Effects of dietary butyric acid on growth, digestive enzyme activity, and haematological responses in GIFT Tilapia

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Abstract

A 60-day indoor feeding trial was conducted to evaluate the effects of dietary butyric acid (BA) supplementation on growth performance, survival, feed utilization, digestive enzyme activities, haematological responses, and whole-body composition of Genetically Improved Farmed Tilapia (GIFT). Four isonitrogenous diets containing 28% crude protein were formulated with graded levels of BA at 0 (control), 0.5, 1.0, and 1.5 g / kg diet. Each diet was randomly assigned to triplicate groups of 30 fish per tank, and fish were fed twice daily at 09:00 and 17:00 hours. Results showed that fish fed the diet supplemented with 1.0 g BA exhibited significantly improved growth performance and feed utilization compared to the control and other treatment groups. Higher amylase and protease activities were observed in fish fed 1.0 g BA, while lipase activity was highest in fish fed 1.5 g BA. Haematological parameters, including haemoglobin concentration, red blood cell (RBC) count, and haematocrit values, were significantly increased ($P<0.05$) in fish fed the 1.0 g BA diet. However, dietary supplementation of BA had no significant effect ($P>0.05$) on the whole-body proximate composition of GIFT. Overall, the study concludes that dietary supplementation with BA at 1.0 g / kg diet can effectively enhance the growth performance, digestive efficiency, and immune health of GIFT without altering body composition.

Keywords: Tilapia, Butyric Acid, Growth Performance, Body Composition, Digestive Enzymes, Haematology.

Introduction

Tilapia is one of the most important aquaculture species, farmed in over 120 countries worldwide. Global aquaculture production reached a record 103 million tonnes in 2024, with aquaculture now contributing more than 53% of total aquatic animal production (FAO, 2026). Nile tilapia (*Oreochromis niloticus*) is known for its tolerance to a wide range of climatic conditions; however, its culture is challenged by poor genetic management and inbreeding issues (Perazza et al., 2025). To address these limitations, the Genetically Improved Farmed Tilapia (GIFT) strain was developed through selective breeding by the WorldFish Centre, Malaysia (WorldFish, 2023). GIFT, an enhanced strain of Nile tilapia, offers substantial advantages in yield and nutritional value, with genomic tools now being applied to accelerate further genetic gains in growth, disease resistance, and environmental tolerance (Ponzoni et al., 2011). In India, GIFT tilapia was evaluated at the ICAR-Central Institute of Freshwater Aquaculture (CIFA) between 1998 and 2000, demonstrating production levels of 5–6 MT per crop over a 4–6-month culture period and superior growth over Indian major carps under polyculture conditions (Arumugam et al.,2023). Following the establishment of the GIFT Satellite Breeding Program by the Rajiv Gandhi Centre for Aquaculture (RGCA), under the Marine Products Export Development Authority (MPEDA), in collaboration with WorldFish, GIFT dissemination has expanded considerably, with over 10 million mono-sex GIFT juveniles produced and distributed to more than 1,600 licensed farmers across India by 2020 (Gaikwad, 2021). The Government of India, through the Pradhan Mantri Matsya Sampada Yojana (PMMSY), aims to scale national tilapia production to 2.4 million metric tonnes by 2030, positioning GIFT as a key candidate species for diversifying freshwater aquaculture (Gaikwad, 2021). Despite these improvements, intensive GIFT farming remains vulnerable to disease outbreaks (Machimbirike et al., 2019). Traditionally, the aquaculture industry has relied heavily on chemical disinfectants and antibiotics to control aquatic diseases. However, the excessive use of these substances has raised significant concerns, including environmental contamination, accumulation of toxic residues in fish tissue, and the promotion of antibiotic-resistant bacteria that pose threats to both aquatic ecosystems and human health (Romero et al., 2012; Hoseinifar et al., 2017; Dawood and Koshio, 2020). These challenges underscore the urgent need for environmentally safe alternatives to enhance fish immunity against pathogens without the adverse effects associated with antibiotics (Dawood et al., 2016; Wang et al., 2017).

