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IMPACT OF FEEDING *ARTEMIA FRANCISCANA* ENRICHED WITH VARIOUS OIL RESOURCES ON GROWTH, BLOOD BIOCHEMICAL AND BEHAVIORAL INDICES, AND SURVIVAL OF *OREOCHROMIS NILOTICUS*

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Abstract

The main objective of the present trial was to examine the efficacy of feeding tilapia fry fish on *Artemia franciscana* diets supplemented with various oil emulsion resources in terms of performance, behavior indices, survival rate, blood biochemical parameters, and immunological response. Four hundred Nile tilapia fry (weighing 0.15±0.05 g and measuring 2.17±0.08 cm) were randomly allocated into four equal groups (each with five repetitions) and acclimatized for fifteen days. The first group served as the control and received unenriched *Artemia franciscana* (G0), while the remaining three groups were fed *Artemia franciscana* diets enriched with different oil resources (0.5 mL oil per liter for 6 hours): soybean oil (G1), sesame oil (G2), and rice bran oil (G3). Behavioral observations were recorded during the 45-day experimental period. At the end of the feeding trial, the chemical composition and fatty acid content of both *Artemia* and fingerlings were analyzed. Furthermore, the growth performance, survival, and immune response of the fingerlings were evaluated. The results indicated noticeable improvements in behavioral measurements (feeding, foraging and schooling), performance (final length, final weight, net weight gain, feed conversion ratio and specific growth rate), survival, and immune response among fry fish supplemented with enriched *Artemia*, particularly those enriched with soybean oil. Additionally, the chemical composition and fatty acid content of both *Artemia* and fish fry were significantly enhanced when oil emulsions are applied, with soybean oil demonstrating the most prominent improvements. Whereas, supplementing fry fish *Artemia* diets with oil resulted in lower liver enzyme activity and higher protein component levels in plasma in comparison to the control group. In brief, feeding Nile tilapia fry fish *Artemia* diets enriched with a soybean oil emulsion (0.5 mL/L) is recommended for promoting high performance, immunological activity, and survival throughout the early stage till fingerlings phase.

Key words: ***Artemia franciscana*, Nile tilapia, behavior, growth, immunity, oil emulsion**

In their native habitats, most fish and shellfish larvae with underdeveloped digestive systems consume monocellular organisms, including phytoplanktonic and zooplanktonic species (Radhakrishnan et al., 2020). Therefore, zooplankton has been identified as a significant natural component of many fish (cod, halibut, tilapia) and shrimp (*Penaeus* sp.) diets (Das et al., 2012). Furthermore, the live feed is especially important for the development of the larval stage in aquaculture since it is more easily digested and consumed, has no deleterious impact on water quality criteria, and contains essential growth components like fatty acids and amino acids (Joshua et al., 2022; Magondu et al., 2022). Specifically, the rotifers and nauplii of brine shrimp *Artemia* has been identified as a common live food species that meets larval feeding dimensions and quantity criteria (Lubzens et al., 1989). According to Herawati et al. (2014), *Artemia* contains a nutritional biomass of 66% protein and 14% lipid, with practically all necessary amino acids and the majority of fatty acids provided at optimal levels. Besides, *Artemia*'s non-selective filter-feeding habit enables suitable substances to enter its cells during the developmental phase and be ingested by aquatic species (Sorgeloos et al., 2001; Wang et al., 2022).

Worldwide, tilapia is the second most commonly farmed fish, with production quadrupling over the last decade due to its adaptability for widespread aquaculture practices, sustainability, marketability, and constant market pricing (Naiel et al., 2023 a). Private or government hatcheries often struggle to meet the demand for cultivated fry because of the fish's infrequent spawning in the natural environments outside of ponds (Naiel et al., 2023 c). The purpose of these controlled-environment fry production facilities is to provide a consistent and ade-

quate supply throughout the aquaculture season (Sharma et al., 2010). Whereas, inadequate feeding methods in hatcheries may cause mass mortality in fish at various phases of growth, particularly in the early developmental stages (Okomoda et al., 2019). Thus, understanding the larval nutritional needs would help to optimize complete diets and feeding regimens, hence improving larval and juvenile quality (Goncalves et al., 2024). Meanwhile, it would aid in the normal development and activation of digestive enzymes, as well as the digestion and absorption of nutritious substances included in microelement diets during the early stages (Marinho et al., 2024).

During the early feeding period of the larvae, all fish required diets containing high level of protein for optimal growth and development (Rajkumar, 2006). The early use of formulated diets has limited effectiveness in larval rearing (Qin and Culver, 1996). However, live feeds are more acceptable in both freshwater and marine aquaculture because of their higher digestibility and nutritional content, availability, and provide a balanced meal compared to formulated diets (Putra et al., 2016). *Artemia* nauplii have several benefits as a live food source, however, it is not complete in nutrients due to its lack of necessary fatty acids (Navarro et al., 1999). Herein, to improve fish larval development and survival, enriching *Artemia* with essential fatty acids (EFA) is recommended (Smith et al., 2002). There are multiple fortified techniques to enrich live foods with highly unsaturated fatty acids (HUFAs) using fresh microalgae or commercial augmentation products, but due to the challenging process of microalgae production, other commercial enrichment products are more commonly used in the enrichment methods (El-Dahhar et al., 2024). Numerous reports have investigated that feeding larval fish brine shrimp nauplii enriched with PUFA has a positive effect on fry performance and development while increasing survival rate, eliminating skeletal anomalies, developing the digestive tract and its enzymatic activity, and boosting resistance against environmental stress (García-Ulloa et al., 2013; Prusińska et al., 2015; El-Dahhar et al., 2024). In addition, various research has investigated diverse fatty acid sources in the larval stages of shellfish species, including commercial diets, plant and animal fats, or oils, as well as the bio-encapsulation technique of live feeds such as *Artemia* and/or rotifer (Narciso et al., 1999; Narciso and Morais, 2001). To date, there is a shortage of understanding regarding the ideal oil emulation source that will be profitable for enriched *Artemia* nauplii with HUFA and its influence on fry development and survival.

