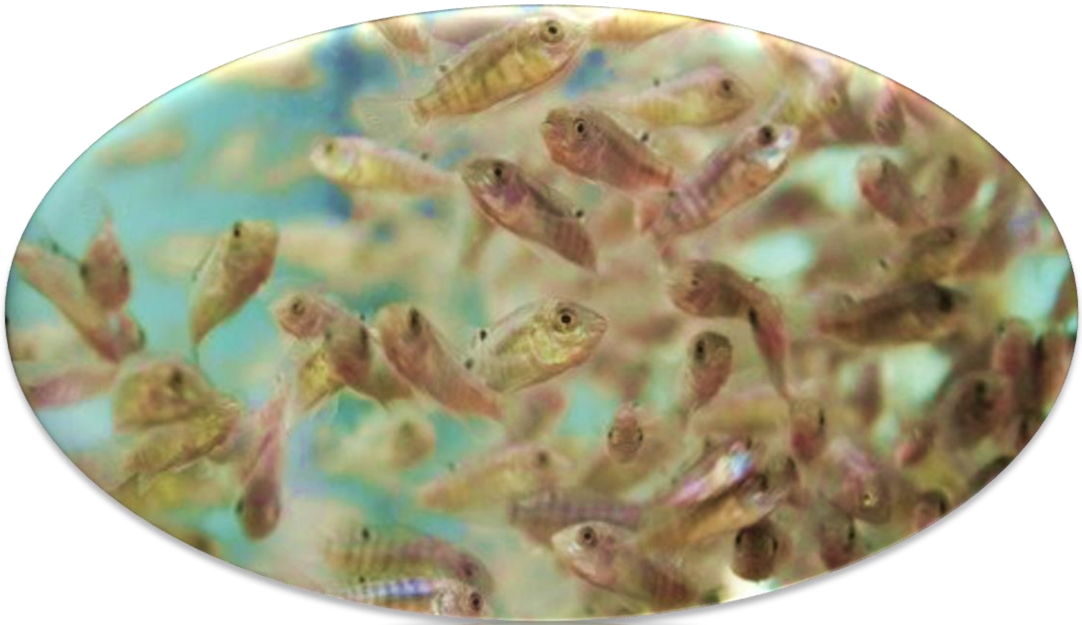
Effects of Feeding Live Feeds *Artemia nauplii* and *Moina macrocopa* (Straus, 1820) as Supplementary Feed on the Growth of Nile Tilapia Fingerlings, *Oreochromis niloticus (Linnaeus, 1758)*

DISSERTATION
Submitted to the University of Kerala
in the partial fulfilment of the requirement for the
BACHELOR OF SCIENCE IN
ZOOLOGY



Department of Zoology
TKM COLLEGE OF ARTS AND SCIENCE, KOLLAM-5

APRIL 2023

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CERTIFICATE

This is to certify that the dissertation entitled ***"Effects of Feeding Live Feeds Artemia nauplii and Moina macrocopa (Straus, 1820) as Supplementary Feed on the Growth of Nile Tilapia Fingerlings, Oreochromis niloticus (Linnaeus, 1758)"*** is a bonafide work done by …………………………………………………….........under my supervision as partial fulfillment of the requirements for the ***Degree of Bachelor of Science in Zoology*** and this report has not been submitted earlier for the award of any degree or diploma or any other similar titles anywhere.

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Examiners: 1.

2.

DECLARATION

I do hereby declare that this dissertation entitled ***"Effects of Feeding Live Feeds Artemia nauplii and Moina macrocopa (Straus, 1820) as Supplementary Feed on the Growth of Nile Tilapia Fingerlings, Oreochromis niloticus (Linnaeus, 1758)"*** is a bonafide work done by me under the supervision of Dr. Mumthas Y., Assistant Professor, Department of Zoology, TKM College of Arts and Science, Kollam as partial fulfilment of the requirements for the award of ***Degree of Bachelor of Science in Zoology***. No part of this has been presented earlier for any degrees or diploma of any university.

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CONTENTS

INTRODUCTION

Aquaculture is essential for achieving the UN's Sustainable Development Goals (SDG) of eradicating poverty (SDG 1), ending world hunger (SDG 2), guaranteeing food security and ensuring access to appropriate nutrition (SDG 8) (UNDP,2015). India's aquaculture sector now ranks second in the world for aquaculture production (SOFIA,2022). Tilapia farming is widespread, occurring in more than 135 countries and territories. The prospect of sustainability in the food and aquaculture industries depends significantly on tilapia. Production of tilapia is increasing because of tilapia’s large size, fast growth, prolific breeding characteristics, palatability, and relatively low cost for production. Despite being a freshwater species, tilapia is capable of withstanding low dissolved oxygen levels, osmotic and alkalinity stress, and low dissolved oxygen concentrations up to a certain range. Within two to three months of birth, these fish can reach maturity, and they can reproduce 75 to 1,000 times every 22 to forty days.

One of the most important aspects of a hatchery is the larval stage, although there are still many of barriers preventing development of larvae. The pre-larval and post-larval phases make up the two stages of the larval stage. The fish body is still translucent in the pre-larval stage but has fully formed by the time it reaches the post-larval stage. For larvae to survive and be successful in culture, increased growth and development are crucial. Fish early development is typically thought of as a continuous accumulation of minuscule change that result in an adult phenotype.

High feed conversion efficiency is related to increased development of tilapia larvae. The availability of food and effective digestion supply the vital energy that enables fish larvae to grow and develop more quickly, enhancing their chances of survival. The larval stage is a transitional time when fish exhibit rapid growth rates along with the differentiation of their organs and tissues. The digestive capacities of fish, which vary by species are impacted by both abiotic and biotic circumstances, are developed during the first month of life. Therefore, it is hypothesized that modus operandi of digestion in fish larvae is different from that in adult fishes.

For fish fry diets, a variety of live feed or zooplankton can be employed. Fish fry production in hatcheries depends on the cultivation and use of these potential organisms. For the raising of fish larvae, Artemia sp. has traditionally been chosen over other live feed organisms, but use of cladocerans has received less attention despite their many advantageous characteristics. The important component of Artemia nauplii is its essential fatty acid (EFA) content, particularly eicosapentaenoic acid (EPA) concentration. Fish larvae fed with nauplii have been shown to thrive, develop, and have a higher probability of surviving (Gopalakrishnan *et al*., 1976; Kadhar *et al.,*2014). Cladocerans have been discovered to be rich in essential nutrients that are easily eaten and digested by fish larvae, complete the nutritional needs of larvae, and enhance water quality by reducing the need for artificial feeding (He et al., 2001).

Quantitative concepts like the length-weight relationship, condition factor, growth, and mortality of fish are crucial for understanding fishing biology. In order to determine the weight that corresponds to a particular length, the length-weight relationship (LWR) method is used. This estimation makes an inference about the weight of fish at a particular length and it generates an assumption regarding the growth pattern of cultivated fish. According to Pauly (1983), larger fish of a given length are thought to be more conditioned, hence the condition factor (K) is largely used to compare the health of fish. It has been demonstrated that the condition factor in fish reflects information about the physiological status of the fish in relation to its welfare through its changes.