Among the various alternatives explored, organic acids, particularly short-chain fatty acids (SCFAs), have shown promise in improving fish health (Ahmed and Sadek, 2015; Omosowone et al., 2018; Aalamifar et al., 2020; Osepchuk et al., 2021). Butyric acid (BA), a SCFA produced through carbohydrate fermentation by anaerobic gut bacteria, has received increasing attention due to its beneficial effects on gut health (Bedford and Gong, 2018). Studies in tilapia suggest that fish intestinal microbiota are capable of fermenting carbohydrates to release SCFAs, including BA, which may help protect fish from pathogenic bacteria (Ghazali et al., 2025). In particular, Youssuf et al. (2025) demonstrated that dietary supplementation with BA improved growth and immune responses in Nile tilapia. However, the application of BA as an alternative to antibiotics in GIFT farming remains unexplored. Given that the GIFT strain exhibits superior feed utilization, enzyme activity, and growth performance, it is hypothesized that GIFT may also exhibit a stronger immune response to BA supplementation compared to conventional Nile tilapia. Therefore, the present study aims to evaluate the effects of a BA incorporated diet on the growth performance, immune response, and health status of GIFT.

Materials and methods

Experimental fish and feeding trial

A total of 500 healthy and uniform-sized fry of GIFT, with an average weight of 0.055 ± 0.024 g and average length of 2.03 ± 0.21 cm, were procured from the Government Fish Seed Farm, Krishnagiri, Tamil Nadu, India. The fry were transported to the Advanced Research Farm Facility, Madhavaram, Tamil Nadu, India (13.1549255°N, 80.2463273°E), where they were acclimatized and conditioned for 30 days. The study was conducted from January to March, corresponding to the winter season in the region. During this period, the fish were fed a commercial diet containing 28% crude protein. After acclimatization, 360 fish with an initial mean body weight of 2.0 ± 0.3 g were randomly distributed into 12 FRP tanks (500 L capacity), with 30 fish per tank and three replicates per dietary treatment, following the method described by Prabu et al. (2020). The feeding trial lasted for 60 days, during which fish were fed to apparent satiation twice daily at 09:00 and 17:00 hours. Daily water exchange was maintained at a rate of 10% in each tank, and continuous aeration was provided using a Hailea high-flow diaphragm air pump. Water quality parameters were monitored daily, and the average values recorded during the trial were: temperature 27 ± 1°C, pH 8.5 ± 0.6, dissolved oxygen 5 ± 0.4 mg/L, and ammonia-N 0.03 ± 0.01 mg/L.

Experimental diets

Dietary ingredients were finely ground and thoroughly mixed using a vertical ingredient mixer (Jinan Sunpring Machinery Co. Ltd, China). The mixed feed was then extruded at a temperature range of 80 to 120°C to

produce 1.5 mm floating pellets using a twin-screw extruder, following the method of Prabu et al. (2020). In total, four experimental diets were formulated for the study. This included one control diet without BA and three treatment diets supplemented with graded levels of BA at 0.5, 1.0, and 1.5 g / kg diet, designated as BA 0.5, BA 1.0, and BA 1.5, respectively (Table 1). The BA used in the diets was a commercially available product procured from HiMedia, Mumbai, India. All prepared experimental diets were stored in airtight plastic containers and maintained at room temperature.

Growth performance and feed utilization

At the end of the feeding trial, all fish were individually counted and weighed to evaluate growth performance and feed utilization parameters. The following metrics were calculated: weight gain, feed conversion ratio (FCR), feed efficiency ratio (FER), average daily gain (ADG), specific growth rate (SGR), protein efficiency ratio (PER), and survival rate.

