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Role of Some Medicinal Plants on Growth Performance and Immune Status in Nile Tilapia (*Oreochromis niloticus*) Against *Aeromonas hydrophila*

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Abstract: The study was conducted to evaluate the effects of Alema feed incorporated with rosemary, Turmeric, and garlic with three different levels of inclusion towards immunity response, growth, and body proximate composition of Nile tilapia. Experiments were conducted under greenhouse conditioning at Sebeta National Fishery and Aquatic Life Research Center. In the experiment, 10 experimental diets were evaluated and Alema feed was used as a control. The result showed that the mean daily growth rate of garlic 8%feed (0.48 ± 0.04^a) was significantly ($p < 0.05$) highest from other experimental diets and the lowest growth rate was observed in diets of rosemary 8% (0.40 ± 0.01^e) and 12% (0.40 ± 0.06^e) inclusions. The FCR values of all experimental diets were greater than 2. Body proximate composition of Nile tilapia showed a statistical difference ($p < 0.05$) between experimental diets. The highest protein and ash content was recorded within the garlic 8% diet, which was found d 19.25 ± 0.22 and 1.68 ± 0.01^a respectively. The crude lipid content of Nile tilapia fillet found in Alema feed (1.61 ± 0.25^a) was the highest in other experimental diets. The hematology responses of Nile tilapia were statically significant ($p < 0.05$) between experimental diets. In hemoglobin content, red blood cell, and platelet count fish feed containing 8% of garlic and 92% Alema feed show significantly high ($p < 0.05$) immunity development than other experimental diets. Overall incorporation of garlic with 8% to commercial fish feed has advantages to improve the immune response of fish.

Keywords: *Oreochromis niloticus*, Medicinal Plants, *Aeromonas hydrophillia*, Fish Feed

1. Introduction

Nile tilapia (*Oreochromisniloticus*) is one of the most known tropical and subtropical freshwater fish and is globally distributed because of its importance in aquaculture [16]. Nile tilapia demonstrates fast growth reproduces easily, consumes a wide range of feed types, able to survive in a wide range of water quality parameters, tolerates environmental stress and has resistance to disease [23]. The gap between food fish supply and demand has resulted in the wide use of aquaculture production worldwide. Fish culture systems have led to many constraints and among them is stress, which as consequence, results in a bunch of conditions such as poor fish performance, alteration of physiological functions, poor digestion and feed utilization, and increased susceptibility to disease assessment [28]. Poor fish meat quality, and in extreme cases lead to mortality [12]. Fish diseases are among many challenges that decrease fish production and cause economic loss in the aquaculture sector. Studies showed that bacterial diseases are the most important cause of heavy losses in aquaculture throughout the world [18, 22]. *Aeromonas hydrophila* is an opportunistic Gram-negative bacteria prevalent in aquatic habitats with cosmopolitan distribution and causes high mortalities in farmed and wild fish. In Ethiopia fish mortality in lake Hora around Debrezeit was caused by *A. hydrophilia* associated with other stressing factors like Ammonia [27]. Recently, due to the inherent negative effects of antibiotics, other alternative antimicrobials from plant origins are increasingly used in aquaculture to control bacterial diseases [24]. The effects of different herbal extracts on the growth and immune defense of fish were studied over the last several decades. Medicinal plants have a role in aquaculture to stimulate the immune system and enhance the disease resistance of fish [20]. Medicinal plants are used in aquaculture not only as chemotherapeutics but also as feed additives [4, 5]. Medicinal plants can be used in aquaculture either as crude, extract, or active component. Among many garlic, rosemary and turmeric have been reported to affect the growth and appetite of fish and have antimicrobial activity. Nowadays, the use of medicinal plant extracts in aquaculture is increasingly used to control bacterial diseases and promote the growth and production of cultured fish species [6]. The use and application of medicinal plants as feed additives to enhance the growth and increase the immune system of *Oreochromis niloticus* is not studied under a controlled system in Ethiopia. Thus, the results obtained from this study can serve as baseline information on the use of selected medicinal plants to enhance the immunity of *Oreochromis niloticus* cultured under greenhouse conditions.