A proper management of the species depends on an understanding of the condition factor and the life cycle of the various fish species. Additionally, it provides data for calculating the gonadal maturation time and monitoring a species' level of feeding activity to see if it is effectively using its food source. Although feeding and nutrition may have an influence on tilapia productivity, the majority of research on nutritional concerns is focused on improving the protein content in artificial feed and its digestibility (Stickney, 2005a). Since it has been hypothesized that traditional meals met the nutritional needs of tilapia fry, there aren't many recent studies on early life nutrition of Tilapia available. However, the experimental use of live feeding has led to superior output growth performance in a variety of commercial freshwater species (Weirich *et al.,* 2000; Camargo *et al.,* 2005).

OBJECTIVES

The objectives of the present study are:

- To evaluate and compare the efficiency of live feeds, *Artemia nauplii* and *Moina macrocopa* as supplementary feed in Tilapia fingerlings *(Oreochromis niloticus*)

- To evaluate the survival rate of Nile tilapia fingerlings (*Oreochromis niloticus)* fed with *Artemia nauplii* and *Moina macrocopa* as supplementary feed.

- To provide information on the growth performance and condition factor (Kf) for Nile tilapia fingerlings (*Oreochromis niloticus*) fed with *Artemia nauplii* and *Moina macrocopa* as supplementary feed in order to assess the fishes' health.

REVIEW OF LITERATURE

Freshwater fish species' dietary and feeding patterns are a subject of continuous research. This is due to the fact that it provides a strong basis for managing culture of fishes. Studies on fish natural dietary patterns also help us to understand the trophic linkages that exist in aquatic environments by revealing details about the structure, stability, and content of food webs. Fish need nutrition for development, reproduction, and other essential physiological processes. The primary food sources for fish in a natural aquatic setting are phytoplankton, zooplankton, plant matter, insects, insect larvae, worms, and smaller fish. Fish frequently exhibit preferences for specific foods found in their surroundings. In any aquatic ecosystem, the presence of food affects fish health and ability to reproduce. The size of the food items, as well as the age and size of the fish, might also affect how they choose their meal. Fish mostly consume foods that fit in their mouths and that their stomachs can digest. Fish have longer stomachs and more complex digestive systems as they get older. The absolute rate of food ingested increases but the feeding rate relative to body weight declines.

Nile tilapia *(Oreochrormis niloticus)* is considered to be a successful intensive aquaculture species in the country due to its relative ease in culture, high market demand, and sustainability to local climate conditions and consequently, is a successful commercial species worldwide. In particular, *O. niloticus* is a scientifically outstanding fish due to its complex social behavior, including parental care and coloration. It has been an important source of carbohydrates, proteins, fats, vitamins, minerals, iron, and calcium, as well as being noted for its eco-friendly aquaculture (McAndrew and Majumdar, 1989). It was observed that tilapia egg production is significantly influenced by water temperature.

High tolerance to environmental conditions and its ability to accept formulated and natural feeds make it economically viable. It has a versatile feeding behavior and is characterized by a generalist and opportunistic omnivorous feeding behavior. Its diet composition may vary within a wide range of temporal and spatial conditions of the environment. Therefore, understanding its food and feeding behavior is a key factor to its successful culture in a controlled environment (Murai and Andrews, 1976).

LARVAL DVELOPMENT OF TILAPIA

A larva is a distinct juvenile form many animals undergo before metamorphosis into adults. The larvae looked transparent (blood circulation and bonny structure were easily visible) with a straight body. The larval development stages of Tilapia are given below:

PERIOD	STAGES	DAY(S) POST-FERTILIZATION	CHARACTERISTICS
Early larva	1	6-7	Opercular movement
Early larva	2	7	Caudal fin ray elements 2; LL = 6.5 ± 0.2 mm
Early larva	3	7-8	Caudal fin ray elements 3; LL = 6.9 ± 0.2 mm
Early larva	4	8-9	Caudal fin ray elements4; LL = 7.2 ± 0.3 mm Late larva
Late larva	5	9-10	Caudal fin ray elements 5; TL = 8.1 ± 0.2 mm
Late larva	6	9-10	Caudal fin ray elements 6; TL = 8.6 ± 0.2 mm
Late larva	7	11-13	Caudal fin ray elements 7; TL = 9.0 ± 0.4 mm

Table 1. Larval stages of Oreochromis niloticus (Source: www.aquanet.com)

ARTEMIA NAUPLII & MOINA MACROCOPA AS LIVE FEEDS IN AQUACULTURE

The continuous production of larvae in aquaculture systems depends on the planktons that have been enclosed with important nutrients (Budhin *et al.,* 2016). Availability of suitable live feed for feeding finfish and shellfish larvae has led to successful propagation of hatchery-reared species (Pronob *et al.,* 2012). Supplying adequate live food for fish larvae is important and the nutritional quality of living food species can be improved by enriching the nutrients (Rasdi and Qin, 2016a; Rasdi and Qin*.,* 2018b). Live feed species in larvae and early post-larvae stages of various fish are more beneficial than artificial feed and shellfish species, since zooplankton are known to be an important food for animals in natural aquatic habitats where they pass on the organic matter to higher trophic levels from phytoplankton and detritus (Das *et al.,* 2007; Parakrama *et al.,* 2012; Abbas *et a.l*, 2015). Larvae consumption of zooplankton constitutes major part of their nutritional intake with zooplankton also playing a major role in recycling nutrients and sources of energy within their respective food web (Kar *et al., 2017;* Miah *et al.,* 2013).

Natural live feed organisms are an important resource in aquaculture practices. Their capacity to move along all the columns of water, smaller size, reproducibility and better nutritional levels make them a better choice than artificial feed, especially at larval stages. These organisms are enriched with most essential micro and macro nutrients, viz., essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1998), thus are nutritionally balanced. However, achieving optimum growth and survival of fish larvae is dependent on application of appropriate live feed organism at appropriate life cycle stage, both qualitatively and quantitatively (Akbary *et al*., 2010). Despite the large-scale usage of artificial feed throughout the world, natural live feed has been found to be essential for proper growth of juvenile forms owing to their higher nutritional values and acceptance (Gogoi *et al*., 2016).

In the tropical countries, including India, natural live feeds mainly comprise of two components: algal and non-algal. Non-algal components comprise of brine shrimps (*Artemia* sp.), rotifers (eg., *Brachionus plicalitis, Brachionus rotundiformis, Keratella* sp., *Asplanchna brightwelli*, *Polyarthra vulgaris*, *Filinia opoliensis* etc.*)* and the freshwater cladocerans (eg., *Moina mongolica, Moina micrura, Daphnia carinata, Ceriodaphnia* sp.) ostracods (*Cypris* sp.), and copepods (*Mesocyclops leuckarti*, *M. hyalinus, Microcyclops varicans*, *Heliodiaptomus viduus* etc.) and their larvae (Palanichamy 1996; Gogoi *et al.,* 2016; Radhakrishnan Kandathil *et al*., 2020).

Choosing appropriate live feed organism at optimum life cycle stage of fish larvae requires the consideration of the following criteria: size of the feed, gape-size of the fry or fingerling's mouth; the nutritional quality of the of the feed and nutritional requirement of the larvae; the feed should essentially be rich in highly unsaturated fatty acids (HUFA); feed should be perceivable and preferred by the juveniles; should be easily digestible; feed organism should reproduce fast and increase in number; and also should be sturdy and eurytolerant (Anuraj *et al*., 2015).

