Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com
NLM ID:101629416
OPEN ACCESS
Zoology

Effect of Dietary Inulin and Lactic Acid Bacteria on Gut Microbiota and Immune Response in *Oreochromis niloticus*

Amara[1]*, Kainaat Azam[2], Iqra Kashaf[3], Fariha[4], Esha Munir[5], Asma Shoukat[6], Sadia Sarwar[7], Muhammad Faisal[8]

[1]Department of Zoology Wildlife and Fisheries, University of Agriculture Faisalabad
[2]Department of Zoology Wildlife and Fisheries, University of Agriculture Faisalabad
[3] National institute of Food science and Technology (NIFSAT), University of Agriculture, Faisalabad
[4]Department of Zoology Wildlife and Fisheries, University of Agriculture Faisalabad
[5]Department of Zoology Wildlife and Fisheries, University of Agriculture Faisalabad
[6]Department of Biosciences, Capital University of Science and Technology
[7]Department of Zoology, University of Okara
[8]Department of Zoology Wildlife and Fisheries, University of Agriculture Faisalabad

DOI: https://doi.org/10.36347/sajb.2026.v14i09.005 | **Received:** 24.06.2026 | **Accepted:** 06.08.2026 | **Published:** 16.09.2026

***Corresponding author:** Amara
Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad

Abstract | **Original Research Article**

The primary aim was to evaluate the effect of dietary inulin and lactic acid bacteria (LAB) on the gut microbiota and immune response of Nile Tilapia (*Oreochromis niloticus*). Experimental group was given inulin and LAB at 2% of body weight. While control group was fed with commercial fish feed. Fish were treated with these three feeds for 2months. Culturing of bacteria and immunological assays were done to evaluate the effect of these feeds on *O. niloticus*. Physico-chemical parameters of the aquarium were maintained end-to-end during the trial period such as DO at 5-7mg/L, temperature at 24-28℃ and pH at 7.0-8.2. The quantitative outcomes from groups were compared using one way ANOVA. In all three-culturing media such as NA, TSA and EMB, the results indicated a significant increase ($P<0.05$) in bacterial colonies in the experimental group T_1 when compared to the T_2 and control group T_0. Compared to the control group, the experimental groups T_1 and T_2. The T_2 group had a significantly higher WBCs count, higher Hemoglobin value, neutrophils and lymphocytes are significantly higher count ($P<0.05$) than the control group. However, the immune system of fish was improved by using the probiotic in the fish diet and it also increases the body's natural resistance to infection and facilitate in prevention of various diseases.

Keywords: Nile tilapia, inulin, lactic acid bacteria, gut microbiota, immune system, bacterial count.

INTRODUCTION

Aquaculture is known as the next agriculture revolution because of its role in the generation of great changes in the production of fish products (Wongprasert *et al*., 2006). Aquaculture is a key to meet fish demands. Undoubtedly this is quickly growing field of food production. This is predicted to reach 62 percent by the time 2030 and it will be become equal to the fish captured from the wild habitat (FAO 2014). Fisheries and aquaculture sector provides a source of livelihood to 59.51 million people (20.53 million people in aquaculture and 38.98 in fisheries), of which 14% are women. Asia is the biggest producer with the highest production of 88.69% (77,812.2 thousand tons), America 4.63% (3,799.2 tons), Europe 3.75% (3,082.6 tons) and Africa 2.67% (2,195.9 tons) (FAO, 2020). The fish can be a good source of essential fatty acids (Ozogul *et al*., 2007). Fish provide about 26.5% of animal protein and this is the main reason fish consider as nutritious food (Delgado, 2002; Louka, 2004; Hussain *et al.,* 2021).

Supplementary diet is the main source of natural feed supply of natural ingredients required by fish for proper fish growth in fish culture system (Karapan, 2002; Ayub *et al.,* 2021). Prebiotics composed of functional oligosaccharides have ability as nutritional supplements that support the host intestine's healthy microbes (Hoseinifar *et al*., 2020). Functional oligosaccharides have been suggested to have health benefits for fish, including more effectively gut physiology and disease resistance through modified gut microbiota (Akrami *et al*., 2013; Fariha *et al.,* 2025) improved metabolic and growth rates (Zhang *et al*., 2014) and integration of immune-protective mechanisms (Wang *et al.,* 2018). Prebiotics made from short-chain oligosaccharides are

able to resist hydrolysis by the host's digestive enzymes and serve as a substrate or source of nutrients for specific microorganisms to ferment in the fish gut, which promotes colonization by certain intestinal bacteria (Nawaz *et al*., 2018; Aqsa Naseem *et al.,* 2026). They exhibit significant potential for enhancing non-specific immunity to infection by pathogenic bacteria, modifying the composition of the gut microbiota and enhancing growth performance in a number of fish species (Wang and Li, 2020; Fiza Nasir *et al.,* 2026).

Inulin is a fructooligosaccharide (FOS) that occurs naturally (Salma Sabir *et al.,* 2026). They are fructans, which may be found in grains to a lesser extent also in dahlia, chicory, Jerusalem artichokes and garlic (White *et al*., 2002; Rabiya Javaid *et al.,* 2025). Inulin, are known to regulate different immune and metabiloc processes in mammals by direct and indirect actions on microbiota (Tawfck *et al*., 2022). As a result of its beneficial effects, inulin is used extensively in aquaculture also and proved to be a growth enchancer for fish (Rohani *et al.,* 2022). The growth and health of fish enhanced by inulin supplements (Becerril *et al*., 2014). As fructose cannot be hydrolyzed by pancreatic digestive enzymes in the proximal intestine of humans or domestic animals, fructans are non-digestible oligosaccharides (Zobel *et al*., 2002). In colon the process of fermentation occurs (Flickinger *et al*., 2003) by healthy bacteria that produce lactic acid, like bifidobacteria, which increases in their relative number and the development of harmful microbes is selectively controlled (Watzl *et al*., 2005; Muhammad Usman Arif *et al.,* 2026).