2. Materials and Methods

The experiment was conducted at Sebeta National Fisheries and Aquatic Life Research Centre (NFALRC), located about 23 km South-west of the capital city, Addis Ababa at 2200 meters above sea level. The area is characterized by a moderately warm climate with a mean annual temperature of about 20°C. The medicinal plants of rosemary, turmeric, and garlic were purchased from the local market Sebeta. Samples were dried and powdered using a milling machine in the NFLARC laboratory and Alema fish feed (commercial fish feed was taken from NFLARC; feedstock The experiment was conducted in black plastic tanks each holding 1m^3 of water in a greenhouse. A total of 30 tanks were used for the ten experimental diets run in triplicate tanks on a completely randomized design. A total of 1500 experimental Nile tilapia fish of initial body weight from 25 gram – 30 gram were collected from NFALRC fish ponds. The fish were stocked in 30 tankers and reared for two weeks for the acclimatization period. During the acclimatization period, fish were fed only Alema feed, which is used as a control feed, and carefully observed for any symptoms of diseases. Water quality parameters such as temperature 26±1°C; dissolved oxygen 9±1mg/L; pH7.7±0.33; NH_3 less than 0.01mg/L and NO_2 less than 0.1mgl/L were controlled by exchanging clean water frequently.

2.1. Feed Preparation

Three types of feed with three levels were prepared from Alema feed incorporated with rosemary, turmeric garlic. All experimental diets were run in triplicate. All feed ingredients were ground to have an excellent uniform particle size and then, the diets were prepared in such a way at each ingredient was first weighed and then mixed to Alema feed thoroughly in a mixer and pellet. 4% rosemary was prepared by using 4 grams with 96 grams of Alema feed, 8% rosemary were prepared by using 8 grams of rosemary and 92 grams of Alema feed, and 12% of rosemary were prepared by using 12 gram of rosemary and 88 gram Alema feed. The other garlic and turmeric inclusion rate of feed were prepared with the same procedure as like rosemary inclusion feed preparation and 100% Alema feed was used as a control. The study was run for 120 days. Fish was fed the same feed (Alema feed) for 15 days for acclimatization and fish were fed different experimental diets for 105 days. Experimental fish was fed 5% of their body weight and fed twice a day at 12-hour intervals. The number of inclusions and preparation of treatment feeds were shown in table 1.

Table 1. *Formulation and preparation of experimental diets.*

Experimental diets	The inclusion rate of ingredients in % (w/w) on dry based			
	Control (Alema feed) %	Rosemary %	Garlic %	Turmeric %
Control (Alema feed)	100	0	0	0
Rosemary 4%	96	4	0	0
Rosemary 8%	92	8	0	0
Rosemary 12%	88	12	0	0
Garlic 4%	96	0	4	0
Garlic 8%	92	0	8	0
Garlic 12%	88	0	12	0
Turmeric 4%	96	0	0	4
Turmeric 8%	92	0	0	8
Turmeric 12%	88	0	0	12

2.2. Laboratory Analysis

In the lab, Kjeldahl digestion, nitrogen analyzer, titration apparatus, Soxhlet apparatus for extraction of oil, muffle furnace for ash determination, drying oven for moisture determination, different size beakers, and measuring cylinders were used. The proximate composition of samples was analyzed using standard methods in the NFALRC laboratory [3]. The body proximate composition of Nile tilapia (*Oreochromis niloticus*) fillet was done after feeding different experimental diets for 120 days. Three Nile tilapia fish were randomly selected from each treatment tank, filleted, and ground with pestle and mortar in the NFALRC laboratory for proximate analysis. The carbohydrate content of samples was obtained based on the following formula.

$$\text{Carbohydrate content} = 100 - (\text{protein} + \text{fat} + \text{ash} + \text{moisture}).$$

Similarly, the energy value of samples was determined by calculation using the relationship between fat, carbohydrate, and protein contents of the Water's Conversion Factors; (4kcal/g) for protein, (9kcal/g) for fat, and (4kcal/g) for carbohydrates based on the following formula: Gross energy (Kcal/g)=$(4 * \text{protein}) + (4 * \text{carbohydrate}) + (9 * \text{fat})$.

2.3. Growth Parameters

A total of 25 experimental fish were stocked in each tanker. The initial weight of the fish ranged between 25-30 grams and the length ranged between 9-11cm. The final weight and length of fish were measured at end of the experiment for all treatments and the growth performances of fish were determined in terms of mean weight gain, daily growth rates (DGR) and specific growth rates (SGR), and survival rates. Growth parameters were calculated following standard equations given below [2].