However, behavioral health indicators are quick and simple to monitor, making them ideal for application on fish farm sites (Pavlov and Kasumyan, 2000). Alterations in food-anticipatory behavior, consumed feed, swimming direction and activity, and rate of respiration are all important indicators of fry health (Ramadan et al., 2018). Meanwhile, behavioral responses, which involve energy cost and impact fish growth, have received less attention (An et al., 2021). In the same context, ecological stress and distress cause biochemical and physiological alterations, which are reflected in behavioral reactions (Naiel et al., 2020, 2023 b). Consequently, it is a key measure for assessing the well-being of fish (Martins et al., 2012).

In this regard, the research trial aims to evaluate the impact of enriched *A. franciscana* with various emulsion oil resources (soybean, sesame, and rice bran) on the nutritional content of live prey (*Artemia* nauplii) as a live feed during Nile tilapia larval weaning. Also, assessing the influence of feeding larvae fish with *Artemia* nauplii supplemented with three different oil emulsions on fingerlings' performance, body composition, behavioral, plasma biochemical, and immunological parameters.

Material and methods

Fish transportation and acclimatization

In April, approximately four hundred Nile tilapia fingerlings were obtained from a private fish farm, Idko, Beheira Governate, Egypt. The initial measurements of the Nile tilapia fingerlings, including their weight and length, were documented as 0.15±0.05 g and 2.17±0.08 cm, respectively. For transportation, the fingerlings were placed in well-oxygenated plastic bags and conveyed to the Faculty of Veterinary Medicine, Alexandria University laboratory. When they arrived, they were allocated immediately into 1000 L fiber tank to adapt to the laboratory settings for two weeks before the experiment's beginning.

In the period of acclimatization, the Nile tilapia fry was fed twice daily with live unenriched *Artemia* nauplii. Instead of employing artificial aeration, daily water changes were implemented to uphold an optimal environment for the fish. This method ensured an adequate supply of oxygen and maintained an environment free from metabolic waste. The environmental conditions of the culture water were controlled within a range of 27.5±1ºC for temperature, 6.9±0.3 for pH, 6.8±0.6 mg L^{-1} for dissolved oxygen, 28.0±2 mg/L for biological oxygen demand (BOD), 125±5 mg L^{-1} for chemical oxygen demand (COD), and 0.13±0.01 mg L^{-1} for ammonia.

Artemia nauplii enrichment

To promote the hatching of *Artemia franciscana* cysts, commercially available vegetable oils such as soybean, rice bran, and sesame oil were employed. These cysts, obtained commercially, were acclimatized to hatch in ideal conditions, which included a salinity of 35 ppt, a temperature of 26.8°C, a pH of 7.5, and a light intensity of 100 lux. After a 24-hour incubation period, the *Artemia* cysts successfully hatched, and the resulting nauplii were gathered from the hatching containers. These nauplii were transmitted to a 10 L glass container at a density of 1500 nauplii per liter. Subsequently, the *Artemia* was enriched with three oil emulsions (soybean in G1, sesame in G2, and rice bran oil in G3) prepared as described by McEvoy et al. (1995) procedure. Briefly, *Artemia* nauplii were individually enriched with the three

oil emulsions at a concentration of 0.5 ml/L for 6 hours, at a room temperature of 27.5±0.2°C. Adequate aeration was maintained to sustain the O_2 level at five ppm. Due to the transparency of the nauplii, the presence of the emulsions could be easily discerned by the yellowish appearance in the gut. A light microscope was used to monitor the time it took for nauplii guts to get saturated with the supplied emulsion oils.

Fish supplementation and experimental design

The Institutional Animal Care and Use Committee at Alexandria University gave their stamp of approval to the study's design (ALEXU-IACUC-013-2023/3/11–3–226). The four hundred Nile tilapia fry were distributed into 4 equal groups, each consisting of 100 individuals (each group has five replicates). The control group (G0) was fed unenriched *Artemia* nauplii, whereas the other three fish fry groups were given *Artemia* nauplii diets enriched with various oil sources, as follows; G1, G2, and G3, supplemented with soybean oil, sesame oil, and rice bran oil, respectively. Fry fish groups were fed twice daily. Then, the uneaten *Artemia* nauplii larvae were removed from the incubator after 30 min from each feeding time. Optimal oxygen levels were maintained during the 45-day supplementation period by consistently administering mild aeration.

Behavioral observations of fingerlings

The behavioral responses were documented through scanning observations conducted twice weekly following Mas-Muñoz et al. (2011) procedure. Two 15-minute sessions per day were carried out during feeding, once in the morning and once in the afternoon (from 08:00 to 08:15 am and from 3:00 to 3:15 pm). The proportion of fish engaging in behavioral patterns including feeding, foraging, resting, swimming, schooling, surfacing, chafing, and aggression was calculated from these observations.

