ASSESSMENT OF FECUNDITY, HATCHING & SURVIVAL RATE OF NILE TILAPIA (*Oreochromis niloticus*) APPLYING FEED WITH GARLIC AND GINGER EXTRACT

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CERTIFICATE

This is to certify that thesis entitled, ***" ASSESSMENT OF FECUNDITY, HATCHING & SURVIVAL RATE OF NILE TILAPIA (Oreochromis niloticus) APPLYING FEED WITH GARLIC AND GINGER EXTRACT "*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degreeof* ***MASTER OF SCIENCE (MS)*** *inBiotechnology,embodiestheresult of a piece of bona fide research work carried out by* ***Dider Haider Nayeem*** *Registration No.* ***19-10308*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation hasdulybeenacknowledged& style of the thesis havebeenapprovedand recommended for submission.

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DEDICATED

TO

My Beloved Parents

who try to

encourage me to

continue my study.

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ASSESSMENT OF FECUNDITY, HATCHING & SURVIVAL RATE OF NILE TILAPIA (*Oreochromis niloticus*) APPLYING FEED WITH GINGER AND GARLIC EXTRACT

Abstract

The study was conducted at commercial hatchery at BRAC Tilapia Hatchery, Magura, Bangladesh and the analytical studies were conducted at Aquaculture Laboratory, Sher-e-Bangla Agricultural University to evaluate the effect of Garlic and Ginger extract in Nile tilapia diets on their reproductive performance (fecundity and hatching), survival rate on produced fry and hematological parameters of fry. A treatment was used as control without any extract. Nile tilapia (*O. niloticus*) with average weight of 267 g ± 3.16 were stocked for spawning into nine hapas at a density of 13 fish (10 females and 3 males) per 10 sq meter hapa, Three hapas with same diameters were used as replicates for each treatment. Hapas were fixed in an earthen pond (5 decimals). Feeding was done at a rate of 0.5% from their total biomass. Water quality parameters (temperature, pH, dissolved oxygen, ammonia) were measured weekly. Production of seed (eggs and fry) was compared with the tested diets. The highest mean of egg number (132.05 pcs) & mean spawn number (98.29 ± 1.11 pcs) per gram were obtained with the diets contained 0.02% Garlic extract (Treatment 2). Survival rate (84.24±0.05%) of fry was also found best with Treatment 2. Tilapia diets supplemented with Garlic extract had best responded on their reproductive performance (Hatching 80.63% ±1.05) and highest value FCR (1.43±0.19) & SGR (1.50 ± .19). The garlic extract treatment showed excellent performance for all the parameter under studied. Hence, the feeding with (0.02%) garlic extract can be used for large scale in tilapia breeding.

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LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviations	Elaboration
%	Percentage
&	And
< / >	Less than / Greater than
±	Two Possible Value/ Range
^{0}C	Degree Celsius
WG	Weight Gain
cm, cm^{-1}	Centimeter, Per centimeter
DM	Dry Matter
DO	Dissolve Oxygen
et al.	And others
etc.	And other similar things
FCR	Feed Conversion Ratio
g	Gram
dec	decimal,
$KMnO_4$	Potassium per Manganate
L, L^{-1}	Litre, Per litre
m^3, m^{-3}	Cubic metre, Per cubic metre
mg/l	Milligram per litre
ml, ml/l	Millilitre, Millilitre per litre
mg/dl	Milligrams per deciliter
mm	Millimetre
NH_3	Ammonia
pH	Puissance of Hydrogen
PI	Protein Intake
Ppt	Parts per Trillion
ppm	Parts per million
SD	Standard Deviation
SGR	Specific Growth Rate
T	Treatment
R	Replications

CHAPTER I

INTRODUCTION

Tilapia is the widely name for the Tilapiine group of Cichlidae species that are indigenous to Africa and the Middle East and are the second most widely grown species in the world, after Carp species (FAO, 2020; Watanabe *et al*., 2002). Numerous tilapia species, including the Mozambique tilapia (*O. mossambicus*), blue tilapia (*O. aureus*), and nile tilapia (*O. niloticus*), are grown in more than 90 countries (Lim & Webster, 2006). According to El-Sayed *et al*. (2005), the handling tolerance, rapid development, tolerance of a wide variety of environmental variables, including pH, temperature, and salinity, and high economic viability of tilapia species make them appropriate for aquaculture (Klett & Meyer, 2002). In a variety of aquaculture production techniques, varying from extensive to intense, tilapia species are widely discussed (cages, tanks, raceways, recirculation systems and earthen ponds (El-Sayed *et al*. 2006).

The main species of tilapia was the Mozambique tilapia *(O. mossambicus*) which was introduced into Bangladesh from Thailand in 1954. Due to its early maturation and prolific breeding tendencies in the ponds, the fish did not thrive and turned out to be a nuisance. As a result, both the fish's producers and consumers referred to them as "nuisance fish." Overall tilapia performance has shown that they are no longer pests and have earned the nickname "aquatic chicken" together with other fast-growing tilapias. Through UNICEF, a promising farmed strain of the Nile tilapia identified as Chitralada was first introduced into Bangladesh in 1974. The second introduction of the fish, again from Thailand, was launched in this country by the Bangladesh Fisheries Research Institute (BFRI, formerly FRI), in 1987. A red mutant tilapia that was developed in Taiwan and brought into Thailand is a cross between albino *O. mossambicus* and *O. niloticus*. Drs. M.G. Hussain and Dewan imported a batch of this red strain of tilapia to Bangladesh in 1988 from Bangkok, Thailand's Asian Institute of Technology (AIT). In July 1994, the World Fish Center (formerly ICLARM) inaugurated the Dissemination and Evaluation of Genetically Improved Tilapia in Asia (DEGITA) project, another promising Genetically Improved Farmed Tilapia (GIFT) strain, an artificial strain of *O. niloticus*, was introduced in Bangladesh from the Philippines.

The International Center for Living Aquatic Resources Management (ICLARM) selected the GIFT type over several generations from a size of a population that included eight different strains of Nile tilapia, *O. niloticus* (Eknath *et al*. 1993). The manufactured GIFT strain reportedly demonstrated on-station experiments with on average 60% higher growth and 50% greater harvesting survivability than the most widely cultivated strain in the Philippines (Eknath *et al*. 1992). Under the direction of a World Fish Center initiative, a search project was launched in Bangladesh, China, the Philippines, Thailand, and Vietnam to evaluate this strain in other Asian nations.

Utilizing biotechnological tools the researchers can determine and conglomerate features in fish and shellfish to boost production and raise excellence. Researchers are looking into genes that will increase the synthesis of substances that help fish species fight against microbial diseases and natural growth hormones for fish. It believes that contemporary biotechnologies should be utilized in conjunction with existing technologies, not as a replacement for them, in order to resolve issues which should be necessary instead of being innovation. There is significant potential for contemporary biotechnology to increase aquaculture and meet consumer needs for aquatic species. Additionally, the uses of biotechnology and genetic modifications have the potential to significantly enhance the quality and yield of fish rose in aquaculture. Aquaculture has growing importance, and biotechnology can assist for improvement of this sector. Aquaculture utilizing biotechnology also benefits the environment. Biotechnology may frequently be a considerable help in meeting the needs of a rising and more industrialized population in the upcoming decade when properly combined with other technologies for the production of food, agricultural products, and services.

For a specific goal, such as enhancing fish standard and effectiveness, supplements are ingestible compounds that are given to feeds in relatively small amounts (alone or in combination). They also help to maintain the physical and chemical quality of the feed as well as the aquatic environment. Numerous studies have demonstrated the benefits of using herbs and spices as feed additives in aquaculture for greater survival and growth. Natural substances originating from plants called phyto-additives or phytogenic feed additives are added to feed to improve the quality and efficiency of the animals. They will contain both nutritive and non-nutritive components and influence the individual's system directly or indirectly. Phytogenic feed additives are frequently divided into the following categories: Technology-based phytoadditives with antioxidant and anti-mold features; zootechnical phytoadditives with immune modulators, gastrointestinal stimulants that work to increase the activity of digestive enzymes, growth promoters of non-microbial origin as well as compounds rising effectiveness as well as quality of dairy products; and nutritional phytoadditives that are exceptionally rich in vitamin.

Due to its nutritional, physiological, and pharmacological benefits, ginger (*Zingiber officinale*) and garlic (*Allium sativum*) are two of the spices that are most frequently employed as bioactive components in farming. *Allium sativum*, a perennial incandescent light herb, may be a member of the Liliaceae family. It has been utilized for years as a flavoring ingredient, a traditional remedy, and a food ingredient to enhance both physical and mental health. Garlic has already been studied using ethanol and aqueous extracts, as well as dried powder. It also contains essential substances like iodine and sediments with beneficial effects on the circulatory, skeletal, and cholesterollemia systems, as well as minerals (phosphorus and calcium), vitamins (A, C, and B complexes), omega-6 fatty acids, carbs, and many more. Garlic has been used as a feed addition in fish feed, according to previous research.

The underlying stem or rhizome of ginger (*Zingiber officinale*) a member of the Zingiberaceae family, is a popular spice used in cuisine all over the world. A wide range of biologically active substances, including alkaloids, flavonoids, polyphenols, saponin, steroids, and tannin, as well as dietary components like fiber, carbohydrates, vitamins, carotenoids, and minerals, can be found in ginger.

Ginger and garlic are frequently used to create stews and soups in most kitchens and eateries around the world. They are frequently added to foods in small amounts to flavor or preserve them, as well as to increase the flow of gastric juice and improve food taste, which helps to arouse the appetite. Due to the physiological and pharmacological characteristics of the various bioactive components found in ginger and garlic, they are regarded as safe herbal medications with negligible or negligible side effects in humans, animals, and fish. Previous research showed that adding a ginger-garlic mixture to the diet of commercial broilers did not boost growth or feed consumption when compared to a control diet. On the other hand, past statements had suggested that feeding Rainbow Trout (*Oncorhychus mykiss*) Compared to a control diet, weight increase, specific rate of growth, and feed conversion ratio were all improved when garlic and ginger were combined. One of the greatest plant source-feed additives for improving the growth promotion, digestion, and flesh quality of the cultured organism is garlic (*Allium sativum*) (Vampires *et al*., 2005; Tatara *et al*., 2008).

To address the nutritional needs of fish for typical physiological processes, such as maintaining an extremely effective natural system, growth, and breeding, aquaculture supplies are prepared with a vast pool of ingredients. An expanding range of non-nutritive feed additives are being employed in aquatic feeds to ensure that the dietary elements are eaten, digested, absorbed, and delivered to the cells. For a specific purpose in aquaculture, tilapia is supplied with feed additives in modest doses. Functional feed additives help tilapia grow and stay healthy. They also strengthen their immune systems and have physiological effects that go beyond what regular feeds can provide. Probiotics, phytogenic compounds, immunological stimulants, enzymes, hormones, mycotoxin binders, organic acids, etc. were the finest functional feed additives for controlling and regulating tilapia activity and improving aquaculture profitability.

Considering the above factors the present experiment was carried out to fulfill the following objectives:

- Assess the ginger and garlic extract on fecundity of brood Nile Tilapia.
- Evaluate the ginger and garlic extract on hatching rate of eggs of Nile Tilapia.
- Estimate the survival rate of Nile Tilapia Fry treating extract.
- Compare the fecundity, hatching and survival rate of Nile Tilapia with Natural system.

CHAPTER II

REVIEW OF LITERATURE

A crucial and essential component of any research project is a survey of the relevant literature. The preparation of the objectives and approach will benefit from a careful analysis of the literature on the subject. A review of writing along those same lines can aid in identifying the subject's research gaps and the necessity of the current investigation. This section, which is introduced as follows, makes an effort to thoroughly review the writing of earlier research papers that are significant to the examination:

Melaku *et al.* (2018) conducted a study on hapa net material and brood stock density's effects on Nile tilapia (*Oreochromis niloticus* L. 1758) fry assembly. They were testing three different types of locally accessible shade nets as breeding hapas in order to determine the optimal brood stock density for maximal fry production and management convenience. Six treatments in duplicate were used in the experiment, which included two brood stock densities (4 fish per m2 and 8 fish per m2) and three different types of shade net materials (30% shadow net, nursery plantation net, and khaki fish net). The hapa method is arguably the most popular way to produce *O. niloticus* fingerlings and fry worldwide. Southeast Asian nations are particularly accustomed to using this strategy. Hapas (net enclosures) are fine-mesh cages made of nylon thread and polyethylene netting in a range of mesh sizes. Hapa nets look like an upside-down screen. The most typical size for producing *O. niloticus* fingerlings and fry is 3x3x1.5m. In comparison to the pond method, the hapa method of producing fingerlings allows for a higher output per unit area and yields fingerlings of more consistent and uniform size. Between each mating cycle, the pond does not need to be regularly drained and prepped for stocking. The hapa technique is the most effective and cost-effective of the three. In this experiment, there was no discernible difference between the different hapa net materials on fry or female. According to the findings, larger brood stock densities (8 fish/m2) significantly increased total mean fry production among farming shade nets utilized for both densities (P0.05). Day 21 of the primary clutch produced much more (p mean fry output of 19319.2 flies). However, in this study, there wasn't any noticeable difference in between third and second clutches.