1) Daily Growth Rate (DGR) in g/day=Final weight (g) – Initial weight (g) / culture period;
2) Weight gain (g) = Final weight (g) – Initial weight (g);
3) Percent weight gain (%WG) $= \frac{Wf-Wi}{Wi} * 100$;
4) Specific Growth Rate (SGR) $= \frac{\ln Wf - \ln Wi}{T}$.

Where Wf= final weight of fish; Wi=initial weight of fish; growth period in days.

1) Feed conversion ratio (FCR) $= \frac{\text{Feed offered for fish (g)}}{\text{Weight gain by fish (g)}}$;
2) Survival rate (%)= (Number of fish harvested/Number of fish stocked) ×100.

Temperature and dissolved oxygen were measured daily using a multi-probe instrument; while other water quality parameters were done every two weeks from each tanker under the greenhouse condition [1].

2.4. Culture of Aeromonas hydrophilla

For bacterial strain isolation, samples were taken from the fish gut, liver, and body, homogenously mixed and powdered. *A. hydrophila* was cultured in Tryptic Soy Broth (TSB) media for 48h at 25°C, and then, 1% formalin (v:v) was added to the bacterial suspension, and the mixture was kept at 4°C for 24h. Finally, inactivated bacteria were collected by centrifuging of media in 4000g for 30 min and the supernatant was discarded, and the pellet was re-suspended in sterile normal saline. This procedure was repeated three times in order to make sure that the formalin and culturing media have been removed thoroughly. Complete inactivation of prepared bacteria was tested and confirmed by its subcultures in TSB for 48h at 25°C. Bacteria stock concentration was adjusted to $1*10^9$ $CFUml^{-1}$ using the McFarland turbidity meter [26]. Morphological, physiological, and biochemical plate and tube tests were done to ensure that the isolated strain is *Aeromonas hydrophila* in the NFALRC microbiology lab.

2.5. Blood Sampling and Chemical Analysis

At the end of the feeding trial, the feeding was discontinued for 24h, and five fish from each tank (i.e., 15 fish per dietary treatment) were randomly sampled for the blood test. Blood samples were collected from the caudal vein using a heparinized syringe (5mL), pooled, and stored in heparinized tubes at 4°C until analyzed to determine blood indices. For blood analyses, hematological parameters including the red blood cells (RBCs), platelet, and hemoglobin (Hb, g/dL) values were immediately evaluated and calculated [10]. RBCs and WBCs were calculated using a Neubauer chamber. All blood samples were done in the Ethiopian national veterinary health institute laboratory, Sebeta.

2.6. Challenge of Aeromonas hydrophila Bacteria

After 105 days, five fish from each tank (i.e., 15 fish per dietary treatment) were injected intramuscularly with 2.1×10^7 cells/fish *Aeromonas hydrophila* bacteria at the base of a dorsal fin and maintained in different tanker under greenhouse conditions for 14 days in order to cheek survival rate and immunity development and mortality of the challenged fish was recorded daily for 14 days. The relative percentage survival (RPS) of each trial was determined using the below equation [10].

$$\text{Relative percentage survival (RPS)} = \frac{1-(\text{\% mortality of treatment feed feeding fish})}{\text{\% mortality of control feeding fish}} * 100$$

2.7. Statistical Analysis

Two-way ANOVA was used to test for the presence of significant differences and Duncan's multiple range test was used to identify which means were significantly different from each other. Differences were considered significant at $p<0.05$. All data were analyzed using the SPSS version20.

3. Results

Table 2. *Percentage proximate composition and gross energy content in kcal/100 g of ingredients in a dry basis (mean ± standard error).*

Ingredients	Parameters						
	Protein %dry (w/w)	**Lipid % dry (w/w)**	**Ash % dry (w/w)**	**Moisture % dry (w/w)**	**Crude fiber % dry (w/w)**	**Carbohydrate % dry (w/w)**	**Gross energy % dry (w/w)**
Alema feed	32.60±0.26[a]	12.30±0.59[a]	9.67±0.21[a]	8.83±0.06[c]	1.65±0.05[c]	37.53±0.36[d]	387.07±0.15[a]
Rosemary	3.37±0.06[d]	1.24±0.01[d]	7.69±0.04[b]	10.36±0.03[a]	1.66±0.05[c]	77.34±0.02[a]	333.65±0.18[c]
Turmeric	9.72±0.08[c]	6.84±0.04[b]	2.83±0.01[d]	8.93±0.02[b]	4.59±0.10[b]	71.87±0.12[b]	387.36±0.16[a]
Garlic	16.43±0.09[b]	2.45±0.04[c]	5.86±0.03[c]	5.50±0.02[d]	3.97±0.02[d]	69.74±0.15[c]	366.59±0.43[b]

Mean in the same column with similar superscripts are not significantly different (p>0.05).