Although in aquaculture practices brine shrimps and copepods are successfully utilised at different life cycle stages, however, easily culturable cladocerans, owing to their jerky movement pattern, are preferred by juvenile fish groups (Akbary *et al.,* 2010; Gogoi *et al*., 2016). Rotifers being small sized are also utilized at larval stages, however, their labour-intensive culture process have been a constraint for their wide usage. Copepods are routinely used successfully in cold water fish culture (Dhont *et al*., 2013). Thus, a comparison between two major live feed organisms *Artemia* sp. and Moina is pertinent to highlight their suitability in commercial aquaculture.

Artemia sp. (Leach, 1819), commonly known as "brine shrimps" or "sea monkeys", are the most routinely used live feed in aquaculture throughout the world (Radhakrishnan Kandathil *et al.,* 2020). It is a primitive arthropod, closely related to shrimp family belonging to the order – Anostraca of the class - Crustacea. Their reproduction process depends on prevailing environmental condition, either by producing nauplii (ovoviviparous mode) or by producing cysts (oviparous) (Criel and Macrae, 2002). Harvested cysts can be preserved for years and can be reutilized according to need. If hydrated in saline water, the quiescent larvae resume arrested metabolism and hatches out as nauplii, which is used as feed (Dhont *et al*., 2013).

Although more than 50 strains of *Artemia* sp. have been identified few species are routinely used viz., *Artemia salina*, harvested from Mediterranean area and *Artemia franciscana*, collected from North, Central and South America (Sorgeloos *et al.,* 2001; Das *et al*., 2013; Dhont *et al*., 2013; Radhakrishnan Kandathil et al., 2020). They are mainly found in hyper saline habitats (Dhont *et al*., 2013). Around 90% of brine shrimps are supplied around the world from Great Salt Lake, Utah, USA (Das *et al.*, 2012). However, the main disadvantage is its cost, unavailability (Akbary *et al*., 2010) and specific nutritional deficiencies (Radhakrishnan Kandathil *et al.*, 2020).

Cladocera, generally called 'water fleas', is a superorder belonging to the class Branchiopoda, super class Crustacea and phylum Arthropoda (Smirnov, 1971). Freshwater cladocerans like *Moina mongolica, Moina micrura, Daphnia carinata, Daphnia lumholtz, Ceriodaphnia* sp. etc. are commonly used as live feed (Palanichamy, 1996). Having high reproduction rates, jerky movements, wide temperature tolerance and the ability to thrive in eutrophic water and organic wastes, they qualify as an important live feed for aquaculture process (Mayer and Wahl, 1997). Inability to tolerate salinity is one of the drawbacks of cladocerans feeds (Das *et al*., 2012).

Artemia sp. or brine shrimps are the most widely used non-algal live feed organism. The main advantage for their wide usage is its storage capacity, as live nauplii can be produced instantaneously "on demand" (Dhert and Sorgeloos, 1995; Sorgeloos *et al.*, 2001) from dry powder like form. In general, around 2, 00,000 to 3, 00,000 nauplii can be hatched per gram of high-quality cysts (Treece *et al.,* 2000). Dormant forms of brine shrimp seeds release free swimming nauplius larvae when kept in water and sodium/calcium hypochlorite solution for standard time periods, (approximately 12-24 hours) (Anuraj *et al*., 2015). The free swimming nauplii regain their metabolic activities and are of 0.14 mm in length on an average (Das *et al*., 2012). All stages of life cycle of *Artemia* larvae can be used as feed for different fish species.

Freshly hatched *Artemia* nauplii appear to be a better food for the larvae of *Penaeus monodon, P. indicus* and *Macrobrachium rosenbergii* (Neelakantan *et al.,* 1988). Cryopreseved *Artemia* seeds can be stored for long periods and transported long distances. It has been recorded that feeding fish larvae with nauplii increases fish growth, development and survival chances (Gopalakrishnan *et al*., 1976; Kadhar *et al.,* 2014). It has been seen that size of *Artemia* larvae varies at different geographical locations. Indian variety of the species could not be successfully employed owing to its smaller size. Thus, in India, *Artemia* seeds have to be imported, increasing the cost of aquaculture (Das *et al.,* 2012).
Cladocerans or water fleas are abundantly found in freshwater ponds with moderate to high productivity levels. Two important genera, *Daphnia* and *Moina,* have been successfully used as live feed in culturing fish larvae since long (Alikunhi, 1952; Das *et al.,* 2012). Use of *Moina* sp. (especially, *Moina macrocopa and Moina salina*) in culture of larval and juvenile stage for finfish and shellfish has been increasing worldwide owing to their rapid growth rate and nutritional quality (especially protein content) (He *et al*., 2001; Ingram *et al*., 2009; Peña‐Aguado *et al*., 2009; Poynton *et al*., 2013). *Moina* sp. has been a successful replacement of *Artemia* sp. for larval feed of finfish and shellfishes (Dodson *et al*., 2010).

Moina sp. can be found worldwide and cultured inexpensively. *Moina micrura* is also used as supplement to artificial feed as well. They have been routinely used in hatcheries and also for ornamental fish culture (Martin *et al*., 2003). *Daphnia* sp., on the other hand, due to their hops and jumps in water are easily predated and preferred by fish larvae. Most encountered species, *Daphnia magna* with a body length of 5mm serves as a preferred food of planktivorous fishes (Lauridsen and Lodge, 1996; Ebert *et al.,* 2005).

Cladocerans, being filter feeders' prey upon, nanoplankton, phytoplankton, bacteria, algae etc. while themselves being devoured by fish larvae help in recycling of nutrients and transference of energy to higher trophic level through trophic chain relationship (Gogoi *et al*., 2014). Thus, they are an integral component for trophic dynamics. Their high fecundity, ability to reach high densities in a short span, broad level of tolerance, low productivity cost and ability to thrive in waste water makes them beneficial as a live feed organism.

The significant nutritional property of *Artemia* nauplii is its essential fatty acid (EFA) content, especially eicosapentaenoic acid (EPA) content. The value of EPA levels in *Artemia* varies on the biogeographic region, climatic features, basis of diet provided, from one strain to another, even between batches of a single strain, thus, estimation of (n-3) HUFA EPA need to be given priority before selecting *Artemia* nauplii as live feed for specific fishes. Otherly, nutrient enrichment of *Artemia* with EFA can be an alternative step (Sorgeloos *et al.*, 2001; Copeman *et al*., 2002; Zakeri *et al.*, 2011; Navarro *et al.*, 2014). This may add or increase the levels of HUFAs, especially EPA (20:5n−3) and docosahexaenoic acid (DHA, 22:6 n−3) (Smith *et al*., 2002).

Artemia nauplii also lack long‐chain PUFA and naturally low in essential HUFAs (Akbary *et al.,* 2010). Ahmadi *et al*., (1990) reported that *Artemia* nauplii contains a good percentage of C18:3n3 and very low amount of C20:5n3 (EPA); therefore, it is considered useful for fresh water applications (Ahmadi *et al*., 1990). The variation in total lipid and protein composition from different strains of *Artemia* sp. is reported to be due to their genetic structure or variation of their diet nutrients (Schauer *et al*., 1980; Agh and Hosseini Ghatre, 2002, Agh and Sorgeloos, 2005). *Artemia* sp. is subjected to different natural algae populations, marine oil emulsion combinations and other supplementary diets enrich their nutrient levels (Woods, 2003; Palma *et al.,* 2011; Figueiredo, 2012).

