Journal of Energy and Natural Resource Management (JENRM), Vol. 9, No. 2, 15-23, 2023
Received: 28 September 2023; Revised: 6 November 2023; Accepted: 18 November 2023

Evaluating the Impact of Varied Probiotic Levels (*Bacillus Subtilis 200*) on Feed Utilization, Growth Performance, and Proximate Composition in African Catfish (*Clarias gariepinus*)

Abubakar Abdul Mutalib[1]; Elliot Haruna Alhassan[1]; Christian Larbi Ayisi[2]

Abstract

In this study, the effects of (*Bacillus Subtilis 200*) on feed utilization, growth and proximate composition on African catfish (*Clarias gariepinus*) were evaluated over an 8-week period. The initial mean weight of *Clarias gariepinus* fingerlings used for this study was 3.47±0.95 g. Experimental fish were fed four different diets supplemented with B. Subtilis 200 at 0g/kg (A), 1g/kg (B), 2g/kg (C), and 3g/kg (D) three times a day until they appeared satiated. The findings revealed a significant difference ($P<0.05$) in final weight (FW) and weight gain (WG) between treatments. An increase in probiotics, resulted in a corresponding increase in FW and WG. Fish fed diet A recorded FW and WG of 72.3 g and 68.95 g, respectively, while fish fed diet B had FW and WG of 77.48 g and 73.70 g, respectively.. Fish fed diet C recorded FW and WG of 83.17 g and 79.86 g, respectively, while 84.73 g and 81.27 g, respectively, were recorded by group D. Feed intake varied significantly ($P<0.05$) between groups, ranging from 69.23 g to 77.21 g. The condition factor, protein efficiency ratio, and feed conversion ratio did not significantly change ($P>0.05$). Similarly, irrespective of the experimental diet, proximate composition (ash, lipid, moisture, and protein) was unaffected ($P>0.05$). The results of this present study imply that *Clarias gariepinus* 200 can be added to *Clarias gariepinus* diets up to 3g/kg without affecting body composition or growth feed utilization.

Keywords

Specific growth rate; Feed efficiency; Probiotics; Protein efficiency ratio; Biochemical composition

[1]1Department of Fisheries and Aquatic Resources Management, Faculty of Biosciences, University for Development Studies, Tamale, Ghana
[2]2Department of Water Resources and Aquaculture Management, School of Sustainable Development, University of Environment and Sustainable Development, Somanya, Eastern Region, Ghana
***Corresponding author**: aclarbi@uesd.edu.gh
DOI: 10.26796/jenrm.v9i2.241
Received: 28 September 2023 ; **Received in revised form:** 6 November 2023; **Accepted:** 18 November 2023; **Published:** 30 November 2023

Contents

1 Introduction **16**
2 Materials and methods **17**
2.1 Experimental fish . . . 17
2.2 Experimental Design . . . 17
2.3 Feed and feeding . . . 17
2.4 Sample collection . . . 18
2.5 Growth and feed utilization . . . 18
2.6 Proximate composition . . . 18
3 Results **18**
3.1 Probiotics' effects on catfish growth performance . . . 18
3.2 Probiotics' effects on catfish condition factor and feed utilization . . . 19
3.3 Probiotics' effects on catfish's biochemical composition 20
4 Discussion **20**
References **21**

1. Introduction

The current expansion and aquaculture's increased intensity has resulted in a rise in outbreak of bacterial diseases of fish (Sundberg et al., 2016; Da Costa Sousa et al. 2019), leading to financial loss (Oroji et al. 2021;Irshath et al., 2023; Hegde et al., 2023). The need to prevent and control disease outbreaks in aquaculture stems from the financial losses these outbreaks cause (Ayisi et al. 2017b). Application of antimicrobial agents and chemotherapeutant such as antibiotics as antidote to increasing outbreak of diseases in aquaculture has led to environmental challenges (Burridge et al. 2010) and drug resistance (Ozorio et al. 2015). Furthermore, it has been reported that using these antimicrobial agents pollutes the water in the aquaculture environment (Alcaide et al., 2005) and might have an impact on human health (Cabello, 2006). As a result, a necessity exists suitable alternatives that would ensure a microbiologically balanced environment (Saenz de Rodriganez et al., 2009), as well as enhance immunity and promote fish growth. Using probiotics is one strategy that is becoming more and more important for preventing infections and disease outbreaks (Das et al. 2021).

