the palm kernel meal treatment (Van Stappen et al., 2024). There were no significant differences in water quality between treatments overall, thus it can be concluded that the observed differences in fecundity and biomass were caused by differences in feed quality, not by aquatic environmental factors.

CONCLUSION

The type of feed had a very significant effect (P<0.01) on the fecundity and biomass of *Artemia franciscana*. Treatment P4, a mixture of spirulina flour, soybean flour, and palm kernel meal flour with a ratio of 1:1:1, gave the best results with an average fecundity of 100.00 ± 4.40 nauplii/broodstock and a final biomass of 1.75 ± 0.07 g/L, significantly different from all other treatments based on the DMRT test (P<0.05). Treatment P1 (spirulina meal) ranked second with a fecundity of 86.00 ± 3.37 nauplii/parent and a biomass of 1.49 ± 0.06 g/L, followed by P2 (soybean meal) with a fecundity of 71.25 ± 2.99 nauplii/parent and a biomass of 1.23 ± 0.05 g/L. Treatment P3 (single palm kernel meal meal) produced the lowest fecundity and biomass (53.75 ± 3.50 nauplii/parent; 0.92 ± 0.05 g/L). Water quality during maintenance was within the range that supports normal life of *A. franciscana* in all treatments. The use of mixed feed was proven to be more effective in supporting reproductive performance and biomass accumulation of *Artemia franciscana* compared to single feeding.

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