The nutritional quality of commonly used Cladoceran genus *Daphnia* and *Moina* varies depending on their life cycle stage, ingested diet, habitat type etc. In general, among

Cladocerans 50 % of the body dry weight is found to be protein, while fat content is 20-27% for adult forms (Rottmann *et al.,* 2003; Gogoi *et al*., 2016). Comparison reveals *Moina* sp. contains better protein and carbohydrate level whereas, *Daphnia* sp. contains better percentage of lipid and ash content. In a study conducted on *Moina macrocopa*, fed with yeast diet, the protein content was found to be 70.87% to 76.26% (Manklinniam *et al*., 2018). However, for commercial production *Moina* sp. cultured with *Chlorella* gives the best nutritional enrichment followed by yeast. Comparison between brine shrimps and cladocerans are highlighted to discuss suitability of them as live feed in aquaculture:

FATTY ACID	ARTEMIA (%)	MOINA (%)
C14:0	0.47	4.25
C16:0	10.5	10.53
C16:1	1.46	21.67
C18:1	6.57	9.1
C18:1n−9	18.9	11.83
C18:1	5.34	–
C18:2n−6	5.29	2.35
C18:3n−3	31.4	20.19
C20:4n−6	0.48	2.66
C20:5n−3	2.19	3.04
C22:6n−3	0.39	1.31
C22:5	0.01	-

Table 2: Composition and percentage of fatty acids in Artemia sp., & Moina sp. (Source: Gladyshev et al., 2016; Rocha et al., 2017; Singh et al., 2019)

MATERIALS AND METHODS

Fish and Experimental Conditions:

The experiment was conducted for 35 days at the Department of Aquatic biology & Fisheries, University of Kerala, Thiruvananthapuram. A total number of 150 (*Oreochromis niloticus*) fry were obtained from fish seed production unit of Neyyar hatchery, Thiruvananthapuram. After transportation, acclimatization of fry was carried out to the lab conditions for a period of one week in 500L capacity Fiber reinforced plastic (FRP) circular tanks. The obtained fish fry was healthy and free from any infection. After one week of acclimatization period, fish with an average body weight of 0.283g were divided into 3 treatment of 3 replicate groups. For the experiment, nine HDPE circular tanks having water holding capacity of 30L were selected. Each tank was washed and disinfected before the introduction of fish. Fifteen fishes were randomly distributed in each tank with three replications.

Fish were fed two times daily at 4% in the morning at 9.00 am and 6.00 pm in the evening for 35 days. Larvae were provided with freshly hatched *Artemia nauplii* for the first treatment (T1) and *Moina macrocopa* for the second treatment (T2). Powdered artificial feed containing 36% CP was fed two times a day at the rate of 7% of body weight per day as for control (T3) (Bag and Mahapatra, 2012). About 20 to 30% water exchange was carried out daily. Uneaten feed and faecal matter were removed by siphoning water before feeding as there was around 12h time for fishes to feed on nauplii. After acclimatization to laboratory conditions, initial length and weight of fishes were recorded before initiation of experiment and after completion of experimental period. Sampling was performed in the 14^{th},21^{th},24^{th},28^{th}, &35^{th} day of the experimental period.

Taxonomic classification

Kingdom	**:**	**Animalia**
Subkingdom	**:**	**Bilateria**
Infrakingdom	**:**	**Deuterostoma**
Phylum	**:**	**Chordata**
Subphylum	**:**	**Vertebrata**
Infraphylum	**:**	**Gnathostomata**
Superclass	**:**	**Actinoptergii**
Class	**:**	**Teleostei**
Superorder	**:**	**Acanthoptergii**
Order	**:**	**Perciformes**
Suborder	**:**	**Labroidei**
Family	**:**	**Cichlidae**
Genus	**:**	**Oreochromis**
Species	**:**	***Oreochromis Oreochromis niloticus (Linnaeus, 1758)***

Source: IT IS (Integrated Taxonomic Information System- Report)-https://www.itis.gov

Kingdom	**:**	**Animalia**
Subkingdom	**:**	**Bilateria**
Superphylum	**:**	**Ecdysozoa**
Phylum	**:**	**Arthropoda**
Subphylum	**:**	**Crustacea**
Class	**:**	**Branchipoda**
Order	**:**	**Diplostraca**
Suborder	**:**	**Cladocera**
Infraorder	**:**	**Anomopoda**
Family	**:**	**Moinidae**
Genus	**:**	**Moina**
Species	**:**	***Moina macrocopa* (Straus, 1820)**

(Source: ITIS NODC taxonomic code, Database (version. 8.0))

Kingdom	**:**	**Animalia**
Subkingdom	**:**	**Bilateria**
Infrakingdom	**:**	**Protostomia**
Superphylum	**:**	**Ecdysozoa**
Phylum	**:**	**Arthropoda**
Subphylum	**:**	**Crustacea**
Class	**:**	**Branchiopoda**
Order	**:**	**Anostraca**
Suborder	**:**	**Artemiina**
Family	**:**	**Artemiidae (Grochowski, 1896)**
Genus	**:**	**Artemia (Leach, 1819)**
Species	**:**	***Artemia salina* (Linnaeus, 1758)**

(Source: ITIS NODC taxonomic code, Database (version. 8.0))

Growth indices

Fish growth performance was calculated using the following formulae:

a) Condition factor,

$$K = W / L^{3} \times 100$$

Where, K= Condition factor, W= Body weight in grams and L= Body length in centimeters

b) Average Daily Gain

(ADG, g/day) *= (Mean final weight - mean initial weight) /time interval (days)*

c) Weight gain

WG = Mean final fish wt (g) -Mean initial fish wt (g)

$$\text{Percent weight gain (\%)} = \frac{\text{Mean final fish wt (g)} - \text{Mean initial fish wt (g)}}{\text{Mean initial fish wt (g)}} \times 100$$

d) Specific Growth Rate

(% day) *= (Log$_e$ final weight – Log$_e$ initial weight) /time interval (days) ×100*

e) Survival Rate

$$(\%) = Nf/Ni*100$$

Where, Nf = total number of larvae at the end of 35 days of experiment
Ni = initial total number of larvae

Statistical analysis:

All statistical analyses for finding differences in GR, SGR, CF and survival of Tilapia fry were determined through Microsoft excel 2019**.** Significant difference was indicated as $p<0.05$, among all the three treatments used.