The growth parameters were calculated using the following formulas (Sathishkumar et al., 2021):

Weight gain (g) = Final weight – Initial weight

Feed Conversion Ratio (FCR) = Dry feed fed (g) / weight gain (g)

Feed Efficiency Ratio (FER) = 1/ FCR

Average daily gain (ADG) (g) = Final weight (g) – Initial weight (g) / Experimental duration

Specific Growth Rate (SGR) (% day^{-1}) = Ln Final weight (g) – Ln Initial weight (g) / Experimental duration × 100

Protein Efficiency Ratio (PER) = Body weight gain (g) / protein intake (g)

Survival (%) = Total number of fish survived / Total number of fish stocked ×100

Digestive enzyme study

Intestinal samples from the fish were homogenized following the procedure described by Gisbert et al. (2016) and stored at –21°C until further analysis. Amylase activity was measured using the method of Bernfeld (1955), and protease activity was determined according to Kunitz (1947), both using a spectrophotometer (Systronics 106) at wavelengths of 560 nm and 280 nm, respectively. Lipase activity was assessed following the method of Cherry and Crandall (1932).

Haematological assay

At the end of the 60-day feeding trial, blood samples (approximately 1 mL) were collected from the caudal vein of four randomly selected fish per treatment group after a 24-hour fasting period. The red blood cell (RBC) count was determined using a Neubauer hemocytometer. Haemoglobin (Hb) concentration was measured using the Cyanmethemoglobin method (Drabkin, 1946), while haematocrit (Ht) was determined by the microhematocrit method (Nelson and Morris, 1989). Erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), were calculated according to Wintrobe (1934) using the following formulas:

MCV (fl) = (Ht×10) / erythrocytes

MCH (pg) = (Hb×10) / erythrocytes

MCHC (g/dl) = (Hb×100) / Ht

Table 1. Formulation and proximate composition of the experimental diets (kg diet)

Ingredients	Control	BA 0.5 g/kg	BA 1.0 g/kg	BA 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 (Defatted)	388	387.5	387	386.5
Vitamin premix[a]	1	1	1	1
Mineral premix[b]	1	1	1	1
Butyric acid	0	0.5	1	1.5
Proximate composition (% dry weight)				
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

Whole-Body Proximate Composition Analysis

The whole-body proximate composition of experimental fish was analysed at the end of the 60-day feeding trial following standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2010). Crude protein content was determined using the Kjeldahl method (Kjeltron, Tulin Equipments), crude lipid by the Soxhlet extraction method (SoxTRON SOX-2, Tulin Equipments), and crude fibre by an analytical method (FibroTRON FRB-2, Tulin Equipments). Moisture content was measured using a hot air oven, and ash content was determined using a muffle furnace.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) of three replicates. Prior to statistical analysis, percentage data were subjected to arcsine transformation to meet the assumptions of normality. One-way analysis of variance (ANOVA) was performed to determine significant differences among the four dietary treatment groups, followed by Duncan's multiple range test at a significance level of $P<0.05$. Statistical analyses were conducted using SPSS version 26.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Growth performance and nutrient utilization

In the present study, GIFT fed with the BA 1.0 diet exhibited significantly ($p<0.05$) higher final weight, weight gain, ADG, SGR, and FER compared to those fed the control and BA 0.5 diets, but not significantly different from the BA 1.5 group (Table 2). Notably, the FCR was significantly ($p<0.05$) lower in the BA 1.0 group than in the control and BA 0.5 groups, indicating better feed utilization. However, no significant difference ($p>0.05$) was observed in FCR between the BA 1.0 and BA 1.5 diets. These findings align with those of Youssuf et al. (2025), who reported improved weight gain and a better FCR (2.83) in Nile tilapia fed a diet fortified with 0.3 g BA / kg diet. However, the current results differ from those of Omosowone et al. (2018), where a 1.5 g BA / kg diet resulted in the highest growth in Nile tilapia. In contrast, our study found that a lower level (1.0 g BA / kg diet) led to the best growth in GIFT, suggesting that GIFT may utilize feed more efficiently and exhibit higher growth potential than conventional Nile tilapia. The PER was significantly ($p<0.05$) higher in fish fed the BA 1.0 diet compared to the control and BA 0.5 groups. This result is supported by Zhou et al. (2019), who observed improved PER in golden pompano fed a diet containing 2 g sodium butyrate/kg diet. Interestingly, the present study showed even higher PER values, potentially due to better palatability and attractiveness of BA compared to sodium butyrate, as previously reported by Abdel-Latif et al. (2020). Survival rates did not differ significantly ($p>0.05$) among treatments (Table 2). Overall, the present study demonstrates that dietary inclusion of 1.0 g BA / kg diet significantly improves growth performance, feed efficiency, and protein utilization in GIFT. Similar growth-promoting effects of BA have been documented in various aquatic species, including *Clarias gariepinus*, *Macrobrachium rosenbergii, Sparus aurata*, *Carassius auratus*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Lates calcarifer*, and *Oreochromis niloticus* (Robles et al., 2013; Sun et al., 2013; Liu et al., 2014; Liu et al., 2017; Aalamifar et al., 2020; Youssuf et al., 2025).