Table 3. *Percentage proximate composition and gross energy content in kcal/100g of experimental diets in a dry basis (mean ± standard error).*

Experimental diets	Parameters						
	Protein%	**Crude lipid%**	**Ash%**	**Moisture%**	**Crude fiber%**	**Carbohydrate%**	**Gross energy%**
Garlic 4%	31.85±0.05^{b}	11.61±0.06^{bcd}	9.37±0.02^{bc}	8.69±0.06^{cd}	1.72±0.01^{d}	38.47±0.02^{d}	386.15±0.01^{ab}
Garlic 8%	31.25±0.05^{c}	11.26±0.03^{e}	9.24±0.01^{c}	8.56±0.03^{de}	1.78±0.02^{c}	39.54±0.22^{c}	385.46±0.12^{ab}
Garlic 12%	30.60±0.06^{de}	10.71±0.17^{f}	9.05±0.02^{d}	8.42±0.01^{e}	1.86±0.03^{b}	41.44±0.33^{a}	384.66±0.12^{abc}
Rosemary 4%	31.86±0.46^{b}	11.56±0.19^{bcd}	9.43±0.05^{ab}	8.82±0.11^{bc}	1.64±0.03^{e}	38.61±0.26^{d}	384.55±0.24^{bc}
Rosemary 8%	30.24±0.09^{e}	11.33±0.14^{de}	9.39±0.05^{bc}	8.93±0.02^{b}	1.64±0.03^{e}	40.35±0.06^{b}	382.70±0.07^{c}
Rosemary 12%	29.14±0.31^{f}	10.65±0.18^{f}	9.27±0.03^{bc}	9.12±0.19^{a}	1.64±0.03^{e}	41.75±0.32^{a}	385.38±4.05^{ab}
Turmeric 4%	31.65±0.21^{b}	11.75±0.17^{b}	9.35±0.13^{bc}	8.84±0.04^{bc}	1.73±0.02^{d}	38.70±0.19^{d}	387.33±1.55^{ab}
Turmeric 8%	30.67±0.23^{d}	11.64±0.04^{bc}	8.85±0.17^{e}	8.89±0.03^{b}	1.84±0.03^{b}	39.55±0.33^{c}	387.03±0.08^{ab}
Turmeric 12%	29.49±0.21^{f}	11.42±0.09^{cde}	8.74±0.05^{e}	8.84±0.04^{bc}	1.94±0.03^{a}	41.47±0.19^{a}	387.00±0.07^{ab}
Alema feed	32.60±0.20^{a}	12.35±0.30^{a}	9.58±0.18^{a}	8.83±0.14^{bc}	1.64±0.03^{e}	37.52±0.36^{e}	386.89±0.10^{ab}

Mean in the same column with similar superscripts are not significantly different (p>0.05).

Table 4. *GrowthparametersofNiletilapia, O. niloticus cultured in tanks under greenhouse conditions (mean ± standard error).*

No	Experimental diets	Growth Parameters				
		Weight gain (g)	**Daily growth rate (g/day)**	**Protein intake rate (%)**	**Feed conversion rate**	**Survival rates**
1	Garlic 4%	6.30±0.10^{d}	0.14±0.03^{c}	0.34±0.02^{c}	2.33±0.04^{g}	88.00±1.00^{b}
2	Garlic 8%	8.10±0.32^{a}	0.18±0.04^{a}	0.45±0.03^{a}	2.45±0.05^{f}	94.67±4.16^{ab}
3	Garlic 12%	7.20±0.26^{c}	0.16±0.03^{b}	0.37±0.05^{bc}	2.63±0.02^{e}	91.67±4.62^{ab}
4	Rosemary 4%	6.30±0.16^{d}	0.14±0.02^{c}	0.38±0.09^{b}	2.70±0.08^{de}	94.33±3.79^{ab}
5	Rosemary 8%	4.50±0.15^{f}	0.10±0.01^{e}	0.21±0.06^{e}	3.73±0.06^{a}	90.33±2.3^{ab}
6	Rosemary 12%	4.50±0.14^{f}	0.10±0.06^{e}	0.26±0.02^{d}	2.78±0.05^{cd}	96.33±1.53^{ab}
7	Turmeric 4%	5.4±0.17^{e}	0.12±0.01^{d}	0.27±0.02^{d}	2.89±0.06^{bc}	92.00±5.20^{ab}
8	Turmeric 8%	4.50±0.15^{f}	0.10±0.07^{e}	0.35±0.04^{bc}	2.89±0.08^{b}	96.00±2.65^{ab}
9	Turmeric 12%	5.40±0.24^{e}	0.12±0.01^{d}	0.23±0.02^{de}	2.89±0.10^{bc}	89.67±6.03^{ab}
10	Alema feed	7.65±0.22^{b}	0.17±0.05^{b}	0.24±0.03^{de}	2.93±0.06^{b}	96.33±2.52^{a}