Probiotics are suitable alternatives since they enhance systemic immunity and improve fish's physiological performance (Dawood et al. 2018). Furthermore, probiotics are also known to improve growth performance (Hai 2015; Chauhan & Singh 2018). Lactic acid bacteria (LAB) (Beck et al. 2015; Liu et al. 2016) and Bacillus spp. (Chai et al. 2016; Giatsis et al. 2016) represent the primary probiotic microbes utilized in aquaculture. A majority of the genius Bacillus are aerobic and catalase positive which differs from sporolactobacillus and clostridia (Gordon et al., 1973). The use of Bacillus has been investigated by some scholars (Abarike et al., 2018, Rodigues et al., 2020; Maas et al., 2021; Das et al., 2021; Shadrack et al., 2021). Probiotics bacillus strains such as subtilis (Das et al., 2021), cereus (He et al., 2017; Yang et al., 2019), amyloliquefaciens (Maas et al., 2021) and coagulans (Amoah et al., 2021) have been studied in various fish and shell fish.

African catfish, *Clarias gariepinus*, is among the world's most significant species of cultivated fish. It is commonly known that *Clarias gariepinus* has a rapid growth rate and requires a high feed intake throughout its life cycle. *Clarias gariepinus* tolerates relatively poor water quality, grows fast and is an omnivorous fish hence it is considered an excellent aquaculture species (Amisah et al. 2009). The purpose of this study was to evaluate the impact of probiotic *Clarias gariepinus* 200 at different inclusion levels on feed utilization, growth performance, and proximate composition of African catfish (*Clarias gariepinus*). This study will improve the body of knowledge regarding the consequences of administering adding probiotics to fish diets.

2. Materials and methods

2.1 Experimental fish

Three hundred catfish (*Clarias gariepinus*) weighing 3.47 ± 0.95 g on average at purchase were acquired from a commercial farm located in Ghana's Eastern Region. The fish were then transported to the rearing facility where prior to the experiment, were acclimatized fto the rearing conditions. Fish were fed a commercial diet three times a day during the acclimation period until they appeared satiated.

2.2 Experimental Design

Fish that had acclimated were divided into twelve tanks at random (four groups in triplicate). Twenty juvenile C. gariepinus were stocked in each tank, which measured 1 m x 1 m x 1 m. For eight weeks, the four experimental diets were fed to the experimental fish. Every week, fresh water was added to replace half of the water. from a common reservoir..

Table 1. Formulation and composition (% dry matter) of the control diet for *Clarias gariepinus*

Ingredients	**Percentage (%)**
Fish meal	20
Soybean meal	30
Wheat bran	15
Maize	13
Salt	2
Vitamins premix	2
Mineral pre-mix	2
Starch	6
Oil	10
Probiotics	0
Proximate composition	
Protein	40.0
Lipid	9.52
Moisture	10.79
Ash	5.38

2.3 Feed and feeding

Four diets under experimentation were were prepared to have varying concentrations of probiotics (B. Subtilis 200). Diets A, B, C and D contained 0g/kg, 1g/kg, 2g/kg and 3g/kg of probiotics. Table 1 shows the basal diet. The diets were prepared using the progressive enlargement method as previously used by Ayisi et al. (2017b). Briefly, dietary ingredients in powdered form were manually combined with water and oil, and then the appropriate amount of B. Subtilis 200 was included to create a soft dough. The resulting dough was pelletized using a meat mincer. The diets were kept in sealed plastic bags. Fish were fed at 8:00 a.m., 12:00 p.m., and 4:00 p.m. every day until they appeared satiated. Uneaten feed were collected, dried and subtracted from the feed administered to calculate feed intake.

Table 2. Probiotics' effects on catfish growth performance

Growth parameters	A	B	C	D	p-value
Initial length (cm)	6.21±0.45	6.37±0.92	6.25±0.58	6.29±0.72	0.9987
Initial weight (g)	3.41±0.73	3.75±0.54	3.28±0.88	3.48±0.39	0.9655
Final length (cm)	20.25±2.55	20.30±2.75	20.17±2.19	20.40±2.88	0.8995
Final weight (g)	72.33±2.41a	77.48±2.17a	83.17±3.07b	84.73±3.19b	0.0077***
Weight gain (g)	68.95±3.25a	73.70±4.15a	79.86±2.78b	81.27±3.05b	0.0401**
Specific growth rate	5.43±0.37	5.42±0.29	5.78±0.25	5.71±0.41	0.8250

Values are means ± SEM (n=3). There are significant differences ($P < 0.05$) between the means with different superscripts in the same column..
$A = 0g/kg, B = 1g/kg, C = 2g/kg, D = 3g/kg$

2.4 Sample collection

To determine the weight gain, survival rate, and specific growth rate, each fish that survived was counted and its length and weight were measured in centimetres (cm) and grams (g), respectively. For the proximate composition analysis, a random selection of five fish per tank was used. The whole body of each fish was used for the analysis.

