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# Influence of Fumaric Acid Fortified Diet on Growth Performance and Haematological Responses of Genetically Improved Farmed Tilapia

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**HM: Conducted the experiment, data analysis, interpretation and prepared original manuscript draft. PC: Conceptualized, designed and supervision of the study. PY: Reviewed and edited the manuscript. MJ: Assistance during experimental trial.**

**Key words**
**Fumaric acid, Feed utilization, Genetically Improved Farmed Tilapia, Growth performance, Haematology**

## ABSTRACT

An eight-week indoor feeding trial was carried out to determine the consequences of fumaric acid (FA) addition in the diet on growth performance, feed utilization and haematological responses of GIFT. Four isonitrogenous (28% of crude protein) diets were formulated with graded levels of dietary fumaric acid (0 (control), 0.5, 1.0 and 1.5 g/kg of diet). Each diet was allocated at random to three groups of thirty fish per experimental tanks, which were fed twice a day (09:00 and 17:00 h). Growth performance and feed utilization were improved in fish fed fumaric acid 1.5 g/kg of diet compared to control and other dietary treatments. Haemoglobin, RBC and haematocrit values were significantly ($p<0.05$) improved in fish fed fumaric acid 1.5 g/kg of diet compared to control. Overall, these findings suggest that fumaric acid (1.5 g/kg of diet) can be effectively utilized for improving growth performance and immunological status of GIFT, highlights its potential as a nutritional strategy for tilapia aquaculture.



## INTRODUCTION

Tilapia is one of the most important candidate fish species cultured in more than 120 countries and contributes more than 10% of worldwide fish production (FAO, 2020). It can tolerate a wide range of climatic conditions. At the same time, it has poor genetic management and inbreeding problems. To mitigate these problems genetically improved farmed tilapia (GIFT) has been produced through selective breeding of tilapia by World Fish Centre. GIFT is an improved strain of Nile tilapia with an enormous benefit for boosting yields and increasing nutritional benefits (Eknath and Hulata, 2009). However, Intensive tilapia farming is susceptible to disease (Machimbirike *et al*., 2019). At present, various chemical disinfectants, antibiotics, and sulfa drugs are repeatedly used in this sector in large quantities to control aquatic diseases by traditional disinfection and sterilization methods. The usage of these chemicals has many undesirable effects like environmental contamination and accumulation of toxic substances in fish muscles which could have an impact on human health and increase the spread of antibiotic-resistant strains by transferring resistant bacteria from the aquatic environment (Dawood and Koshio, 2020; Romero *et al*., 2012; Hoseinifar *et al*., 2017).

As a result, fish immune responses to invasive infections must be strengthened immediately, and ecologically benign immunostimulants should be used instead of antibiotics (Wang *et al*., 2017; Dawood *et al*., 2016). In this context, various organic acids have been utilized as immunostimulants in fish farming by several authors (Aalamifar *et al*., 2020; Abdel-Tawwab, 2016; Ahmed and Sadek, 2015; Da Silva *et al*., 2013, 2016; Nhan *et al*., 2010; Owen *et al*., 2006; Omosowone *et al*., 2015). Among various organic acids, fumaric acid (FA) is non-toxic organic acid. Since 1946, FA has been utilized as

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a food additive (Malone, 2000; Xie *et al*., 2003), because of its ability to reduce bacteria in feed, it has been used in food preservation (Luckstadt, 2008). Dietary organic acids have been used as alternatives to other feed additives in aquaculture diets to improve growth performance of many fish species (Ramli *et al*., 2005; Owen *et al*., 2006; Freitag and Lückstädt, 2007; Ng *et al*., 2009; Luckstadt and Kuhlmann, 2011). But the use of FA as an alternative to antibiotics in GIFT farming is not yet studied. Since the GIFT strain has better feed utilization, the present study hypothesized that GIFT would reveal a better immune response than Nile tilapia. Hence, the present study is aimed to evaluate the influence of FA incorporated diet on the growth performance and immune response of GIFT.