Probiotics act in multiple ways to support increases in aquaculture production, by promoting enhanced growth (Silva *et al*.,2012) improved feed utilization and nutrition (Zhou *et al*., 2010) reducing diseases (Irianto and Austin, 2002) and developing immune responses. Probiotics not only improve the health status of cultured animals but also help to ensure the safety of consumers (Prasad *et al*., 2003; Tehreem Fatima *et al*., 2026) The most commonly used bacterial probiotics strains are *Bacillus sp*., *Lactobacillus sp*., *Bifidobacterium* sp., *Pseudomonas sp*., *Streptococcus sp*., *Arthrobacters sp*., *Microbacterium sp*., *Phaeobacter sp*., *Streptomyces sp*., *Enterococcus sp*., *Lactococcus sp*., *Micrococcus sp*., etc., yeast probiotics are *Saccharomyces cerevisiae*, *Debrayomyces hansenii*, cell wall of yeast etc. Provision of selected species of autochthonous bacteria as growth modulators in live feed significantly improved the growth and survival of the halibut larvae (Bjornsdottir *et al*., 2003; Muhammad Nabeel Sharif *et al.,* 2025).

Lactic Acid Bacteria (LAB), a beneficial probiotic, which are widespread in nature, Gram-positive, typically catalase-negative and capable of producing lactic acid (Cheng *et al*., 2014). In aquaculture, the role of LAB as an immunostimulant has been demonstrated for its contribution to growth acceleration, antioxidant capacity, immunological reaction and resistance to disease, in addition to management of the microbial balance and pathogen inhibition (Hoseinifar *et al*., 2019). Groups of microbes have been present in the intestine of fish in a complex and dynamic manner (Semmova *et al*., 2012). Numerous investigations have shown that the dominant resident flora in fish gut microbiome is LAB (Hoseinifar *et al*., 2019). The LAB are obtained from cow milk, chees and yougert (Tarkhani *et al*., 2020b). Various physicochemical parameters and other growth parameters affect fish protein requirement in the fish diet (Cowey, 1976; Hassan et al., 2021).

Thus, the present study was conducted to examine that which dietary supplementation has more effect on gut microbiota and immune response in *Oreochromis Niloticus*.

METHODOLOGY

Investigational Specie

Nile Tilapia (*Oreochromis niloticus*)

Experimental Design

Healthy fingerlings of *O. niloticus* with an average weight of 4-9g were collected from Fisheries Research Farms. The fish were distributed randomly into three groups, two was the treatment group each aquarium has (n=20) and the other was the control group (n=20). The experimental group was given inulin and lactic acid bacteria feed at the rate of 2% body weight once a day. Similarly, the control group was given commercial fish feed. The feeding trial continued for eight weeks.

Sterilization

The process of killing microbial life in a clean environment is sterilization. Sterilization could be done through moist or dry sterilization. Here, we used moist sterilization method, and culturing media and all glassware were kept in an autoclave at 200lb, 121°C for 15 minutes, wrapped in aluminum foil during sterilization. The inoculation needles were sterilized on a red-hot flame.

Feeding Trail and Sampling Procedure

During feeding trail, aquarium's water chemistry indices were maintained regularly. After 2 months of feeding trail, fish were dissected to obtain gut. Intestinal samples were preserved in Eppendorf tubes already filled with 0.5ml saline solution and obtained samples were shaken well in an electric shaker to make a homogenized solution. The blood samples were collected from fish subjected to euthanasia by adding clove powder solution in water. Blood was collected in vacutainer containing EDTA.

Total Bacterial Count using Different Media

Solutions of different agar media such as NA, TSA and EMB were used to prepare accurate jelly-like culture media. For this step, all three agars were

measured using weight balance (0.01g least count) and dissolved with 100ml distilled water in 250ml conical flask using a clean glass stirrer at the following concentrations nutrient agar 2.80g, TSA 4.00g and EMB 3.75g.

Conical flasks were then closed with aluminum wrap and placed in the autoclave at 121ºC for 15 minutes. Bacteria in the gut of fish were cultured using media like Eosin Methylene Blue, Nutrient Agar and Tryptic Soy Agar according to the method of Ogunshe and Olabode, (2009) and Adejonwo *et al.,* (2020). Samples were placed on culture media (solidified) using sterile inoculating loop and then the loop was moved zig-zag (Quadrant Sticking Method) on to the plate (Sanders, 2012). Finally, petri plates were placed in an incubator set at 37ºC for one day. After 24 hours of incubation, these petri plates were then placed on the colony counter and CFUs were counted by the method of Clarke et al., 2010. This was helpful to find out the effect of inulin and LAB feed and commercial feed on the gut microflora.

Gram Staining

Gram staining is used to characterize and identify bacterial populations found in the fish gut microbiota by distinguishing Gram-positive and Gram-negative bacteria. It is also used to detect and classify bacterial strains by examining their morphological characteristics under a microscope. It essentially differentiates cells by the fact that their cell walls absorb more dye than those with a thin cell wall made up of a few layers of peptidoglycan. Gram-positive bacteria maintain their primary color and appear purple or blue under a microscope, whereas gram-negative bacteria appear red or pink.