Mean in the same column with similar superscripts are not significantly different (p>0.05).

Table 5. *Percentage proximate body composition and gross energy content in kcal/100g of Nile tilapia fillet on a wet basis (mean± standard error).*

Experimental diets	Parameters%(w/w) wet based					
	Protein	**Ash**	**Crude lipid**	**Moisture**	**Carbohydrate**	**Gross energy**
Garlic 4%	18.54±0.20^{bc}	1.71±0.06^{a}	1.44±0.02^{bcd}	79.41±0.43^{cd}	1.67±0.02^{a}	86.50±0.24^{d}
Garlic 8%	19.25±0.22^{a}	1.68±0.01^{a}	1.47±0.02^{abcd}	80.22±0.12^{b}	1.15±0.18^{a}	82.81±0.19^{g}
Garlic 12%	18.20±0.02^{c}	1.67±0.02^{a}	1.59±0.02^{ab}	79.34±0.05^{cd}	1.78±0.25^{a}	86.56±0.22^{fd}
Rosemary 4%	18.38±0.03^{c}	1.66±0.02^{a}	1.45±0.03^{abcd}	80.26±0.18^{b}	1.71±45.96^{a}	82.74±0.11^{g}
Rosemary 8%	18.30±0.08^{c}	1.64±0.04^{a}	1.37±0.03^{d}	79.31±0.29^{cde}	1.47±0.02^{a}	85.22±0.12^{e}
Rosemary 12%	18.41±0.06^{bc}	1.66±0.04^{a}	1.36±0.02^{d}	78.94±0.08^{de}	1.61±0.23^{a}	87.88±0.08^{b}
Turmeric 4%	18.49±0.39^{bc}	1.70±0.03^{a}	1.43±0.04^{bcd}	78.84±0.17^{e}	1.52±0.07^{a}	88.47±0.16^{a}
Turmeric 8%	18.24±0.14^{c}	1.65±0.02^{a}	1.41±0.11^{cd}	79.51±0.35^{c}	1.65±0.02^{a}	84.65±0.26^{f}
Turmeric 12%	18.26±0.16^{c}	1.67±0.02^{a}	1.57±0.05^{abc}	81.57±0.47^{a}	1.30±0.06^{a}	78.26±0.07^{h}
Alema feed	18.72±0.17^{b}	1.49±0.11^{b}	1.61±0.25^{a}	78.87±0.10^{f}	1.85±0.07^{a}	87.25±0.05^{c}

Mean in the same column with similar superscripts are not significantly different (p>0.05).

Table 6. *Hematological characteristics of Nile tilapia (Oreochromis niloticus) fed the different experimental diets (mean ± standard error).*

No	Experimental diets	Hematology Parameters		
		Hemoglobin (Hb, g/dL)	**RBC (×10^{6}/mm^{3})**	**Platelet**
1	Garlic 4%	17.67±4.80^{a}	28.03±0.45^{de}	72.00±0.50^{bcd}
2	Garlic 8%	5.43±1.40^{e}	33.00±0.40^{c}	66.00±0.50^{e}
3	Garlic 12%	11.83±1.76^{bc}	26.00±3.00^{de}	74.00±4.00^{abc}
4	Rosemary 4%	9.67±2.08^{bcd}	28.50±1.50^{d}	70.00±2.00^{d}
5	Rosemary 8%	10.83±3.25^{bcd}	37.33±4.51^{b}	60.67±3.51^{f}
6	Rosemary 12%	8.33±0.58^{cde}	18±0.80^{f}	70.73±0.93^{cd}
7	Turmeric 4%	9.83±0.29^{bcd}	44.00±0.50^{a}	54.07±0.50^{g}
8	Turmeric 8%	7.80±0.00^{de}	24.10±0.36^{d}	76.00±0.50^{a}
9	Turmeric 12%	12.67±0.76^{b}	25.00±4.00^{de}	75.17±2.75^{ab}
10	Alema feed (control)	18.00±0.00^{a}	34.03±0.45^{bc}	66.07±0.31^{e}