## MATERIALS AND METHODS

*Experimental fish and feeding trial*

Healthy and uniform sized 500 numbers of GIFT fry (average weight of 0.05±0.02 g and average length of 2.03±0.21 cm) were procured from Government Fish Seed Farm, Krishnagiri, Tamil Nadu, India and transferred to the Advanced Research Farm Facility, Madhavaram, Tamilnadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India. The fishes were acclimatized and conditioned for 30 days and fed with a commercial feed containing crude protein at 280 g/kg of diet. After acclimatization, 360 fishes with an initial mean body weight of 2.0±0.3 g were randomly distributed in 12 FRP tanks, with 30 fish per FRP tanks (500 lit capacity), with three replicates per diet. During the 60-day feeding trial, the fish were fed until apparent satiation two times per day (09:00 and 17:00). Water was exchanged at the rate of 10% every day in each FRP tank. Aeration was continuously provided throughout the experimental period using an air blower (Hailea hi-flow diaphragm air pump). During the feeding trial, water quality parameters were monitored daily and the mean values were as follows: water temperature 27±1°C, pH 8.4±0.6, dissolved oxygen 5±0.6 mg/L and ammonia –N 0.03±0.01 ppm.

*Experimental diets*

Using a vertical ingredient mixer (Jinan Sunpring Machinery Co Ltd, China), dietary ingredients were finely ground and fully blended and then extruded at 60 to 70°C to prepare 1.5 mm floating pellets using a twin-screw extruder. A total of four experimental diets were formulated in this present study including one control diet without inclusion of FA and three treatment diets with graded levels of FA at 0.5, 1.0 and 1.5 g/kg of diet which were named FA 0.5, FA 1.0 and FA 1.5, respectively (Table I). A commercially available FA was procured from Hi media. All the experimental diets were stored in air tight plastic containers and kept at room temperature.

**Table I. Formulation and biocomposition of the experimental diets (Kg of diet).**

| Ingredients | Control | FA 0.5 g/kg | FA 1.0 g/kg | FA 1.5 g/kg |
|---|---|---|---|---|
| Fish meal | 80 | 80 | 80 | 80 |
| Soybean meal | 100 | 100 | 100 | 100 |
| Corn gluten meal | 150 | 150 | 150 | 150 |
| Corn flour | 180 | 180 | 180 | 180 |
| Wheat flour | 100 | 100 | 100 | 100 |
| Rice bran (Defat) | 388 | 387.5 | 387 | 386.5 |
| Vitamin premix[a] | 1 | 1 | 1 | 1 |
| Mineral premix[b] | 1 | 1 | 1 | 1 |
| Fumaric acid | 0 | 0.5 | 1 | 1.5 |
| **Chemical composition (% dry matter)** | | | | |
| Moisture | 8.6 | 8.8 | 8.7 | 8.8 |
| Crude protein | 27.79 | 27.48 | 27.81 | 27.61 |
| Crude lipid | 5.75 | 5.6 | 5.9 | 5.8 |
| Crude fibre | 2.78 | 2.6 | 2.41 | 2.52 |
| Ash | 8.78 | 8.56 | 8.5 | 8.8 |

[a]Composition of vitamin premix (quantity per kg): Vit. A – 1, 00, 00,000 IU, Vit. B1-5,000 mg, Vit. B2 – 5,000 mg, Vit. B3- 6,000 mg, Vit. B5 – 6,000 mg, Vit. B6 – 6,000 mg, Vit. C – 60,000 mg, Vit. D3 – 20,00,000 IU, Vit. E – 10,000 EU, Vit. H – 200 mg. [b]Composition of mineral premix (quantity per kg): Magnesium – 2,800 mg, Iodine – 7.4 mg, Iron – 7,400 mg, Copper – 1,200 mg, Manganese – 11,600 mg, Zinc – 9,800 mg, Chlorides Cobalt – 4 mg, Potassium – 100 mg, Selenium – 4 mg, Calcium Carbonate – 27.25%, Phosphorous – 7.45 mg, Sulphur – 0.7 mg, Sodium – 6 mg, Calpan – 200 mg, Aluminium – 1,500 mg and Choline Chloride – 10,000 mg. FA, Fumaric acid.

*Growth parameter and nutrient utilization*

At the end of trial, all the fishes were individually counted and weighed to estimate their weight gain (WG), feed conversion ratio (FCR), feed efficiency ratio (FER), average daily gain (ADG), specific growth rate (SGR), protein efficiency ratio (PER) and survival rate (SR) were calculated according to Raj *et al*. (2008).