2.5 Growth and feed utilization

Since the study's goal was to assess how probiotics affected catfish growth and feed utilization, the following metrics were employed to gauge those effects, following Ayisi et al. (2017b).. Final weight (g) − initial weight (g) = weight gain (WG). Specific growth rate (SGR %) is defined as (ln FW (g) - ln IW) (g) /T × 100. Where T is the total number of days spent testing (feeding) Condition factor (K) = [Body Weight / Total Length 3] × 100%. Total Length is in cm. Feed Intake (g) / Weight Gain (g) = Feed conversion ratio (FCR). The total amount of feed consumed (g) during the 56-day trial is known as feed intake (FI). Wet weight gain (g) / Protein intake (g) = Protein efficiency ratio (PER).

2.6 Proximate composition

Fish samples from experimental treatments (five fish from each tank) were transported to the lab for protein, lipid, moisture, and ash analyses. In order to analyze these parameters, standard protocols of AOAC (2003) which were previously described by Mehbood et al. (2017) were applied. To determine the moisture content, samples were dried at 105 °C until they reached a constant weight.. To ascertain the amount of ash present, samples were burned for 12 hours at 550 °C in a muffle furnace. Amoah et al. (2021) had previously employed the Kjeldahl method for the analysis of crude protein content. This same procedure was used in this present study. Crude protein was calculated by applying the Folch et al. (1957) method. This method allowed for the determination of each sample's nitrogen content, which was then multiplied by 6.25.. For each value, the percentage dry weight is used.

3. Results

3.1 Probiotics' effects on catfish growth performance

Table 2 displays the impact of probiotics on catfish growth. Final length was indifferent irrespective of the probiotic level in the feed (p=0.8995). Final length ranged between 20.17±2.19 and 20.40±2.88. Final weight (FW) and weight gain (WG),were significantly different with p-value of 0.0077 and 0.0401 respectively. As probiotic levels increased, there was a corresponding increase in FW. Group fed diet D recorded the highest WG (81.27±3.05) whilst group fed diet A recorded the least WG (68.95±3.25). Weight gain increased with increasing levels of probiotics. Feeding fish with different levels of probiotics did not alter SGR significantly (P=0.8250), and ranged from

5.42±0.29 (diet B) to 5.71±0.41 (diet D). Groups fed diet A and diet C recorded SGR of 5.43±0.37 and 5.78±0.25, respectively.

3.2 Probiotics' effects on catfish condition factor and feed utilization

The effects of probiotics on condition factor, feed utilization as well as hepatosomatic index of catfish is presented in Table 3. The amount of feed intake varied significantly between treatments (p=0.0042). Feed intake increased as probiotics level increased. The range of feed intake was 69.23±0.21 to 77.21±0.84. There was no discernible variation in the feed conversion ratio (p=0.9953). Nonetheless, it was noted that when probiotic levels increased, the feed conversion ratio (FCR) decreased. FCR ranged between 0.95±0.21 and 0.99±0.09

There was no discernible difference in the protein efficiency ratio (p=0.8755). However a non-significant increase in PER was reported as probiotic levels increased in experimental diets. PER ranged between 2.46±0.45 (Diet A) and 2.63±0.15 (diet D). The PER values for the diet B and C-fed groups were 2.60±0.25 and 2.60±0.25, respectively.

Table 3. Probiotics' effects on catfish condition factor and feed utilization

Parameters	A	B	C	D	p-value
Feed intake (g)	69.23±0.21a	72.35±0.37a	76.84±0.75b	77.21±0.84b	0.0042**
Feed conversion ratio	0.99±0.09	0.98±0.14	0.96±0.12	0.95±0.21	0.9953
Protein efficiency ratio	2.46±0.45	2.55±0.37	2.60±0.25	2.63±0.15	0.8755
Condition factor	0.87±0.15	0.92±0.18	1.01±0.21	0.99±0.16	0.9398

Values are means ± SEM (n=3). There are significant differences ($P < 0.05$) between the means with different superscripts in the same column.
$A = 0g/kg, B = 1g/kg, C = 2g/kg, D = 3g/kg$

3.3 Probiotics' effects on catfish's biochemical composition

Regarding the biochemical composition of the treatments, there was not a noticeable distinction (P>0.05). Moisture content ranged between 74.19±3.09 and 76.26±2.55 whilst crude protein ranged between 15.49±2.54 to 17.22±3.11. The moisture content of fish fed diet D was highest, while that of fish fed diet B was lowest.. Crude lipid content decreased as probiotic levels increased whilst ash content increased with increasing probiotic content. Fish fed diets A, B, C and D recorded ash content of 4.32±1.43, 5.88±1.00, 5.79±0.45 and 6.75±1.13 respectively.