Growth performance parameters of fingerlings

The total fry length at the start (Initial length, IL, cm) and end (Final length, FL, cm) of the feeding experiment was measured from the snout tip to the caudal fin end. Whereas, the performance and efficiency of consumed feed parameters were measured by weighing fingerlings from each replicate at the beginning and end of the trial period and calculated using the following formula:

Net weight gain (NWG, g) = (FW – IW)

Specific growth rate (SGR, g d^{-1}*) = (Log FW – Log IW) × 100/ Feeding days (45 d)*

Food conversion ratio (FCR, g/g) = Total consumed feed/ NWG

Survival percentage (SR, %) = (Fish number that survived the experiment/ Fish (number) at the beginning of the experiment) × 100

Chemical composition and fatty acid content analysis of *Artemia* nauplii and fish fry

The enriched and un-enriched *Artemia* samples, as well as each fingerling specimen, underwent analysis to determine the levels of chemical constituents that are total protein (Lowry et al., 1951), carbohydrate (Roe, 1955), lipid (Folch et al., 1957), ash, and total moisture content (APHA, 1995). Furthermore, the analysis of fatty acid content was taken out as per the procedure detailed by Nichols et al. (1993) for both the enriched and un-enriched *Artemia*, as well as for each group of fingerlings. The fatty acid methyl esters underwent separation using a Hewlett Packard 5890 gas chromatograph fitted with a fused silica capillary column (12 m length × 0.22 mm inner diameter) coated with BPX70. The oven temperature was held at 180°C, and helium was utilized as the carrier gas at a flow rate of 2 ml min^{-1}.

Plasma samples and measurements

At the end of the experimental period, blood was taken from the caudal peduncle using a non-coagulant-containing tube, allowed to coagulate, and subsequently subjected to centrifugation. The plasma biochemical and immunological parameters were evaluated in the following manner:

The protein concentration in plasma was assessed using the Bradford (1976) technique, using bovine serum albumin as the standard. The albumin content was determined following the method outlined by Doumas et al. (1997). While, the quantity of globulin was then calculated by subtracting the albumin from the total protein, as detailed by Kumar et al. (2005). Lysozyme activity was measured using the turbidimetric method described by Ellis (1990) employing a *Micrococcus lysodeikticus* suspension solution (Sigma-Aldrich, USA). The analysis depends on the lysis of Gram-positive bacteria that is susceptible to lysozyme (*Micrococcus lysodeikticus*). Glucose levels were determined using the glucose oxidase method reported by Yuen and McNeill (2000). In addition, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assessed following the methods specified by Thomas (1998) and Rosalki et al. (1993).

Data analysis

The data was analyzed using SPSS (Statistical Package for Social Sciences software, version 25) using one-way ANOVA. To assess the significant difference between treatments, Tukey tests were employed. The data was presented as means ± S.E.M., with P values <0.05 considered significant in all tests, unless specified otherwise.

Results

Behavioral responses of Nile tilapia fingerlings

Table 1 demonstrates the percentage of fish exhibiting various behavioral responses. There was a clear improvement in the behavioral responses of fingerlings fed an enriched *Artemia* nauplii diet, with the most progress in the soybean oil group. The highest feeding, foraging, and

schooling proportions were in G1, followed by the other treated groups with the least in control. However, the swimming proportion was the least in G1 and highest in control. Furthermore, the abnormal behavioral responses such as surfacing, chafing, and aggression were the least in G1, then the other groups, the control group with the highest.

The growth and feed efficiency criteria of tilapia fingerling

Table 2 presents the growth parameters, including length, weight, survival, net weight gain, FCR, and SGR, of Nile tilapia fingerlings fed with various oil-enriched *Artemia* nauplii. The fingerlings that were fed enriched *Artemia* displayed an increase in overall length and weight across all experimental groups associated with the control group. Specifically, the G1 group, which was fed with soybean oil-enriched *Artemia*, attained the highest weight (5.92±0.10 g) and length (6.22±0.12 cm) compared to the other groups. Additionally, G1 demonstrated the highest survival rate compared to the control, with G3 and G0 presenting survival rates of 82±1.75% and 79±2.35%, respectively. Furthermore, the G1 group exhibited the highest net weight gain (5.85±0.18), FCR (3.81±0.05), and specific growth rate (12.03±0.9), followed by G3, G2, and the control group.

The chemical and lipid analysis of *Artemia* nauplii and fish fry

Table 3 illustrates the chemical composition of *Artemia* nauplii enriched with different oils. It is noteworthy that soybean oil-enriched *Artemia* nauplii (G1) showcased the highest levels of protein (53.6±0.565%), carbohydrate (17.95±0.919%), and lipid (21.05±0.212%) content compared to the other treatment groups. Conversely, rice bran oil-enriched *Artemia* (G3) demonstrated the highest moisture (8.2±0.141%) and ash (7.35±0.353%) levels relative to G1 and G2. The nutritional value of the live feeds followed the order of soybean oil-enriched *Artemia* >sesame oil-enriched *Artemia* > rice bran oil-enriched *Artemia* > control. Thus, the results suggest that the enrichment process enhances the nutritional value of *Artemia*.