Slide Preparation

After sterilizing the culture plates. Colonies were selected using a flame-sterilized platinum loop. The samples were distributed, air-dried, then heat-fixed by passing the slides over a flame. Gram staining was performed on the heat-fixed bacterial smear. Under the light microscope, a vast family of protease-producing gram-positive and gram-negative bacteria was observed.

Immunological parameters

For leukocyte count, blood sample was mixed with Giemsa stain and cells were counted in hemocytometer under light microscope using the method published by Oluyemi *et al.,* (2008). Differential leukocyte count was made by preparing blood smears stained with Giemsa stain, methanol, aqua distillate and methylene blue. Neutrophils, monocytes, lymphocytes, basophils and eosinophils were identified using microscope at 100X oil immersion, all cells were morphologically different from each other and were counted as described by Hidayaturrahmah, (2015).

Physico-Chemical Parameters

Consistent monitoring of water parameters was confirmed by maintaining the regulatory control according to the method followed by Manzoor et al., 2023. Electronic devices such as a pH meter and HANNA water testing kit like HANNA HI-8424 were used to measure pH levels, temperature, alkalinity, water hardness and dissolved oxygen (DO). Measurements are given in table 2.

Statistical analysis

The data was subjected to statistical interference at the end of the research analysis. The results were compared using one-way analyses of variance (ANOVA) followed by Tukey's test.

RESULTS

Limnological Parameters

Fish and other biological organisms grow differently depending on the temperature and dissolved oxygen, which affects their biological productivity. In aquatic habitats, pH is a crucial limnological parameter that controls chemical equilibrium and biological processes. Total alkalinity and total hardness contribute to water quality and consequently ecosystem health. Table 1 shows the average values of T0, T1 and T2 of physico-chemical parameter of 8 weeks.

Table 1: average T0, T1 and T2 physico-chemical parameters.

Week	pH	Temperature (˚C)	DO (mg/L)	Total Alkalinity (mg/L)	Water Hardness (mg/L)
16-10-2013	7.4	27.9	4.3	94	168
23-10-2013	7.0	28.4	4.7	101	135
30-10-2023	7.2	27.3	4.1	100	162
06-11-2023	7.8	27.6	4.6	110	155
13-11-2023	7.1	26.3	5.16	97	150
20-11-2023	7.5	27.1	4.71	94	148
27-11-2023	7.3	27.4	4.68	99	157
04-12-2023	7.4	25.6	4.4	117	144
Mean± SD	7.33±0.25	27.2±0.88	4.58±0.32	101.5±8.05	152.37±10.43

Total Bacterial Counting

The total microbiological content and CFU of intestinal samples from *O. niloticus* grown on different agar media revealed information on the variety and quantity of microorganisms present in the fish's digestive system. The gut microbiota is affected by changes in diet

and these microbial communities help in immune modulation and thus aid in aquaculture management strategies. Fish fed with inulin and LAB diet had impacted the gut colonies as shown in table 2.

Table 2 total viable count of *o. niloticus* cultured on Different Agar Media

Agar	T_0	T_1	T_2	P-value	Significance/non significant
NA	197.6±7.35	297.8±6.39	240.7±11.23	0.00	Significant
TSA	107±11.94	185.1±10.46	133.4±7.19	0.00	Significant
EMB	106.1±9.71	182.3±10.97	118.8±7.91	0.00	Significant