Mean in the same column with similar superscripts are not significantly different (p>0.05).

During the experimental period, water quality parameters were controlled for each treatment. To avoid any stress from water quality parameters, continuous aeration and water exchange were implemented. As a result, a temperature of

26±1°C; dissolved oxygen, 9±1mg/L; pH7.7±0.33; NH_3 less than 0.01mg/L, and NO_2 less than 0.1 mg/L were recorded throughout the experimental period.

4. Discussion

Prevention mechanism of fish disease and good feed development is one of the most critical things for the success of aquaculture. In this study, the growth performance and hematology parameters of *Oreochromis niloticus* were compared between experimental diets containing different types of medicinal plants (rosemary, garlic, and turmeric) incorporated with Alema feed. In table 4. The growth parameters of Oreochromis niloticus was statistically significant (p<0.05). Results of the growth experiments showed that the highest daily growth rate (0.18g/day) was recorded on fish fed with garlic 8% followed by Alema fed (0.17g/day) and the lowest was recorded in fish given turmeric 8% (0.10g/day), Rosemary 8% (0.10g/day) and Rosemary 12% (0.10g/day) Table 4. In this study mixture, of 8% garlic and 92%, Alema feed is the best combination for better growth rate of *Oreochromis niloticus.* In similar studies, the daily growth rate of 0.16g/day to 0.18g/ day for evaluation of growth performance and body proximate composition of three strains of *Oreochromis niloticus* under greenhouse condition was obtained [11]. The daily growth rate of the present study is lower than the daily growth rate reported by other authors for *Oreochromis niloticus*. For instance a daily growth rate of 0.29 ± 0.02 gram/fish for mixed-sex *Oreochromis niloticus* in the pond culture system was obtained [8]. On other study, a daily growth rate ranging from 0.52 to 2.30 for *Oreochromis niloticus* in happa condition was obtained [16]. Likewise, the daily growth rate was found from 0.23 to 0.33 g/day and 0.68 to 0.86g/day for *Oreochromis niloticus* in the pond culture experiment [1, 15]. The apparently lower growth rate of the fish in our experiment might be due to the low protein content of the experimental feed. Because the experimental feed was formulated with the incorporation of medicinal plants which have low protein content and may be due to stress of fish due to large variation of temperature at day and night time in experimental place of greenhouse condition. Feed conversion ratio (FCR) expresses the weight of feed (g or kg) used to produce 1g or 1kg of fish flesh [29]. It is an important indicator of the quality of fish feed where lower FCR indicates better utilization of feed and extraction of nutrients from the feed and further converting it into flesh. The feed conversion ratio reported in the present study ranges from 2.45 to 3.73. A Significant difference (p<0.05) was observed in the feed conversion ratio of the experimental diets. The values recorded in this study are higher than the FCR reported by other authors who reported FCR values ranging from 1.44 ± 0.02 to 1.68±0.05 in the growth comparison experiment [25]. In a similar study, lower FCR values were recorded from 1.72 to 1.76 [7]. Furthermore, the FCR of 2.45 to 3.73 in the present study was also higher than the acceptable recommended FCR value of 1.2-1.5 for aquaculture. The difference in FCR value might probably be due to the quality of feed and different culture environments [12]. The efficient utilization of diet may vary even within diet type and environmental factors. The FCR values reported in the present study is lower than other studies, who reported FCR value ranging from 3.6 to 4.4 for *Oreochromis niloticus* strains in the pond culture system [1]. Similarly, a daily growth rate of 4.6g/ day to 4.7g/day for evaluation of growth performance and body proximate composition of three strains of *Oreochromis niloticus* L.,(1758) under greenhouse condition was obtained [19]. Thus, the incorporation of medicinal plants like turmeric, garlic, and rosemary with a specified amount gives a better growth rate for *Oreochromis niloticus*. The overall survival rate of the fish was high ranging from 88%–96% per tank indicating the high tolerance of the fish in a confined system. Our results agreed well with a previous study conducted on the feeding rate of *Oreochromis niloticus* which reported survival rates ranging from 80% to 96% [11]. Few mortality of the fish in this experiment is likely due to mechanical damage during the cleaning of the tanker where the fish were taken out. In the future, it is important to avoid such stress on fish by using an appropriate cleaning design. As shown in table 5 the proximate composition (protein, lipid, ash, and moisture) of *Oreochromis niloticus* fillet was a statically significant difference between treatment feeds (p<0.05). The present study of data is compared to both natural water and aquaculture system results so far. In the present study, the protein, lipid, ash, and moisture content of *Oreochromis niloticus* was found to be in the range of 18.82– 9.25, 1.36–1.61%, 1.49–1.71%, and 78.84–81.57%, respectively. In general, in the present study, the protein content was found to be lower while crude lipid and ash content were higher than other research findings, which is found to be the crude protein, crude lipid, ash, and moisture content of *Oreochromis niloticus* was 18.8%, 0.6%, 1.4%, and 79% respectively [14]. In Lake Tana, the crude protein, crude lipid, ash and moisture content of *Oreochromis niloticus* were 14.77%, 2.39%, 1.51%, and 73.62%, respectively [9]. The result of the present study disagreed with a former study this may be due to the difference in the environmental conditions. The other reason for disagreement with the result is the age of fish, quality of feed they feed, and experimental season difference.