Fig 1. Procured Tilapia fingerlings

Fig 2. Experimental setup for larval feeding

Fig 3. Tilapia fingerlings in HDPE tank

Fig 4. Measuring length of Tilapia fingerlings

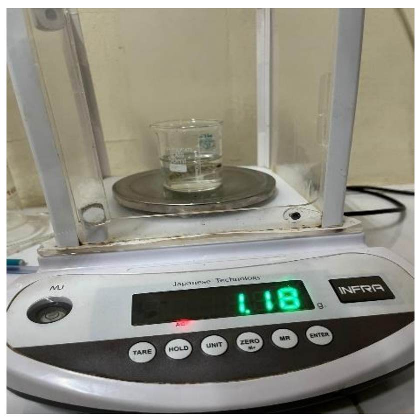

Fig 5. Weighing tilapia in electronic precision balance

Fig 6. Siphoning and water exchange of Tilapia fingerlings

Fig 7. Moina macrocopa as supplementary feed

Fig 8. Artemia nauplii as supplementary feed

Fig 9. Powdered artificial feed

RESULTS

The present study described the effect of live feeds *(Artemia nauplii and M. macrocopa)* as supplementary feed fed with powdered formulated feed on the fingerlings of Nile tilapia *(Orechromis nilotics),* and the effect of live feeds (*Artemia nauplii and M. macrocopa)* on the growth parameters of Nile Tilapia fry. The mean lengths for Nile tilapia (*Oreochromis niloticus)* were 3.25 ± 0.186 cm, 3.129 ± 0.149 cm, 3.25 ± 0.135 cm while the mean weights were 0.73 ± 0.46 g, 0.530 ± 0.33 g,0.601 ± 0.84 g, for three different feeding treatments T1 (*Artemia nauplii*), T2 (*Moina macrocopa*), T3 (Powdered artificial feed). The growth performance of tilapia fingerlings in terms of condition factor, average daily weight gain (g*day^{-1}), specific growth rate (%*day^{-1}) were calculated at the end of the experiment.

During the experimental period (35 days), no significant differences were observed between the treatment means regarding the water quality control variables: temperature (°C), dissolved oxygen (mg/l), electrical conductivity (μS/cm^2), and pH. Variables of temperature (°C), dissolved oxygen (mg/l), electrical conductivity (μS/cm^2), and pH were in the ideal conditions for the development of the species at this stage. Factors such as feeding frequency can configure a direct correlation with the water conditions of the growing environment through the efficiency of food utilization and the amount consumed. The interferences that occurred in the performance were not enough to change these parameters, so that all treatments produced conditions that allow the good development of the tilapia, implying that the possible changes in performance occurred due to the use of the feed.

1.Condition factor

The condition factor (K) of *O. niloticus* fingerlings in different treatments ranged from 1.73 to 2.18. The highest condition factor was found in the T1 (2.18 ± 0.09) fed with Artemia nauplii and there was no significant variation in condition factor of fingerling in T2 and T3 i.e., 1.73 and 1.74 respectively. The condition factor (K) of fingerlings in treatment T1 was significantly ($p<0.05$) higher than other treatments (Figure 10). The K value obtained for *O. niloticus* in all treatments was greater than one which suggests that the fish was in good condition.

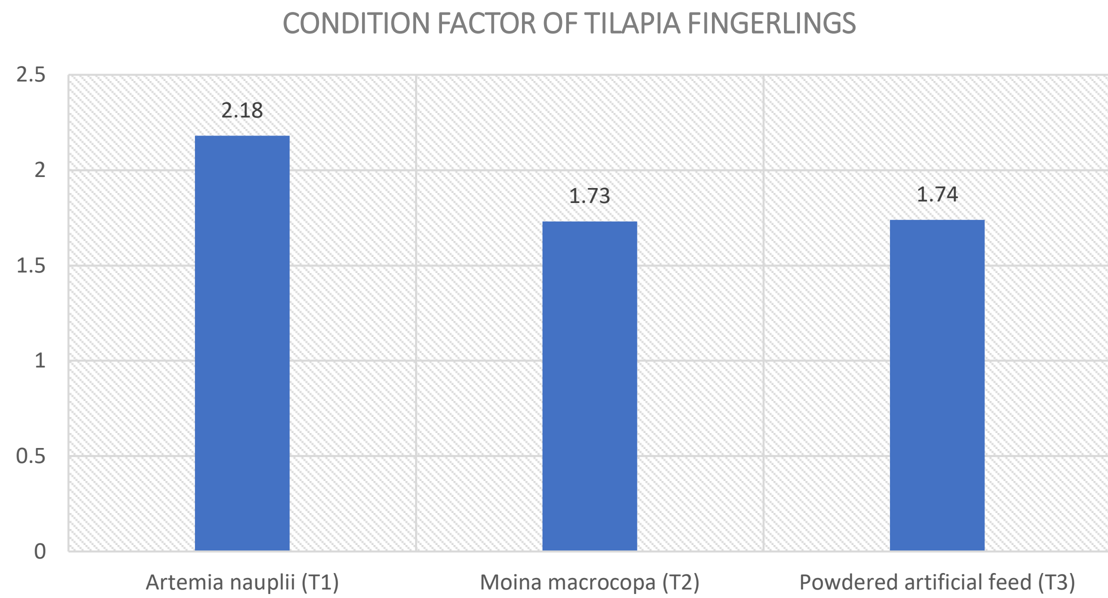

Figure 10. Condition factor (%) of Tilapia fingerlings fed with live feeds.

2.Daily weight gain and average weight gain

Fig. 11 represented the highest average weight gain of Tilapia fry was found in T1 and the lowest was in T2. The AWG value of T1 (0.94 ± 0.01) was significantly higher than that of T2 (0.53 ± 0.01) at 5% level. On the 14th and 35th days average weight gain of fish fed with *M.macrcopa* was found significant compared to artificial feed.

This controlled experiment was also conducted to assess the daily weight gain of tilapia frequently at 7 days intervals. In this study, daily weight gain of tilapia for T_1 was 165.08±5.65 g and for T_2 was 106.790±1.29 g, respectively. The difference in daily weight gain is notably remarkable between the two treatments. The daily weight gain of tilapia was higher in T_1 than in T_2. This frequent observation was performed to find out where the maximum growth was taking place in the production cycle of two different feeding systems. Daily weight gain of Tilapia fingerlings was maximum observed in second week of sampling of which the highest peak was for T1 treatment. In terms of weight gain, in most sampling stages, the performance in T_1 was significantly ($p < 0.05$) higher than in T_2. In terms of growth trend, after about 14 days, a different trend of weight gain was observed.

The higher weight gain in T_2 was observed from the 3 sampling. However, it was a marginal increment of growth after about 35 days of experiment in *M.macrocopa* fed Tilapia fingerlings.