Dada *et al*. (2015) conducted a study on Production Performance Fed Tilapia (*Oreochromis niloticus*) Enhancement Three Feed Supplements in Commercial Feeds. In this experiment, Nile tilapia *Oreochromis niloticus* fingerlings' growth, nutritional consumption, body composition, and hematologic profile are evaluated in relation to the effects of 3 different feed additives (average initial weight of seven .18-7.35 g). three industrial feed additives Fish were fed the supplements for 56 days at 5% weight after being provided Aqua booster (Fish Vet, Inc. product, Ohio Dayton), Aqua superliv (Ayurvet Limited product, India), and Aqua pro (Smart Microbial, Inc., USA) at 0.5 g/100 g of feed additive each. When compared to fish fed the control diet, those fed diets with industrial food supplements had noticeably better growth performance, feed consumption, and body composition. The group supplied dietary Aqua superliv showed the simplest growth, whereas groups fed dietary Aqua booster showed the highest protein efficiency ratio and groups fed dietary Aqua superliv the highest specific growth rate. The fish fed had the highest protein content, at 70.87 percent while the moisture content was similar among treatments in the fish fed dietary Aqua pro, The effects of feed additives were particularly seen in the ash and fat concentrations. The results showed that for weight gain (8.85 0.92 g) and feed consumption (FCR=0.26), using Aqua superliv at a concentration of 0.5 g/100 g diet was the simplest.

Chand *et al*. (2013) conducted a study on *Allium sativum* (garlic) and *zingiber officinale* (ginger) have antibacterial effects against *Staphylococcus aureus, typhoid bacillus, Escherichia coli,* and *Bacillus cereus*. In the research, the antimicrobial activity of garlic and ginger extracts against four distinct bacteria—*Escherichia coli, typhoid bacillus, Staphylococcus aureus,* and *Bacillus cereus*—was assessed. The antibacterial efficacy of garlic and ginger extracts was assessed using the disk diffusion method and the agar diffusion method, respectively. Ginger extract only exhibited antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus*, but garlic extract shown excellent performance on antimicrobial activities against all four test organisms. Additionally, the zone of inhibition produced by the spice at the same dose against the test microorganism using the disk diffusion method was lower than that produced by the agar well diffusion method. Penicillin G exhibited the lowest zone of inhibition against *Escherichia coli*, 0.000.00, and the highest zone of inhibition against *Staphylococcus aureus*, 40,000,00. Allicin was found at the active ingredient in garlic and it works as an antimicrobial agent by inhibiting DNA and protein synthesis moderately and inhibiting RNA synthesis completely as an initial target (Shobana et al., 2009; Rahman et al., 2011). Moreover, garlic contains a lot of anionic substances like nitrates, chlorides, and sulfates as well as other moisture plant substances that may have antibacterial characteristics. (Shobana et al., 2009)

Ali *et al*. (2010) conducted a study on Effectiveness of garlic as a feed addition on Nile tilapia brood stock body weight and reproductive efficiency. In that study, Nile tilapia (*Oreochromis niloticus*) one-year-olds were stocked for mating into six hapas at a density of 200 fish (150 female and 50 male) per hapa, two hapas per treatment. Their average weight was 240 g, or 3.16 pounds. In an earthen pond, hapas were fixed. Fish were given 0.5% of their total biomass to the diets under test. Variables of water quality were evaluated. The studied diets were compared to the breeding programs (i.e., the production of eggs and fry).The results revealed that spawned females/wk, fecundity as the number of eggs produced per female and fry production per fish were significantly affected by dietary Garlic level. The highest values were obtained with the diets contained 0.5% or 1.0 % Garlic levels. Feed conversion as a feed required to produce 1000 fry was the best with the Garlic supplementation diets. Garlic supplements added to tilapia diets improved the fish's ability to reproduce and utilize nutrition. The use of 0.5% garlic level may be suggested by comparing between the provided values.

Mahmoud *et al.* (2019) conducted a study on Effect of Ginger and Garlic Dietary supplements on Nile Tilapia Muscle Oxidative Stress, Whole Muscle Mass, and Specific Growth rate (*O. Niloticus*). In that study, the fish were fed a standard basal diet for 60 days at 3% body weight, a food augmented with 1.5% ginger powder, and another diet enriched with 1.5% garlic powder. Additionally, relative to other test group, the feed conversion ratio (FCR) of Nile tilapia fish fed a control basal diet improved. There were no discernible variations between the experimental groups in the fish's basic chemical makeup. Using garlic as a feed supplement greatly reduced lipid peroxidation and had an antioxidant property, but combining ginger and garlic at 1.5% had no discernible impact on Nile tilapia growth parameters or muscle mass.

Abaho *et al*. (2021) conducted on a study the state of and potential applications for plant extracts in the management of tilapia reproductive. Because male individuals in that experiment grew more quickly than female and mixed-sex populations, it was common practice to limit prolific breeding through the use of all-male tilapia cultures. All-male tilapia are currently produced by most farmers using 17-methyltestosterone (MT), despite the fact that synthetic hormones have been associated with dangers to human health and the environment. Plant-based products have received a lot of attention lately as MT substitutes since they are less expensive, safer, and more environmentally friendly. Although there is rising attention in using plant extracts to reduce the frequency of spawning in tilapia production, the knowledge that is currently accessible has not been compiled to create eligibility requirements. Additionally, restrictions on the extracts' commercial use were found. 20 plant species' seeds, roots, and leaves have been extracted so far. But there is potential for *Tribulus terrestris, Mucuna pruriens,* and *Carica papaya* to stop unintended mating in tilapia production technologies. The majority of the extracts were given orally and included in fish diets. The primary bioactive substances in the phyto-extracts that cause sex inversion and reduce fertility in tilapia were saponins and flavonoid contents. However, the absence of standardized information on extract manufacturing, ideal doses, and mechanism of action hindered the commercialization of plant extracts. Future research should therefore focus on these technical constraints and emphasize the financial benefits of using plant extracts commercially in tilapia farming.

Fazio *et al*. (2022) conducted on a study *Oreochromis niloticus* (Nile tilapia) haematological and biochemical parameters are affected by supplemented meal with olive leaf extract. In that experiment, look at how *Oreochromis niloticus's* haematological and biochemical parameters respond to enriched feed with olive leaf extract. In comparison to the control group's diet, fortified feed was made with different extracts at concentrations of 1%, 1.5%, and 2% in the diet. The results showed that fish fed on 1% of extract had considerably higher levels of haematological indices such WBC, RBC, Hg, PCV, and plasma protein, whereas fish fed on higher concentrations of extract had lower levels of triglycerides and cholesterol. It has been established that olive leaf extract can be utilized to improve the resistance, development, and health of fish. Olive leaves have historically been used to boost immunity to a number of illnesses.

Abwao *et al*. (2021) conducted a study on Nile tilapia, *Oreochromis niloticus*, selective breeding: a Kenyan aquaculture development approach for improved genetic diversity. They found that appropriately planned selective breeding programs both in government and private hatcheries would be the key to supplying high-quality seeds for long-term aquaculture expansion. Presently, given rearing circumstances, the seedlings generated show a slow pace of growth. Using both recent and historical global experience and findings, this paper analyzes the significance of genetic transformation of *Oreochromis niloticus* using selective breeding. For farmers to receive high-quality seeds for increased body weight and the establishment of profitable aquacultures, tilapia genetic enhancement is essential. Heritable qualities such as weight, survival, and disease resistance will be enhanced through selective breeding for lengthy genetic gain and phenotypic development. To guarantee adherence to the procedures for stock improvement and sustainable growth of aquaculture, an in-situ strain increase in performance program that includes the creation of breeding nuclei and programs for monitoring and evaluating hatcheries, backed by the current standard operating guidelines for tilapia seed production, should be in place.

Mabrouk *et al.* (2011) conducted a study on Sensitivity of fingerling Nile Tilapia (*Oreochromis niloticus*) to varying degrees of natural soybean meal substitution utilizing garlic and onion. In this test, twelve experimental diets developed to incorporate 35% crude protein and 429.93 Kcal energy value per 100g-1 were used to investigate the effects of replacing dietary fish meal with soybean meal flavored with garlic and onion on Nile tilapia (*Oreochromis niloticus*) mono-sex fingerlings' growth performance, feed utilization, and overall body composition. Diets were processed in three groups with degrees of fish meal and soybean meal substitution. Group 1 is made up of 25% fish meal (FM) and 75% soybean meal (SBM); Group 2 is made up of 50% FM and 50% SBM; and Group 3 is made up of 75% FM and 25% SBM. Each group received four treatments that varied in level of garlic and onion: Three test interventions were used as negative control diets (D1, D5, D9); three were treated with 4% garlic (D2, D6, D10); three were supplied with 6% onion (D3, D7, D11); and three were supplied with a 10% combination (4% garlic and 6% onion: D4, D8, D12). Fish were raised for 84 days in 36 glass aquariums, three of each treatment. Each tank contained ten tilapia fingerlings, with a mean starting weight of 3.12 ± 0.3g/fish. The findings demonstrated that simple growth performance values and feed efficiency were considerably (P0.05) higher than single inclusion in terms of growth efficiency and feed conversion efficiency. When fingerlings were fed any of the D8 diets, the protein productivity value (PPV %), energy utilization (EU %), and feed conversion ratio all showed higher values (FCR). The findings show that the diet of 50% FM + 50% SBM with 10% of a combination of garlic and onion had improved growth performance and diet optimum utilization for 84 days fingerlings of mono-sexual Nile tilapia.

Iseghohi *et al*. (2019) conducted a study on Garlic and ginger extracts' antimicrobial effects on a few clinical specimens. In this study, experiments were carried out to determine the inhibitory impact of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extracts at concentrations of 0, 1, 2, 7, and 10% on Escherichia coli and Listeria mono cytogenes in vitro. The concentration of extracts rose along with the inhibitory action. In regards to E, the garlic extract was easier rather than L. mono cytogenes. Ginger extract proved successful in limiting the growth of *E. coli* at concentrations of 0.5, 1.0, and 2.0%. Ginger extract was shown to have a minimum inhibitory efficacy of 8.7 and 8.5 for E. L. coli and E. monocytogenes, however in the context of garlic extract, the figures were 9.1 and 8.0. Simultaneously both spices have been found to have a high possibility for acting as natural preservatives.

Duponchelle *et al.* (2000) conducted a study on Fecundity and egg shape differences in Cote d'Ivoire's artificial lakes' female Nile tilapia, *Oreochromis niloticus*. In that study, female *Oreochromis niloticus* fecundity and oocyte size were examined over the course of two year cycles in six small agropastoral and three big hydropower reservoirs in Cote d'Ivoire. There were significant variations in fecundity and oocyte size among the same group as well as between group’s years after years. The quantity of female oocytes produced and their size were shown to be inversely correlated. For a continued spawn weight during the first year of the research, but not during the second, this inverse association was present. Fecundity exhibits seasonal variation, according to monthly mean residuals of regressions between fecundity and feminine body weight. The peak of fecundity coincided with the greatest available resources and flooding prominence, which could have a massive effect on the fitness of both parents and children.

