Growth performance, haematological assessment and chemical composition of Nile tilapia, Oreochromis niloticus (Linnaeus, 1758) fed different levels of Aloe vera extract as feed additives in a closed aquaculture system

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ABSTRACT

A 105-day experimental trial was conducted to assess different levels of dietary Aloe Vera leaf extract supplementation on water quality parameters, proximate composition, growth performance and haematological parameters of fry Oreochromis niloticus. Four different percentages of dietary leaf extract powder of Aloe vera (ALE) with a basal feed, designated as, i.e., T0 (Control group; without ALE), T1 (1% ALE), T2 (2% ALE), and T3 (3% ALE). Fish fry was reared in concrete tanks (7.0 m, 1.6 m, 1.0: L, W, H; water volume 11.2 m3/tank), with an average initial weight 4.04 ± 0.03 g/fry, and each treatment was triplicated. Fry was randomly distributed at a stocking rate of 450 individuals/tanks. The water quality parameters revealed that temperature, pH, salinity, dissolved oxygen (DO) and nitrates were found in a promising range as given by FAO/WHO limits. However, the record values obtained for Electric Conductivity (EC), Total dissolved solids (TDS), and alkalinities were not found in all tanks’ suitable range according to FAO/WHO limits. The results revealed a significant impact of different percentages of dietary ALE supplementation on fry’s body composition and haematological parameters. Moreover, the final body weight, final body length, average daily weight gain (g), net weight gain (g) and specific growth rate (%) were significantly higher (p < 0.05) in T1 and T2 compared with T0 and T3 treatments. The poorest feed

Abbreviations: ALE, Aloe vera; PER, Protein Efficiency Ratio; ANOVA, Analysis of Variance; IBL, Initial Body Length; FBL, Final Body Length; DE, Digestible Energy; DM, Dry Matter; DP, Digestible Protein; EAA, Essential Amino Acid(s); ADWG, Average Daily Weight Gain; FA, Fatty Acid(s); EFA, Essential Fatty Acid; WG, Weight Gain; FCR, Feed Conversion Ratio; FM, Fish Meal; IBW, Initial Body Weight; FBW, Final Body Weight; K, Condition Factor; PO, Crude Palm Oil; RO, Rapeseed Oil; SGR, Specific Growth Rate; VO, Vegetable Oil(s); VSI, Viscerosomatic Index; HSI, Hepatosomatic Index.

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1. Introduction

Aquaculture is one of the fastest emerging food-producing sectors globally and is flourishing day by day (Habib et al., 2020). Tila-pia is one of the popular aquaculture species known as “aquatic chicken” due to its rapid growth, great adaptability to survive even in harsh environmental conditions, more resistance against a specific disease, high protein composition in meat (El-Sayed, 2006; Abdel-Aziz et al., 2021). Oreochromis niloticus is commonly known as “Nile tilapia”, and is the third most famous cultured species for providing good quality fish protein in the human diet worldwide (FAO, 2012). More recently, tilapia production has been sharing up to 75% of total aquaculture production globally due to its ability to tolerate a wide range of several environmental factors. Therefore, considered a significant cultural species in freshwaters or even in brackish water conditions of hatcheries and fish farms in 100 countries worldwide, including Pakistan (Esselman 2009), FAO (2012) reported that tilapia is the most dominant group of warm water fishes used for aquaculture purposes in various tropical regions globally. Tilapia can tolerate a wide range of temperatures; even can survive for few days in temperature below 10 °C and increases up to 40 °C. Nile tilapia is multiple spawners that is reproduced throughout the year, and gonads mature even during early life stages, which also depend upon the availability of food rich protein and lipid contents. Among 70 aquaculture tilapia species, nine species are widely used for aquaculture purposes, including i.e. Tilapia zillii and Oreochromis niloticus.