4. Discussion

Numerous investigations have revealed improved growth rate in fish fed probiotics (Giri et al., 2013; Guidoli et al. 2018; Gobi et al., 2018; Sousa et al., 2019; Silva et al. 2020). Probiotics enhance their host's health and subsequently improve growth by balancing microbial flora in the intestines (Fuller 1989). This study evaluated the probiotics' effects on the utilization of feed and growth of African catfish (*Clarias gariepinus*). The probiotic-fed groups showed a significant improvement in their growth performance (P<0.05). This is consistent with earlier research by Al-Dohail et al. (2009), which reported that feeding diets enhanced with L. acidophilus significantly increased the growth of African catfish. Similarly, Putra et al. (2017) recorded significant improvement of growth in African catfish fed probiotics. The increased growth performance recorded in groups fed probiotics could be as a result of proper balance of the intestinal microbial flora arising from increased nutrient digestibility, better absorption, and enhanced enzyme activities.

Feed intake increased significantly with increasing probiotics content (P<0.05). This is consistent with a prior study by Nwanna and Tope-Jegede (2017) that found that when *Clarias gariepinus* was fed varying concentrations of L. plantarum, the mean feed intake increased. Contrary to this study, there was reduction in feed intake as *Clarias gariepinus* in diets of *Clarias gariepinus* was increased (Lawal et al. 2019). The variations in the age of the species and feed protein content may be the cause of the discrepancies in the results. Feed conversion ratio (FCR) was indifferent irrespective of the levels of probiotics. This implies all groups had similar conversion abilities to utilize feed.

In this study, there was no discernible change in the feed conversion ratio between the treatments. FCR in fish fed probiotic diets were similar to the control diet. This implies that probiotics did not raise the levels of gastrointestinal bacteria responsible for nutrient decomposition, hence additional vitamins, enzymes, and amino acids were not produced. Increasing probiotic content in diets increased protein content. Increased nutrient deposition may be the cause of this. There was no discernible

Table 4. Effects of probiotics on biochemical composition of catfish

Parameters	A	B	C	D	p-value
Moisture (%)	75.25±3.47	74.19±3.09	75.18±4.16	76.26±2.55	0.9792
Crude protein (%)	15.49±2.54	16.23±2.15	17.22±3.11	17.05±2.16	0.9595
Crude lipid (%)	4.90±0.93	4.82±1.02	4.78±0.58	3.28±0.27	0.3769
Ash (%)	4.32±1.43	5.88±1.00	5.79±0.45	6.75±1.13	0.4464

Values are means ± SEM (n=3). There are significant differences (P < 0.05) between the means with different superscripts in the same column.
$A = 0g/kg, B = 1g/kg, C = 2g/kg, D = 3g/kg$

difference among treatments, contrary to earlier reports that documented significant differences among groups in tilapia (El-Haroun et al. 2006; Bagheri et al. 2008). However, this study is consistent with previous research (Merrifield et al., 2010; Hassaan et al., 2018) which found no discernible variation in protein content among fish fed varying amounts of probiotics. Azarin et al. (2015) and Hassaan et al. (2018) previously revealed a rise in the amount of protein. and a decrease in lipid content, which could be explained by variations in their breakdown, the rate at which they deposit in muscle, and varying growth rates (Abdel-Tawwab et al., 2006).

This study recommend that catfish farmers could apply B. Subtilis 200 at 3g/kg of diet to maximize growth and feed utilization without compromising proximate composition. It is recommended that further studies be conducted on how probiotics affect genes that regulate growth as well as how the influence intestinal microbiota.

Conflict of Interests Statement

No conflicts of interest are disclosed by the authors

Data Availability

Upon reasonable request, all study-related data will be made available.

References

[1] Abarike, E.D., Cai, J., Lu, Y., Yu, H., Chen, L., Jian, J., Tang, J., Jun, L., Kuebutornye, F. K., 2018. Effects of a commercial probiotic BS containing Bacillus subtilis and Bacillus licheniformis on growth, immune response and disease resistance in Nile tilapia, Oreochromis niloticus. Fish Shellfish Immun. 82, 229–238

[2] Alcaide, E., Blasco, M.D., & Esteve, C. (2005) Occurrence of drug resistant bacteria in two European eel farms. Applied and Environmental Microbiology, 71, 3348–3350.