CHAPTER III

MATERIALS AND METHODS

This chapter describes the various tools and techniques about research location, Nile Tilapia breeding, hatchery procedure, feeding technique, fecundity & hatching rate, analysis of growth parameters & blood parameters, etc for the achievement of the research's goals. The flowchart of the overall research work is given below:

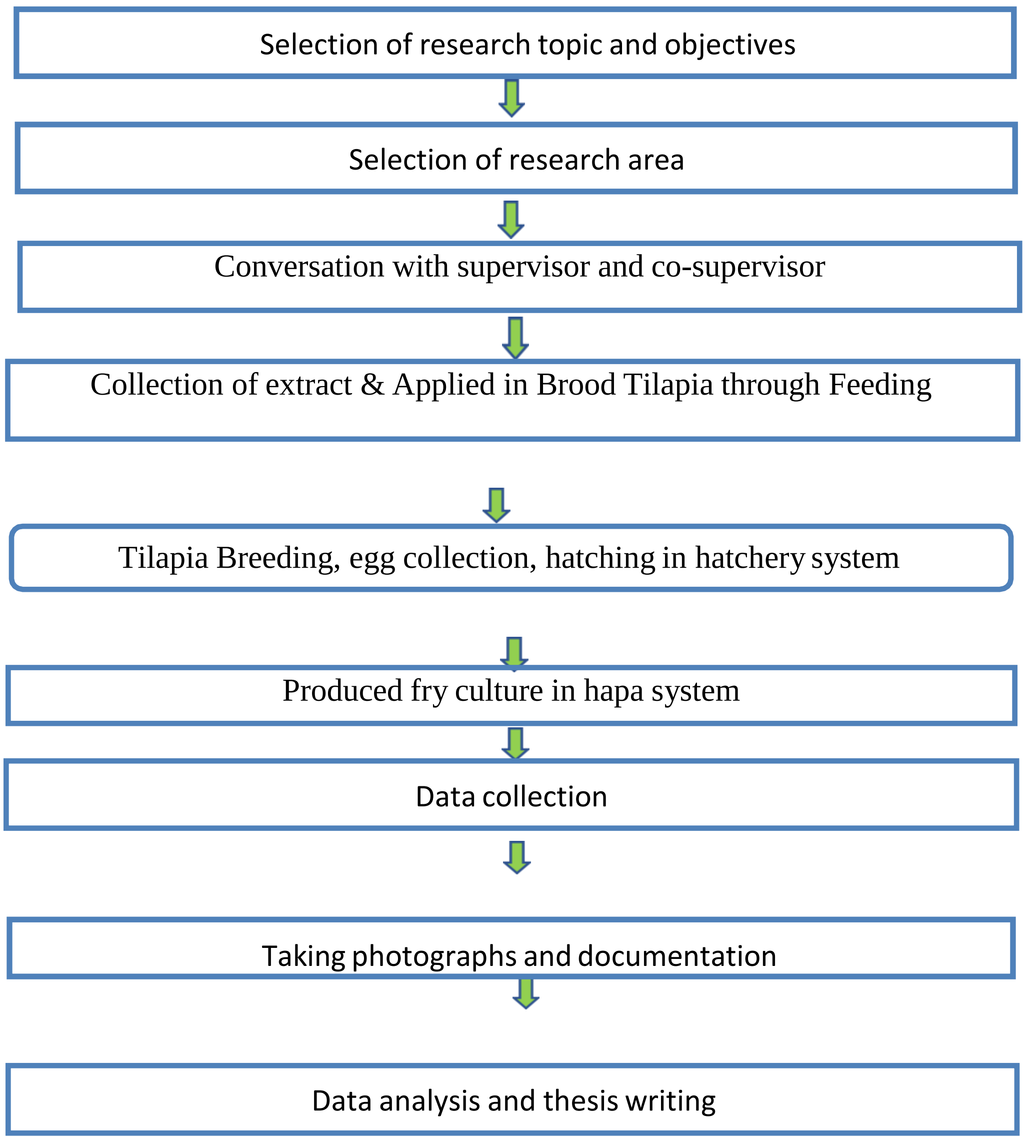

Figure 01: Flowchart of the overall research work

3.1 Study area

The study was performed in the BRAC Tilapia Hatchery (BRAC Fisheries Enterprise, BRAC), Magura, Bangladesh. Genetically Improved Farmed Tilapia (GIFT) strain Nile tilapia (*O. niloticus*) was chosen as breeding species in hapa method applying feed with ginger and garlic extract for the first time in Bangladesh to assessing its fecundity, hatching rate and evaluating its survival rate of fry, growth and other parameters.

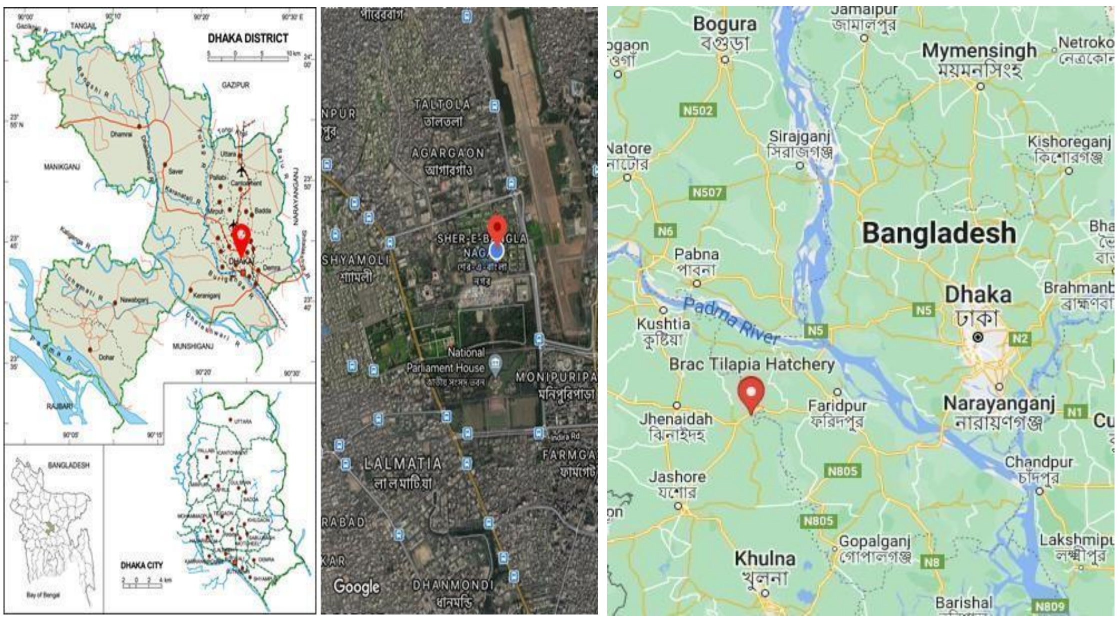

Figure 02: Map and satellite view of the research location

3.2 Duration of the study

The time period for the study was March to August for natural breeding purpose of Nile tilapia (*O. niloticus*) according to the supervisor's schedule. The experiment was set considering breeding behavior of Nile Tilapia, nursery management and hatching opportunity in that hatchery and lab.

3.3 Experimental design and setup

Treatments	Replications	Extract Use On feed	Fish Species	Fish Population Density
1	3 (10 sq.meter hapa for each Replications)	No	(GIFT) strain Nile tilapia (*O. niloticus*)	10 female:3 Male /Replication
2	3 (10 sq.meter hapa for each Replications)	Garlic	(GIFT) strain Nile tilapia (*O. niloticus)*	10 female:3 Male /Replication
3	3 (10 sq.meter hapa for each Replications)	Ginger	(GIFT) strain Nile tilapia (*O. niloticus*)	10 female:3 Male /Replication

Size of hapa = 10 Sq. meters (5 meter × 2 meter × 1 meter)

Size of fish= Initial average weight 267 g ± 3.16/fish,

Total average weight of fish= 3.5 kg/ hapa

3.4 Pre-stocking management

Pre-stocking management refers to several ongoing managerial activities done before stocking various fish species in hapa. Pre-stocking management concentrates on adequate pond preparation to eliminate the reasons for poor survival, unsatisfactory growth, and other issues, as well as to assure prepared accessibility of natural food in sufficient amount and quality for the spawn/Fry/fingerlings to be supplied. The following are included in the management's pre-stocking component & successive actions:

3.4.1 Pond preparation

Prior to the start of the experiment, the ponds were dried, the dykes were repaired, and undesired aquatic species were manually removed. Pond dykes underwent renovation and restoration. All of the ponds undergone liming (CaO) at a rate of 1 kg/decimal before 7 days of brood set-up in hapa. Total 5 kg lime was used in 5 decimal experiment ponds.

Figure 03: Pond preparation for the experiment

3.4.2 Bird protection

Bird protection net was established on the upper portion for eradicating mortality by birds.

3.4.3 Water supply

A deep tube-well inlet water supply system was used to feed the pond with water after it had been limed for 7 days; rainfall was also used to supply the pond with water. Daily water supplied by inlet because of water imbibes through mud. Water absorbance through pond mud is a greatest problem in Magura region.

3.4.4 Hapa setting

Properly hapa setting was one of the most important tasks for this experiment. : At first we were set up 4 bamboo pillars on the pond .Then tighten the hapa rope with pillars accordingly. We have to remember that bottom of the hapa can’t touch with the mud of pond. Hapa bottom was higher than pond bottom & upper portion was high 1 ft. from the water level of the pond. Keep a little distance between two hapa for collecting eggs, sampling, changing hapa etc. Total 9 Hapa was set up for this experiment.

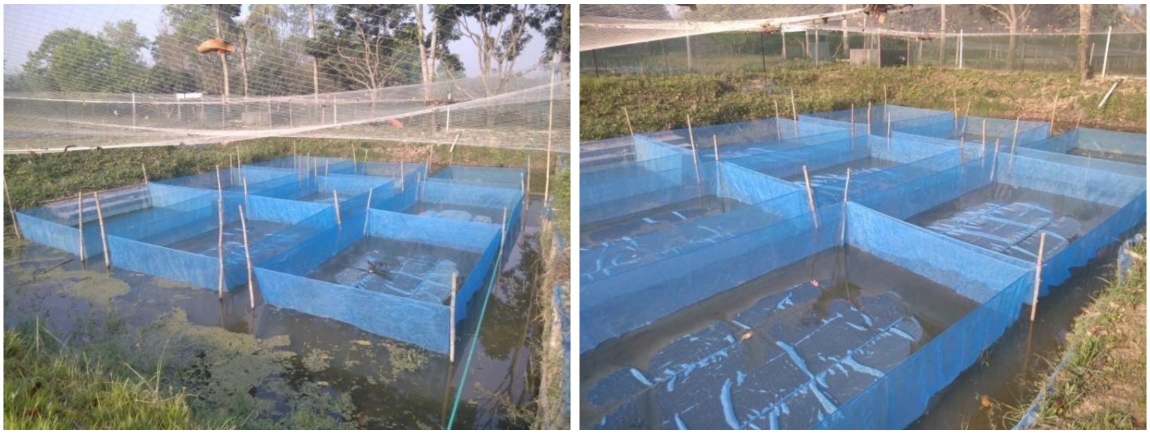

Figure 04: Hapa setting for evaluate garlic and ginger extract diets with feed

3.4.5 Treatment: Three treatments were applied for the experiment.

Treatment 1 (T_1): Oil coated commercial feed was used without any extract.

Treatment 2 (T_2): One kg oil coated feed mixed with 20 ml (SAU-1) garlic extract liquid before 1 hour application of feed.

Homogenously mixed the feed 20 ml extract with spray & spread that feed in room temperature for 30 minutes & after that preserved it in an air tight polythene bag.

Treatment 3 (T_3): One kg feed was homogenously mixed properly with 20 ml (SAU-2) ginger extract with spray all over for 30 minutes and spread that in room temperature. After that preserved it in an air-tight polythene bag. GIFT tilapia (*O. niloticus*) was stocked 10 sq meter hapas. In that pond total treatment number is 3. Each treatment has 3 replications which was set up in hapa .Total 9 hapa was set for this experiment which also identified through numbering like T1R1, T1R2, T1R3, T2R1, T2R2, T2R3, T3R1, T3R2, and T3R3. Fish population density was 13 fish per 10 sq meter hapa, stocking fish biomass total 3.5kg /10 sq hapa. The initial average weight of *O. niloticus* was 267 g ± 3.16 and initial average length of *O. niloticus* was 14 cm.

3.4.6 Collection of brood

The prerequisite for developing the highest-quality fry in any fish culture is healthy brood. Broods were gathered for this experiment from the BRAC Tilapia hatchery in Magura. For excellent results, organoleptic tests were performed, which meant that healthy, attractive, and disease-free broods were chosen for collecting. Broods were collected ranged from 261 g to 275 g & length ranged from 12cm to 16cm.

3.4.7 Disinfection of broods

The BRAC Tilapia Hatchery's broods should be cleaned and sterilized after being removed. In this research, potash (potassium per manganite / $KMnO4$) was used to disinfect brood Nile tilapia (*O. niloticus*). In the drums used to transport the broods, 1gm/L of potash was added. After adding potash to the drum, the broods were delivered to the experimental hapas within a minute in accordance with the prescribed procedures. The broods were evenly distributed and properly sorted before being stocked in the hapas. Each brood's initial weight was determined and noted.

3.5 Fish sampling

Fish were sampled at fortnightly interval. Firstly, fish were harvested by scoop net and randomly 10 fish were weighted by digital electric balance to measure the growth parameter .During sampling fish were handled very carefully. At the end of the 12 weeks experimental period, all fish were counted and weighed individually to determine weight gain.