Furthermore, most of these species are native to western Africa (FAO, 2012). In sustainable aquaculture practice, the management of fish nutrition is necessary to minimize costs and maximize growth performance is needed to handle fish nutrition for the best possible growth performance and fish health (Hassan et al., 2021a, 2021b). Today, the world is faces a shortage of fish meal production due to its increased demand in aquaculture and prices. Therefore, many least developing countries are now not using such expensive fish meal as a major source of protein in fish feed and replacing it partially or totally with cheap other animal origin protein sources such as poultry by-products, blood meal and meat, bone meals (FAO, 2012; Hussain et al., 2021). These fish feeds are sometimes deficient, particularly in a few crucial amino acids like isoleucine, lysine and methionine. Tilapia meat can also be used as a major replacing ingredient in the production of fish feed to improve the quality of fish feed in proper ratio to maintain essential amino acid composition in their diet for commercially farmed fish species (Admasu et al., 2017).

The use of herbal immune stimulants in the aquaculture sector, has spread worldwide, specifically to improve the immune system and increase fish tolerance to a range of infectious diseases. Many studies have shown that plant immune stimulants can enhance specific and non-specific immune protective mechanisms and increase or decrease fish losses against various pathogens. (Yin et al., 2009). Aloe vera is among the essential plants in the Liliaceae family, native to tropical and subtropical regions. (Mandrioli et al., 2011). This plant has a wide range of pharmacological impacts, including skin lesions and wound healing, anti-viral, antibacterial, and other effects. However, the results of immune stimulation on warm-blooded animal development have not been confirmed. Furthermore, little is known about the effects of immunogenicity and anti-toxicity. No research has been conducted lately to examine the possible effects of Aloe vera extract mixtures in aquaculture. Reports on their combination with other herbs/natural products, on the other hand, support the finding that the benefits of herbal extracts in fish could be enhanced, mainly when applied as mixtures. Case in point, a dietary mix of thyme was reported to have significantly improved growth, overall health, and resistance of Sparidentex hasta fry against Photobacterium damsela (Jahanjoo et al., 2018). Positive synergistic effects of different Aloe vera extracts mixed with other herbal extracts were also reported in O. niloticus and Litopenaeus vannamei (Huang et al., 2018).

Several studies have been revealed that various endogenous and exogenous factors, including water pH, salinity, temperature, total dissolved solids, dissolved oxygen concentration and fish feeding composition and its frequency, can affect the proximate composition of fish found in any ecosystem. Furthermore, several genetic and life-related factors, including size, age, gender, and physiology are the endogenous factors governing the fish body composition (El-Sayed et al., 2006). Some factors include dietary protein sources, culturing conditions, and water quality, which is also responsible for changing fish body composition, e.g., small-sized fry stages required more protein in their diets than adult stages. Moreover, the protein composition in the fish diet is an essential factor affecting fish growth and protein composition (Ahmad et al., 2004; Abdel-Tawwab & Ahmad, 2009). The proper development and production of some commercially important tilapia species need 25 to 56% dietary protein in its diet (FAO, 2012). Being an omnivorous fish, Nile tilapia can also use less expensive plant origin sources as major feed ingredients in their diet (Chowdhury, 2011). Therefore, the present study was based on analyzing the impact of diets containing different percentages of Aloe vera leaf extract powder (ALE) on water quality parameters growth and protein composition of Nile tilapia O. niloticus cultured in a close aquaculture system.

2. Materials and methods

2.1. Experimental Design

This study was conducted in National Agricultural Research Centre (NARC) located in Islamabad, Pakistan. It consisted of four treatments in three replicates and were designated as the following: T0 (control group: a basal feed without ALE), T1 (1% of ALE in fish feed), T2 (a diet supplemented with 2% ALE), and T3 (a diet supplemented with 3% ALE) fry were cultured (7.0 m, 1.6 m, 1.0 m) each; length, width, depth) and volume 11.2 m3 with an average initial weight of 4.04 ± 0.03 g and were randomly distributed at a stocking rate of 450 individuals/ tanks for total 105 days. Each fish tank was dried, cleared, and add cattle dung 50% of the total organic fertilizers, Urea 1.25 kg, poultry manure 45.0 kg, and di-ammonium phosphate (DAP) 2.5 kg and left the tanks for 15 days before experimental study and culture of Nile tilapia by following the methods of Haraz et al. (2018).
2.2. Fish feed formulation

Fish feed was prepared to contain 30% crude protein (CP) from Oryza Organics fish fed twice a day/week at 8:30–9:00 AM and 4:30–5:00 PM. to fry stages at the ratio of 3% of total body-weight of fish. A daily meal was readjusted at every fortnight sampling according to the wet-weight gain by following the methods of Admasu et al. (2017).