Table 1. Behavioral measurements of Nile tilapia fry fish fed *Artemia franciscana* diets enriched with different oil resources

Items	Experimental groups				P value
	G0	G1	G2	G3	
Feeding	0.25±0.03 c	0.55±0.03 a	0.43±0.03 b	0.37±0.03 b	0.024
Foraging	0.35±0.03 c	0.65±0.03 a	0.53±0.03 b	0.46±0.03 b	0.005
Resting	0.62±0.03 a	0.33±0.03 c	0.49±0.03 b	0.40±0.02 bc	0.017
Swimming	0.67±0.03 a	0.36±0.03 c	0.47±0.03 b	0.57±0.03 ab	0.001
Schooling	0.16±0.02 d	0.47±0.03 a	0.37±0.03 b	0.27±0.03 c	0.001
Surfacing	0.52±0.03 a	0.22±0.02 c	0.34±0.03 b	0.33±0.03 b	0.042
Chafing	0.47±0.03 a	0.17±0.02 d	0.27±0.03 c	0.37±0.03 b	0.011
Aggression	0.42±0.03 a	0.14±0.02 b	0.24±0.03 b	0.21±0.03 b	0.001

G0 = The control group received unenriched *Artemia franciscana* diets.
G1 = The fish group fed *Artemia franciscana* diets enriched with soybean oil.
G2 = The fish group fed *Artemia franciscana* diets enriched with sesame oil.
G3 = The fish group fed *Artemia franciscana* diets enriched with rice bran oil.
Means having different letters within the same row are significantly different (P<0.05).

Table 2. Growth performance and survival rate of Nile tilapia fry fish fed *Artemia franciscana* diets enriched with different oil resources

Items	Experimental groups				P value
	G0	G1	G2	G3	
IL (cm)	2.15±0.37	2.18±0.28	2.17±0.18	2.13±0.26	0.354
FL (cm)	3.74±0.08 d	6.22±0.12 a	5.45±0.11 b	4.82±0.09 c	0.001
IW (g)	0.14±0.15	0.15±0.55	0.15±0.45	0.14±0.31	0.624
FW(g)	3.77±0.19 c	5.92±0.11 a	4.38±0.09 b	4.35±0.17 b	0.024
SR (%)	79±2.35 d	91±2.4 a	85±2.65 b	82±1.75 c	0.003
NWG (g)	3.65±0.15 c	5.85±0.18 a	4.17±0.03 b	4.25±0.05 b	0.011
FCR (g/g)	5.85±0.15 a	3.81±0.05 c	4.54±0.14 b	5.08±0.25 a	0.002
SGR (%/d)	7.10±0.12 c	12.03±0.9 a	8.25±0.4 b	8.43±0.6 b	0.028

G0 = The control group received unenriched *Artemia franciscana* diets.
G1 = The fish group fed *Artemia franciscana* diets enriched with soybean oil.
G2 = The fish group fed *Artemia franciscana* diets enriched with sesame oil.
G3 = The fish group fed *Artemia franciscana* diets enriched with rice bran oil.
IL = initial length; FL = final length; IW = initial weight; FW = final weight; SR = survival rate; NWG = net weight gain; FCR = feed conversion ratio; SGR = specific growth rate.
Means having different letters within the same row are significantly different (P<0.05).

Table 3. Chemical analysis (%DW) of enriched and unenriched *Artemia franciscana*

Items (%)	Experimental groups				P value
	G0	G1	G2	G3	
CP	54.7±0.47 a	53.8±0.13 a	52.2±0.32 a	37±1.51 b	0.013
Carbohydrate	18.85±0.72 b	17.55±0.07 bc	16.05±0.07 c	21.45±0.64 a	0.011
CL	22.04±0.21 a	21.85±0.07 ab	19.75±0.21 b	9.45±0.78 c	0.041
Ash	5.30±0.14 b	3.45±0.07 c	8.55±0.35 a	3.65±0.35 c	0.015
Moisture	8.85±0.07 ab	8.60±0.14 b	9.5±0.14 a	7.45±0.21 c	0.001

G0 = The control group received unenriched *Artemia franciscana* diets.
G1 = The fish group fed *Artemia franciscana* diets enriched with soybean oil.
G2 = The fish group fed *Artemia franciscana* diets enriched with sesame oil.
G3 = The fish group fed *Artemia franciscana* diets enriched with rice bran oil.
CP = crude protein; CL = crude lipid.
Means with different letters within the same column differ significantly (P<0.05).

Table 4. Fatty acid composition of enriched and unenriched *Artemia franciscana* (expressed in mole %)