Digestive enzyme activities

Digestive enzyme activities of GIFT fed with varying levels of BA are presented in Table 3. Amylase and protease activities were significantly higher ($p<0.05$) in fish fed the BA 1.0 diet than in the control and other treatment groups, consistent with reports of increased amylase and protease activity in golden pompano fed 2 g sodium butyrate/kg diet (0.613 U/mg and 16.6 U/mg, respectively; Zhou et al., 2019) and enhanced protease activity in seabass fed 5 g BA/kg diet (Aalamifar et al., 2020). Lipase activity increased with higher dietary BA levels, with the BA 1.5 group showing significantly higher activity than the control, though it did not differ significantly from the BA 1.0 group. Overall, the inclusion of 1.0 g BA/kg diet effectively enhanced amylase and protease activities in GIFT, while 1.5 g / kg promoted higher lipase activity. This may be associated with improved growth performance in GIFT. It has been hypothesized that organic acids like BA can enhance digestive enzyme activity by lowering the pH of the digesta (Castillo et al., 2014). The mild acidifying effect of BA could have stimulated intestinal enzyme activities. Supporting this, recent studies confirm that dietary organic acids enhance digestive enzyme activity and intestinal morphology in Nile tilapia by lowering gut pH and stimulating enzyme secretion (Ruenkoed et al., 2025).

Haematological parameters

Haematological parameters serve as effective and sensitive indicators for detecting physiological and pathological changes in fish. The normal ranges of various blood parameters in GIFT have been reported by several researchers (Prabu et al., 2020; Sathishkumar et al., 2021). However, the haematological responses of GIFT to BA-supplemented diets have not yet been thoroughly investigated. In the present study, the haematological changes in GIFT fed with BA-incorporated diets were evaluated. Fish fed the 1 g BA/kg diet showed significantly ($p<0.05$) higher

Table 2. Growth performance of GIFT fed diets supplemented with graded levels of butyric acid

Parameters	Control	BA 0.5 g/kg	BA 1 g/kg	BA 1.5 g/kg
Initial weight (g)	2.08±0.04 [a]	2.09±0.03 [a]	2.08±0.06 [a]	2.08±0.02 [a]
Final weight (g)	6.33±0.31 [b]	6.49±0.30 [b]	8.27±0.83 [a]	7.27±0.64 [ab]
Weight gain (g)	4.25±0.30 [b]	4.40±0.30 [b]	6.19±0.87 [a]	5.19±0.65 [ab]
Feed Conversion Ratio	3.14±0.21 [a]	3.02±0.20 [ab]	2.18±0.33 [c]	2.59±0.35 [bc]
Feed Efficiency Ratio	0.32±0.02 [b]	0.33±0.02 [b]	0.45±0.07 [a]	0.39±0.05 [ab]
Average Daily Gain (g)	0.0708±0.005 [b]	0.0734±0.005 [b]	0.1031±0.015 [a]	0.0865±0.011 [ab]
Specific Growth Rate (% day^{-1})	1.85±0.07 [b]	1.89±0.08 [b]	2.29±0.20 [a]	2.07±0.16 [ab]
Protein Efficiency Ratio	1.16±0.08 [b]	1.22±0.09 [b]	1.70±0.25 [a]	1.43±0.18 [ab]
Survival (%)	95.00±5.00 [a]	95.33±5.03 [a]	98.33±2.89 [a]	98.67±1.15 [a]