2.3. Aloe vera feed

Dried leaves of Aloe vera in 80% methanol at room temperature were extracted and filtered. Then this filtrate was dehydrated in a rotary for getting a dark-greenish residue, which was later used as feed additive as 1%, 2% and 3% along with the major ingredient percentages recorded in Table 1 and 2 to enhance the immune system of fish and improve its health condition by following the methods of Gabriel et al. (2015), as given below in Tables 1 & 2.

2.4. Water quality parameters

Water temperature (°C), salinity, dissolved oxygen (ppm) and pH were recorded every day, and they were measured by (Celsius glass thermometer), and Handheld refractometer, mobile digital DO-meter (Model: HI9146), and digital pH meter, while alkalinity (mg/l) and nitrate (mg/l) were determined with the chemical methods according to (APHA, 1995).

2.5. Analysis of the proximate composition of fish

At the end of this experimental study, about ten specimens of fingerling fishes from each fish pond were capture for the chemical analysis of their carcass composition.

2.6. Growth and morphological indices

Sampling was performed weekly. At the time of each sample, the fish growth parameters were calculated (Makori et al., 2017; Hassan et al., 2020; 2021c). Indices for the evaluation of growth performance have been determined as follows:

Net weight gain (NWG) = Average final body weight (AFW) (g) - Average initial body weight (AIW) (g)

Average daily weight gain = ((Final body weight-Initial body weight)/Days)

Specific growth rate (SGR) = ((Final body Weight-Initial body weight)/Days) \times 100

Feed Conversion Ratio (FCR) = Feed intake (g)/Wet weight gain (g)

Feed Conversion Efficiency (FCE) = (Weight gain/Feed intake) \times 100

Hepatosomatic index HSI, % = (liver weight/body weight) \times 100

Viscerosomatic index VSI, % = (weight of viscera and associated fat tissue/body weight) \times 100

2.7. Hematological parameters

The total red blood cells (RBC) were calculated using an improved method of Neopor emission. Blood was diluted with 5% EDTA with a ratio of 1: 250. Red blood cells were measured by the hemocytometer chamber and then calculated to \(10^6\) mm\(^3\). For total white blood cells, blood was diluted with 1: 100 Dacie fluid and measured using a hemocytometer and then calculated on \(x10^3\) mm\(^{-3}\). Both measurements were run under a 100 X microscope (Olympus).

The haemoglobin (Hb, g dL\(^{-1}\)) concentrations were determined by the cyanomethaemoglobin method (Klontz, 1994). All the values of red blood cell indices, the mean values of cell haemoglobin (MCH pg), cell haemoglobin concentration (MCHC %), and cell haemoglobin volume (MCV fl) were calculated according to Wintrobe formulae (Anderson and Klontz, 1965). The percentage composition of leukocytes was determined based on their identification characters listed (Ivanova, 1983)

2.8. Statistical analysis

The experimental results were analyzed by one-way analysis of variance (ANOVA) using SPSS version 26. The differences among the treatments were determined by Duncan Waller at a significant level (\(p < 0.05\)).

3. Results

3.1. Water quality parameter

The water quality parameters were analyzed for all experimental tanks. Means of water temperature, dissolved oxygen (DO), salinity, pH and nitrates were in range of standard value as given

| Table 1 |
| --- |
| Fish feed composition with different doses of dried Aloe vera leaf extract used for four experimental trials. |

| Ingredient used/100 g feed | Control group diet % | Experimental group diets % |
| --- | --- | --- |
| Fish meal (g) | 39.01 | 39.01 |
| Corn gluten (30 % CP) | 40.54 | 40.54 |
| Cotton seed meal (g) | 40.95 | 40.95 |
| Rice polish (g) | 28.50 | 28.50 |
| Wheat bran (g) | 36.72 | 10 |
| Sun flower oil (mL) | 0.00 | 0.00 |
| Vitamins-mineral premix (quantity in g/2.5 kg) | 46.60 | 9.32 |
| Dried leaf extract of Aloe vera (g) | 30.05 |

