

GROWTH PERFORMANCE OF LIVE FISH FEED: *Artemia salina* IN DIFFERENT SUPPLEMENTAL FEEDS IN AQUARIUM CULTURE

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ABSTRACT

Artemia is an excellent live feed for newly hatched fish and crustaceans gained a unique position in the aquaculture system. Brine shrimp is a non-selective filter feeder, so different locally available source feeds should be evaluated as potential food source for their control mass culturing. The experiment was carried out to study the effect of different supplement feeds on the growth performance of *Artemia* at Central Fisheries Promotion and Conservation Centre, Baalaju, Kathmandu from December 2021 to February 2022. The experiment was carried out in complete randomized design (CRD) with four treatments, each treatment consisting four replications. Four treatments consist different combination of supplement feed i.e., T1: (spirulina + rice bran), T2: (soyabean + rice bran), T3: (Mustard oilcake + rice bran), T4: (Yeast + rice bran). All supplement feed contains 25% CP and feed diet was supplied by 2% of body weight. In terms of growth and yield performance parameters, significant effect was recorded on average length, individual weight and total biomass. Also, significant effect was recorded on survivability and density. However, no significant effect was recorded on CP content of *Artemia* biomass. T1 recorded the maximum average length (8.7 ± 0.21 mm), individual weight (0.0028 ± 0.0001 gm), and biomass (1.79 ± 0.069 gm/L) whereas lowest average length (7 ± 0.08 mm), individual weight (0.0013 ± 0.0002 gm) and biomass (0.61 ± 0.005 gm) was recorded in T4. Also, T1 recorded the maximum density (633.8 ± 3.62), and survivability (70.8 ± 0.5) whereas lowest density (467.9 ± 4.26) and survivability (58.0 ± 1.71) found in T4. Hence it can be concluded that the combination of spirulina and rice bran was best supplement diet and can be applied in the cultivation of *Artemia* for best growth performance and biomass Production in aquarium condition.

1. INTRODUCTION

Artemia is a genus of aquatic crustaceans also known as brine shrimp. *Artemia* populations are found worldwide in inland saltwater lakes, but not in oceans. *Artemia* are able to avoid cohabiting with most types of predators, such as fish, by their ability to live in waters of very high salinity up to 25% (Daintith, 1996). *Artemia* is a typical primitive arthropod with a segmented body to which is attached broad leaf-like appendages.

Artemia is widely used by aqua-culturist as an excellent live food for crustacean and fish (Azad *et al.*, 2018). It constitutes the principal ration and frequently the only food for the larvae and juveniles of many cultured species, such as freshwater prawns (*Macrobrachium sp.*), shrimp (penaeids), and lobsters (*Homarus sp.*), crabs and various finfish (Bardach *et al.*, 1972). *Artemia* Nauplii constitutes the most widely used food item among the live diets used in the larviculture of fish and shell fish (Van Stappen, 1996). *Artemia* considered as

an aquaculture live food, has a high nutritional content (Wache and Laufer, 1997). *Artemia* was found by several investigators to be an excellent food for newly hatched fish and shrimp larvae (Sorgeloos *et al.*, 1998). Being live feed, the larvae of *Artemia* are readily taken by aquatic animals under nursery conditions without fouling aquarium water (Islam *et al.*, 2019).

Inadequacy, and absence of live feed increase the chances of mortality of fish seed in hatcheries. Besides this, the inconsistent supply of the live feed in Nepalese market has limited the access to live feed for hatcheries. In Nepal, there is trend of feeding fish seed with *Artemia* at its juvenile stage with no any instances of feeding *Artemia* in its adult stage. Culturing *Artemia* up to its adult stage helps to feed fish seed with the different stages of *Artemia* i.e., nauplii, juvenile, and adult. This can address the constraints of inconsistent supply in the market and ensure its constant availability

in the hatchery. So, better combination is important for culturing *Artemia* to boost its growth and survivability.

Thus, the objective of this research is to compare the growth performances of live feed *Artemia salina* under the different locally available feed sources to find best combination.

