

# THE EFFECT OF PROBIOTICS (*Lysinibacillus fusiformis* LFUG) ON THE GROWTH PERFORMANCE AND SURVIVAL OF JUVENILE NILE TILAPIA (*Oreochromis niloticus*)

BY

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A RESERCH REPORT SUBMITED TO THE FACULTY OF NATURAL RESOURCES AND ENVIRONMENTAL SCIENCES IN PARTIAL FILFULLMENT OF THE REQUIREMENT FOR THE AWARD OF A DEGREE IN BACHEROLORS OF SCIENCE IN FISHERIES AND WATER RESOURCES MANAGEMENT OF BUSITEMA UNIVERSITY.





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## DECLARATION

I Lota Brian hereby declare that this is my own original work and has never been submitted to any other institution or University for the award of a degree.

Signature ..... Date.....

### APPROVAL

This study was carried out under supervision and the report is now ready for submission with the approval of the following people.

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### **DEDICATION**

I dedicate this piece of work to my academic supervisor Mr. Philip Rwezawula, my field supervisor Ms. Catherine Agoe, my beloved mother Ms. Kauta Ruth, my Uncle Mr. Waweyo Charles, my brother Nzogi Paul, and to all my fellow classmates.

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## List of Acronyms.

TSB Tryptic soy broth
FCR Feed conversion Ratio
K Fulton condition factor
DO Dissolved oxygen
PH Potential of hydrogen
CFU Colon forming unit.
FAO Food and agriculture Organization
SOP Standard operating procedures
ABW Average body weight
SGR Specific growth rate
WGWeight gain
ARDCK Aquaculture research and development center Kajjansi

#### Abstract

Probiotics play a crucial role in improving the water quality, growth, and resistance of fish against pathogenic organisms. They have shown great potential for application in aquaculture and could be safer alternatives to antibiotics. The present study was conducted to evaluate the effect of Lysinibacillus fusiformis LFUG dietary inclusion on the growth performance and survival of juvenile Oreochromis niloticus, and on the physiochemical water quality parameters. Two different concentrations of the bacteria (1X10<sup>6</sup> (LFUGT1) and 1X10<sup>8</sup> (LFUGT2) CFUml<sup>-g</sup>) were incorporated in a commercial diet (Koudijs) to make the experimental test diet whereas the negative control diet had no bacteria added (normal saline only). Nile tilapia juveniles of average body weight 1.723±0.4g were subjected to the respective experimental diets for a period of fifty days with 10-day sampling intervals for weight (g), total length (cm) and standard length (cm). Fish fed on diets supplemented with the bacteria showed significantly better growth performance than those that were not (control) (P < 0.05). Fish in the LFUGT2 treatments significantly added more weight (5.649±1.253) than LFUGT1 (5.286±1.236) the Control (4.407±1.817) after the 50 days of feeding on the test diets. There wasn't any negative effect of the bacteria on the health of the fish fed on feeds enriched with the different concentrations since there was no significant difference in the survival rates as compared to the control. (Control - 92.7±1.5%, LFUGT1 -94.6±0.6% and LFUGT2 - 95.3±2.5%). Furthermore, the Ammonia, Nitrites and Nitrates were better controlled respectively in LFUGT2 (0.25±0.01mg/l, 0.5±0.01mg/l and 15±5mg/l) and LFUGTT1( $0.5\pm0.02$ mg/l,  $0.5\pm0.01$ mg/l and  $10\pm5$ mg/l) than that in the control treatment (1±0.02mg/l, 0.6±0.02mg/l and 20±5mg/l). Throughout the experiment, the mean temperature and dissolved Oxygen (DO) were (Temp 26±0.5°C, 26±0.2°C, and 26±0.4°C, DO 4.3mg/l, 4.9mg/l and 4.5mg/l) for control, LFUG T1 and LFUG T2 respectively. Therefore, Lysinibacillus fusiformis dietary inclusion at the two concentrations used in this study enhanced growth of juvenile Nile tilapia, improved water quality, and did not have any biosafety concerns for the 50 days of the experiment. Thus, the bacterium is a promising candidate for use in Nile tilapia fish farming as a probiotic.