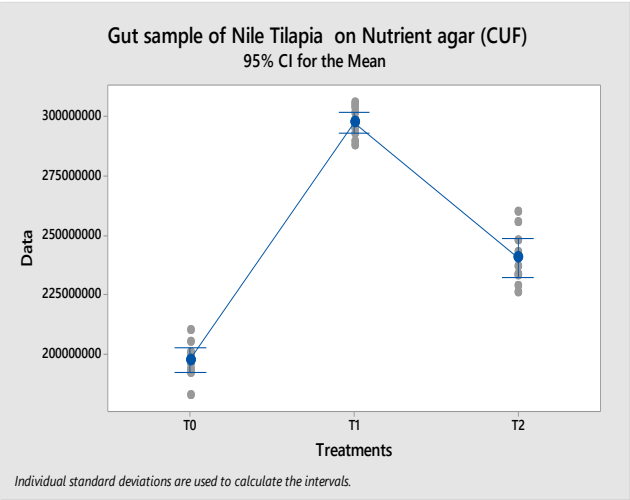

Figure 1: NA colony count comparison

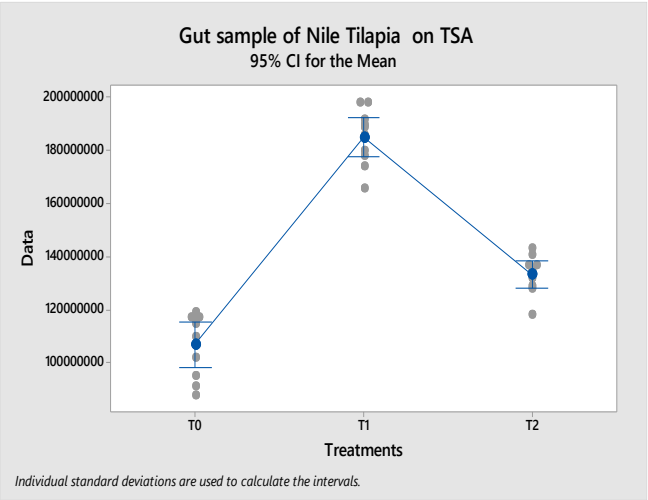

Figure 2: TSA colony count comparison

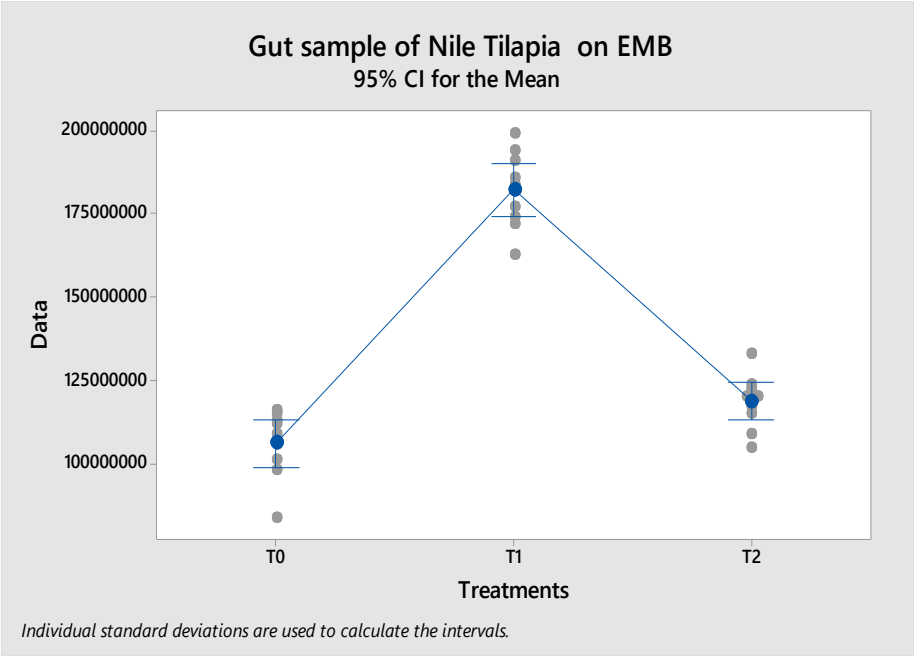

Figure 3: EMB colony count comparison

Immunological Parameters

Leukocytes, the scientific term for white blood cells, are immune system cells that help defend the body against foreign invaders and infectious diseases. Lymphocytes are a type of white blood cell that plays a key role in the adaptive immune response. Monocyte are phagocytic cells capable of engulfing and digesting pathogens and cellular debris. Neutrophils are highly phagocytic, meaning they can engulf and destroy pathogens, such as bacteria and fungi. They also release toxic substances to help kill microbes.

Table 3 immunological indices control vs experimental

Immunological parameters	T_0	T_1	T_2	P-values	Significant/non significant
WBCs	29.56±0.88	30.5±0.44	32.02±0.79	0.00	Significant
Hb	5.08±0.07	5.84±0.42	5.26±0.17	0.002	Significant
PLT	20.7±0.52	22.76±0.87	25.72±2.18	0.001	significant
Lymphocytes	64.77±2.04	70.94±1.81	72.21±0.90	0.00	significant
Monocytes	22.51±0.48	24.07±0.44	25.21±0.21	000	significant
Neutrophils	1.26±0.41	2.68±0.64	3.60±0.36	0.00	significant
Eosinophils	2.54±0.43	3.34±0.37	4.18±0.32	0.00	significant