2. MATERIALS AND METHODS

2.1 Location of experimental set up, period, design and unit

The experiment was carried out in the research laboratory of Central Fisheries Promotion and Conservation Center (CFPCC), Baalaju, Kathmandu which is located at Bagmati province of Nepal. Geographically the site is located at 27°44'0" N latitude, 85°18'0" E longitude and an altitude of 1400 meters above sea level. The experiment was conducted for 90 days from December 1, 2021 to February 30, 2022. The experiment was conducted in completely randomized design (CRD) having four treatments and four replications. In total

there were 16 experimental units. The randomization of treatments was done by creating random numbers in the MS Excel sheet using (RAND) command.

2.2 Treatments details

The total of four treatments were included in this experiment. The treatment consists of different combination of supplement feeds as Spirulina and rice bran, Soyabean meal and rice bran, Mustard oil cake and rice bran, Yeast and rice bran. The feed consists of both carbohydrate and protein source. The 25 % supplement feed was formulated by two feed sources according to the Protein requirement of *Artemia*. The 25% CP feed was formulated according to Pearson square method and feed was supplied according to the body weight of *Artemia nauplii*.

2.3 Proximate analysis

Proximate composition of different supplement feeds is given in Table 1.

Table 1. Proximate composition of different supplement feeds.

Treatment (Mean)	Moisture(M) %	Crude Protein (CP)%	Fat(F)%	Total Ash (TA)%	Fiber (Fb) %	Method of analysis
T1	11.82	25	10.27	7.74	6.255	NIRS
T2	12.225	25	7.45	7.94	8.36	NIRS
T3	12.625	25	7.32	7.59	8.505	NIRS
T4	12.125	25	7.52	7.84	8.255	NIRS

*T1= Spirulina + rice bran, T2 = Soyabean meal + rice bran, T3 = Mustard oil cake + rice bran, T4 = yeast +rice bran, NIRS- Near Infra Red Spectroscopy

2.4 Sample collection

The crude salt and different supplement feeds were collected from market. The saline water was made 30 ppt and proximate analysis of feeds was analyzed by NIRS and Kjeldahl's method at Animals feeds and quality control laboratory at Harihar Bhawan, Lalitpur Nepal. *Artemia* cyst used in this experiment was collected from laboratory of Fisheries promotion and conservation Centre, Kathmandu Nepal.

2.5 Experiment set up

Four V-shaped containers made of plastic material were set up in the laboratory for hatching cyst. For culturing

Artemia sixteen aquaria were set up for rearing *Artemia nauplii*. The necessary equipment (aerators, heaters, thermometer, refractometer, Hanna pH meter, microbalance, burette, pipette etc.) were collected and set in the laboratory for measuring the water quality parameters and biomass of *Artemia* at different concentrations. The containers were kept at a position of the laboratory where sunlight could not bring any deleterious effects. Heaters were used to maintained temperature. Artificial aerator was used for maintaining the optimum level of oxygen. Florescent lamp was fitted to provide sufficient light during hatching. The aquaria were filled with 20-liter saline water having

concentrations 30 ppt. 3 gm/ liter *Artemia* cyst was provided in four containers containing 3-liter saline water having concentration of 30 ppt. Total biomass was measured by using microbalance (electrical analytical balance, OSK 11325A).

2.6 Hatching

Four 'V' shaped plastic containers containing 3-liter of saline water (30 ppt) were used for hatching. The decapsulated *Artemia* cyst 3 gm/ liter was provided to each tank with continuous vigorous aeration. Heater was provided to maintain temperature 25 degree centigrade. Constant florescent lamp was provided. After 48 hours hatching nauplii were harvested by turning off the air flow of the aerator. Then, nauplii were attracted by light, they were concentrated at the bottom of the 'V' shaped container where sufficient light was ensured. The hatched nauplii was extracted from the container onto a beaker with 100 ml distilled water. The content was mixed well and 1-ml sample was taken by pipet and put into Rafter Counter for counting. The counted nauplii @1 individual/ ml was transferred into culture tank by pipette.