3.6 Post-stocking management

The post-stocking management stage encompasses the activities or upkeep from stocking to harvesting. Such as feeding, sampling, applying chemicals, harvesting, and so forth. Several different workouts were carried out over the study period in this experiment using a culture of brood Nile tilapia (*O. niloticus*). Every cultural activity was carried out as and when it was essential.

3.6.1 Feeding

Maintaining proper feeding is important for optimum results. On a daily basis, feeding was adequately maintained, and two feedings in six-hour intervals were completed. Feed was initially supplied at a rate of 0.8% of body weight, then at a rate of 0.5% throughout the duration of the trial with 26.24% protein in feed.

3.6.2 Use of extracts

For experimental objectives, two different types of extract were combined with the diet in this investigation. Both the extracts of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) were employed as feed supplements. Before applying feed to the hapa for an hour, extract was combined with it (20 ml/1000 g). Since tilapia fish are surface feeders, food was distributed at the water's surface.

3.6.3 Water quality parameters

All of a fish's bodily functions take place in water. Because fish must have access to water in order to breathe, feed, develop, excrete waste, maintain a salt balance, and breed. Aquaculture success depends on knowing the physical and chemical properties of water. The achievement or failure of an aquaculture operation depends on the quality of the water. The following water characteristics were investigated.

A) Temperature

In the case of Nile tilapia breeding, temperature may be a serious problem (*O. niloticus)*. The ideal water temperature for tilapia breeding is between 21˚ c to 31˚ c. Its equilibrium affects a variety of water properties. An appropriate temperature range helps to grow fish, promote reproduction, and maintain 21˚ c other microbial groups. During the study period, temperature range was monitored every morning. Digital multi meters were used to acquire the data.

a) Temperature measure b) Ammonia measuring kit c) DO measure

Figure 05: Temperature, pH, Ammonia and Dissolved Oxygen was tested during the experimental period

B) pH

For aquatic organisms, particularly fish cultivation, pH is a crucial element. Water's acid and base concentrations are determined by pH. Metal concentrations, vitamin shortages, and other issues are related to pH levels. Weekly measurements of pH were taken throughout the trial using a Hanna pH digital meter. For this, water from the Hapas treatment facility was collected in a beaker, and a pH sensor was applied to it. The pH level was kept within the range of 7.0 to 8.5. pH level was measured by Hanna pH meter .

C) Ammonia (NH_3)

The most harmful substance for a productive fish culture is ammonia. The rate of fish survival in tilapia breeding is significantly influenced by nitrogenous compounds. In essence, ammonia is produced by the deposition of additional feed on the Hapa bottom level and the excretions of cultured species. Ammonia Level was measured by Sk+F Ammonia Test kit.

D) Dissolved oxygen (DO)

Dissolved oxygen is the most important element for the survival of aquatic organisms. Dissolved oxygen (DO) may be a crucial environmental indicator in aquaculture since fish inhale oxygen similarly to humans do. Fish mortality is primarily caused by low levels of DO below the acceptable level, and it is known that low oxygen levels are their most egregious foe. At least 5.0 ppm DO levels should be maintained just for fish culture. During this experimental work, daily water system through an inlet was utilized for keeping up DO level. DO level data was measured by Lutron Pen Type DO Meter (Dissolved Oxygen Meter) PDO519, Made in Taiwan.

3.6.4 Egg collection from treatments

After Tilapia broods breeding set-up in hapa, Broods were suitably done their reproductive activity. One month later of brood set-up, Egg collection was done weekly basis. Egg collection was done by collecting hapa brooding female per replication of treatments. At first Total hapa was gather in one side pulling through a bamboo then tie up the net with bamboo stick. Fertilized eggs collected from the mouth of a female from the breeding hapas. Collect all eggs based on their color in different bowl & transfer immediately to the hatchery for wash properly. Eggs were mainly 4 types in color-Light yellow, Orange, Reddish/ash, Swim-up (Figure 07).

a) Collection process b) Hapa pulling through bamboo

Figure 06: Egg collection process from female brood mouth

a) Yellow color egg

(b) Orange color egg

(c) Ash or swim-up color egg

Figure 07: Egg collection from brood female mouth

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At first egg was washed through a sieve & washed into 5 PPT salt water then after washed into 20 PPM formalin water. Recorded all data through treatment wise were kept in jar (6 L) incubation system in hatchery with a water flow rate of 2 L per minute. Jar also identified with marking treatment number to avoid mixing eggs. Before Putting eggs in jar all data were measure & recorded through treatment wise.

3.6.5 Spawn transfer & fry production

Hatching tray was siphoned two times in a day for collecting dust. After 72 hours in jar hatching eggs were transferred to hatching tray which was made of fiber. Then transfer the spawn to rearing tray which also made of plastic or fiber .Hatching spawns were reared 2 or 3 days in these trays until the spawn absorbed the yolk-sac. Fry were obtained through hatching of eggs after an incubation period of 90-96 hours. After absorbing yolk-sac. Spawn were ready to transfer nursing hapa for rearing to different sizes fry. Treatment wise spawn were measured through weight machine & count them manually by spoon using a bowl. 1 kg salt was used in nursing hapa for disinfecting fry from any disease.

a) Hatching Jar b) Identified jar & tray c) Rearing tray

Figure 08: Hatching jar and rearing tray of BRAC tilapia hatchery for the experiment

3.6.6 Fry feeding

Nursery ready floating feed were used 4 times per day according to their 20-25% body weight. It was applied up to 20 days enriched with > 35% protein.

a) Produced fry b) Feeding nursing feed to fry

Figure 09: Feed of fry in different treatment

3.6.7 Survival rate of fry measure

Nursing stage take 15-20 days. After completion of nursing stage all treatment hapa was checked through manually. Measured all hapa through weight machine & count them manually by using spoon. All data were recorded treatment wise.

3.6.8 Collection and preservation of blood

Blood was drawn from a randomly chosen fish from each treatment during sampling. To remove water and slime, the fish were caught and carefully wiped with a dry cloth. Fish's caudal peduncle was used to draw blood using a syringe. The needle was immersed in a 2% heparin solution during blood collection to prevent clotting. In order to determine the hematological parameter, the blood was then put into an EDTA (Ethylene Diamine Tetra Acetic Acid) tube and kept at 4°C (Figure 10). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration

(MCHC) were assessed, together with the leukocyte (WBC), erythrocyte (RBC), packed cell volume (PVC), hemoglobin (Hb), and mean corpuscular volume (MCV).

a) Collection of blood of Nile tilapia fry b) Preservation of blood in EDTA tube

Figure 10: Blood sampling

3.7 Reproductive performance parameters

These parameters were measured as follows:

1) Egg number/ female = $\frac{\text{Egg number}}{\text{female number}}$

2) Egg number/g = $\frac{\text{Egg number}}{\text{gram of eggs}}$

3) Fry number/ female = $\frac{\text{Fry number}}{\text{Female number}}$

4) Hatching % = $\frac{\text{Fry number}}{\text{Egg number}}$

5) Spawned females % = $\frac{\text{Spawned females number} \times 100}{\text{Total females number}}$

6) Number of spawning/fish = $\frac{(\text{Spawned female No/wk}) \times (\text{No of wk})}{\text{Total females number}}$

7) Average weight of spawn = Average of the total subsample / week.

8) Egg weight, g/fish = $\frac{\text{Egg weight,g/hapa}}{\text{Females No./hapa}}$

3.8 Growth parameters

Growth is an integrated physiological response that takes into accounts both internal physiological status and external environmental factors like food quality and quantity, temperature, and water quality (health, stress, reproductive state). Growth parameters are used to evaluate future growth, development, and disease risk from the initial level of any species culture while taking a variety of factors into account. The dimensions include things like length, weight, size, and shape.

3.8.1 Average body weight gain

The average body weight gain (g/fish) was estimated according to the following equation:

Average weight gain (g) = Mean final weight (g) - Mean initial weight (g)

3.8.2 Weight gain (%) = $\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$

3.8.3 Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was determined by the following formula:

Feed Conversion Ratio (FCR) = $\frac{\text{Amount of feed}}{\text{Fish weight gain}} \times 100$

3.8.4 Specific growth rate (SGR %)

Specific growth rate (SGR % per day) = $\frac{ln\ \text{W2} - ln\ \text{W1}}{T2 - T1} \times 100$

Where, W1 = the initial live body weight (g) at time T1 (day)

W2 = the final live body weight (g) at time T2 (day)

T2 –T1 = No. of days of experiment

3.8.5 Survival rate

The survival rate of Nile tilapia fry for each treatment was examined based on the number of fries survived at the end of the experiment using the following formula:

$$\text{Survival rate \%} = \frac{\text{Final number of fish survived}}{\text{Number of actual fish stocked}} \times 100$$

3.9 Hematological parameters

Hematological parameter like Leukocyte (WBC), erythrocyte (RBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined. The haemoglobin content determined by Sahlin-Hellige method (Srivastava *et al*.2011). The RBC and WBC counts were determined by an improved Neuberger hemocytometer (Rahmdel *et al*., 2018).

3.9.1 Estimation of haemoglobin

Haemoglobin estimation was done by using a digital Hg meter. One drop of blood sample collected from each replication was applied on the test strips connected to the Hg meter and values were recorded. The values were expressed in g/dl.

3.9.2 Estimation of red blood cell (RBC)

The blood sample was taken in an Eppendorf tube of initial concentration (5%). An aliquot of 1 µL sample was added in 39 µL EDTA to make 200 folds dilution of blood with the help of micropipette and pipetting to make a homogenized solution. The sample was taken in a hemocytometer and observed under a light microscope.

Five small squares were randomly observed and counted the RBC. It was calculated by the following formula:

$$\text{Cells}/\mu\text{L} = \frac{\text{Average no.of cells} \times \text{dilution factor (200)}}{\text{Volume factor (0.1)}}$$

3.9.3 Estimation of white blood cell (WBC)

The blood sample was taken in an Eppendorf tube of initial concentration (5%). An aliquot of 1 µL sample was added in 19 µL solution to make 20 folds dilution of blood with the help of micropipette and pipetting to make a homogenized solution. The solution was kept at rest for 1- 2h. The sample was taken in a hemocytometer and observed under a light microscope. Four large squares were randomly observed and then counted the WBC was calculated by the following formula:

$$\text{Cells}/\mu\text{L} = \frac{\text{Average no.of cells} \times \text{dilution factor (200)}}{\text{Volume Factor (0.1)}}$$

3.9.4 Mean corpuscular volume (MCV)

MCV stands for mean corpuscular volume. The MCV was measured by using a formula. In calculating MCV, the PCV is multiplied by ten and divided by the red blood cell count measured in millions of cells per cubic millimeter of blood.

MCV(fl) = (PCV ÷ RBC) x 10

3.9.5 Mean corpuscular hemoglobin (MCH)

The mean corpuscular hemoglobin, or "mean cell hemoglobin" (MCH), is the average mass of haemoglobin (Hg) per red blood cell (RBC) in a sample of blood. It is identified as part of a standard complete blood count. It was calculated by dividing the total mass of hemoglobin by the number of red blood cells in a volume of blood.

MCH(pg) = (Hg ÷ RBC) x 10

3.9.6 Mean corpuscular haemoglobin concentration (MCHC)

The MCHC (mean corpuscular haemoglobin concentration) is the average amount of hemoglobin present in red blood cells per unit volume (weight/volume or g/dl) (RBC). It can also be thought of as the proportion of the RBC that is made up of hemoglobin.

MCHC(%) = (Hb ÷ PCV) x 100

3.10 Statistical analysis

During the experimental period, all of the information was gathered, noted, and organized in a computer spreadsheet. The statistical software Statistix 10 (2013) was used to do a one-way ANOVA on the data. Standard Deviation used in every graph to show the differences between the treatments.

CHAPTER IV

RESULTS & DISCUSSION

The results of the experiment are presented in different sub-headings.

4.1 Water quality parameters

A healthy aquatic environment and better aquatic organism production depend on the measurement of water quality parameters. Water quality is one among the important factors determining growth and survival of fish. Water quality parameter includes all physical and chemical characteristics of water. Good quality water system is prerequisite for good fish production (Rahman *et al*., 2017). Different physio-chemical parameters like temperature (°C), dissolved oxygen (mg/l), proton concentration (pH), and ammonia-nitrogen (mg/l) were recorded throughout the experimental period, which were presented within the sections bellow:

4.1.1 Water temperature

The water temperature of the treatment hapa was ranges from 24.3 to 28.60^{o}C (Table 1) during the experimental period. Maximum value of the temperature was recorded in 3rd week (28.60± 0.02^{o}C) at treatment Ginger Extract whereas the minimum value of temperature was recorded in 1st Week (24.3± 0.03^{o}C) at treatment Ginger Extract, respectively. However, there was no significant difference (P>0.05) in water temperature of the hapas during the experimental period.