| Table 2 |
| --- |
| Tilapia Fish feed ingredients and composition used during 105 days experimental trials. |

| Ingredients | Control group diet % | Experimental group diets % |
| --- | --- | --- |
| Wheat bran (g) | 14 | 3.36 |
| Rice polish (g) | 12 | 2.88 |
| Cotton seed meal (g) | 40.95 | 1.64 |
| Corn gluten (g) | 28.50 | 2.85 |
| Fish meal (g) | 56.72 | 10 |
| Sun flower oil (mL) | 0.00 | 0.00 |
| Mineral premix (g) | 46.60 | 9.32 |
| Total | 30.05 |

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by FAO/WHO; however, the electrical conductivity (EC), total dissolved solids (TDS). According to the FAO/WHO, the total alkalinity was not lied in standard values according to the FAO/WHO as shown in Table 3 and Figs. 1–8.

| Water Parameters          | Mean ± S.D | Range       | WHO/FAO limits                      | References               |
|---------------------------|------------|-------------|-------------------------------------|--------------------------|
| Temperature (°C)          | 22.78 ± 0.05 | 22.7–22.8   | 20 to 36 °C                         | (FAO, 2012)              |
| Dissolved Oxygen (DO) mg/L| 5.66 ± 2.83 | 5.66–5.7    | 3 to 5 mg/L                         | (El-Sayed, 2006)         |
| Salinity (g/L)            | 0.79 ± 0.11 | 0.68–0.9    | 0.5 to 2.5 g/L                      | (Yanbo et al., 2006)     |
| pH                        | 7.70 ± 0.12 | 7.6–7.8     | 5.5 to 9.5                          | (Rebouças et al., 2016)  |
| Electric Conductivity (EC) | 2354.5 ± 65.03 | 2307.6–2445.5 | 100 to 2000 μS/cm                  | (Stone et al., 2013),     |
| Total Dissolve Solids (TDS) mg/L | 1353.8 ± 186.37 | 1077.1–1464.8 | <400 ppm                           | (Boyd et al., 2016)      |
| Nitrates mg/L             | 0.20 ± 0.01 | 0.19–0.21   | 0.2 to 219 mg/I                     | (Boyd et al., 2007)      |
| Alkalinity mg/L           | 379.0 ± 28.07 | 338.4–401.6 | 20 mg/l                             | (Boyd et al., 2016)      |

Fig. 1. Water temperature (T) of four experimental fish tanks.

Fig. 2. Dissolved oxygen of four experimental fish tanks.

Fig. 3. Salinity of four experimental fish tanks.

Fig. 4. Water pH of four experimental fish tanks.

Fig. 5. Electric conductivity of four experimental fish tanks.

Fig. 6. Total dissolved solids of four experimental fish tanks.
3.2. Proximate composition

Table 4 presented the chemical composition of fish carcasses at the end of the study. The crude protein was high and ranged between 60 and 70%, while crude lipid content ranged 2.5–2.7%, ash content was 2.5–2.8, and moisture was much high range from 93.0 to 95.0% in the body composition of Oreochromis niloticus, as given below.

| Ingredients (%) | Initial | C(T0) | T1 | T2 | T3 | Permissible range (WHO) | References |
|-----------------|---------|-------|----|----|----|-------------------------|------------|
| Moisture        | 95.78 ± 0.24 | 93.5^a ± 0.21 | 94.0^b ± 0.20 | 95.0^a ± 0.5 | 93.0^b ± 0.56 | 78.9 ± 0.5 | (FAO,2012) |
| Ash             | 2.50 ± 0.11   | 2.7 ± 0.20   | 2.8 ± 0.1 | 2.5 ± 0.22 | 2.7 ± 0.57 | 2.6 ± 0.2 | (Boyd et al., 2016) |
| Crude Lipid     | 2.10 ± 0.16   | 2.6 ± 0.1    | 2.7 ± 0.10 | 2.7 ± 0.25 | 2.5 ± 0.40 | 5.0 ± 12.0 | (El-Sayed, 2004) |
| Crude Protein   | 60.14 ± 2.16  | 65.0^a ± 4.01 | 67.0^b ± 0.5 | 70.0^a ± 0.30 | 60.0^b ± 1.05 | 30.0 ± 40.0 | (El-Sayed, 2006) |

Values are the mean ± SD of groups in the same row with different superscripts are significantly different (p < 0.05).