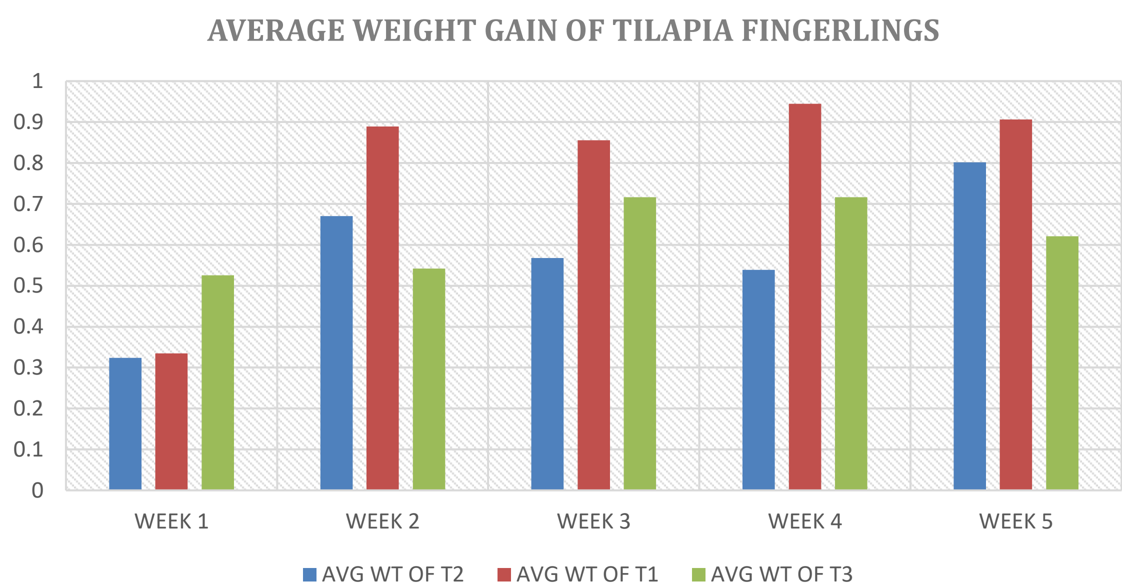

***Figure 11** Weekly average weight gain of Tilapia fingerlings fed with live feeds*

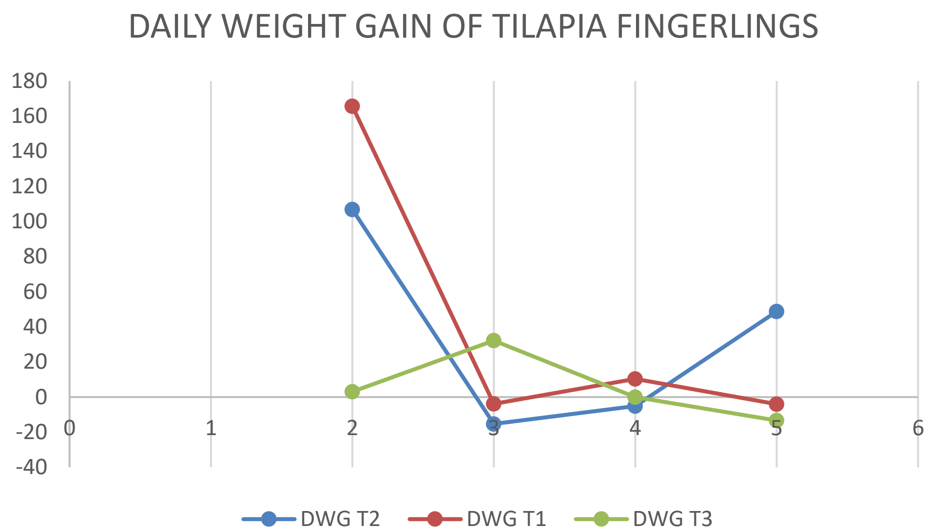

Figure 12 Daily weight gain of Tilapia fingerlings fed with live feeds

3.Specific growth rate

Specific growth rate is defined as growth rate over time. The SGR values of experimental fish in treatment T1, T2, T3 were 1.33 %, 1.54%, 0.827 % respectively. Although the SGR in T3 was lower but no significant difference was found among the three treatments. The present study resulted that better SGR gain in T2 than all other treatments and ranked (T2>T1 >T3). The highest SGR value was observed in T2 and the lowest one was T3. There was no significant difference (p>0.05) of SGR among the different treatments (Figure 13). The value of specific growth rate is used to compare growth on a daily basis. The significantly higher growth rate and specific growth rate indicate the effective role of protein in the growth performance. The highest SGR (1.54%) was recorded in treatment T2 after 35 days of experiment which was significantly different from control, T1 and T3. The treatments mean revealed that the best SGR was recorded in T2 followed by T1 and lowest in control. Thus, the treatment T2 showed best SGR which was significantly higher as compared to control as well as all other treatments.

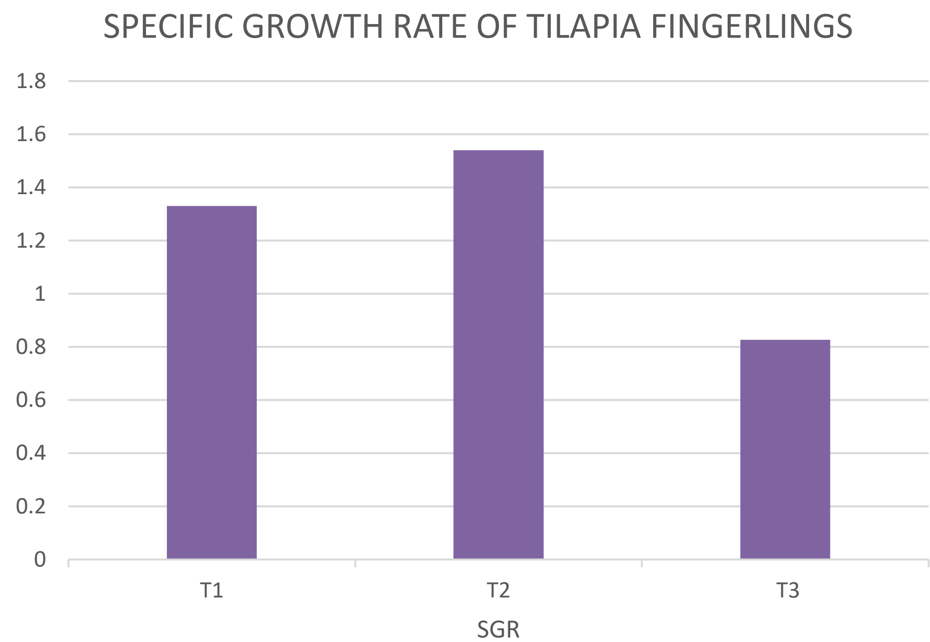

Figure 13 specific growth rate of Tilapia fingerlings fed with live feeds

4.Survival rate

Fig.14 shows, after culture period of 35 days, the survival rates of Tilapia fry in the T1, T2 and T3 were 86.60 ±1.5%, 77.70 ±1.5% and 60 ±1.5% respectively. Survival of fingerlings fed Artemia, Moina and artificial diet decreased significantly on week 2. Survival in all treatments stabilized during week 3 until week 4; however, survival decreased further in fingerlings fed powdered artificial feed.