*Haematological assay*

At the end of the feeding trial (60th day), blood sample was collected from the caudal vein of four fish from each treatment after they were starved for 24 h. The red blood cell (RBC) counts were determined by a Neubauer hematocytometer. Haemoglobin (Hb) concentrations were determined by Cyanmethaemoglobin method (Drabkin, 1946) and haematocrit (Ht) was determined by the microhematocrit method (Nelson and Morris, 1989).

According to Wintrobe (1934) the erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated.

*Statistical analysis*

All the data were presented as the mean values ± standard deviation (SD) of three replicates. All the percentage values were subjected to arcsine transformation prior to statistical analysis. One-way ANOVA, followed by Duncan's test at the significant level of 0.05 was used to compare the differences among the four dietary groups. The data were statistically analysed by SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

*Growth performance and nutrient utilization*

The dietary supplementation of FA in the diets of GIFT significantly improved the growth performance and feed utilization. FA plays an important role in improving the growth performance, nutrient utilization and immune enhancement (Owen *et al*., 2006; Omosowone *et al*., 2015; Luckstadt and Kuhlmann, 2011). Similarly in the present study, the highest mean WG of GIFT was recorded in the fish fed with FA 1.5 g/kg of diet followed by FA 1 g/kg and FA 0.5 g/kg. There was a significant WG from lower to higher concentration of FA 0.5 g/kg to FA 1.5 g/kg. The highest SGR of GIFT was recorded in the fish fed with FA 1.5 g/kg of diet followed by FA 1 g/kg and FA 0.5 g/kg. There was a significant difference between FA 0.5 and FA 1.5 g/kg and lowest was observed in control. Previously, Das Neves *et al*. (2021) reported an improvement in growth, feed efficiency of Nile tilapia juveniles fed up to 15 g/kg FA diets. Similarly, Abd-Elala and Ragaa (2015) reported that *O. niloticus* fed on 0.2% organic acid showed significant improvements in their feed intake, live WG, SGR, PER and FCR compared to control. Similarly enhanced nutrient and mineral digestibility leading to maximum growth performance in GIFT was reported by Luckstadt and Kuhlmann (2011).

Increase in the dietary FA level also resulted in a significant increase in growth rate and lower FCR. The FA 1.5 g/kg of diet produced better WG and lower FCR than FA 1.0 g/kg of diet in the present study given in Table II. In contradictory, Omosowone *et al*. (2015) observed better growth performance in African catfish *Clarias gariepinus* when the FA level is supplemented at FA 1.0 g/kg and growth rate decreased with increasing addition of FA. The study showed lowest growth performance was observed with the diet containing 0.5 mg/kg of FA in GIFT. Overall, the present study revealed best growth performance of GIFT at 1.5 g/kg of FA incorporated diet.

*Haematology parameters*

Haematological characteristics are used as an effective and sensitive index to screen physiological and pathological changes in fishes. The normal ranges of various blood parameters in GIFT have been reported by different investigators (Prabu *et al*., 2020; Sathishkumar *et al*., 2021). However, FA supplemented diet fed GIFT haematological response not yet documented. Hence, the present study also intended to analyse the haematological changes of GIFT when fed with FA incorporated diet.

The results of the FA diet showed increase in haematological value of GIFT fed with FA 1.5 g/kg of diet when compared to other experimental diets (Table III). FA 1.5 g/kg diet fed fish showed significantly ($p<0.05$) higher RBC, haematocrit, Hb, MCV and MCHC value compared to control, significant difference was noticed within the treatments.