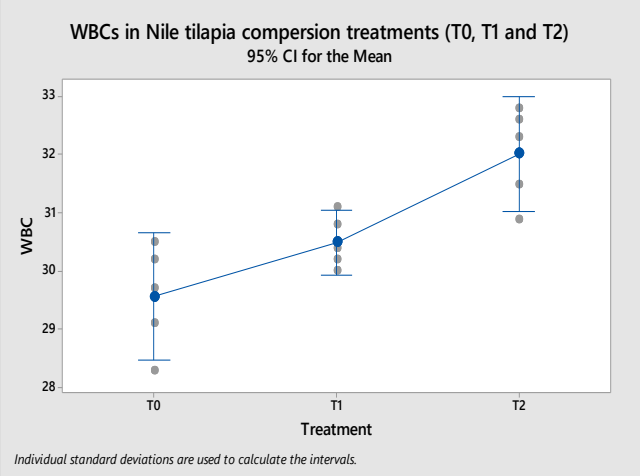

Figure 4: WBCs mean comparison

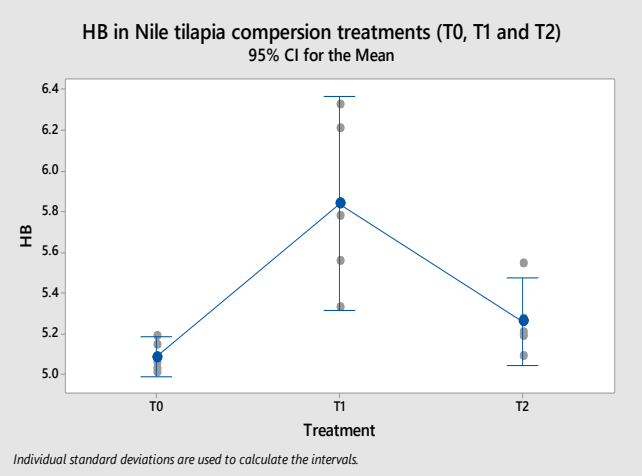

Figure 5: Hb mean comparison

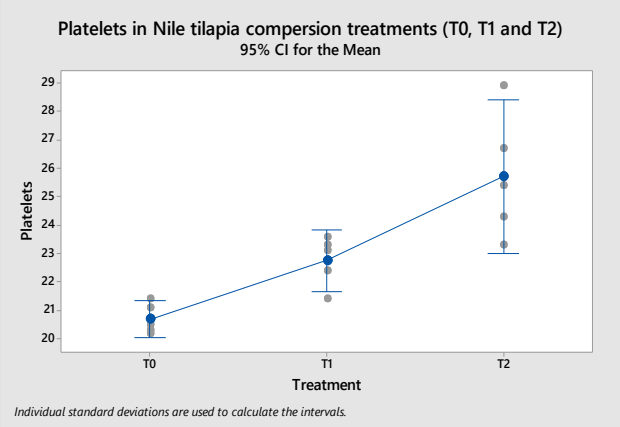

Figure 6: platelets mean comparison

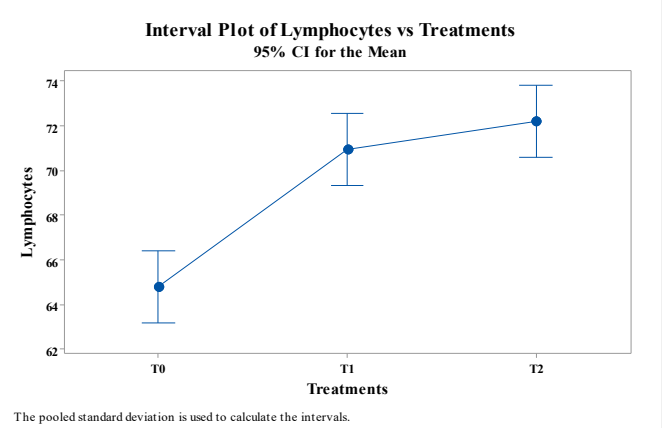

Figure 7: lymphocytes mean comparison

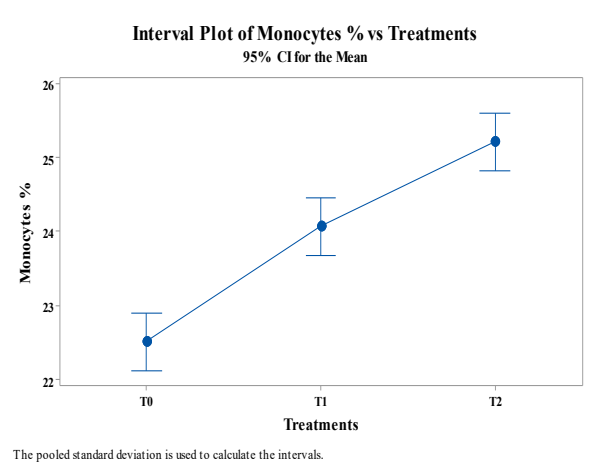

Figure 8: monocytes mean comparison

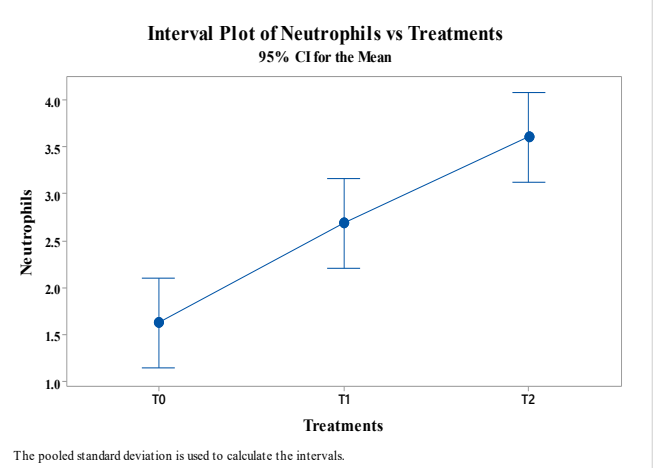

Figure 9: neutrophils mean comparison

DISCUSSION

The aim of current study was to evaluate the role of dietary inulin and LAB in the gut microbiota and immune response in *Oreochomis niloticus*. Inulin is used extensively in aquaculture also and proved to be a growth enchancer for fish. LAB as an immunostimulant has been demonstrated for its contribution to growth acceleration, antioxidant capacity, immunological reaction and resistance to disease, in addition to management of the microbial balance and pathogen inhibition

The results shows that the higher number of colonies were observed in the gut of *o. niloticus* T_1 group as compared to the T_2 and T_0. The t1 shows significant results as compared to the T_2. The results were also resembled with findings of (Liu *et al*., 2024).

The findings of the current study are also supported by Reza *et al.,* (2009) was showed that inulin enhanced the growth performance, intestinal microflora and body composition of juvenile Beluga (*Huso huso*). The study of Boonanuntanasarn *et al.,*(2018) also provded supports for the findings of the current inquiry. They investigated the effects of dietary inulin and Jerusalem artichoke (JA) on intestinal microbiota and morphometry of Nile tilapia fingerlings. They demonstrated that a greater intestinal villi height was observed in fish fed inulin and JA at both levels. There results showed that dietary inulin and JA feeding enhanced the intestinal microbiota and morphology of Nile tilapia fingerlings.