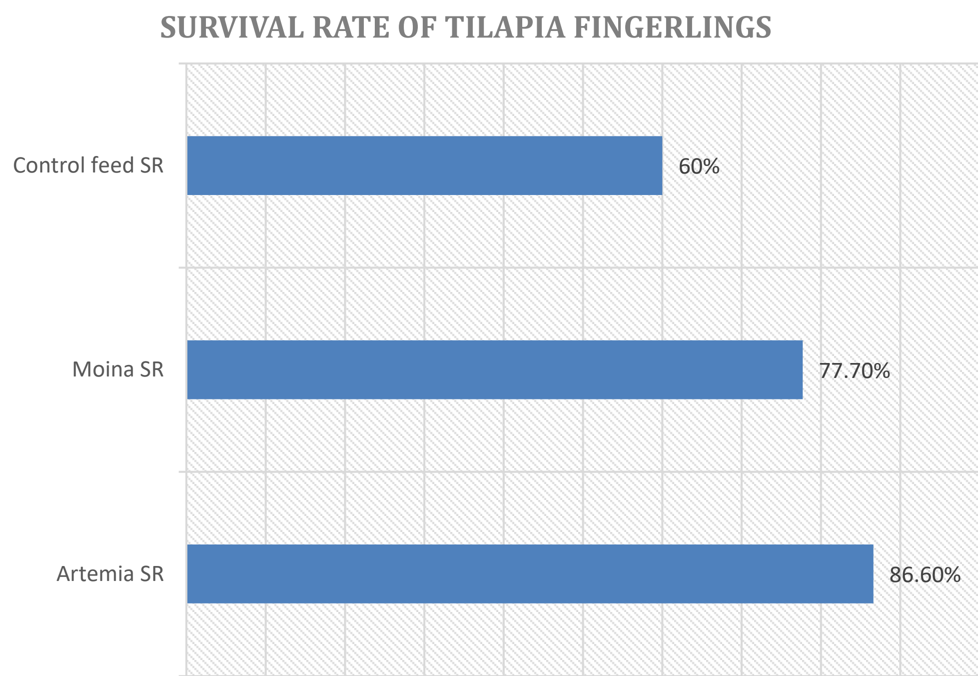

Figure 14 survival rate of Tilapia fingerlings fed with live feed

DISCUSSIONS

In the present study, the fingerlings are fed with live feeds (Artemia *nauplii & Moina macrocopa*) and powdered artificial feed in T_1, T_2 &T_3 respectively. A difference in weight gain was found between the treatments. The mean weight of T1, T2 and T3 was 0.73 ± 0.46 g, 0.530 ± 0.33 g,0.601 ± 0.84 g respectively at the end of the experiment. The weight gain was higher in T_1 which might be because fingerlings are fed with Artemia nauplii which improved their ingestion in taking powdered artificial feed.

It is evident from the present study that live food organisms are more effectively utilized by the tilapia fingerlings than the dry artificial feed. This phenomenon has been attributed by various workers to the presence of enzymes in natural feeds that hasten the digestive processes in first-feeding larvae (Verreth et al.,1993). In this experiment, the dietary inclusion of Artemia nauplii in the commercial diet produced bigger fingerlings after 30 days of feeding compared with the animals fed with the commercial diet sole, coinciding with the results reported by Garcia-Ulloa *et al.,* (2006) rearing red tilapia fry.

Overall growth results for the animals fed with the *Artemia nauplii and M. macrocopa* diet were significantly better than those obtained with the powdered artificial diet suggesting that growth performance of Nile tilapia fingerlings was improved mainly due to the mixed ingredients that affected its nutritional quality. Mihelalakis *et al.,* (2001) pointed out that quality of feed is one of the most important factors determining the fate of food consumed and subsequent growth.

Moina was shown to significantly enhance growth and survival of some freshwater and marine fish species (Fermin and Recometa, 1988; Villegas, 1990). A high growth performance of African catfish C. gariepinus fed M. dubia was reported by Adeyemo et al. (1994). The complete replacement of Artemia by Moina in the larval rearing of O.*niloticus* is possible (Fermin and Bolivar, 1991), but the growth of Tilapia fingerlings given Moina in the present study was lower than that of Artemia fed Tilapia fingerlings.

Differences in research findings may be because of the nutritional quality of the Moina, which varies according to culture conditions (Watanabe et al., 1983). Verreth et al. (1993) reported that differences in fish growth observed between species and between regions were related to the feeding strategy, the growth parameter measured, and the age of the larvae at the time of measurement.

Feeding Moina to fish fry as a mono diet can generally give comparatively better results than feeding a mixed diet or feeding other live feeds, although results are dependent on the specific requirements of fish cultured. Okunsebor et al. (2010) showed best performance of Heteroclarias fry (African catfish) when fed on Moina alone when compared with a mixed diet (Moina and Artemia). In fact, the best mean weight gain of goldfish fry (Carassius auratus) was recorded for fry fed with Moina in comparison to other diets tested (Okunsebor and Ofojekwu, 2003; Samuel, 2014). However, Moina can be used alone or, in combination with Artemia when fed to *Macrobrachium rosenbergii* without any adverse effect on production (Alam et al., 1991; Rahman et al., 2003).

Although Artemia being the most frequently used for larval feeding, the seasonal variation and expensive cost often limit their use in aquaculture hatcheries, and hence it is a requisite to develop low-cost live feed organisms which are easy to culture like Moina in order to minimize risk to aquaculture practice during early larval development stages (Singh et al., 2012). Moreover, the use of Moina as fish larval feed has yielded commendable results with indigenous species in various regions of the world (Bryant and Marty, 1980; Adeyemo et al., 1994; Samuel, 2014). Moina has been utilized as an ideal food organism for carp, shrimp and catfish larvae (Singh et al., 2019; Islam et al., 2017). In India and Thailand, Moina has also been used in shrimp hatcheries (Rahman et al., 2003).

Artemia is famous as solitary live feed for commercially cultured larvae fish for several decades, and their amino acid profile has been well approved (Watanabe et al., 1978, 1983; Solomon et al., 2006; Dararat et al., 2012). On the other hand, the amino acid profile of freshwater zooplankton has been poorly documented, although these species are increasingly relevant as larvae feeds in hatcheries for freshwater fish (Solomon et al., 2006). The nutritional value of Moina differs according to the culture media used and life- cycle. Adult Moina usually have higher protein composition than juveniles. However, Moina has a higher nutritional value when compared with Artemia and, furthermore both species breed and grow faster (Mubarak et al., 2017) making Moina comparable to that of Artemia (Mubarak et al., 2017).

The moisture content of Moina species, approximately 87.9 - 89.0%, is similar to other live foods such as Daphnia (88.1 - 89.3%), *Branchionus plicatilis* (86.4 - 91.8%) and *Tigriopus japonica* (86.0 - 87.3%) (Watanabe, 1978, 1983; Solomon et al., 2006). With respect to crude protein content, Moina (59.95 - 62.6%) had the highest protein content when compared with Daphnia (62.6%) and *B. plicatilis* (52.15 - 60.57%) (Watanabe, 1983; Solomon et al., 2006). Bogut et al. (2010) claimed that *D. magna* contained 39.24% and 33% which is relatively lower than Moina in fresh and dry weight, which is lower than the equivalent values determined for Moina. Solomon et al. (2006) stated that the moisture content of *M. micrura* was around 89%, while crude protein was 52.4% of dry weight. *M. micrura* recorded higher amino acid concentrations than *Diaphanosoma excisum* and *B. calyciflorus*, except for alanine, tyrosine, threonine, proline and serine amino acids (Solomon et al., 2006).