S. No.	Fatty acid	Experimental groups				P value
		G0	G1	G2	G3	
1	C14:0	3.35±0.07 b	4.33±0.01 a	3.18±0.11 b	3.32±0.12 b	0.015
2	C16:0	20.15±0.21 a	13.48±0.25 c	8.45±0.7 d	16.05±0.14 b	0.002
3	C18:0	4.85±0.21 b	6.25±0.21 a	3.2±0.14 c	3.70±0.14 c	0.004
4	C20:0	1.45±0.30 b	1.55±0.21 b	0.5±0.07 c	1.89±0.23 a	0.023
5	C22:0	1.55±0.23 a	1.19±0.03 b	0.44±0.04 c	1.18±0.14 b	0.001
6	C24:0	1.58±0.23 a	0.35±0.03 d	0.88±0.02 c	1.11±0.06 b	<0.001
7	SFA	30.46±0.13 a	25.03±0.43 c	18.55±0.41 d	27.16±0.33 b	0.004
8	C16:1(n-7)	2.36±0.31 a	0.51±0.02 c	1.45±0.25 b	2.35±0.15 a	0.014
9	C18:1(n-9)	28.60±1.12 c	24.14±0.07 d	35.66±0.55 a	31.70±0.41 b	<0.001
10	MUFA	32.09±0.44 b	24.57±0.12 c	37.14±0.15 a	36.03±0.45 a	0.005
11	C18:2(n-6)	26.75±0.54 d	39.51±0.11 a	35.14±0.06 b	31.22±0.32 c	0.001
12	C18:3(n-3)	1.89±0.12 d	6.81±0.22 a	3.41±0.07 b	2.11±0.13 c	0.001
13	EPA	2.88±0.28 c	4.71±0.22 a	2.95±0.04 c	3.78±0.28 b	0.031
14	DHA	0.90±0.14 b	1.30±0.14 a	0.81±0.08 b	0.49±0.02 b	0.021
15	PUFA	34.35±0.53 d	51.32±0.23 a	45.34±0.13 b	38.09±0.22 c	0.006
16	Others	2.85±0.76 b	0.29±0.03 d	4.06±0.51 a	1.90±0.13 c	<0.001

G0 = The control group received unenriched *Artemia franciscana* diets.
G1 = The fish group fed *Artemia franciscana* diets enriched with soybean oil.
G2 = The fish group fed *Artemia franciscana* diets enriched with sesame oil.
G3 = The fish group fed *Artemia franciscana* diets enriched with rice bran oil.
SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; PUFA = polyunsaturated fatty acids.
Means with different letters within the same row are significantly different (P<0.05).

Table 4 provides a detailed breakdown of the fatty acid profile of enriched *Artemia* nauplii, presented in mole percentages. Soybean oil-enriched *Artemia* exhibited the highest content of PUFA (51.32%), followed by sesame oil-enriched *Artemia* nauplii (45.34%).

The rice bran oil-enriched *Artemia* nauplii comprised 38.09% of the sample, whereas the freshly hatched *Artemia* nauplii accounted for 33.65%. Notably, essential fatty acids such as eicosapentaenoic acid (20:5n-3), docosahexaenoic acid (22:6n-3), linoleic acid (18:2n-6), and linolenic acid (18:3n-3) were found to be significantly higher in the selected oil-enriched *Artemia* nauplii compared to the freshly hatched *Artemia*. Furthermore, significant differences in the composition of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were observed between unenriched *Artemia* nauplii and various oil-enriched *Artemia* nauplii at a P-value of less than 0.05.

The results presented in Table 5 outline the chemical composition of Nile tilapia fingerlings after the 45-day experimental period. It is evident that the enriched *Artemia* nauplii significantly influenced the gross chemical components of the Nile tilapia fingerlings. The highest protein content was observed in fingerlings fed with soybean oil-enriched *Artemia* (51.34±0.07%), tracked by those

fed with sesame oil-enriched *Artemia* (51.14±0.07%) and rice bran oil-enriched *Artemia* (48.24±0.07%). Additionally, the highest carbohydrate and lipid content was recorded in the group fed with soybean oil-enriched *Artemia* (G1) compared to the other groups.

Furthermore, Table 6 illustrates that the fatty acid composition of Nile tilapia fingerlings was notably impacted by the enriched *Artemia* nauplii. The essential fatty acids in fingerlings fed with various oil-enriched *Artemia* displayed higher levels of linoleic acid and linolenic acid associated with those given unenriched nauplii. Specifically, the PUFA content in fingerlings fed unenriched nauplii was 32.83%, whereas those fed enriched nauplii containing selected oil emulsions exhibited reasonably higher PUFA content of 45.17% (G1), 39.15% (G2), and 41.89% (G3). Overall, the essential fatty acid content (PUFA, MUFA, and SFA) in the experimental groups was discovered to have higher levels compared to the control group.

Blood parameters of tilapia fingerlings

The findings outlined in Table 7 provide a comprehensive overview of the plasma biochemical and immune parameters of Nile tilapia fed diets supplemented with various oil emulsion-enriched *Artemia franciscana*. Importantly, a noteworthy discrepancy in glucose levels was noted among the experimental groups ($P<0.05$). Fish that were fed enriched diets displayed higher glucose levels compared to the control group ($P<0.05$). Furthermore, total protein and albumin levels in fish fed enriched diets were significantly higher than those in the control ($P<0.05$). Remarkably, the most pronounced increase in lysozyme activity was observed in treatments G1 and G2, whereas the lowest lysozyme activity was recorded in fish fed the control diet. Moreover, the activity of liver enzymes (ALP, AST, and ALT) was markedly reduced in Nile tilapia fed diets supplemented with various oil emulsion-enriched *Artemia franciscana* in comparison to Nile tilapia fed the control diet ($P<0.05$).

Table 5. Body chemical analysis of Nile tilapia fry fish fed *Artemia franciscana* diets enriched with different oil resources

Items	Experimental groups				P value
	G0	G1	G2	G3	
CP	38.51±0.76 c	51.34±0.06 a	51.14±0.06 a	48.24±0.03 b	0.001
Carbohydrate	26.53±0.22 a	23.75±0.08 c	24.90±0.06 b	26.50±0.27 a	0.045
CL	8.15±0.08 c	10.34±0.07 a	10.05±0.24 a	9.55±0.25 b	0.005
Ash	5.62±0.07 b	7.91±0.05 a	7.79±0.07 a	5.54±0.08 b	0.001
Moisture	6.43±0.06 c	9.31±0.13 a	9.04±0.71 a	8.41±0.18 b	0.006

CP, crude protein; CL, crude lipid.
Means with different letters within the same column differ significantly ($P<0.05$).