The results of this study support prior research by Foysal *et al.,*(2020) which showed that the probiotic significantly modified the gut microbiota, improved innate immune response and disease resistance of Nile Tilapia. The outcomes of the current investigation are supported by the observation made by Abarike *et al.,*(2020). They investigated the effect of herbal-probiotic supplements comprising a mixture of Chinese Herbs (CH) and or commercially prepared probiotic *Bacillus species* (BS) on the physiological response of Nile Tilapia. They discovered that the herbal-probiotic improves the levels of hematocrit, red blood cells, haemoglobin and white blood cells in fish blood.

Abou-El-Atta *et al.,* (2019) investigation also supports this finding. They pointed out that dietary probiotic *Lactobacillus plantarum* and whey protein concentrate (WPC) in practical diets for Nile Tilapia, *Oreochromis niloticus.* They recommended the use of probiotic *L. plantarum,* improve the growth, antioxidant, immunity responses and tolerance of Nile tilapia to *A. sobria* infection.

CONCLUSION

The study highlights the effects of the inulin on gut microbiota as it enhances the beneficial bacteria in the gut of *o. niloticus*, but on the other hand the LAB notable increases WBC counts hemoglobin, lymphocytes, neutrophils and esophile as compared to the control group and T_1 group.

REFERENCES

- Abou-El-Atta, M. E., Abdel-Tawwab, M., Abdel-Razek, N., & Abdelhakim, T. M. (2019). Effects of dietary probiotic Lactobacillus plantarum and whey protein concentrate on the productive parameters, immunity response and susceptibility of Nile tilapia, Oreochromis niloticus (L.), to Aeromonas sobria infection. *Aquaculture nutrition*, *25*(6), 1367-1377.
- Akrami, R., Iri, Y., Rostami, H. K., & Mansour, M. R. (2013). Effect of dietary supplementation of fructooligosaccharide (FOS) on growth performance, survival, lactobacillus bacterial population and hemato-immunological parameters of stellate sturgeon (Acipenser stellatus) juvenile. *Fish & shellfish immunology*, *35*(4), 1235-1239.
- Ayub, A., Rasool, F., Khan, N., Qaisrani, S. N., Parveen, S., Anjum, K. M., ... & Zulfiqar, T. (2021). Limiting amino acids supplementation in low crude protein diets and their impacts on growth performance and carcass composition in Labeo rohita (rohu) adult fish. *Brazilian Journal of Biology*, *83*, e249422.
- Bjornsdottir, R., Karadottir, E. G., Johannsdottir, J., Thorarinsdottir, E. E., Smaradottir, H., Sigurgisladottir, S., & Gudmundsdottir, B. K. (2010). Selection of bacteria and the effects of bacterial treatment of Atlantic halibut (Hippoglossus hippoglossus L.) eggs and larvae. *Aquaculture*, *302*(3-4), 219-227.
- Chu, W., Zhou, S., Zhu, W., & Zhuang, X. (2014). Quorum quenching bacteria Bacillus sp. QSI-1 protect zebrafish (Danio rerio) from Aeromonas hydrophila infection. *Scientific reports*, *4*(1), 5446.
- Cowey, C. B. (1976). Use of synthetic diets and biochemical criteria in the assessment of nutrient requirements of fish. *Journal of the Fisheries Board of Canada*, *33*(4), 1040-1045.
- Delgado, C. L., Rosegrant, M. W., Wada, N., Meijer, S., & Ahmed, M. (2002). Fish as food: projections to 2020 under different scenarios.
- FAO. 2014. The state world Fishries and Aquaculture. The food and Agriculture Organization of The United Nation (FAO) Rome: 233.
- Hassan, H. U., Ali, Q. M., Ahmad, N., Masood, Z., Hossain, M. Y., Gabol, K., ... & Kamal, M. (2021). Assessment of growth characteristics, the survival rate and body composition of Asian Sea bass Lates calcarifer (Bloch, 1790) under different feeding rates in closed aquaculture system. *Saudi Journal of Biological Sciences*, *28*(2), 1324-1330.
- Hoseinifar, S. H., Hosseini, M., Paknejad, H., Safari, R., Jafar, A., Yousefi, M., ... & Mozanzadeh, M. T. (2019). Enhanced mucosal immune responses, immune related genes and growth performance in

common carp (Cyprinus carpio) juveniles fed dietary Pediococcus acidilactici MA18/5M and raffinose. *Developmental & Comparative Immunology*, *94*, 59-65.