2.7 Inoculation and feeding

The counted *Artemia nauplii* were inoculated in the rearing aquarium after hatching. The water of the aquarium was previously enriched with different supplement feeds. The different supplement feed was provided twice a day by 2 % of nauplii body weight. Feeding was done early morning and evening time. Nauplii became pre- adult after 5-8 days of hatching. Moderate and continuous aeration was provided by aerators to keep the food in suspension and supply of oxygen to *Artemia*. The growth rate and weight of *Artemia* was measured every 3rd day from 9th day. The experiment lasted for 21 days.

2.8 Harvesting

Adult *Artemia* was completely collected on 21 days manually by using 120 μ m size net. Harvested adult *Artemia* was dried for proximate analysis (CP %) by NIRS method.

2.9 Determination of water quality

The important water quality parameters such as temperature, dissolved oxygen and pH were measured periodically. A laboratory thermometer, electrical pH-meter and DO meter were used to measure temperature,

pH and dissolved oxygen respectively.

2.10 Measuring technique

A homogenous distribution of *Artemia* in water of the tanks was ensured by giving continuous vigorous aeration before sampling. The 100 ml water sample was taken from each aquarium to estimate the density, individual weight, average length, survivability, CP % and biomass of *Artemia* by different technique.

The body length was measured by using a microscope equipped with a drawing mirror and calculated using a digitizer. Adult *Artemia* was measured by measuring scale. The randomly selected individual weight was measured by micro weighing machine. The survivability was calculated by Counting and comparing individuals with initial stocking. For density, the individuals were counted manually from a randomly selected sample from each treatment. The harvested individuals were Measured by micro weighing machine. The total biomass was measured by digital electrical balance (electrical analytical balance, OSK,11325A).

The harvested adult artemia was taken from each treatment and dried. The crude protein was calculated by NIRS method on dry basis at Central, Fisheries promotion and conservation center, laboratory which is located at Baalaju, Kathmandu Nepal.

2.11 Statistical analysis

All the collected data were calculated and tabulated according to replication and treatments. MS excel was used for tables and SPSS version 20 and Gen stat 15th edition for statistical analysis. The growth Parameters of *Artemia* during experimental period in same temperature and salinity with different feed was tested using one way Analysis of Variance. ANOVA was done to test the significance of difference for each parameter. Calculation of the significant difference at 5% level of significance made by the mean comparison. The comparison of mean was done by DUNCAN at 5% level of significance.

3. RESULTS AND DISCUSSION

The experimental finding obtained during the investigation entitled "Growth performance of *Artemia Salina* in different supplemental feeds in aquarium condition at Balaju Kathmandu" were analysed. The results are presented and discussed below;

3.1 Water quality parameters

For an adequate production of *Artemia* in controlled conditions, water quality parameters should be maintained within an optimal range (salinity between 30-65 ppt, oxygen above 2 mg/L, temperature between 19-25 °C, and pH between 6.5-8.0). No significant effect was obtained on temperature, dissolved oxygen and pH of water with different supplements feeds (Table 2).

Table 2. Water quality parameters during experiment period.

Treatments	Water quality parameters		
	Dissolved Oxygen (mg/l)	Temperature (Degree Celsius)	pH
T1	5.525	26.40	6.425
T2	5.850	26.70	6.450
T3	5.050	26.80	6.775
T4	5.975	27.80	6.950
Significance	NS	NS	NS
CV	4.3%	6.4%	6.9%
SE	± 0.2574	± 1.713	± 0.460
Df	12	12	12
L.S.D.	0.3965	2.639	0.708

3.2 Effect of different supplement feed on survivability, density and crude protein content of artemia.

The effect of different supplement feed on Survivability was found highly significant ($p < 0.05$) as shown in Table 3. The highest survivability rate ($70.8 \pm 0.57\%$) was found in supplement feed with spirulina and rice bran which is statistically significant with other supplement feeds whereas lowest survivability rate (58 ± 1.71) was found in yeast and rice bran which is statistically at par with the supplement feeds mustard oil cake with rice bran and soyabean meal with rice bran. Similarly, the effect of different supplement feed on density was found highly significant ($p < 0.05$) as shown in table 3. The highest density (633.8 ± 3.62) was found in supplement feed spirulina and rice bran which is highly significant with others supplement feeds whereas lowest density (467.9 ± 4.26) was found in supplement feed yeast with rice bran which is statistically at par with mustard oil cake with rice bran. Also, there is no significant ($p > 0.05$) effect among the treatments with crude protein content in *Artemia* biomass.