Table 6. Fatty acid composition of Nile tilapia fry fish fed *Artemia franciscana* diets enriched with different oil resources (expressed in mole %)

S. No.	Fatty acid	Experimental groups				P value
		G0	G1	G2	G3	
1	C14:0	1.15±0.12 b	0.75±0.08 a	0.76±0.01 a	0.69±0.04 a	0.022
2	C16:0	15.22±0.16 b	14.85±0.25 a	14.79±0.45 a	14.82±0.11 a	0.023
3	C18:0	9.66±0.05 bc	8.62±0.25 a	9.84±0.06 bc	9.30±0.42 b	0.005
4	C20:0	4.33±0.05 c	0.90±0.08 a	2.80±0.02 b	2.45±0.08 b	0.001
5	C22:0	5.40±0.21 c	4.41±0.35 b	3.31±0.04 a	3.61±0.16 a	0.011
6	C24:0	4.03±0.26 b	0.76±0.13 a	3.65±0.04 b	0.58±0.07 a	0.006
7	SFA	32.76±0.13 a	26.04±0.16 d	31.04±0.26 b	27.57±0.36 c	0.001
8	C16:1(n-7)	0.15±0.01 a	0.19±0.07 a	0.18±0.07 a	0.16±0.16 a	0.745
9	C18:1(n-9)	32.31±2.12 b	27.65±0.07 a	28.35±0.49 a	31.5±0.42 b	0.014
10	MUFA	32.44±0.49 a	29.93±0.52 c	30.84±0.59 b	31.55±0.66 ab	0.008
11	C18:2(n-6)	21.05±0.57 a	34.01±1.14 c	24.58±0.05 b	23.62±0.40 b	0.013
12	C18:3(n-3)	7.25±0.14 b	6.90±0.28a	8.65±0.07c	11.36±0.37d	0.001
13	EPA	2.34±0.13 a	3.70±0.01 b	4.02±0.17 c	4.09±0.04 c	0.021
14	DHA	3.26±0.35 b	2.65±0.22 a	3.61±0.35 bc	4.67±0.13 c	0.051
15	PUFA	32.83±0.11 d	45.17±0.28 a	39.15±0.44 c	41.89±0.14 b	0.005
16	Others	1.98±1.20 c	0.89±0.03 a	1.29±0.05 b	1.18±0.49 b	0.001

G0 = The control group received unenriched *Artemia franciscana* diets.
G1 = The fish group fed *Artemia franciscana* diets enriched with soybean oil.
G2 = The fish group fed *Artemia franciscana* diets enriched with sesame oil.
G3 = The fish group fed *Artemia franciscana* diets enriched with rice bran oil.
SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; PUFA = polyunsaturated fatty acids.
Means with different letters within the same row are significantly different ($P<0.05$).

Table 7. Plasma biochemical and immune parameters of Nile tilapia fry fish fed *Artemia franciscana* diets enriched with different oil resources

Parameters	Experimental groups				P value
	G0	G1	G2	G3	
GLU (mg dL^{-1})	120±1.31 b	129.51±1.15 a	128.92±0.95 ab	128.41±1.01 ab	0.006
TP (g dL^{-1})	3.83±0.06 b	4.37±0.24 a	4.42 ± 0.07 a	4.24±0.04 a	0.024
ALB (g dL^{-1})	1.32±0.04 b	2.24±0.01 a	2.30±0.02 a	2.31±0.01 a	0.003
GLOB (g dL^{-1})	2.51±0.01 a	2.13±0.34 b	2.12±0.44 b	1.93±0.14 b	0.014
LZY (U mL^{-1})	26.59±0.7 c	36.71±0.53 a	37.79±0.81 a	33.6±0.37 b	<0.001
ALP (U/L)	945.5±1.6 a	626.9±1.56 c	856.4±1.42 b	642.3±1.38 c	0.011
AST (U/L)	789.7±1.16 a	521.3±1.70 c	661.8±1.98 b	469.5±1.30 d	<0.001
ALT (U/L)	23.4±1.29 a	15.08±1.89 c	17.88±0.45 b	11.14±0.33 d	<0.001

G0 = The control group received unenriched *Artemia franciscana* diets.
G1 = The fish group fed *Artemia franciscana* diets enriched with soybean oil.
G2 = The fish group fed *Artemia franciscana* diets enriched with sesame oil.
G3 = The fish group fed *Artemia franciscana* diets enriched with rice bran oil.
GLU = glucose; TP = total protein; ALB = albumin; GLOB = globulin; LZY = lysozyme; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.
Mean values with different letters are significantly different (P<0.05).

Discussion

Sustainable aquaculture practices and production are vital for the success of the aquaculture industry in regard to the expanding global demand for animal protein sources (Naiel et al., 2023 d). Aquaculture sustainability requires the development of three key areas: social, economic, and environmental challenges, with ecological challenges mostly related to fish nutrition (Hixson, 2014). Over the years, major efforts have been devoted to developing cost-effective aquafeed formulations to further enhance the growth of farmed fish (Turchini et al., 2019). Larvae of numerous aquatic organisms either completely rely on zooplankton live food as a baseline diet, or they survive much better when begun on live food (Samat et al., 2020).