Values were expressed as mean ± SD (n=3) and values with different superscripts indicate significant differences ($p<0.05$)

Table 3. Digestive enzyme activities of GIFT fed diets supplemented with graded levels of butyric acid

Enzyme activities	Control	BA 0.5 g/kg	BA 1.0 g/kg	BA 1.5 g/kg
Amylase (U/mg protein)	0.537±0.015 [c]	0.550±0.005 [c]	0.600±0.001 [a]	0.577±0.001 [b]
Protease (U/mg protein)	0.224±0.025 [c]	0.296±0.003 [b]	0.379±0.018 [a]	0.324±0.008 [b]
Lipase (U/mg protein)	0.349±0.061 [b]	0.434±0.05 [ab]	0.441±0.054 [ab]	0.550±0.076 [a]

Values were expressed as mean ± SD (n=3), and values with different superscripts indicate significant differences ($p<0.05$).

Table 4. Haematological responses of GIFT fed diets supplemented with graded levels of butyric acid

Parameters	Control	BA 0.5 g/kg	BA 1.0 g/kg	BA 1.5 g/kg
RBC (million/ cu mm)	1.39±0.21 [b]	1.82±0.81 [ab]	2.13±0.31 [a]	1.64±0.32 [ab]
Haemoglobin (g/dl)	5.02±0.68 [c]	6.73±0.57 [ab]	7.80±0.46 [a]	5.87±0.45 [ab]
Haematocrit (%)	19.30±2.86 [b]	26.80±3.30 [a]	30.77±3.15 [a]	25.63±1.30 [a]
MCV (fl)	134.83±6.75 [c]	147.10±6.60 [b]	159.80±2.81 [a]	156.67±3.05 [ab]
MCH (pg)	33.77±1.60 [b]	37.33±2.50 [b]	43.77±2.15 [a]	35.97±2.15 [b]
MCHC (g/dl)	24.56±1.73 [b]	25.37±3.15 [ab]	27.83±1.25 [a]	26.42±2.40 [ab]

Values were expressed as mean ± SD (n=3), and values with different superscripts indicate significant differences ($p<0.05$).

Table 5. Whole body proximate composition (% dry weight) of GIFT fed diets supplemented with graded levels of butyric acid

Parameters	Control	BA 0.5 g/kg	BA 1.0 g/kg	BA 1.5 g/kg
Moisture (%)	5.46±0.51 [a]	5.5±0.54 [a]	4.64±0.57 [a]	4.9±0.41 [a]
Crude protein (%)	51.40±2.15 [a]	51.56±2.13 [a]	52.03±4.39 [a]	51.35±2.14 [a]
Crude fat (%)	14.25±0.99 [a]	14.54±0.95 [a]	14.60±0.61 [a]	14.48±1.58 [a]
Crude fibre (%)	1.88±0.19 [a]	1.96±0.44 [a]	1.72±0.50 [a]	1.99±0.34 [a]
Ash (%)	30.20±3.17 [a]	30.0±3.17 [a]	31.29±3.03 [a]	31.35±2.04 [a]

Values were expressed as mean ± SD (n=3), and values with same superscripts indicate no significant differences ($p>0.05$).

RBC count, haematocrit, haemoglobin, and MCHC than the control group, with haematological values increased overall relative to the other experimental diets (Table 4). However, no significant differences were observed among the other treatment groups. These findings are consistent with the report by Reda et al. (2016), where dietary inclusion of formic acid significantly improved erythrocyte count, haemoglobin level, haematocrit, and mean corpuscular haemoglobin in *O. niloticus*. In the current study, the observed improvement in haematological parameters may be attributed to the immunomodulatory effects of BA, potentially enhanced by the presence of mineral mixtures (such as calcium, phosphorus, iron, and copper) in the feed constituents. Similarly, Aalamifar et al. (2020) reported that *L. calcarifer* fed with BA-supplemented diets also showed significantly ($p<0.05$) enhanced immune responses.