Table 3. Mean value of survivability, density, CP % content of Artemia by feeding different supplement feeds

Treatment	Parameters ± SE		
	Survivability%	Density ind./L	C.P%
Spirulina +Rice bran	70.8a±0.57	633.8a±3.62	66.7±0.17
Soyabean meal +Rice bran	61.1b±1.20	514.9b±4.41	66.5±0.09
Mustard oil cake +Rice bran	58.5b±0.78	473.6c±5.04	66.5±0.11
Yeast + Rice bran	58.0b±1.71	467.9c±4.26	66.5±0.10
Sig.	**	**	Ns
LSD (0.005)	3.559	13.46	0.3875
F (3,15) Value	<0.001	<0.001	0.39
P value	26.92	311.30	0.761
CV (%)	3.7	1.7	0.4
Grand mean	62.14	522.6	66.59

3.3 Effect of different supplement feed on average length, individual weight, biomass and total biomass of artemia.

The effect of different supplement feed on average length was found highly significant ($P < 0.05$) as shown in table 3. The highest average length (8.7 ± 0.21 mm) was found in supplement feed Spirulina and rice bran which is followed by supplement feeds soyabean meal with rice bran whereas lowest average length (7.0 ± 0.08 mm) was found in the supplement feed yeast and rice bran which was also statistically at par with mustard oil cake. Similarly, the effect of different supplement feed on individual weight was found highly significant ($P < 0.05$) as shown in table 3. The highest individual weight (0.0028 ± 0.0001 gm) was found in Supplement feed spirulina and rice bran which is statistically significant with other supplement feeds. Similarly, the lowest individual's weight (0.0013 ± 0.002 gm) was found in supplement feed yeast and rice bran which is statistically significant with other supplement feeds. Also, the effect of different supplement feed on total biomass was found highly significant ($p < 0.05$) as shown in table 3. The highest biomass concentration (66.7 ± 0.17) was found in Spirulina and rice bran which is statistically significant with other supplement feed. Similarly, the lowest biomass concentration (12.2 ± 0.11) was found in yeast and rice bran which is statistically at par with supplement feed mustard oilcake and rice bran.

The purpose of the present study was to analyses four

Table 4. Mean value of average length, individual weight, biomass, total biomass, of *Artemia* in different supplement feeds.

Treatment	Parameters Mean± SE)			
	Average length(mm)	Individual weight(gm)	Biomass gm/L	Total biomass gm/20 L
Spirulina + Rice bran	8.7a ± 0.21	0.0028a±0.0001	1.79a±0.09	35.7a±1.38
Soyabean meal + Rice bran	8.2a ± 0.40	0.0021b±0.0009	1.11b±0.05	22.2b±1.02
Mustard oil cake + Rice bran	7.1b ± 0.06	0.0016c±0.0056	0.79c±0.03	15.8c±0.60
Yeast + Rice bran	7.0b±0.08	0.0013d±0.0020	0.61c±0.05	12.2c±0.11
Sig.	**	**	**	**
LSD (0.005)	0.719	0.0002320	0.1412	2.824
F (3,15) Value	<.001	<.001	<.001	<.001
P value	12.41	75.62	127.29	127.29
CV (%)	6	7.6	8.5	8.5
Grand mean	7.81	0.001992	1.077	21.45

different types of supplement diet and their effects on the growth performance, survivability, density and total biomass yields and also which of these feeds yields best results for the production of adult brine shrimp. The different supplement feed used in these experiments have very different chemical composition, the quantity of food required for maximum growth, survival rate, density and total biomass yields of *Artemia* varied from one type of feed to another feed.