Artemia nauplii, known for their high digestive enzymes that stimulate larval appetite (Zeng et al., 2018), are valued as highly nutritious living capsules containing essential nutrients and energy sources such as lipids, proteins, vitamins, fatty acids, amino acids, minerals, and carbohydrates crucial for the growth and sustenance of various aquaculture species (Mona et al., 2017). In addition, numerous studies have investigated the n-3 and n-6 fatty acid constraints of various marine finfish and shellfish species, leading to the development of standard lipid emulsions (Milke et al., 2004; Natnan et al., 2022; Singh et al., 2024). The current trial focuses on analyzing the chemical composition and fatty acid profile of enriched *Artemia* nauplii and assessing the impact of feeding these enriched nauplii to Nile tilapia fingerlings on their behavior, growth performance, and immune response.

The present study findings showed a considerable increase in the feeding and foraging of fingerlings fed on *Artemia franciscana* enriched by various emulsion oil sources. Similarly, Grageda et al. (2008) reported that larvae fed live *Acartia* recorded the best consumption behaviors, even though all fish given other types of live feeds had equivalent rates of intake and satiation times. The improvement in feeding and foraging behaviors might be related to the higher palatability of live feed supplemented with oil (Kandathil et al., 2020).

Conversely, the fish given *Artemia* supplemented with soybean emulsion oil demonstrated lower swimming activities compared to the other groups. The present findings disagreed with Pandey et al. (2008) findings, which showed that feeding larvae with *Acartia* significantly reduced swimming activity compared with larvae provided *Artemia* (both unenriched and DHA-enriched). In the current study, the lower swimming activity seen in the fish fed with *Artemia* enriched with soybean oil was related with a higher weight in this group. Moreover, swimming is an energy-loss activity in certain situations, particularly during the larval stage, when efficient energy-saving strategies are lacking (Grageda et al., 2008). The observed activity entailed substantial oxygen consumption, with levels ranging from 2 to 15 times higher than the resting state in certain species of fish larvae, for instance, brown trout, Pacific sardine, whitefish, and certain cyprinids (McKay and Jeffs, 2023). Additionally, fish strive to maintain a schooling position to facilitate food detection and/or enhance their chances of survival and tranquility (Hellinger et al., 2017).

The ability of a fish to acquire desired food is the most essential factor influencing a fish's weight (Yousif, 2002); consequently, the existence of a smaller proportion of aggressive fish in the oil-enriched groups supports this knowledge. Furthermore, the oil's volatile components may be responsible for the anti-aggression reactions via diminishing serotonin reuptake and increasing the duration in which serotonin is available for neurotransmission pathways reactions (Riyazi et al., 2007). Hence, it

was observed that lower aggressiveness activities might be preserving energy for growth and reducing stress impact on larvae (Petrović et al., 2020). Also, Ishibashi et al. (2013) reported that the aggressive behavior of larvae increased during the live pray feed scarcity. Meanwhile, Fox et al. (2006) reported that aggression activities were reduced with increased feed availability in several fish species. Whilst, the higher proportion of chafing behavior may be attributed to the high pH in fish rearing water, which encourages the formation of more slippery mucus (Sanderson et al., 1996). As a consequence, promoting more chafing behavior is essential for removing this layer from the surface to acquire more oxygen for respiration (Al-Arifa et al., 2011).

The present findings suggested that fish fed enriched *Artemia* exhibited a statistically significant improvement in their growth and feed efficiency metrics in comparison to the fingerlings fed unenriched *Artemia*. The recent findings were shown to be in accordance with prior findings in *Poecilia latipinna* (Ahmadifard et al., 2019), *Carassius auratus* (Elshafey et al., 2023), *Sander lucioperca* (Yanes-Roca et al., 2020) and *Dicentrarchus labrax* (El-Sayed et al., 2022). Previous reports have shown that *Artemia* is a rich source of polyunsaturated fatty acids (PUFA), which are required for the development and metabolism of many aquatic species (Elshafey et al., 2023). Likewise, PUFA, like docosahexaenoic acids (DHA) and eicosapentaenoic acids (EPA), have been demonstrated to have a substantial positive impact on the performance and survival rate of goldfish larvae (Bransden et al., 2005; Das et al., 2007). Accordingly, our findings supported that enriched *Artemia* nauplii work well in Nile tilapia larval rearing.

The results of *Artemia* chemical analysis revealed that unenriched and soybean oil-enriched *Artemia* nauplii had a high protein content, whereas unenriched *Artemia* nauplii had low lipid and moisture content. While, it seemed that both the unenriched and enriched *Artemia* nauplii contained approximately the same quantity of carbohydrates. The present results were shown to be similar to prior findings (Seixas et al., 2008). Generally, carbohydrates play a crucial role, being more essential than protein and lipids, as they are required for locomotion, metabolism, and coping with stress conditions (Wilson, 1994). The soybean oils have a high level of linoleic acid, which is transformed during the metabolism process into lecithin, and this encourages the metabolic process and the absorption of fats (Yang et al., 2022), which might be responsible for the high lipid and low protein levels in enriched *Artemia* nauplii.