Since it directly influences food intake, reproduction, metabolism, oxygen consumption, growth, moulting, and survival, water temperature is likely among the most significant environmental factors in fish culture. Through its influence on a water body's physical, chemical, and biochemical properties, temperature plays a crucial part in aquatic production.

Table 01: Water temperature (°C) of different treatments in the experiment period

Time (week)	Treatments(°C) Control (T_1)	Garlic Extract (T_2)	Ginger Extract (T_3)
1st	28.2	26.4	24.8
2nd	27.2	27.4	25.3
3rd	24.3	27.5	28.6
4th	25.9	27.2	28.1
5th	27.3	25.1	27.2

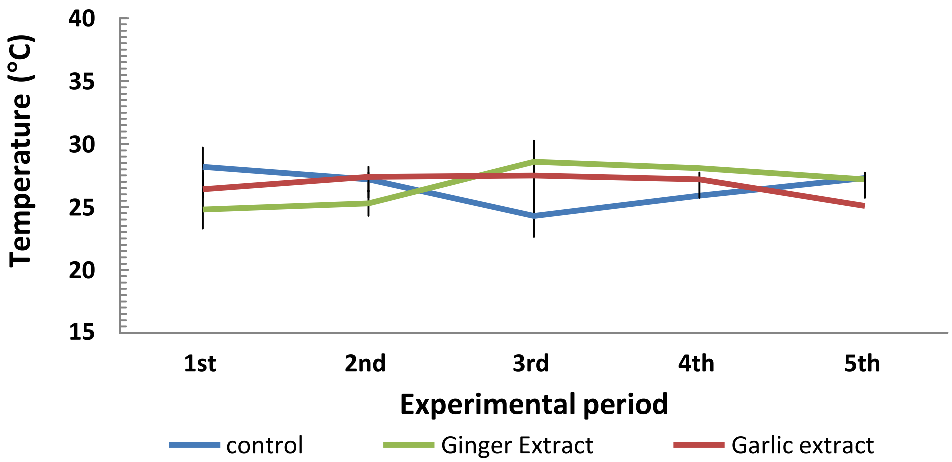

Figure 11: Water temperature (°C) in different treatments during the experimental period (Mean ±SD)

4.1.2 Water pH

The mean (±SD) values of pH in different treatments during the experimental period are presented in (Table 2**)**. The average pH level during the experimental period ranged from 7.0 to 8.30. Maximum pH value was determined in 5th week at treatment 1 (8.30± 0.03) and the minimum pH value was observed in 1st week at treatment 1 (7.0 ± 0.02). pH in water body generally regulates considerably the water chemistry. Any sudden fluctuation of pH like neutral to acidic or alkaline causes the death of many aquatic species. According to Tamlurkar *et al.* (2017) the pH 6.5 to 9.0 is suitable for pond fish culture and pH more than 9.5 is unsuitable. DoF (2018) reported that the suitable range of pH of a water body for fish culture should be 6.5 to 8.5.

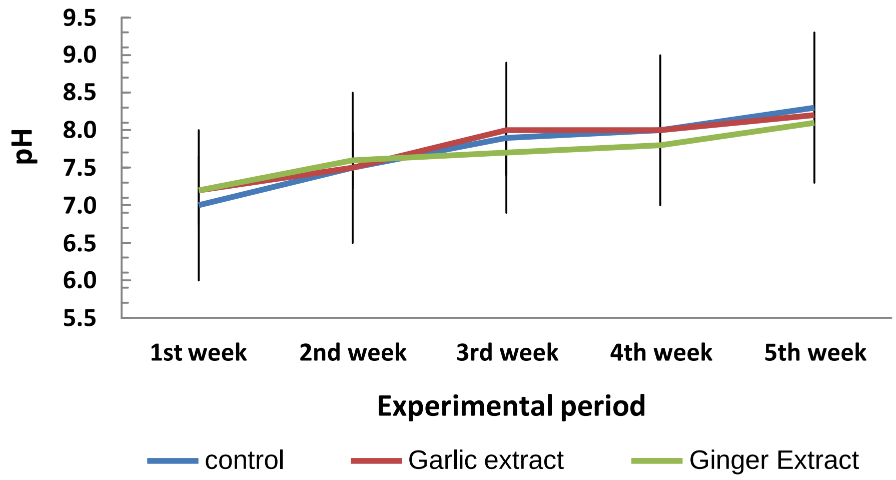

Figure 12: Water pH in different treatments during the experimental period (Mean ±SD)

4.1.3 Dissolved oxygen

The range of DO concentration was found between 4.9 ± 0.05 to 7.2± 0.03mg/l (Table 03).There was no significant difference of mean values in DO concentration within a week in different treatments.

For fish farming, dissolved oxygen (DO) is likely the most crucial element in water quality. Fish and other aquatic animals require DO, and a pond's capacity to support aquatic life depends on its level of DO. In this experiment, the dissolved oxygen level was greater than 5.0 mg/l throughout the rearing period in the current study; the DO remained within the acceptable range for Nile tilapia *(O. niloticus*).

Table 02: Water DO (mg/l) of different treatments in experiment period

Time	Treatments		
	Control	Garlic Extract	Ginger Extract
(week)	(T_1)	(T_2)	(T_3)
1st	7.2	6.2	6.1
2nd	6.9	6.7	6.6
3rd	6.5	6.6	6.8
4th	5.2	5.6	5.8
5th	5.1	4.9	5.3

According to an experiment done by Dong et al. (2019), water with a DO level between 5.0 and 7.0 ppm is favorable for production whereas water with a DO level below 5 mg/1 is unproductive. According to DoF (2018), the DO range for fish cultivation would be 5-8 mg/l. While Kohinoor *et al*. (2012) found that in Indian large carp cultivated ponds, the highest growth occurred at dissolved oxygen levels of 4.23 to 5.32 mg/l. Rahman *et al.* (2014) determined that a productive pond's dissolved oxygen content should be 5.00 mg/l or above.

4.1.4 Ammonia

An important factor in fish culture is the ammonia level in the water body. Ammonium ion (NH_4^+) is relatively innocuous; however unionized ammonia (NH_3) is extremely detrimental to fish. Lower values of total ammonia under cultivation conditions mean improved water quality for fish. The ammonia levels in the water in this study's several treatments ranged from 0.17 mg/l to 0.43 mg/l (Table 4). However, there was no noticeable change in ammonia levels among the various experimental aquariums during the trial.

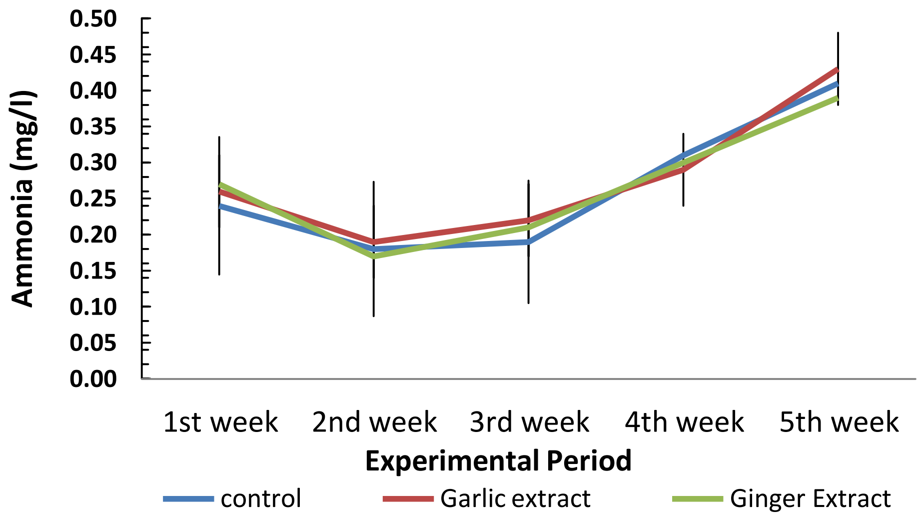

Figure 13: Ammonia in different treatments during the experimental period (Mean ±SD)

4.2 Egg collection

The fertilized eggs are often held in the mouth of the mother fish for natural incubation. Therefore, it is necessary to twice a week check the mouths of all the females in each hapa to gather the fertilized eggs/larvae with yolk sac in anticipation for their artificial incubation. Plastic trays with such a 2.0 liter capacity and a medium type can be used to create a straightforward method for incubating tilapia eggs (30 x 25 x 8 cm). The bottles and trays were placed over the concrete platform that has been created and connected to the recirculating system, where fresh water (28°C) is delivered by gravity directly from a header tank. In this approach, each female fish's mouth is used to gather fertilized eggs or larvae with yolk sacs, which are then incubated separately. The larvae are moved to a series of trays shortly after hatching and maintained apart until their yolk sac resorption stage i, e. first feeding fry stage.

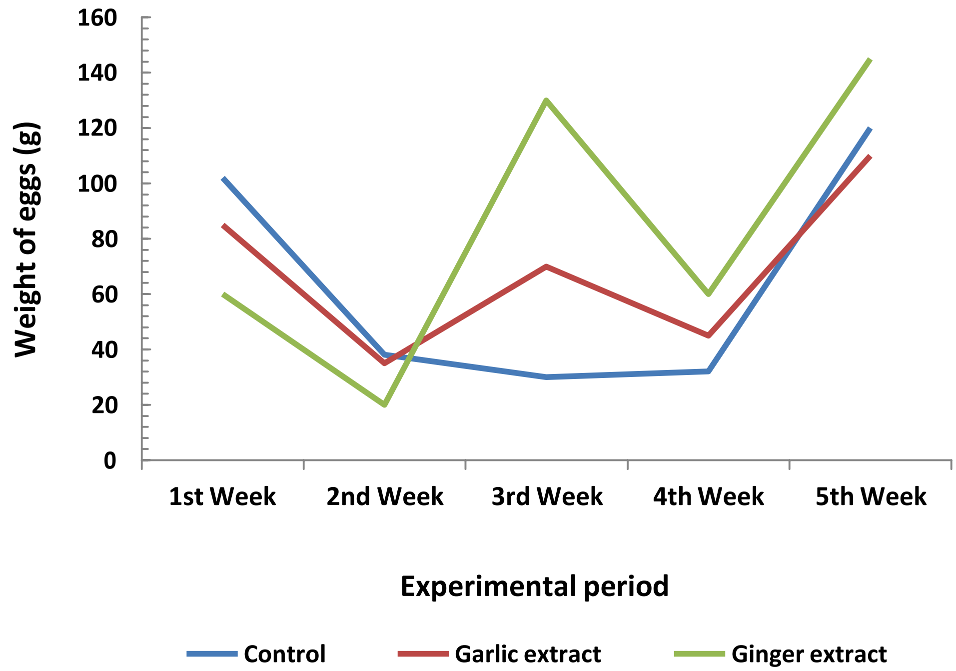

Figure 14: Weight of eggs collected per week from Nile Tilapia Brood (*O. niloticus*)

In that Figure, there are 5 weeks of collection, where the maximum average weights of eggs are collected from treatment ginger extract. The minimum average weight of eggs is collected from treatment control. Although in 1st and 2nd week Ginger extract treatment has lower performance then its production performance was higher in rest of the period. Garlic extract always shown the middle performance on collecting weight of eggs in every week.

4.3 Average weight of eggs

The maximum weight (83gm) was found in Ginger Extract treatment and minimum weight (64.4gm) was in control treatment. In that study, we find more total weight of egg in ginger extract treatment than other treatments.

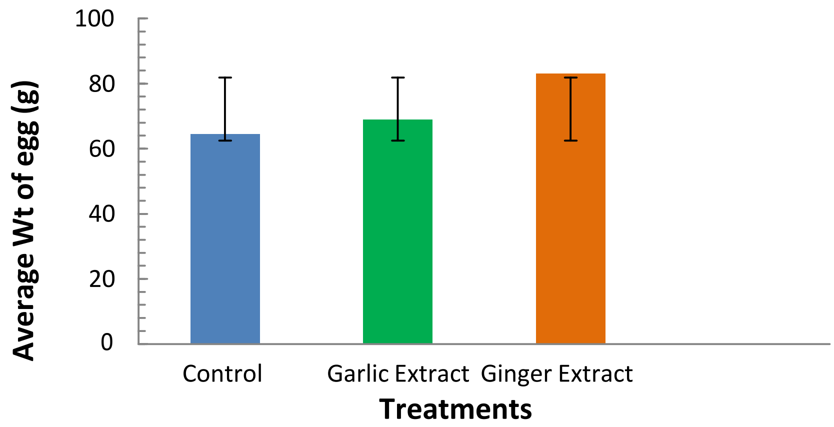

Figure 15: Average weight of eggs in different treatments of Nile tilapia brood (*O. niloticus*)

4.4 Total female brood laying eggs found

The presence of a brooding female was crucial for hatchery purposes. The production of eggs can be increased by increasing the proportion of brooding females. The pie chart shows that after 5 weeks of collection, the maximum 35% of brood females were laid in the treatment Ginger Extract. The minimum percentage (33%) of females laid eggs was found in the control treatment. Finding additional brooding females was crucial for all commercial hatcheries order to improve production.