**Table II. Growth performances of GIFT fed graded levels fumaric acid (FA) supplemented diets.**

| Parameters | Control | FA 0.5 g/kg | FA 1 g/kg | FA 1.5 g/kg |
|---|---|---|---|---|
| Initial weight (g) | 2.08±0.04 [a] | 2.09±0.06 [a] | 2.09±0.01 [a] | 2.08±0.04 [a] |
| Final weight (g) | 6.33±0.31 [b] | 6.54±0.13 [b] | 6.56±0.17 [b] | 7.80±0.16 [a] |
| Weight gain (WG, g) | 4.25±0.30 [b] | 4.45±0.14 [b] | 4.48±0.17 [b] | 5.72±0.17 [a] |
| Feed conversion ratio (FCR) | 3.14±0.21 [a] | 2.98±0.09 [a] | 2.97±0.11 [a] | 2.32±0.07 [b] |
| Feed efficiency ratio (FER) | 0.32±0.02 [b] | 0.34±0.01 [b] | 0.34±0.01 [b] | 0.43±0.01 [a] |
| Average daily growth (ADG, g/animal/day) | 0.071±0.004 [b] | 0.074±0.002 [b] | 0.075±0.002 [b] | 0.095±0.002 [a] |
| Specific growth rate (SGR) (%) | 1.85±0.08 [b] | 1.90±0.06 [b] | 1.90±0.05 [b] | 2.20±0.04 [a] |
| Protein efficiency ratio (PER) | 1.16±0.08 [b] | 1.21±0.04 [b] | 1.23±0.05 [b] | 1.59±0.05 [a] |
| Survival rate (%) | 95.00±5.00 [a] | 98.33±1.52 [a] | 96.33±3.21 [a] | 96.00±1.73 [a] |

Values were expressed as means ± SD of three replicate trough per treatment (n=3) and values with different superscripts indicate significant differences as determined by Duncan test ($p < 0.05$).

**Table III. Hematological responses of GIFT tilapia fed graded levels of fumaric acid supplemented diets.**

| Parameters | Control | FA 0.5 g/kg | FA 1 g/kg | FA 1.5 g/kg |
|---|---|---|---|---|
| RBC (million/ cu mm) | 1.39±0.21 $^{b}$ | 1.54±0.32$^{ab}$ | 2.04±0.35 $^{a}$ | 2.05±0.3$^{2\ a}$ |
| Haemoglobin (g/dl) | 5.02±0.68 $^{c}$ | 4.47±0.65$^{bc}$ | 5.80±0.66 $^{ab}$ | 6.43±0.6$^{5\ a}$ |
| Haematocrit (%) | 19.30±2.86$^{b}$ | 21.60±0.91 $^{b}$ | 26.40±1.93 $^{a}$ | 27.47±0.9$^{7\ a}$ |
| MCV (fl) | 134.83±6.75$^{b}$ | 138.43±4.68 $^{ab}$ | 137.37±5.76$^{ab}$ | 148.03±6.0$^{5\ a}$ |
| MCH (pg) | 33.77±1.60$^{a}$ | 34.47±1.76 $^{a}$ | 34.73±2.10 $^{a}$ | 35.63±1.3$^{2\ a}$ |
| MCHC (g/dl) | 24.56±1.73$^{b}$ | 23.17±1.30$^{ab}$ | 27.90±1.51 $^{ab}$ | 29.50±1.5$^{1\ a}$ |

Values were expressed as means ± SD of three replicate troughs per treatment (n=3), and values with different superscripts indicate significant differences as determined by Duncan test ($p$ <0.05).

The haematological findings of the present study is in agreement with Reda *et al*. (2016) reported in which inclusion of FA diet significantly increased total erythrocyte count, Hb, haematocrit and MCH in *O. niloticus*. In the current study, FA might have increased the immunomodulatory effect in GIFT body through the highest liberation of calcium, phosphorous, iron, and copper from the feed constituents, particularly FA complexes. Similarly, Omosowone *et al*. (2015) also reported that FA incorporated diet fed African catfish showed significantly (p<0.05) higher immune response.

## CONCLUSION

The present study conclude that genetically improved farmed tilapia fed with 1.5 g/Kg of FA incorporated diet significantly improved the growth, nutrient utilization and haematological response. Further study is recommended to test the maximum inclusion level of FA in the diet of GIFT.

## DECLARATIONS

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*IRB approval*

This research work has been approved by Advisory committee and Institutional Review Board of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India.

*Ethical statement*

The experiment was conducted following the procedures of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Government of India on care and use of animals in scientific research. The study was approved by Ethical Committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.

*Statement of conflict of interest*

The authors have declared no conflict of interest.



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