- Hoseinifar, S. H., Yousefi, S., Van Doan, H., Ashouri, G., Gioacchini, G., Maradonna, F., & Carnevali, O. (2020). Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. *Reviews in Fisheries Science & Aquaculture*, *29*(2), 198-217.
- Hussain, M., Hassan, U.H., Siddique, M.A., Mahmood, K., Abdel-Aziz, M.F., Laghari, M.Y., Abro, N.A., Gabol, K.,
- Irianto, A., & Austin, B. (2002). Probiotics in aquaculture. *Journal of fish diseases*, *25*(11), 633-642.
- Karapan, A. (2002). Studies to optimize poly unsaturated fatty acid composition of tilapia for human consumption in SE Asia. *Aquaculture News*, *28*, 6-7.
- Louka, Nicolas, Frédéric Juhel, Victurnien Fazilleau, and Pierre Loonis. "A novel colorimetry analysis used to compare different drying fish processes." *Food control* 15, no. 5 (2004): 327-334.
- Manzoor, K., Rasool, F., Parveen, S., Hafeez-ur-Rehman, M., Mahmood, S., & Ayub, A. (2023). Impact of feed with varying crude protein (CP) levels on growth performance of different fish species cultured under similar regimes. *International Journal of Applied and Experimental Biology*, *2*(1), 33-42.
- Nawaz, A., Irshad, S., Hoseinifar, S. H., & Xiong, H. (2018). The functionality of prebiotics as immunostimulant: Evidences from trials on terrestrial and aquatic animals. *Fish & shellfish immunology*, *76*, 272-278.
- Ogunshe, A. A., & Olabode, O. P. (2009). Antimicrobial potentials of indigenous Lactobacillus strains on gram-negative indicator bacterial species from Clarias gariepinus (Burchell.) microbial inhibition of fish-borne pathogens. *African Journal of Microbiology Research*, *3*(12), 870-876.
- Ozogul, Y., Özogul, F., & Alagoz, S. (2007). Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: A comparative study. *Food chemistry*, *103*(1), 217-223.
- Pool-Zobel, B., Van Loo, J., Rowland, I., & Roberfroid, M. B. (2002). Experimental evidences on the potential of prebiotic fructans to reduce the risk of colon cancer. *British Journal of Nutrition*, *87*(S2), S273-S281.
- Prasad, L., Baghel, D., Kumar, V., Laxmi, P., Dharmendra Singh, B., & Vikas, K. (2003). Role and prospects of probiotics use in aquaculture. *Aquacult*, *4*(2), 247-251.
- Reyes-Becerril, M., Ascencio, F., Gracia-Lopez, V., Macias, M. E., Roa, M. C., & Esteban, M. Á. (2014). Single or combined effects of Lactobacillus sakei and inulin on growth, non-specific immunity and IgM expression in leopard grouper (Mycteroperca rosacea). *Fish physiology and biochemistry*, *40*, 1169-1180.
- Hussain, M., Hassan, H. U., Siddique, M. A. M., Mahmood, K., Abdel-Aziz, M. F. A., Laghari, M. Y., ... & Rizwan, S. (2021). Effect of varying dietary protein levels on growth performance and survival of milkfish Chanos chanos fingerlings reared in brackish water pond ecosystem. *The Egyptian Journal of Aquatic Research*, *47*(3), 329-334.
- Rohani, M. F., Islam, S. M., Hossain, M. K., Ferdous, Z., Siddik, M. A., Nuruzzaman, M., ... & Shahjahan, M. (2022). Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. *Fish & shellfish immunology*, *120*, 569-589.
- Semova, I., Carten, J. D., Stombaugh, J., Mackey, L. C., Knight, R., Farber, S. A., & Rawls, J. F. (2012). Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. *Cell host & microbe*, *12*(3), 277-288.
- Silva, E. F., Soares, M. A., Calazans, N. F., Vogeley, J. L., do Valle, B. C., Soares, R., & Peixoto, S. (2012). Effect of probiotic (B acillus spp.) addition during larvae and postlarvae culture of the white shrimp L itopenaeus vannamei. *Aquaculture Research*, *44*(1), 13-21.
- Wongprasert, K., Asuvapongpatana, S., Poltana, P., Tiensuwan, M., & Withyachumnarnkul, B. (2006). Serotonin stimulates ovarian maturation and spawning in the black tiger shrimp Penaeus monodon. *Aquaculture*, *261*(4), 1447-1454.
- Tarkhani, R., Imani, A., Hoseinifar, S. H., Ashayerizadeh, O., Moghanlou, K. S., Manaffar, R., ... & Reverter, M. (2020). Comparative study of host-associated and commercial probiotic effects on serum and mucosal immune parameters, intestinal microbiota, digestive enzymes activity and growth performance of roach (Rutilus rutilus caspicus) fingerlings. *Fish & Shellfish Immunology*, *98*, 661-669.
- Tawfick, M. M., Xie, H., Zhao, C., Shao, P., & Farag, M. A. (2022). Inulin fructans in diet: Role in gut homeostasis, immunity, health outcomes and potential therapeutics. *International Journal of Biological Macromolecules*, *208*, 948-961.
- White, L. A., Newman, M. C., Cromwell, G. L., & Lindemann, M. D. (2002). Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. *Journal of animal science*, *80*(10), 2619-2628.
- Wang, A. R., Ran, C., Ringø, E., & Zhou, Z. G. (2018). Progress in fish gastrointestinal microbiota research. *Reviews in Aquaculture*, *10*(3), 626-640.
- Watzl, B., Girrbach, S., & Roller, M. (2005). Inulin, oligofructose and immunomodulation. *British Journal of Nutrition*, *93*(S1), S49-S55.