#### **CHAPTER ONE: INTRODUCTION**

#### 1.1 Background

Aquaculture plays a critical role in meeting the increasing demand for fish protein worldwide (FAO, 2020). Globally, there is a growing demand for fish and fish products caused by the rapid population growth and the increased preference for the consumption of healthier foods (Sherif, A.H. (2023). Nile tilapia (Oreochromis niloticus) is one of the most widely cultured and produced species in freshwater aquaculture with global production reaching over 1.5 million metric tons (FAO, 2020). This is due to its rapid growth, high adaptability to various environments, good resistance to rough handling, and high nutritional value (FAO, 2020; El-Sayed, 2006). In Uganda Tilapia production has significantly increased over the past few years due to the adoption of semiintensive and intensive aquaculture technologies. However, these farming systems have subjected the fish to stressful conditions that suppress their immunity, hence exposing them to various pathogens. Infectious diseases pose a significant challenge to tilapia aquaculture, resulting in substantial economic losses. According to Assefa & Abunna (2018), more than 50% of the overall losses in aquaculture is attributed to fish diseases. Besides, the global annual economic losses in aquaculture are estimated at approximately 9 billion USD (Novriadi, 2016). Conventional treatment methods for bacterial infections in aquaculture, such as the use of antibiotics and chemotherapeutics to enhance disease resistance, survival, and growth performance in aquaculture has been recently questioned. This is due to the emergence of antimicrobial resistant (AMR) strains that pose a serious threat to the environment and consumers of aquatic organisms (Cabello, 2006; Sørum, 2006). Hence, the need for alternative sustainable and safer approaches is warranted.

Probiotic, prebiotic, and symbiotic use in tilapia production is considered a viable, safe, and environmentally friendly alternative that enhances growth performance, feed utilization, immunity, disease resistance, and fish survival against pathogens and environmental stress (Vignesh. R *et al*, 2011). Probiotics are live microorganisms that confer health benefits when administered in adequate amounts through an appropriate route (Hill *et al.*, 2014). *Lysinibacillus fusiformis* is a bacterium that has shown strong antimicrobial properties against various pathogens and has also enhanced overall growth and health of fish (Santos *et al.*, 2016). Consequently, this research aimed at assessing the effect of probiotics on the growth performance and survival of juvenile Nile Tilapia, and on the physiochemical water quality parameters. This would contribute

#### REFERENCES

- Assefa, A., & Abunna, F. (2018). Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary medicine international*, 2018.
- FAO (Food and Agriculture Organization of the United Nations) (2006c) Regional Review on Aquaculture Development. 1. Latin America and the Caribbean – 2005. FAO, Rome
- FAO. (2020). The State of World Fisheries and Aquaculture 2020. Food and Agriculture Organization of the United Nations.
- Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., . . . Morelli, L. (2014). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology, 11(8), 506-514.
- Klesius, T., Klesius, E., & Huff, W. (2017). Enteric red mouth disease in tilapia: A review. Journal of Aquatic Animal Health, 29(3), 231-243.
- 6. Novriadi, R. (2016). Vibriosis in aquaculture. *Omni-Akuatika*, 12(1).
- Sherif, A.H., & Kassab, A.S. (2023). Multidrug-resistant Aeromonas bacteria prevalence in Nile tilapia broodstock. BMC Microbiology, 23(1), 80. [https://doi.org/10.1186/s12866-023-02827-8 7](https://doi.org/10.1186/s12866-023-02827-8)
- Wye Sze Lee, O., Puvanasundram, P., Lim, K. C., & Karim, M. (2022). In vitro Assessment of Multistrain Probiotic on Its Safety, Biofilm Formation Capability, and Antimicrobial Properties Against Aeromonas hydrophila. *Pertanika Journal of Tropical Agricultural Science*, 45(4).
- FAO 2023. Fishery and Aquaculture Country Profiles. Uganda, 2020. Country Profile Fact Sheets. Fisheries and Aquaculture Division [online]. Rome. Updated Jan 31, 2022 [Cited Thursday, October 26th, 2023].
- 10. FAO 2012, The state of world Fisheries and Aquaculture.
- 11. Agus Iriant & Brian Austin, 2002. Journal of fish diseases 25(11) p633-642.
- FAO, The State of World Fisheries and Aquaculture, Contributing to food security and nutrition for all, Food and Agriculture Organization of the United Nations, Rome, Italy, 2016.