According to Han et al. (2001) and Narciso and Morais (2001), the freshly born *A. franciscana* had a comparatively high eicosapentaenoic acid (EPA) content but low docosahexaenoic acid (DHA) and arachidonic acid (ARA) content. Therefore, adding a supplement during *Artemia* enrichment may assist balance and optimizing the fatty acid profile (Sargent et al., 1997; Castell et al., 2003). Specifically, supplementation by emulsion oil attains to increase and rebalance the fatty acid profile, notably raising DHA level, to achieve the ideal essential fatty acid ratio, which is 10 DHA:5 EPA:1 ARA (Jafaryan et al., 2009; Divya et al., 2014). Furthermore, it is acknowledged that the nutritional value of *Artemia* is not stable, but changes both temporally and geographically, which may diminish the EPA, ADH, and ARA content of *A. franciscana* owing to fluctuation in the enrichment time (Nieves-Soto et al., 2021).

As previously stated, the enrichment duration, specific dose, and emulsion method, particularly during the early stages of nauplii development, are key factors that have a significant impact on the nutritional content of nauplii (Léger et al., 1986, 1987). In this investigation, the fatty acid profiles of numerous experimental oil emulsions applied for enriching *Artemia* nauplii, including soybean, sesame, and rice bran oil emulsions, indicated considerably higher quantities of PUFA than the unenriched *Artemia* nauplii. Specifically, the maximum PUFA level was noticed in the *Artemia* nauplii group that received soybean oil emulsion, whereas the unenriched *Artemia* nauplii exhibited significantly lower levels. The aforementioned results were found to be consistent with prior findings by Clawson and Lovell (1992). The obtained findings may be attributed to the fact that soybean oil has a high level of PUFA (about 62%), which increased the PUFA content in *Artemia* nauplii treated with soybean oil (Sargent et al., 1997).

The research findings suggested that the metabolic profile of Nile tilapia fingerlings was significantly affected by the various emulsion oil-enriched *Artemia* nauplii sources. In particular, fingerlings fed *Artemia* enriched with soybean oil had the highest levels of fat and protein content, followed by the fish group receiving *Artemia* enriched with sesame oil and rice bran oil. The present findings were found to be consistent with previous investigations (Merchie et al., 1995; Arulvasu and Munuswamy, 2009; Fereidouni et al., 2013). It is identified that supplementing *Artemia* with emulsion oil may improve its nutritional value, notably its protein, lipid, fatty acid, and essential amino acid profiles (Zheng et al., 2021). According to Yu et al. (2021), the higher tissue protein and lipid levels of the fish group administered *A. salina* nauplii compared to the unfed group most likely suggest proficient protein and lipid absorption, particularly considering that *A. salina* nauplii are abundant in proteins and fats. This proves that applying an ingredient that can sustain the live pray population would boost the host's nutritional value (El-Sayed et al., 2022).

In the same context, it is widely acknowledged that the fatty acid configuration of higher animals reflects that of their feed, a principle assumed to apply to invertebrate animals as well (Fracalossi and Lovell, 1994). This study found that the fatty acid composition of Nile tilapia fry mirrored that of their diets. The investigation into the nutritional effectiveness of various oil emulsion diets for the survival and growth of Nile tilapia yielded highly positive results, especially in terms of chemical compo-

sitions. By the end of the feeding trial, fingerlings administered oil-emulsion *Artemia* displayed higher levels of plasma proteins, albumin, and lysozyme activity than those provided unsupplemented *Artemia*, demonstrating the efficiency of enriched *Artemia* employing oil emulsion for fish health. In contrast, the fish assigned *Artemia* supplemented with oil had greater glucose levels than the control group, which was consistent with comparable results by Metwally (2009), and Talpur and Ikhwanuddin (2012). Notably, dietary fatty acids and carbohydrates may impact each other's metabolism in fish because lipids may be converted to glucose via gluconeogenesis and glucose can be accumulated as fats in tissues (Li et al., 2019).

Lysozyme is a well-known innate immune system component that contributes to nonspecific fish immunity and has been recognized for its anti-inflammatory and bactericidal features (Negm et al., 2021). The present study found that Nile tilapia fingerlings fed *Artemia* supplemented with soybean or sesame oil exhibited higher lysozyme activity than the control group. In this regard, our findings are consistent with previous research (Gatesoupe, 1994; Patra and Mohamed, 2004; Rezaei et al., 2019). Although the majority of the research on lipids and fish health has explored the influence of alternative oil sources, there is a lack of evidence on the impacts of particular fatty acids. For instance, Kiron et al. (1995) stated that immunological responses were significantly elevated in rainbow trout that were fed pure fatty acids. While, Makol et al. (2009) emphasized that dietary linoleic acid (CLA) boosted the seabass fish's defensive system against bacterial infection by promoting an alternative complement pathway and stimulating lysozyme activity. Consistently, a balance of dietary n-3 and n-6 of PUFA, such as those found in *Artemia* enriched with emulsion oil, would activate immunological defensive systems against infectious diseases (Fracalossi and Lovell, 1994).

Conclusion

In conclusion, our findings demonstrated that enriched *Artemia franciscana* with soybean oil emulsion (0.5 mL oil per liter for 6 hours) significantly enhanced Nile tilapia fingerling behavior, performance, blood biochemical indices, survival rate, and immunological activity. Therefore, it can be assumed that *Artemia franciscana* enriched with soybean oil emulsion might be an excellent addition to the fry fish diet, especially during the early weaning period.

Author contribution

All authors have an equal contribution to the conceptualization, implementation, and outputs of this research work presented in this manuscript.

Data availability

Available under reasonable request from the corresponding author.

Consent for publication

The authors approve processing this manuscript for publication.

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