As shown in Table 6. The hematology parameters (Hemoglobin, RBC, and Platelet) of *Oreochromis niloticus* fillet was a statically significant difference between treatment feeds (p<0.05). In the present study, the Hemoglobin, RBC, and Platelets count of *Oreochromis niloticus* was found to be in the range of 5.43–18.0 (Hb, g/dL), 18–44 ($\times10^6/mm^3$), and 60-76 respectively. Likewise, after fed medicinal plants, the hematology parameters of fish such as Hemoglobin and RBC were 0.4-0.9 Hb, g/dL and 1.49–4.37 x $10^6/mm^3$ [13]; 8.6-10.4 Hb, g/dL and 2.9-4.4 x $10^6/mm^3$ [17], and 5.93-10.93) Hb, g/dL and 29.42-37.08)$\times10^6/mm^3$ [21]. In the present study, the highest hematology parameters were obtained due to feeding the fish for 120 days, while the lowest result was obtained when the fish fed for 30 days [13]. The obtained result was disagreeing between different authors mainly due to the use of different size fish, experimental feeding period, and difference in active ingredients within the same medicinal plants in different agro ecology zone**.**

5. Conclusion and Recommendations

From the present study, the following points can be concluded and recommended. Fish fed with garlic 8%, with a protein content of 31.25% showed the highest daily growth rate (0.18g/day) as compared to fish supplied with control and other experimental diets. The high FCR values (>2.0) observed in all experimental diets might be due to greenhouse environmental conditions, and high temperature fluctuation within 24 hours that may stress the fish. Moreover, the physicochemical parameters such as DO, and pH recorded in the experimental tanks were generally found to be close to the lower limit required for the growth and production of *Oreochromis niloticus*. The low daily growth rate of *Oreochromis niloticus* might be attributed to the combined effects of the low protein content of feed and environmental conditions. The hemoglobin contents of garlic 8% after challenging with *Aeromons hydrophila*was 5.43 d/Gl which indicates a healthy fish. The protein, ash, and carbohydrate content of *Oreochromis niloticus* fillet were the highest in feeds of garlic at 8%. Thus, it can be concluded that the fortification of Alema feed with 8% garlic was advantageous in terms of growth rate, immunity response, and quality fillets of fish. This study has limitations in getting reference *Aeromonas hydrophila* and we recommend further study using standard *Aeromonas hydrophila* in controlled environmental conditions.

Authors' Contributions

Seferu Tadesse conceptualized the study, write a proposal, did proximate composition of feed formulation, and wrote the final full write-up. Marshet Adugna did hematology parameters of fish and edits the final write-up of the experiment. Fikadu Hailemichael and Legese Hagos isolated and identified *Aeromonas hydrophila.*

Conflict of Interest

The authors confirm that there is no conflict of interest.

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