- Zhang, C. N., Li, X. F., Jiang, G. Z., Zhang, D. D., Tian, H. Y., Li, J. Y., & Liu, W. B. (2014). Effects of dietary fructooligosaccharide levels and feeding modes on growth, immune responses, antioxidant capability and disease resistance of blunt snout bream (Megalobrama amblycephala). *Fish & shellfish immunology*, *41*(2), 560-569.
- Zhou, X., Tian, Z., Wang, Y., & Li, W. (2010). Effect of treatment with probiotics as water additives on tilapia (Oreochromis niloticus) growth performance and immune response. *Fish physiology and biochemistry*, *36*, 501-509.
- Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. *JoVE (Journal of Visualized Experiments)*, (63), e3064.
- Hidayaturrahmah. 2015. The characteristics of the shape and size of the blood cells of anabas fish (*Anabas testudineus*) and Snake head (*Chana sriata*). Enviro Scienteae. 1 (1): 88-93. (in Indonesia).
- Oluyemi, K. G., Adeparusi, E. A., & Olanrewaju, J. (2008). Basic haematological parameters in African catfish, Clarias gariepinus (Burchell, 1822) fed ascorbic acid supplemented diets. *Research Journal of Animal Sciences*, *2*(1), 17-21.
- Abarike, E. D., Jian, J., Tang, J., Cai, J., Sakyi, E. M., & Kuebutornye, F. K. (2020). A mixture of Chinese herbs and a commercial probiotic Bacillus species improves hemato-immunological, stress, and antioxidant parameters, and expression of HSP70 and HIF-1α mRNA to hypoxia, cold, and heat stress in Nile tilapia, Oreochromis niloticus. *Aquaculture Reports*, *18*, 100438.
- Reza, A., Abdolmajid, H., Abbas, M., & Abdolmohammad, A. K. (2009). Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, Huso huso (Linnaeus, 1758). *Journal of the World Aquaculture Society*, *40*(6), 771-779.
- Foysal, M. J., Alam, M., Kawser, A. R., Hasan, F., Rahman, M. M., Tay, C. Y., ... & Gupta, S. K. (2020). Meta-omics technologies reveals beneficiary effects of Lactobacillus plantarum as dietary supplements on gut microbiota, immune response and disease resistance of Nile tilapia (Oreochromis niloticus). *Aquaculture*, *520*, 734974.
- Boonanuntanasarn, S., Tiengtam, N., Pitaksong, T., Piromyou, P., & Teaumroong, N. (2018). Effects of dietary inulin and Jerusalem artichoke (Helianthus tuberosus) on intestinal microbiota community and morphology of Nile tilapia (Oreochromis niloticus) fingerlings. *Aquaculture nutrition*, *24*(2), 712-722.
- Liu, J., Lu, X., Fang, F., Wu, K., Wu, J., & Gao, J. (2024). Comparison of the in-vitro effect of five prebiotics with different structure on gut microbiome and metabolome. *Food Bioscience*, 103810.
- Nasir, F., Ali, M. Z., Hayat, K., Fatima, T., Waheed, K., Abbas, S. Anwar, U., Waris,M., F., Laser-Induced Break Down Spectroscopy Of Water. Multidisciplinary Surgical Research Annals, 4(1), 223-235.
- Naseem, A., Arshad, B., Khan, M., Akram, A., Naqvi, S. R., Azam, N.,& Hazrat Bilal, F. Investigating the Effect of Biogas Slurry on the Morpho-Physiological Traits of Mustard Plant. (2026). *Multidisciplinary Surgical Research Annals*, *4*(1), 153-172.
- Sharif, M. N., Khaliq, A., F., Khalil, M. N., Qadir, M. M., Chohan, A. A., Najeeb, M., & Saif, S. (2025). Contaminated waters: Unveiling the environmental and health [i]impacts of global water pollution. Kashf Journal of Multidisciplinary Research, 2(05), 64-85.
- Fariha, Arif, S., Ali, H., Naseer, A., Noreen, U., Fatima, F., Ashraf, S., & Maryam, S. (2025). Determining the relationship between metal contamination and peroxide activity in grass carp from indus river. Journal of Medical & Health Sciences Review, 2(2).
- Javaid, R., Summaiya, A. F., Khan, A. A., Taj, J., Siddique, N., Fariha, A. T., & Hussain, M. (2025). Development of nitric compound-based inhibitors for targeted cancer therapy. Frontier in Medical & Health Research.
- Tahreem Fatima, Mubarah Shahid , Aqsa Waqar , Humara Noreen , Muhammad Asadullah Khan, Uswah Anwar, Amara Saghir , Mujahid Khan, Muhammad Umar ,Fariha, Zia ul-Haq To Study The Pretreatment Effect Of Green Laser Irradiation On Germination And Yield Of Tomato Seeds (Solanum lycopersicum) (2026). *Multidisciplinary Surgical Research Annals*, *4*(1), 295-306.
- Muhammad Usman Arif, Bilal Haider , Hamna Zahid, Rimsha Maqbool, Aqsa Javaid, Namrah Shahzad, Maham Fatima, Habib Ur Rehman, Physicochemical and Sensory Characteristics of Vacuum Aged Buffalo Meat: https://doi.org/10.5281/zenodo.20095032. (2026). *Annual Methodological Archive Research Review*, *4*(5), 43-58.
- Salma Sabir , Asma Shoukat, Syeda Yousra Mahmood , Syeda Hijab Fatima, Shamsa Khizar, Rafia Maryyam Rafaqat, Safia Naz , Muhammad Faisa,SEQUENCE ANALYSIS OF MTND2 GENE IN FAMILIES SEGREGATING MATERNALLY INHERITED CVDS FROM DISTRICT MANSEHRA: https://doi.org/10.5281/zenodo.20094958. (2026). *Annual Methodological Archive Research Review*, *4*(4), 400-424.
- Aleem, M., Soomro, A. A., Anwar, S., Ahmad, I., Bilal, H., Javed, A., ... & Shafiq, A. (2026). Effects Of Thiourea And Micronutrients On Growth And Yield Of Bell Pepper Application. *Research Consortium Archive*, *4*(2), 1784-1803.
- Sharif, U., Soomro, A. A., Masood, H., Fatima, S. H., Shoukat, A., Khan, R., & Faisal, M. (2026). Role Of Pheromones In Attraction Of Bactrocera Cucurbitae. *Research Consortium Archive*, *4*(2), 2301-2315.