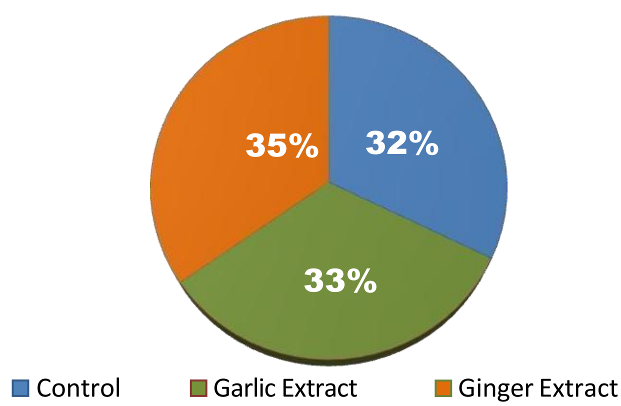

Figure 16: Total female brood laying eggs found of Nile tilapia (*O. niloticus*) in different treatments

4.5 Female brood egg color

Eggs are laid by females. Depending on the length of incubation, ripe and fertilized eggs are pale yellow, orange, ash, or swim-up in color, ovoid in shape, and measure 1.0–2.0mm, 1.5–3.0mm in diameter and 2.3–2.8mm in length. A female can laid a few hundred to a few thousand eggs (800 pcs - 2200 pcs egg). The Nile tilapia is a mouth brooder that is maternal.

The males build the nests where the females lay their eggs, which they then carry in their mouths until they hatch. Once the fingerlings are big and strong enough, they are maintained close (10 mm). Tilapia has chromatophores or light-reflecting cells, in their scales. They are capable of changing colors as a result, letting the female realize they are in the "breeding spirit."

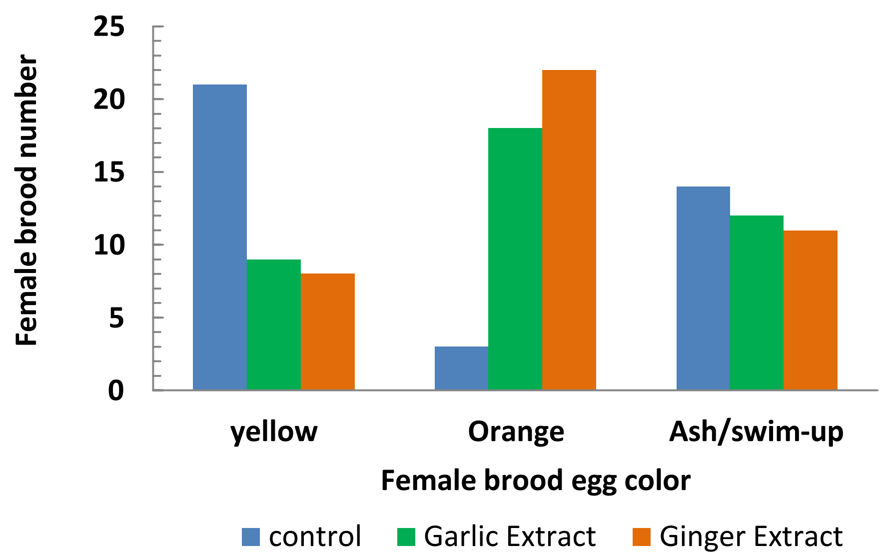

Figure 17: Brood female egg color of Nile tilapia (*O. niloticus*) in different treatments

In Figure 17, we find that the maximum pale yellow eggs were found in treatment control & minimum in Ginger extract treatment. The Orange color eggs were maximum in ginger extract treatment & minimum in treatment control. Ash or Swim-up eggs were found maximum in treatment control & minimum in ginger extract treatment.

4.6 Fecundity

The quantity of ova that are likely to be laid by a fish during the spawning season" is the definition of fecundity. Depending on the fish's size and age, different fish species produce varied numbers of eggs. The treatment with garlic extract had the highest average egg number per gram and the control treatment with the lowest average egg number per gram. Fecundity and egg size are extremely variable features that can vary both within and between populations over the course of a single year. In that experiment, we find maximum average egg number per gram in garlic extract (132.05 pcs) and minimum value in control treatment (116.58 pcs). Numerous studies on tilapias have produced contradictory findings on food quality and, in particular, the impact of dietary proteins on reproduction. For breeders of *O. niloticus* and red hybrid (*O. niloticus O. mossambicus*), respectively, Santiago *et al*. (1985) and Chang *et al*. (1988) observed an increased young production for breeders fed with greater protein levels.

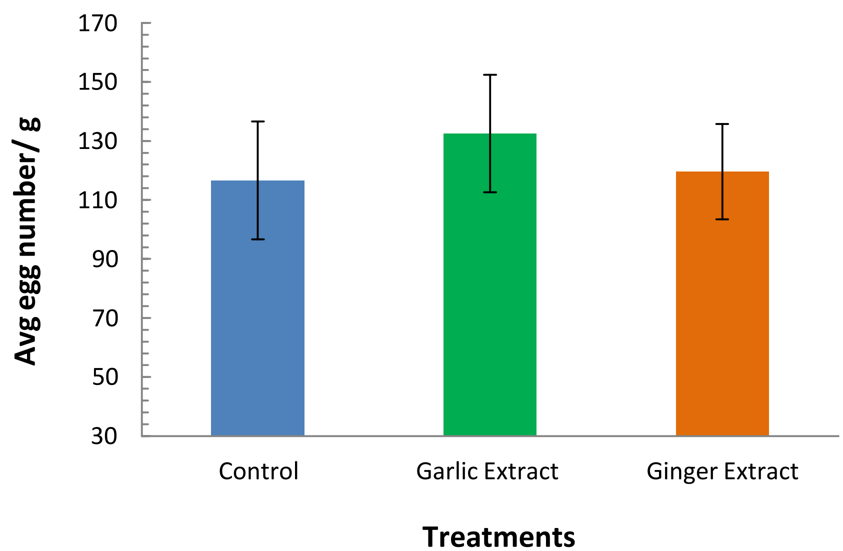

Figure 18: Average egg number/g of Nile tilapia (*O. niloticus*) in different treatments

4.7 Average weight of spawn

Both intensive and extensive aquaculture systems are used to raise tilapia. The majority of farmers favor outside clay ponds. When farming is done properly, females spawn every 17 days. Market-sized fish can be produced in seven to ten months if water quality and temperature conditions are optimized. Depending on the species, one female tilapia will spawn in between 4 weeks and 4 months if the conditions are ideal. Even with the extremely poor survival rates females can lay up to 1,200 eggs in a single spawn. Some species lay eggs in nests, but others are mouth brooders that carry fry in the mouth. Average Weight of spawn found maximum (94.4gm) in Ginger extract after 5 weeks Egg Collection & minimum (76gm) was found in control treatment (Figure 19).

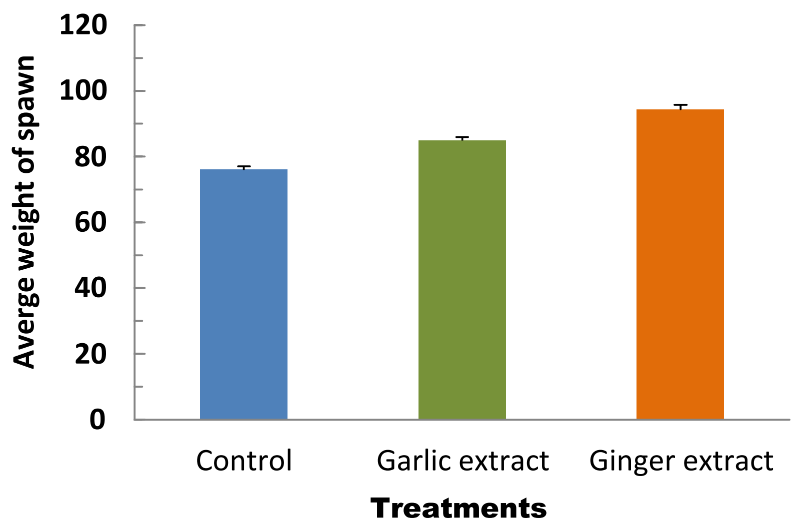

Figure 19: Average weight spawn of Nile tilapia (*O. niloticus*) in different treatments

4.8 Spawn rate

In this investigation, the treatment using garlic extract had the highest average spawn rate per gram in Garlic extract treatment 98.29 ± 1.11 pcs, then ginger extract treatment has 87.71± 1.04 pcs & control treatment has 86.65± 1.00 pcs. Spawn rate increased progressively as female body weight increased. The increase in egg weight generated by huge, elderly tilapia was hypothesized by Watanabe and Kuo in 1985. While De Silva (1986) insisted that the rise was connected to body length, Rana *et al*. (1988) discovered that a fish's egg production was more closely related to body weight. However, Cisse *et al*. (1988) found no evidence of a relationship between spawner weight and the quantity of spawning.

Table 03: Spawn rate /g of Nile Tilapia (*O. niloticus*) in different treatments

Time (week)	Treatments		
	Control (T_1)	Garlic Extract (T_2)	Ginger Extract (T_3)
1st	102	104.4	103.38
2nd	105.3	109.74	75.13
3rd	88.65	126.05	115.82
4th	85.5	87.31	84.32
5th	51.76	63.97	59.92
Average	86.65	98.29	87.71

4.9 Hatching rate

According to the findings of observations made about the hatching rate of tilapia eggs (*O. niloticus*) ranged from 76.0% to 80.63%. With rising salinity, tilapia eggs will hatch more frequently, but at a certain point, the value of the hatching rate hits a maximum and the yield declines. Figure 22 shows the percentage of tilapia fish eggs that hatch. The hatching rate graph reveals that treatment ginger extract & control, with an average hatching rate of 76.01% ±14.20, generates the lowest hatching rate have no significant difference among themselves , while treatment garlic extract, with an average hatching rate of 80.62%± 9.51 produces the highest hatching rate. Early stages on the eggs were the reason of the treatment controls and ginger extract's has low hatching rate such that the egg cannot grow and ultimately perish.

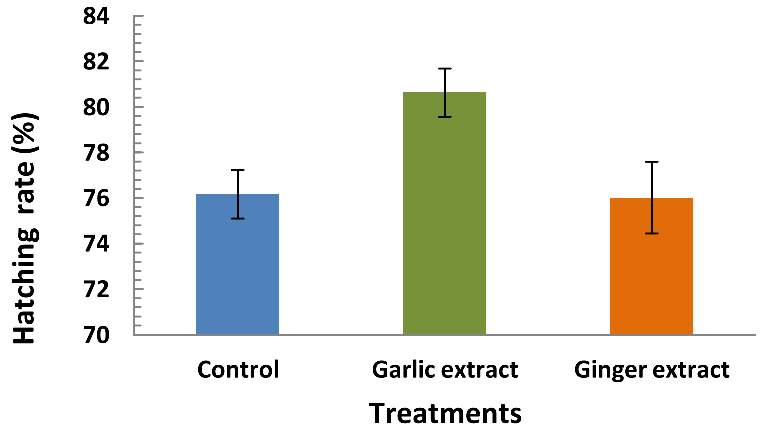

Figure 20: Hatching percentage of Nile tilapia (*O. niloticus*) in different treatments

4.10 Survival rate of fry

A crucial factor in fish culture is the survival rate. It specifies the percentage of living things left over from the research time period. In this study, the average survival rates in the control, garlic extract, and ginger extract treatments were reported to be 78.25± 0.09%, 84.24±0.05%, and 81.63±0.13% respectively. Compared to the treatment control, the survival rate was considerably higher in the garlic and ginger extracts.

Table 04: Survival rate (%) of Nile Tilapia (*O. niloticus*) in different treatments

Time (week)	Treatments		
	Control (T_1)	Garlic Extract (T_2)	Ginger Extract (T_3)
1st	86.22%	89.06%	55.31%
2nd	59.77%	88.23%	94.59%
3rd	84.31%	72.64%	88.80%
4th	80.41%	86.64%	86.90%
5th	80.55%	84.64%	82.55%
Average	78.25%	84.24%	81.63%

4.11 Feed conversion ratio (FCR)

The feed conversion ratio (FCR) is a crucial factor for fish culture. It is the quantity of feed required to raise one kilogram (kg) of fish. Estimating the amount of feed needed during the growing cycle is helpful. A farmer can assess the ratio to determine whether an aquaculture business will be profitable. In the present study it was found that FCR were 1.53±0.09, 1.43±0.19 and 1.48±0.14 in control, garlic extract and Ginger extract treatments respectively. The treatment-2 which was made by garlic extract showed best performance among the three treatments. If the lower FCR value is found fish culture was more profitable than.