Whole body proximate composition

Dietary BA supplementation had no significant effect ($P>0.05$) on the whole-body proximate composition (moisture, crude protein, crude fat, crude fibre, and ash) of GIFT (Table 5), although fish fed 1 g BA/kg diet showed a marginally higher crude protein content than the other groups, consistent with the improved PER and protease activity observed in this study. These findings agree with Omosowone et al. (2018), who reported a similar increase in carcass crude protein in *O. niloticus* fed BA-supplemented diets. Overall, BA supplementation in the present study did not exert any negative effects on the whole-body composition of GIFT. On the contrary, it demonstrated positive impacts on growth performance and immune response, suggesting potential benefits of BA inclusion at appropriate dietary levels.

Conclusion

The present study revealed that dietary inclusion of BA at 1g/kg diet was efficiently utilized by GIFT and significantly improved their growth performance. Additionally, BA supplementation enhanced haematological responses and intestinal digestive enzyme activity, indicating a positive impact on fish health and nutrient utilization. However, no significant changes were observed in the whole-body proximate composition, suggesting that BA did not adversely affect the nutritional quality of the fish.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Ethical statement

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

References

1) Aalamifar, H., Soltanian, S., Vazirzadeh, A., Akhlaghi, M., Morshedi, V., Gholamhosseini, A., Torfi Mozanzadeh, M. (2020). Dietary butyric acid improved growth, digestive enzyme activities and humoral immune parameters in Barramundi (*Lates calcarifer*). Aquaculture Nutrition, 26(1): 156-164. https://doi.org/10.1111/anu.12977
2) Abdel-Latif, H. M., Abdel-Tawwab, M., Dawood, M. A., Menanteau-Ledouble, S., El-Matbouli, M. (2020). Benefits of dietary butyric acid, sodium butyrate, and their protected forms in aquafeeds: a review. Reviews in Fisheries Science & Aquaculture, *28*(4):421-448. https://doi.org/10.1080/23308249.2020.1758899
3) Ahmed, H. A., & Sadek, K. M. (2015). Impact of dietary supplementation of sodium butyrate and/or protexin on the growth performance, some blood parameters, and immune response of *Oreochromis niloticus*. International Journal of Agriculture Innovations and Research, 3(4): 985-991.
4) AOAC, 2010. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
5) Arumugam, M., Jayaraman, S., Sridhar, A., Venkatasamy, V., Brown, P. B., Abdul Kari, Z., ... & Ramasamy, T. (2023). Recent advances in tilapia production for sustainable developments in Indian aquaculture and its economic benefits. Fishes, *8*(4): 176. https://doi.org/10.3390/fishes8040176
6) Bedford, A., Gong, J. (2018). Implications of butyrate and its derivatives for gut health and animal production. Animal Nutrition, 4(2): 151-159. https://doi.org/10.1016/j.aninu.2017.08.010
7) Bernfeld, P. (1955). [17] Amylases, α and β. Methods Enzymol 1:149–158. http://dx.doi.org/10.1016/0076-6879(55)01021-5
8) Castillo, S., Rosales, M., Pohlenz, C., & Gatlin III, D. M. (2014). Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum *Sciaenops ocellatus*. Aquaculture, 433: 6-12. https://doi.org/10.1016/j.aquaculture.2014.05.038
9) Cherry, I. S., Crandall Jr, L. A. (1932). The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. American Journal of Physiology-Legacy Content, 100(2): 266-273. https://doi.org/10.1152/ajplegacy.1932.100.2.266
10) Dawood, M. A., Koshio, S. (2020). Application of fermentation strategy in aquafeed for sustainable aquaculture. Reviews in Aquaculture, *12*(2): 987-1002. https://doi.org/10.1111/raq.12368
11) Dawood, M. A., Koshio, S., Ishikawa, M., Yokoyama, S., El Basuini, M. F., Hossain, M. S., Moss, A. S. (2016). Effects of dietary supplementation of Lactobacillus rhamnosus or/and Lactococcus lactis on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. Fish & Shellfish Immunology, 49: 275-285. https://doi.org/10.1016/j.fsi.2015.12.047
12) Drabkin, D. L. (1946). Spectrophotometric studies: XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. Journal of biological chemistry, 164(2): 703-