The main amino acid identified for *M. micrura* was lysine, and glutamine is the main non-essential amino acid (Solomon et al., 2006). Amino acid levels in the profile of Moina were higher than for Artemia except for alanine, glycine, proline, serine, and tyrosine (Watanabe et al., 1983; Solomon et al., 2006). *M. micrura* was reported to contain sufficient crude protein and amino acid concentration to provide basic requirements of larvae and postlarvae feed that are essential to the survival and growth of fry and larval cultivated species (Solomon et al., 2006). Meanwhile, *M. mongolica* had most essential amino acids less than Artemia and *B. plicatilis,* but, methionine content in *M. mongolica* (1.5%) was greater than Artemia (0.9%) and B. plicatilis (0.8%) (Tong et al., 1988; Budhin et al., 2016; Khudyi et al., 2018). Therefore*, M. mongolica* can serve as a supply medium in terms of methionine levels for fish larvae. For unsaturated fatty acids, *M. mongolica* recorded high eicosapentaenoic acid levels (EPA) (12.7%) of total fatty acids, while in Artemia and *B. plicatilis* EPA level was recorded low, i.e., 2.1% and 1.9%, respectively (Tong et al., 1988; Budhin et al., 2016; Singh et al., 2019). It can be summarized that the nutritional content of Moina was higher as other live foods and can be a reliable and affordable replacement for costly marine Artemia. In fact, Lavens and Sorgeloos (1996) believed that Artemia cysts are less nutritious than other zooplanktons (Solomon et al., 2006).

Statistical analysis of variance indicated that the growth per day in percent body weight gain was significantly higher in all the treatments as compared to control (T3). Poor growth and survival of Tilapia fingerlings given artificial feed proved that prepared diets are not yet suitable for feeding fingerlings.

The condition factor is used in studies of fisheries biology to indicate the well-being degree of fish in the environment in which they live and to verify if they make good use of the foods available (Weatherly, 1987). The development of tilapia in a habitat can be determined by total biomass, increase in length and weight (Medri*, et. al*., 1990). Sexual differences, age, changes in seasons, gonad maturity levels, nutritional levels and maturity of fishes can influence the condition factor (K) value (Lagler, 1952; Kotos, 1990). Condition factor (K) of this work showed the less variation and good performance at T1. Here K increased according to the treatment but at T1 the K value (2.184) was higher than that of other treatments. The lower value of the K factor (1.731) was found at T2. The high K values obtained in these findings compared to the results of this study could be attributed to nutritional quality of feed.

The length-weight relationship (LWR) is an important tool that provides information on growth patterns and growth of animals. The b values in length-weight relationships have been used to determine the growth pattern of fish species. According to Alam et al. [14], the value of b is an exponent indicating an isometric growth when equal to 3 and indicating an allometric growth when significantly different from 3. From this study, *O. niloticus* fingerlings were observed to have a slightly negative allometric growth pattern with b value less than 3.0 (For T1;2.94, T2;2.367, T3;2.58). It clearly shows that b value of *O.niloticus* treated with Artemia nauplii shows a near isometric growth with respect to other treatments.

CONCLUSION

In the present study, significantly positive effect was found by using Artemia nauplii & *M. macrocopa* as live zooplankton feed on the growth of Tilapia fry. On the other hand, Nile Tilapia fed with only powdered artificial feed showed the poorest growth and body weight gain. At the conclusion of the trial, the mean weights of T1, T2, and T3 were 0.73 0.46 g, 0.530 0.33 g, and 0.601 0.84 g, respectively. The fact that T1 fingerlings were fed Artemia nauplii, which enhanced their intake of powdered artificial feed, may have contributed to the weight growth being higher in T1. Condition factor (K) of this work showed the less variation and good performance at T1. The average daily gain, specific growth rate, survival rate and condition factor were better in live feed treatment of Tilapia culture than those of artificial feeds. Therefore, Artemia nauplii & *M. macrocopa* can be used as supplementary feed for Tilapia fingerlings rearing in hatchery and nursery.

SUMMARY

India's aquaculture sector now ranks second in the world for aquaculture production (SOFIA,2022). Tilapia farming is widespread, occurring in more than 135 countries and territories. The prospect of sustainability in the food and aquaculture industries depends significantly on tilapia. Production of tilapia is increasing because of tilapia's large size, fast growth, prolific breeding characteristics, palatability, and relatively low cost for production. The objectives of the present study are to evaluate and compare the efficiency of live feeds, *Artemia nauplii* and *Moina macrocopa* as supplementary feed in Tilapia fingerlings (*Oreochromis niloticus*), to evaluate the survival rate of Nile tilapia fingerlings (*Oreochromis niloticus*) fed with Artemia nauplii and *Moina macrocopa* as supplementary feed and to provide information on the growth performance and condition factor (Kf) for Nile tilapia fingerlings (*Oreochromis niloticus*) fed with Artemia nauplii and *Moina macrocopa* as supplementary feed in order to assess the fishes' health.

The experiment was conducted for 35 days at the Department of Aquatic biology & Fisheries, University of Kerala, Thiruvananthapuram. A total number of 150 (Oreochromis niloticus) fry were obtained from fish seed production unit of Neyyar hatchery, Thiruvananthapuram. After transportation, acclimatization of fry was carried out to the lab conditions for a period of one week in 500L capacity Fiber reinforced plastic (FRP) circular tanks. The obtained fish fry was healthy and free from any infection. After one week of acclimatization period, fish with an average body weight of 0.283g were divided into 3 treatment of 3 replicate groups. For the experiment, nine HDPE circular tanks having water holding capacity of 30L were selected. Each tank was washed and disinfected before the introduction of fish. Fifteen fishes were randomly distributed in each tank with three replications.

Fish were fed two times daily at 4% in the morning at 9.00 am and 6.00 pm in the evening for 35 days. Larvae were provided with freshly hatched Artemia nauplii for the first treatment (T1) and Moina macrocopa for the second treatment (T2). Powdered artificial feed

containing 36% CP was fed two times a day at the rate of 7% of body weight per day as for control (T3) (Bag and Mahapatra, 2012). About 20 to 30% water exchange was carried out daily. Uneaten feed and faecal matter were removed by siphoning water before feeding as there was around 12h time for fishes to feed on nauplii. After acclimatization to laboratory conditions, initial length and weight of fishes were recorded before initiation of experiment and after completion of experimental period. Sampling was performed in the 14^{th},21^{th},24^{th},28^{th}, &35^{th} day of the experimental period.

In the present study, the fingerlings are fed with live feeds (Artemia *nauplii & Moina macrocopa*) and powdered artificial feed in T_1, T_2 &T_3 respectively. A difference in weight gain was found between the treatments. The mean weight of T_1, T_2 and T_3 was 0.73 ± 0.46 g, 0.530 ± 0.33 g,0.601 ± 0.84 g respectively at the end of the experiment. The weight gain was higher in T_1 which might be because fingerlings are fed with Artemia nauplii which improved their ingestion in taking powdered artificial feed.

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In the present study, significantly positive effect was found by using Artemia nauplii & *M. macrocopa* as live zooplankton feed on the growth of Tilapia fry. On the other hand, Nile Tilapia fed with only powdered artificial feed showed the poorest growth and body weight gain. At the conclusion of the trial, the mean weights of T1, T2, and T3 were 0.73 0.46 g, 0.530 0.33 g, and 0.601 0.84 g, respectively. The fact that T1 fingerlings were fed Artemia nauplii, which enhanced their intake of powdered artificial feed, may have contributed to the weight growth being higher in T1. Condition factor (K) of this work showed the less variation and good performance at T1. The average daily gain, specific growth rate, survival rate and condition factor were better in live feed treatment of Tilapia culture than those of artificial feeds. Therefore, Artemia nauplii & *M. macrocopa* can be used as supplementary feed for Tilapia fingerlings rearing in hatchery and nursery.

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