Survivability depends on many factors like nature of feeds and environment of culture medium. The optimum temperature, dissolved oxygen and pH remained constant. So, survival rate directly depends on nature, quality and source of supplement feeds. This result possibly due to high protein content (57%) of diet. The protein content in spirulina is complete since it contains all essential amino acids forming 47% of protein weight (Bujard-E *et al.*, 1970). Also, Spirulina is a rich source of vitamins, minerals, poly unsaturated fatty acids, enzymes and pigments. It also enhances the nonspecific immune system, increasing the state of readiness of natural defenses. Beside this Spirulina protein is highly digestible because of absence of cellulose walls (Dillon and phan, 1993). The combination of nutrients, pigments and immunostimulants explains reports of dietary spirulina yielding better growth, appearance, reduction of stress and high survival rate. Similarly, rice bran is very cheap products available worldwide

which supports better growth in *Artemia* and is very suitable for batch culturing because it's contents of water-soluble components are minimal (Sorgeloos *et al.*, 1980). As a result of its fibrous structure, less than 50% of the initial crude product can be processed into *Artemia* food by homogenizing and sieving treatments. Similarly, in soyabean meal high ammonia levels were build up and high mortality were noted. This is due to fact that, soyabean powder appears to contain high amounts of soluble proteins which cannot be ingested by *Artemia* but provokes water pollution by bacterial development. (Sorgeloos *et al.*, 1980). This clearly indicates the suitability of spirulina with rice bran for best diet of *Artemia* in terms of growth performance and survivability.

The best yield found in spirulina containing feed is due to spirulina is nutritionally significant because of high concentrations of natural nutrients, bio-modulatory and immune-modulatory functions (Khan *et al.*, 2005). It contains various B-vitamins and minerals in high amount of calcium, iron, magnesium, manganese, potassium and zinc (Binkava *et al.*, 2002; Gireesh *et al.*, 2004). Spirulina contains ten times more proteins of dry weight than soyabean and three times more than that of beef proteins (Dillon *et al.*, 1995).

Khan *et al.* (2005) reported that spirulina species have antibacterial and antiparasitic activity. According to Bujard-E *et al.* (1970) protein content in spirulina is

complete since it contains all essential amino acids, poly unsaturated fatty acids (PUFA), and forming 47 % of total protein weight. Spirulina has several pharmacological activities such as anticancer, antiviral, antibacterial, metallo-protective, and immunostimulant effects. Thus, spirulina have both therapeutic and nutritional application. Mechanisms of anticancer, antiviral and antimicrobial effects of spirulina is due to its endonuclease which repair damaged DNA. Calcium sulfated polysaccharides which inhibit the invitro replication of viruses and fatty acids especially high content of γ linolenic acid which have a crucial role in growth and individual mass. Also, the metallo-protective role of spirulina may be attributed to the presence of beta-carotene, vitamins C, E, enzymes, superoxide dismutase, selenium and brilliant blue polypeptide pigment phycocyanin. Cyanobacteria have been found to be a good source of protein for fish (Nandesha *et al.*, 2001).

In addition, spirulina have no cell walls which results better digestion and absorption (Desikachary,1959). This clearly shows that spirulina have positive effect on growth and yield of *Artemia*. From the above result, it is concluded that spirulina is a rich source of protein, vitamins, minerals and pigments. It also enhances the nonspecific immune system, increasing the state of readiness of natural defenses. The combination of nutrients, pigments and immunostimulants explains

reports of content dietary spirulina yielding better growth, reduction of stress and better appearance. Hence, spirulina gave the best result in growth performance and total biomass. Also, this result reveals that spirulina could be used as protein and lipid source diet for *Artemia* growth and production to have a better culturing and gain market acceptance. The extremely poor survival and growth of *Artemia* cultured on fresh baker's yeast clearly showed that, this diet is an inadequate food for brine shrimp. The observation of intact yeast cells in the fecal materials of *Artemia* fed fresh baker's yeast reveal a problem with digestibility of yeast by *Artemia*.

4. CONCLUSION

The result indicated that different Supplemental feeds had a significant effect on growth parameters and total yields but did not affect on crude protein content in biomass of *Artemia* which are crucial parameters for Production purposes. Hence it can be concluded that the combination of spirulina and rice bran was best supplement diet and can be applied in the cultivation of *Artemia* in aquarium condition for best growth performance and biomass Production in controlled condition.

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