723. https://doi.org/10.1016/S0021-9258(17)41272-5
13) Food and Agriculture Organization (FAO), 2026. The State of World Fisheries and Aquaculture 2026 (SOFIA 2026). Food and Agriculture Organization of the United Nations, Rome.
14) Gaikwad, A., Padiyar, A. P., Datta, S., Shikuku, K. M., Mohan, C. V., Trong, T. Q., Phillips, M. (2021). Dissemination and scaling strategy for genetically improved farmed tilapia (GIFT) in India, 2020–2030. Monographs
15) Ghazali, S. Z., Gani, N. A. S. M., Madiran, N. A., Ismail, N., Hasan, V., Novriadi, R., Kamarudin, A. S. (2025). Metagenomic analysis of gut microbiome in Nile tilapia, *Oreochromis niloticus*: Insights from cultured and lake populations. Egyptian Journal of Aquatic Research. https://doi.org/10.1016/j.ejar.2025.10.005.
16) Gisbert, E., Mozanzadeh, M. T., Kotzamanis, Y., Estévez, A. (2016). Weaning wild flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish meal substitution. Aquaculture, 462: 92-100. https://doi.org/10.1016/j.aquaculture.2016.04.035
17) Hoseinifar, S. H., Sun, Y. Z., Caipang, C. M. (2017). Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquaculture Research, 48(4): 1380-1391. https://doi.org/10.1111/are.13239
18) Kunitz, M. (1947). Crystalline soybean trypsin inhibitor: II. General properties. The Journal of General Physiology, 30(4): 291-310. https://doi.org/10.1085/jgp.30.4.291
19) Liu, M., Guo, W., Wu, F., Qu, Q., Tan, Q., Gong, W. (2017). Dietary supplementation of sodium butyrate may benefit growth performance and intestinal function in juvenile grass carp (*Ctenopharyngodon idellus*). Aquaculture Research, 48(8): 4102-4111. https://doi.org/10.1111/are.13230
20) Liu, W., Yang, Y., Zhang, J., Gatlin, D. M., Ringø, E., Zhou, Z. (2014). Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil. British Journal of Nutrition, 112(1): 15-29.
21) Machimbirike, V. I., Jansen, M. D., Senapin, S., Khunrae, P., Rattanarojpong, T., Dong, H. T. (2019). Viral infections in tilapines: More than just tilapia lake virus. Aquaculture, 503: 508-518. https://doi.org/10.1016/j.aquaculture.2019.01.036
22) Nelson, D. A., Morris, M. W. (1989). Basic methodology. Hematology and coagulation, part IV. Clinical diagnosis and management by laboratory methods, 578-625.
23) Omosowone, O., Dada, A., Adeparusi, E. (2018). Comparison of dietary butyric acid supplementation effect on growth performance and body composition of *Clarias gariepinus* and *Oreochromis niloticus* fingerlings. Iranian Journal of Fisheries Sciences, 17(2): 403-412. https://doi.org/10.22092/ijfs.2018.115901
24) Osepchuk, D.V., Yurina, N.A., Yurin, D.A., Kuzminova, E.V., Semenenko, M.P., Gneush, A.N., Maksim, E.A., Danilova A.A. 2021. Liver condition and blood biochemical value of sturgeon fish when fed combined feed with increased fat content. Journal of Livestock Science 12: 312-316; doi. 10.33259/JLivestSci.2021.312-316
25) Perazza, C. A., da Fonseca, F. S., Máximo Tagashira, L. A., Onaka, E. M., dos Santos, V. B., Reis Neto, R. V., Hilsdorf, A. W. S. (2025). Genetic and phenotypic characterization of Nile tilapia (*Oreochromis niloticus*) in Brazil: implications for germplasm conservation and selective breeding programs. Critical Insights in Aquaculture, 1(1): 2560212.https://doi.org/10.1080/29932181.2025.2560212
26) Ponzoni, R. W., Nguyen, N. H., Khaw, H. L., Hamzah, A., Bakar, K. R. A., & Yee, H. Y. (2011). Genetic improvement of Nile tilapia (*Oreochromis niloticus*) with special reference to the work conducted by the WorldFish Center with the GIFT strain. Reviews in Aquaculture, 3(1): 27–41. https://doi.org/10.1111/j.1753-5131.2010.01041.x
27) Prabu, E., Felix, N., Uma, A., Ahilan, B., Antony, C. (2020). Metabolic responses of juvenile GIFT strain of Nile tilapia (*Oreochromis niloticus*) to dietary L-tryptophan supplementation. Aquaculture Nutrition, 26(5): 1713-1723. https://doi.org/10.1111/anu.13122
28) Reda, R. M., Mahmoud, R., Selim, K. M., El-Araby, I. E. (2016). Effects of dietary acidifiers on growth, haematology, immune response and disease resistance of Nile tilapia, *Oreochromis niloticus.* Fish & Shellfish Immunology, 50: 255-262. https://doi.org/10.1016/j.fsi.2016.01.040
29) Robles, R., Lozano, A. B., Sevilla, A., Márquez, L., Nuez-Ortín, W., Moyano, F. J. (2013). Effect of partially protected butyrate used as feed additive on growth and intestinal metabolism in sea bream (*Sparus aurata*). Fish Physiology and Biochemistry, 39(6): 1567-1580.
30) Romero, J., Feijoó, C. G., Navarrete, P. (2012). Antibiotics in aquaculture–use, abuse and alternatives. Health and environment in aquaculture, 159(1): 159-198.
31) Ruenkoed, S., Pholoeng, A., Nontasan, S., Panprommin, D., Mongkolwit, K., Wangkahart, E. (2025). Assessing the impact of acidifiers on growth performance, innate immune capacity, response to ammonia nitrogen