- 13. J. Kubečka, D. S. Boukal, M. Čech et al., "Ecology and ecological quality of fish in lakes and reservoirs," Fisheries Research, vol. 173, pp. 1–3, 2016.
- 14. A. Rico, K. Satapornvanit, M. M. Haque et al., "Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: A critical review," Reviews in Aquaculture, vol. 4, no. 2, pp. 75–93, 2012.
- T. L. F. Leung and A. E. Bates, "More rapid and severe disease outbreaks for aquaculture at the tropics: Implications for food security," Journal of Applied Ecology, vol. 50, no. 1, pp. 215–222, 2013.
- 16. E. J. Peeler and N. G. Taylor, "The application of epidemiology in aquatic animal health opportunities and challenges," Veterinary Research, vol. 42, no. 1, article no. 94,
- 17. J. Romero, C. G. Feijoó, and P. Navarrete, "Antibiotics in aquaculture Use, abuse and alternatives," Health and Environment in Aquaculture, pp. 160–198, 2012.
- V. Kumar, S. Roy, D. K. Meena, and U. K. Sarkar, "Application of probiotics in shrimp aquaculture: importance, mechanisms of action, and methods of administration," Reviews in Fisheries Science and Aquaculture, vol. 24, no. 4, pp. 342–368, 2016.
- 19. R. Harikrishnan, C. Balasundaram, and M.-S. Heo, "Fish health aspects in grouper aquaculture," Aquaculture, vol. 320, no. 1-2, pp. 1–21, 2011.
- R. Gudding, "Vaccination as a Preventive Measure," in Fish Vaccination, R. Gudding, A. Lillehaug, and O. Evensen, Eds., vol. 1st, pp. 12–21, John Wiley & Sons, Inc, Oxford, UK, 2014.
- M. Dadar, K. Dhama, V. N. Vakharia et al., "Advances in Aquaculture Vaccines Against Fish Pathogens: Global Status and Current Trends," Reviews in Fisheries Science & Aquaculture, vol. 25, no. 3, pp. 184–217, 2016.
- 22. K. P. Plant and S. E. LaPatra, "Advances in fish vaccine delivery," Developmental & Comparative Immunology, vol. 35, no. 12, pp. 1256–1262,
- 23. A. Adams, Fish Vaccines, Springer Science+Business Media, Basel, Switzerland, 1st edition,
- R. Gudding and W. B. Van Muiswinkel, "A history of fish vaccination: Science-based disease prevention in aquaculture.," Fish and Shellfish Immunology, vol. 35, no. 6, pp. 1683–1688, 2013.

- 25. S. Lien, B. F. Koop, S. R. Sandve et al., "The Atlantic salmon genome provides insights into rediploidization," Nature, vol. 533, no. 7602, pp. 200–205, 2016.
- 26. G. Earle and W. Hintz, "New approaches for controlling saprolegnia parasitica, the causal agent of a devastating fish disease," Tropical Life Sciences Research, vol. 25, no. 2, pp. 101–109, 2014.
- R. Gudding, "Vaccination as a Preventive Measure," in Fish Vaccination, pp. 12–21, John Wiley & Sons, Inc, oxford, 1st edition, 2014.
- J. W. Pridgeon and P. H. Klesius, "Major bacterial diseases in aquaculture and their vaccine development," CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, vol. 7, article no. 48, pp. 1–16, 2012.
- S. Craig and P. Klesius, "Replicating vaccines," in in Fish Vaccination, R. Gudding, A. Lillehaug, and Ø. Evensen, Eds., pp. 29–38, John Wiley Sons, Inc, Oxford, 1st edition, 2014.
- Leung, T. L., & Bates, A. E. (2013). More rapid and severe disease outbreaks for aquaculture at the tropics: implications for food security. *Journal of applied ecology*, 215-222.
- 31. Yunarty ., Anton ., Diana Putri Renitasari, Toto Hardianto, Ardana Kurniaji, Utilization of Sugarcane Bagasse (Saccharum officinarum Linn.) as a Carbon Source in Biofloc System of Vaname Shrimp Litopenaeus vannamei, Pakistan Journal of Biological Sciences, 10.3923/pjbs.2024.90.99, 27, 2, (90-99), (2024).
- Ferreira, A. L., Amorim, M. P., Souza, E. R., Schorer, M., Castro, G. H., & Pedreira, M. M. (2019). Probiotic, antibiotic and combinations in Nile tilapia juveniles culture. Anais da Academia Brasileira de Ciências, 91, e20180169.