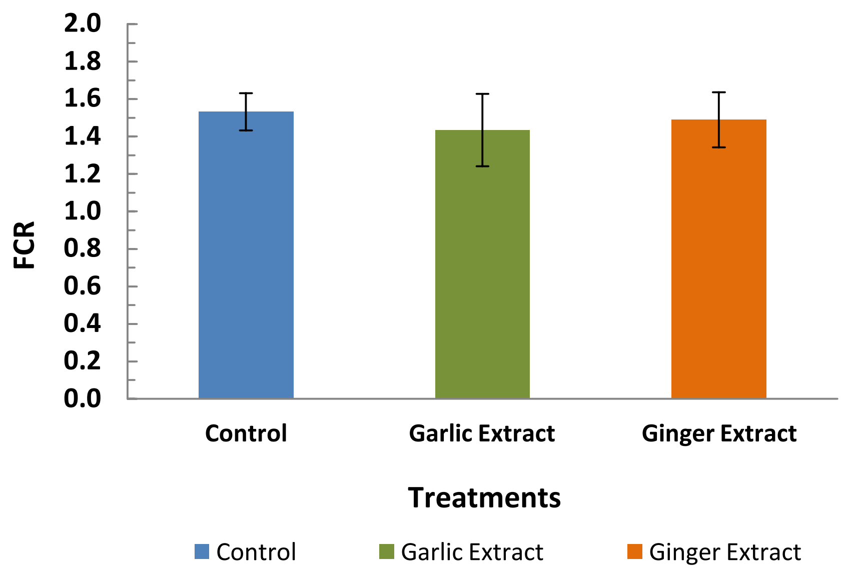

Figure 21: Feed conversion ratio (FCR) of Nile Tilapia (*O. niloticus*) in different treatments

4.12 Specific growth rate (SGR % per day)

In this study, there was significant difference in SGR among the treatments ($P<0.05$). It was found that garlic extract has the higher SGR than control & ginger extract treatment. The highest (150 ± 9.1) SGR value was found in Garlic extract treatment and lowest (141 ± 0.94) value was found in control treatment.

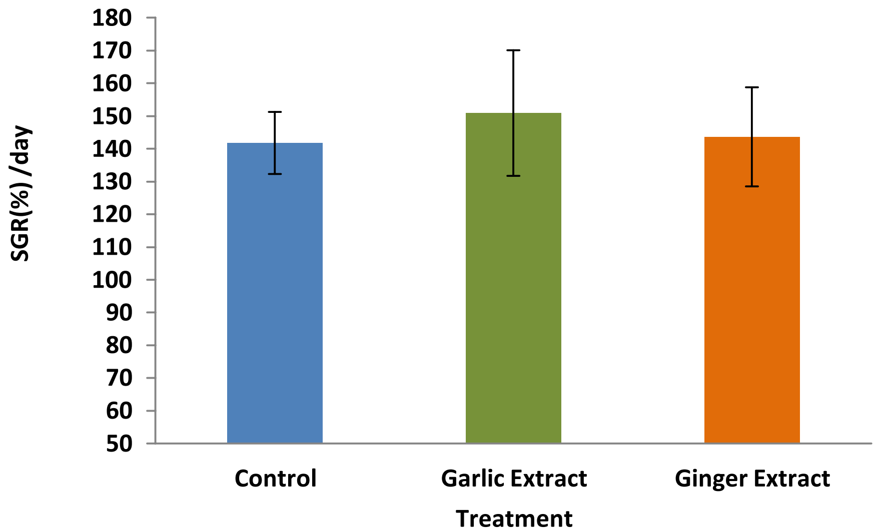

Figure 22: Specific growth rate (SGR, % per day) of Nile tilapia (*O. niloticus*) different treatments

In an experiment with *Clarias gariepinus* fry, Fagbenro *et al*. (2010) discovered that the specific growth rate decreased as the raw plant protein increased by 30% and decreased further than 20%. The results of the current experiment almost exactly match those of earlier researches. Low levels of lysine, anti-nutritional elements like protease and arginase inhibitors, and fiber content, which have a detrimental impact on pellet quality and feed digestibility, may be the cause of the increase in SGR in high inclusion levels.

4.13 Mean weight gain

The Mean weight gain of cultured Tilapia was 129±8.6, 137.33±17.46 and 130.66±13.76 g in c o nt r ol , garlic extract and ginger extract treatments respectively. From the figure 26, it is clearly said that garlic extract gave best outcome (137 g) among all treatments of same stocking density. The least outcome (129g) was found in treatment control.

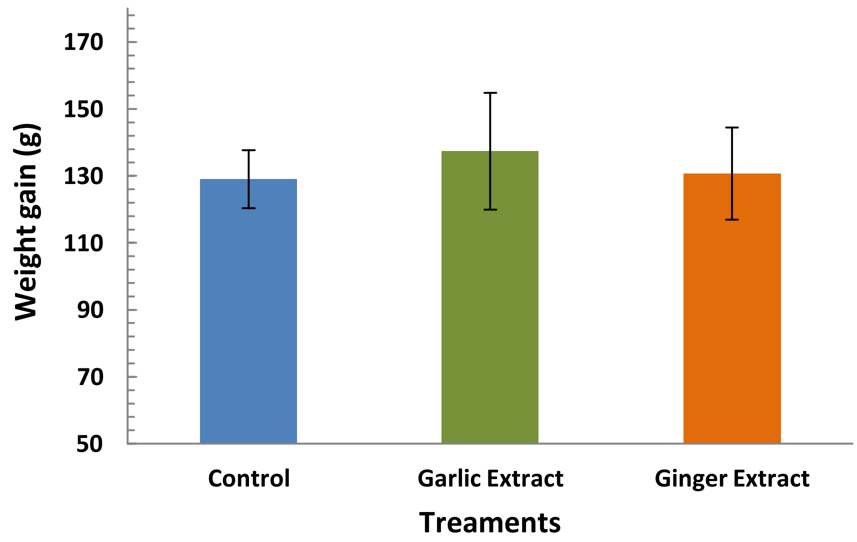

Figure 23: Mean weight gain (g) of Nile tilapia (*O. niloticus*) with different treatments

4.14 Proximate composition of feed

The feed we applied during the experimental period contained 26.24% crude protein, 7.53% ash, 7.40% crude fiber, 38.08% carbohydrate and 5.60% fat. Moisture content was 15.15% and 84.85% dry matter content was available in the feed which we applied to the Brood Nile Tilapia. By comparing the analysis of proximate composition of feed with the aforementioned research it can be state that the nutritional value of feed was optimal to provide proper nutrition to the Nile Tilapia.

Table 05: Proximate composition of applied feed

Parameters	Nutritional composition
Moisture	15.15
Dry Matter	84.85
Crude Protein	26.24
Total Ash	7.53
Acid Insoluble Ash	0.42
Crude Fiber	7.4
Crude Fat	5.6
Carbohydrate	38.08

4.15 Blood parameters

Blood serves as the most convenient indicator of the general condition of the animal body. Subsequently, haematological studies are promising tools for investigating physiological changes caused by environmental pollutants (Zaghloul, 2001 and Zaghloul *et al*., 2005)

4.15.1 Haemoglobin

The values of hemoglobin content were recorded after 12 weeks of study period in this experiment. There was significant different ($P<0.05$) among the treatments. The highest hemoglobin value (6.2± 0.08 g/dl) was found at ginger extract and the lowest (4.6 ± 0.20 g/dl) was in control treatment. (Figure 24), in garlic treatment there is a gradual decrease of Hg than ginger extract.

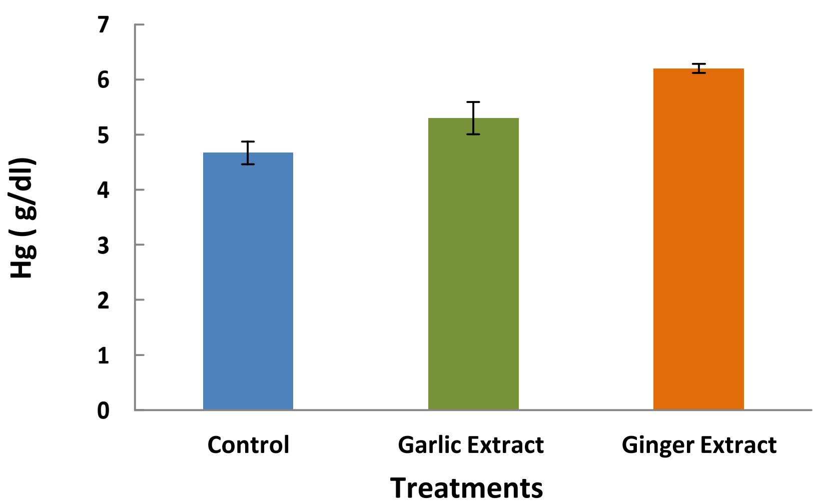

Figure 24: Hemoglobin (Hg) content in Nile tilapia (*O. niloticus*) with different treatments

Fish respond to stress by using a range of compensatory mechanisms, including as increased breathing frequency and decreased Hg production, to reduce their increased oxygen requirement. The decrease of Hg with numerous stressors, including ammonia and low temperature, was noted in the current review. Given that the Hg was decreasing regardless of the kind of stress and the species, it appears that this decline has been a worldwide reaction to stress. The Nile tilapia (*O. niloticus)* had a monthly mean hemoglobin (Hg) value that varied from 4.05 g/dL in January to 10.43 g/dl in October. The observation is less than the values Adakole *et al.* examined (2012).

4.15.2 White blood cell (WBC)

WBC plays a key role in immunological response, infection control, and body defense against invading pathogens. WBC is another reliable marker of physiological characteristics. One of the first lines of defense against infectious agents brought on by microbial and chemical causes is the white blood cell (WBC), which is well known (Talpur and Ikhwanuddin, 2012).The values of WBC count were recorded after 12 weeks of study period in this experiment. The WBC count was significantly different ($P<0.05$) among the treatment. The higher Average WBC count ($61.26 \times 10^3/\mu l$) was found in garlic extract treatment and ($57.7 \times 10^3/\mu l$) was found in ginger extract treatment whereas the lower count ($53.46 \times 10^3/\mu l$) was found in control treatment.

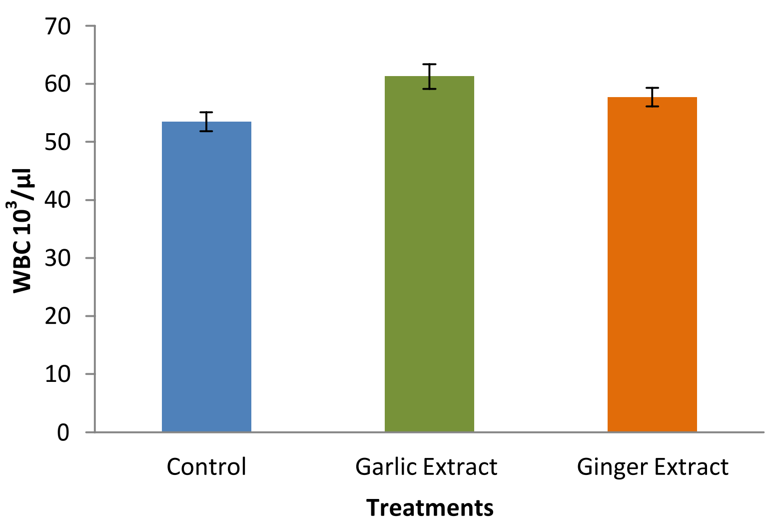

Figure 25: White blood cell (WBC) counts in Nile tilapia (*O. niloticus*) fed with different treatments

According to Ahmed et al., the monthly mean white blood cells (WBC) of Nile tilapia (*O. niloticus*) varied from 58.67×10^3/mL in January to 88.00×10^3/mL in September (2013).

The low value seen during the dry season may be due to the lake's little volume of water, which causes an increase in WBC counts in the natural world.

According to earlier reports by Zaghloul *et al*. (2001), the rise in fish WBC may reflect a change in the defensive system against the effect of the highly poisonous and bio-accumulated heavy metals in fish tissues (2005). Due to the presence of stresses or other factors, fish will typically exhibit a rise or decrease in their WBC count (Kori- Siakpere *et al*. 2006). In the current study, lower numbers were linked to decreased activation of the fish's defensive mechanism in response to the stressor (Parrino *et al*. 2018). Hematological changes may be caused by stress factors such as ant nutrients, foods with greater fiber content, and foods with high-quality amino acids (Jimoh *et al.* 2020).