3.3. Growth performance and feed utilization parameters

Results obtained in Table 5 indicated that the growth performance of Nile tilapia significantly varied with a difference in dietary ALE supplementation. The highest growth was recorded in T1, while the FBW (82.6 ± 0.64 g), FBL (18.10 ± 0.10 cm) ADWG (0.7 ± 0.01 g), SGR (3.03 ± 0.06), NWG (78.70 ± 0.80 g) followed by dietary ALE (T3) and (T0) while these parameters were the least with fish fed a diet containing 3% ALE (T3) where:- ADWG, NWG, SGR, 0.67 ± 0.01 (g), 70.7 ± 0.62 (g), 2.96 ± 0.50 %d-1 respectively. There was a significant difference among treatments at level (p < 0.05) in FCR. The best FCR was recorded in (T1) and (T2) compared to other treatments. No significant changes were found among T0, T3 and T3 in HSI and VSI. They were significantly higher than T1.

3.4. Haematological analysis

Blood samples of fish were collected from each treatment with the help of syringes in vacationers in which EDTA (anticoagulant) and the following parameters were calculated for each treatment: TLC (total leukocyte count), Neutrophil, lymphocytes, monocytes, eosinophil, platelets, total RBC, and haemoglobin. While in total, proteins, albumins and globulins were also calculated. Haemoglobin concentration was recorded maximum in T2 with 10.85/µL of blood among all treatments, while minimum in T0 with 7.65/µL. The values recorded regarding to the TLC and RBC were maximum in T2 with 76000, and 2.28/µL neutrophils concentration were analyzed maximum in T1 with 8%, while minimum in T0 with 6%. Lymphocytes in T2 were recorded maximum with 90.5% and minimum in T3 with 89.5%. Platelets count for all the treatments in decreasing manner as T1 > T2 > T0 > T3. The highest value of total proteins and globulins were recorded in T2 with 7.6 and 2.7, while the lowest values were 6.4 and 2.3 g/L in T1, respectively. Albumins were analyzed and recorded maximum during liver function test in T0 with 4.8, while minimum in T1 with 4.14 g/L as shown in Table 6.
Haematological analysis of Oreochromis niloticus in four experimental fish ponds during the study period

Table 5
Effects of Aloe vera on growth, feed utilization and survival indices of Oreochromis niloticus

| Parameters                      | Treatments |
|---------------------------------|------------|
|                                 | T0         | T1         | T2         | T3         |
| Initial body weight BW (g)      | 4.0 ± 0.01 |
| Final body weight FW (g)        | 77.0 ± 0.21 |
| Specific growth rate (g/d)      | 2.98 ± 0.04 |
| Feed conversion ratio           | 0.68 ± 0.12 |
| Feed conversion efficiency      | 7.059 ± 0.02 |
| Average daily weight gain (g/day)| 0.69 ± 0.00 |
| Net weight gain (g)             | 73.0 ± 0.20 |
| Hepatosomatic index             | 3.10 ± 0.1 |
| Viscerosomatic index            | 4.20 ± 0.3 |
| Survival %                      | 89.76 ± 0.02 |

Table 6
Haematological analysis of Oreochromis niloticus in four experimental fish ponds during the study period

| Blood Parameters                | Treatments |
|---------------------------------|------------|
|                                 | T0         | T1         | T2         | T3         |
| Hemoglobin/μL                   | 7.65 ± 0.04 |
| TLC/μL                          | 48,900 ± 8.44 |
| Neutrophils %                   | 6 ± 0.99 |
| Lymphocytes%                    | 90 ± 1.16 |
| Monocytes %                     | 1.5 ± 0.66 |
| Eosinophils%                    | 2.5 ± 0.66 |
| Platelets count/μL              | 135,500 ± 10.043 |
| Total RBC/μL                    | 1.42 ± 0.05 |
| HCT (%)                         | 23.05 ± 0.29 |
| MCV                             | 163.1 ± 2.52 |
| MCH                             | 53.95 ± 0.56 |
| MCHC                            | 28.65 ± 0.26 |
| WBC count/μL                    | 5.9 ± 0.8 |
| albumin (g/L)                   | 4.8 ± 0.22 |
| Globulin (g/L)                  | 2.3 ± 0.99 |
| Total protein (g/L)             | 6.4 ± 0.86 |