stress, digestive enzyme activity, intestinal histology, and gene expression of Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology, 162: 110315. https://doi.org/10.1016/j.fsi.2025.110315

32) Sathishkumar, G., Felix, N., Prabu, E. (2021). Effects of dietary protein substitution of fish meal with bioprocessed poultry by-product meal on growth performances, nutrient utilization, whole-body composition and haemato-biochemical responses of GIFT tilapia reared in floating cages. Aquaculture Research, 52(11): 5407-5418. https://doi.org/10.1111/are.15410

33) Sun, L., Liu, Z., Hao, G., Zhou, L., Lu, S. Q., Zhang, J. S., Xiao, T. (2013). Effects of sodium butyrate on growth and intestinal cell proliferation of *Carassius auratus*. Journal of Fishery Sciences of China, 20(4): 893-901.

34) Wang, W., Sun, J., Liu, C., Xue, Z. (2017). Application of immunostimulants in aquaculture: current knowledge and future perspectives. Aquaculture Research, 48(1): 1-23. https://doi.org/10.1111/are.13161

35) Wintrobe, M. M. (1934). Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia haematologica, 51(32): 32-49.

36) WorldFish. (2023). Genetically Improved Farmed Tilapia (GIFT). WorldFish Centre. https://worldfishcenter.org/pages/gift/

37) Youssuf, H. A., Shehab, A., Hamed, A. M. A. E., Raslan, W. S., Magdy, Y., Mohamed, M. G., Matter, A. F. (2025). Potential impact of L-threonine and encapsulated butyric acid (ButiPEARL) on growth performance, serum biomarkers, intestinal histomorphometry and economic indices of Nile tilapia fingerlings. *BMC* Veterinary Research, 21(1): 222.

38) Zhou, C., Lin, H., Huang, Z., Wang, J., Wang, Y., Yu, W. (2019). Effect of dietary sodium butyrate on growth performance, enzyme activities and intestinal proliferation-related gene expression of juvenile golden pompano *Trachinotus ovatus*. Aquaculture Nutrition, 25(6): 1261-1271. https://doi.org/10.1111/anu.12940