4.15.3 Red blood cell (RBC)

The values of RBC count were recorded after 12 weeks of study period in this experiment. The RBC count was significantly different ($P<0.05$) among the treatments. Average RBC count was higher in ginger extract (1.22 $x10^6$/ml) and lower RBC count observed at control treatment (0.89 $x10^6$/ml).

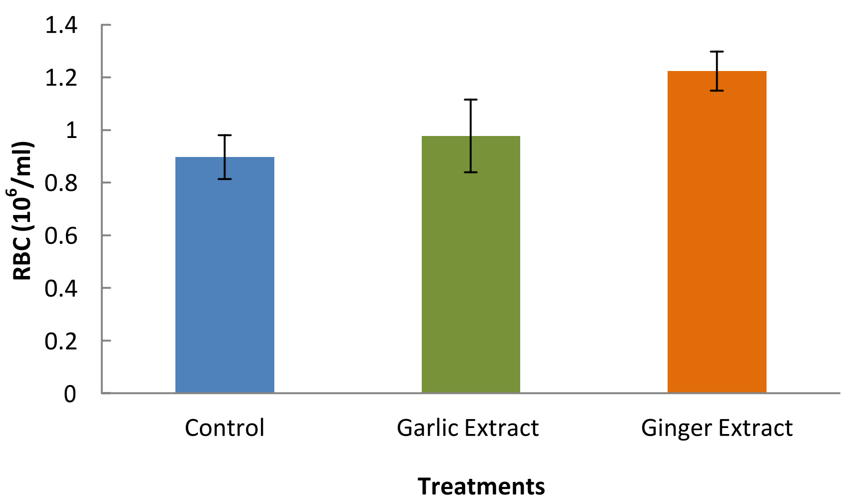

Figure 26: Red blood cell (RBC) counts in Nile tilapia (*O. niloticus*) fed with different treatments

The average RBC count in fish is 1.56 (or 10^6/mm3), with normal RBC counts ranging from 0.4 to 5.2. Fish have less RBC per unit volume compared to higher vertebrates.

Sharp snout sea bream fingerlings were used in an experiment by Mérida *et al*. (2010) to see whether sunflower meal (SFM) might replace fish meal. The results showed no significant variations in the RBC content between the treatments. RBC did not differ between control and HG fish when fish were fed on diets supplemented with EDTA, coriander (*Coriandrum sativum*) extract, -glucan, and carvacrol. The trends in growth and RBC were comparable among studies. The results of these studies do not conflict with those of the current investigation. The stress caused by the diet's anti-nutrient, increased fiber content, and high-quality amino acid content could be the cause of the trend in RBC count observed in those studies (Zhou *et al*. 2016).

4.15.4 Haematocrit

After 12 weeks of study period & collecting blood sample from the treatments, HCT count was higher ginger extract (18.8±0.29%) and lower HCT count observed at treatment control (14.3±0.28 %).

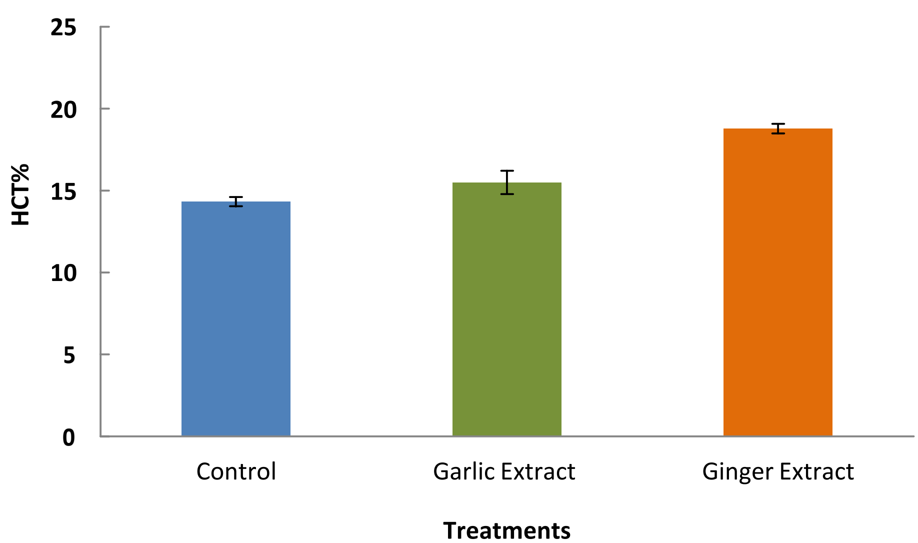

Figure 27: Haematocrit (HCT %) in Nile tilapia (*O. niloticus*) fed with different treatments

The RBC volume in the plasma is displayed by the HCT (%). It is commonly acknowledged upon that higher HCT, which displays increased viscosity, is beneficial to health. The range of this component in various studies was 9 to 18 g/dl, with 13 g/dl being the average. The decrease of HCT was seen in fish under varied stress conditions. Plasma levels play a key role in controlling HCT. To balance off the tissues' higher oxygen needs, the decreased HCT level (increased plasma level) improves the passage of proteins and salts through the bloodstream.

Usually, hematocrit is utilized as a broad measure of fish health. Hematocrit readings frequently vary in situations of significant dietary alterations and/or overt illnesses, according to Rumsey *et al.* (1994) research.

4.15.5 ALP, triglycerides and albumin

ALP: Blood alkaline phosphate levels drop (P 0.05), and a lower value was noted in the control treatment (75±2.94). Higher value was found in garlic extract (84.3±2.86) alkaline phosphatase (ALP) a liver-bound enzyme reacted differentially to the presence of glycin in the meals, declining sharply while maintaining within the normal range for fish, according to Muhammad *et al.* (2010). An enzyme that identifies the plasma membrane and endoplasmic reticulum is alkaline phosphatase. Significant elevation of these enzymes in the blood has been reported to signify, among other things, tissue necrosis or cell injury, according to Ioannou *et al*. (2006).

Triglyceride: Despite the fact that, the reduced triglyceride concentration seen in ginger extracts (186.13±2.16 mg/dl). According to Abudabos *et al.* (2013), broiler chicken diets with up to 3% *Ulva lactuca* had decreased triglyceride levels. There is currently no research on other farmed fish that have lower cholesterol levels because extracts were included in their meals. Higher triglyceride value was found in control treatment (205.7±3.09 mg/dl)

Albumin: The albumin levels found in this investigation were consistent with those of Khalafalla and El-Hais *et al.* (2015), who found no changes between Nile tilapia (*O. niloticus*) treated with ginger & garlic extract and control treatment for these parameters.

Table 06: Blood parameters (ALP, Triglyceride & Albumin) in different treatments (Mean ±SD)

	Treatments		
Blood Parameters	Control (T_1)	Garlic Extract (T_2)	Ginger Extract (T_3)
ALP (mmol/dl)	75 ±2.94	84.3±2.86	78.7±1.24
Triglyceride (mg/dl)	205.7±3.09	193.3±1.63	186.13±2.16
Albumin (g/dl)	1.63±0.04	1.88±0.05	1.42±0.05

4.15.6 MCV, MCH, MCHC

The majority of the blood parameters in our studies displayed better values.

The normal ranges of hematological parameters in Nile tilapia were MCV (120.33 fl-167.67fl), MCH (45.03 fl-63.21fl) & MCHC (28.47 pg-50.32 pg)

The findings showed that garlic extract substantially decreased mean cell volume (MCV) and ginger extract treatment in mean cell hemoglobin (MCH), but did not affect mean cell hemoglobin concentration (MCHC). The garlic extract with the lowest mean cell volume was 159.3 fl. Control treatment had the highest mean cell volume (MCV) level (176 fl).

Between the control and garlic extract treatments, the mean cell hemoglobin concentration was the same (34.360.38 to 34.80.24 g/dl). The lowest value, however, was associated with ginger extract therapy (32.10.8 g/dl). Red cell indices, such as MCV, MCH, and MCHC, show signs of anemia or blood poisoning in animals (Demir *et al.* 2014). The presence of macrocytic anemia, a serious illness that is characterized by RBC enlargement, destruction, and impaired osmoregulation, is indicated by higher MCV readings (Javed *et al*. 2016). MCH and MCHC count changes may indicate diseases affecting hematopoietic tissues such the liver and spleen. Rahmdel and others (2018). This might be caused by a decrease in cellular blood iron, a decrease in blood's ability to carry oxygen, and eventually a stimulation of erythropoiesis (Shah *et al*. 2013).

Table 07: Blood parameters (MCV, MCH & MCHC) in different treatments (Mean ±SD)

	Treatments		
Blood Parameters	Control (T_1)	Garlic Extract (T_2)	Ginger Extract (T_3)
MCV (fl)	176 ±0.44	159.3±0.53	166.7±0.37
MCH (fl)	60.5±0.37	58±0.61	54.16±0.55
MCHC (pg)	34.36±0.38	34.8±0.24	32.1±0.83

CHAPTER V

SUMMARY AND CONCLUSION

The present research was carried out at BRAC Tilapia Hatchery, Magura, Bangladesh. During the period from March to august three treatments were made to assess the fecundity, hatching and survival rate of produced fry. Garlic and ginger extract were applied with feed to investigate the potentiality of each extract on tilapia production and different morph biochemical parameters. The key findings are given below

In this experiment, Applying 0.02% garlic extract has increase fecundity than control & ginger extract. Egg number per gram was highest in garlic extract (132.05 pcs) and followed by ginger extract treatment (119.56 pcs) and control treatment (116.58 pcs) . Brooding female was found more in ginger extract than control & Garlic extract treatment. Ginger Extract treatment has 41 brooding female, Garlic extract treatment 39 brooding females & in controls treatment 38 brooding females has found through 5 weeks of collection. Hatching rate of Nile tilapia (*O. niloticus*) eggs was ranged from 76.0%-80.63%. Where we find garlic extract treatment has 80.63% ±1.05, ginger extract 76.01%±1.5 & in control treatment 76.16% ±1.06.Spawn rate per gram also an important factor for commercial hatcheries which highest in garlic extract treatment 98.29 ± 1.11 pcs then ginger extract treatment 87.71± 1.04 pcs & control treatment has 86.65± 1.00 pcs. Survival rate of fry is important for fish culture. Survival rates of fry in the control, garlic extract, and ginger extract treatments were reported as 78.25±0.09%, 84.24±0.05% and 81.63±0.13% respectively. FCR find in the treatments were 1.53±0.09, 1.43±0.19 and 1.48±0.14 in control, garlic extract and ginger extract respectively. The Mean weight gain of cultured Nile tilapia was 129±8.6, 137.33±17.46 and 130.66±13.76 g in cont ro l, garlic extract and ginger extract treatments.

Complete Blood Count (CBC) which evaluates the values of MCV, MCHC, alkaline phosphate, triglyceride, and albumins provided valuable information for fishery biologists in the assessment of fish immune status. Highest WBC result showed (61.26 $\times 10^3/\mu l$) in garlic extract treatment and RBC in ginger extract (1.22 x106/ml) respectively. The highest hemoglobin value (6.2± 0.08 g/dl) was found at ginger extract, garlic extract treatment (5.3± 0.29 g/dl) & in control treatment (4.6± 0.20 g/dl). The highest hematocrit was measured in ginger extract (18.8±0.29%) and lowest in control (14.3±0.28 %).The highest MCHC was measured in garlic extract treatment (34.8±0.24pg) and lowest in ginger extract treatment (32.1±0.83pg). Albumin highest in garlic extract treatment 1.88±0.05 g/dl & lowest value in ginger extract treatment 1.42±0.05 g/dl.

All the parameters were showed the highest result in garlic extract treatment with compare to control & ginger extract treatment. From the above result, 0.02% garlic extract supplemented with feed showed best result compare to control & ginger extract treatment which increased the egg production, hatching rate, survival rate of fry and immune system of Nile tilapia.

CHAPTER VI

RECOMMENDATIONS

The study focused on the potential of the Brood Nile Tilapia to improve reproductive efficiency through increased fertility, egg hatching percentage, and fry survival rate using the hapa breeding technique. Present study should that feed supplemented with 0.02% Garlic extract treatment produce highest amount of eggs and subsequently higher hatching rate and survival rate of Nile Tilapia.

The following recommendation might be taken into consideration in light of the findings and conclusion:

- Find out the growth performance of Nile tilapia (*O. niloticus)* fry for a more extended period could be done for more research.
- Additional research could be done with different doses of garlic extract
- Further research could be done with both mixed 10 ml garlic & 10 ml ginger extract with supplemented feed
- Treatment with other extract could be used for further research

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