4. Discussion

Aloe vera has been utilized as a popular folk remedy for as long as civilization has existed. In aquaculture, it has recently been reported that it has the potential to act as an alternative growth booster, anti-stressor, immunological stimulant, appetizer, and digestive stimulant (Heidarieh et al., 2013). The present study revealed that dietary extract of Aloe vera has the potential to act as an alternative growth booster, anti-stressor, immunological stimulant, appetizer, and digestive stimulant (Heidarieh et al., 2013). The present study revealed that dietary extract of Aloe vera has the potential to act as an alternative growth booster, anti-stressor, immunological stimulant, appetizer, and digestive stimulant (Heidarieh et al., 2013). The present study revealed that dietary extract of Aloe vera has the potential to act as an alternative growth booster, anti-stressor, immunological stimulant, appetizer, and digestive stimulant (Heidarieh et al., 2013).

The present study revealed the significant impact of different percentages of dietary Aloe vera leaf extracts on the body composition and haematological parameters of fry stage found in four groups as, T3 > T0 > T1 > T2, respectively. In this study, the highest FBW, ADWG, NWG, and SGR recorded in T3 compared to other treatments and the best FCR recorded in a similar trend to our finding (Ali et al., 2005).
between 100 and 2000 µS/cm. For maintaining the normal water pH range or buffering, a minimum value of alkalinity, 20 mg L⁻¹. CaCO₃ is suitable for fish culture, as reported by Boyd et al. (2016), had also given the appropriate amount of total dissolved solids (TDS) in fish ponds was 400 ppm. Yanbo et al. (2006) found 0.3 mg/l nitrates in fresh water ponds were found suitable for Nile tilapia growth. Though Nile tilapia are well-known examples of freshwater fishes, some of its strains are euryhaline and can tolerate high salinity or even can grow and reproduce well if salinity reach > 36 ppt; however, the optimal growth and reproduction performance were attained at high at 19 ppt (El-Sayed,2006). Thus, tilapia fish has been suggested as marine origin fishes in terms of evolution (Beveridge and Mc Andrew, 2000).

Nowadays, Nile tilapia is considered a commercially critical cultural species as the other tilapia species because of its wide tolerant range of pH, 6.2-8.3, and even survive in low DO (dissolved oxygen) contents. That is ranged from 4.81 and 6.79 mg/L (Njiru et al. 2006; Yongco and Outa 2016). So, all concerns regarding the water parameters of fish ponds were directly proportional to their gross production. Thus, the physicochemical conditions of the fish pond have an enormous correlation with fish farming or fish culture (Bryan et al., 2011). A broad range of positive and negative impacting factors are independent of each other and are generally necessary for the optimal growth of fish. It has been reported that fish growths are highly dependent on feed consumption and quality. In addition, there are other biotic (i.e., age and sex, genetic vari- ances) and abiotic factors (i.e., stocking density, water temperature, dissolved amount of oxygen) (Emshland et al., 2007; Bhatnagar and Devi, 2013). Thus, it is essential to keep the DO level up to 5 ppm and salinity as 10 to 25 ppt for most euryhaline species, as suggested by Bhatnagar et al. (2004) Bhatnagar and Singh (2010) for supporting the fish production.

5. Conclusion

This study concluded that water parameters and dietary ALE supplements had shown a substantial effect on the growth, immune responses, and body composition of Nile tilapia O. niloticus. Thus, our study would also benefit in provoking information to the fish farmers about the culturing of good quality tilapia species and fulfils the protein requirement in human diets.

Data availability statement

All data analyzed during this study are included in this published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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