[3] Al-Dohail MA., Hashim, R., & Aliyu-Paiko, M. (2009). Effects of the probiotic, Lactobacillus acidophilus, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (Clarias gariepinus, Burchell 1822) fingerling. Aquaculture Research, 40(14), 1642-1652. https://doi.org/10.1111/j.1365-2109.2009.02265.x

[4] Amisah, S., Oteng, M.A., & Ofori, J.K. (2009). Growth performance of the African catfish, Clarias gariepinus, fed varying inclusion levels of Leucaena leucocephala leaf meal. Journal of Applied Sciences & Environmental Management, 13(1), 21 - 26

[5] Amoah K., Dong X-H., Tan B-P., Zhang S., Chi S-Y., Yang Q-H., Liu H-Y., Yang Y-Z., and Zhang H (2021). Effects of three probiotic strains (Bacillus coagulans, B. licheniformis and Paenibacillus polymyxa) on growth, immune response, gut morphology and microbiota, and resistance against Vibrio harveyi of northern whitings, Sillago sihama Forsskal ´ (1775). Animal Feed Science and Technology 277: 114958

[6] AOAC. (2003). Official methods of analysis, 17th ed. Association of Official Analytical Chemists, Washington D.C., USA.

[7] Ayisi, C.L., Apraku, A., & Afriyie, G. 2017. A Review Of Probiotics, Prebiotics, And Synbiotics In Crab: Present Research, Problems, And Future Perspective. Journal of Shellfish Research,. 36(3), 1–8.

[8] Ayisi, C.L., Zhao, J. and Rupia, E.J. 2017. Growth performance, feed utilization, body and fatty acid composition of Nile tilapia (Oreochromis niloticus) fed diets containing elevated levels of palm oil. Aquaculture and Fisheries, 2(2), 67-77. https://doi.org/10.1016/j.aaf.2017.02.001.

[9] Azarin H., Aramli, M. S., Imanpour, M. R., & Rajabpour, M. (2015). Effect of a probiotic containing Bacillus licheniformis and Bacillus subtilis and ferroin solution on growth performance, body composition and haematological parameters in Kutum (Rutilus frisii kutum) fry. Probiotics and antimicrobial proteins, 7, 31-37

[10] Bagheri, T., Hedayati, S.A., Yavari, V., Alizade, M., & Farzanfar, A. (2008). Growth, Survival and Gut Microbial Load of Rainbow Trout (Onchorhynchus mykiss) Fry Given Diet Supplemented with Probiotic during the Two Months of First Feeding. Turkish Journal of Fisheries and Aquatic Sciences, 8, 43-48.

[11] Beck, B. R., Kim, D., Jeon, J., Lee, S.-M., Kim, H. K., Kim, O.-J., et al. (2015). The effects of combined dietary probiotics Lactococcus lactis BFE920 and Lactobacillus plantarum FGL0001 on innate immunity and disease resistance in olive flounder (Paralichthys olivaceus). Fish Shellfish Immunol. 42, 177–183. doi: 10.1016/j.fsi.2014.10.035

[12] Burridge L., Weis J.S., Cabello F., Pizarro J. & Bostick K. (2010) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306, 7–23.

[13] Cabello, F.C. (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ. Microbiol., 8, 1137–1144.

[14] Chai, P-C., Song, X-L., Chen, G-F., Xu, H., Huang, J. (2016). Dietary supplementation of probiotic Bacillus PC465 isolated from the gut of Fenneropenaeus chinensis improves the health status and resistance of Litopenaeus vannamei against white spot syndrome virus. Fish & Shellfish Immunology, 54, 602-611. https://doi.org/10.1016/j.fsi.2016.05.011.

[15] Chauhan, A., and Singh, R. (2018). Probiotics in aquaculture: A promising emerging alternative approach. Symbiosis, 1–15.

[16] Da Costa Sousa N, do Couto MVS, Abe HA, et al. Effects of an Enterococcus faecium-based probiotic on growth performance and health of Pirarucu, Arapaima gigas. Aquac Res. 2019;00:1–9. https://doi.org/10.1111/are.1433

[17] Das, S., Mondal, K., Kumar A.P., Sengupta, C. (2021). Evaluation of the probiotic potential of Streptomyces antibioticus and Bacillus cereus on growth performance of freshwater catfish Heteropneustes fossilis. Aquaculture Reports. 20:100752.

[18] Dawood, M.A., Koshio, S., Esteban, M.A., 2018. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev. Aquac. 10, 950–974. https://doi. org/10.1111/raq.12209.

[19] El-Haroun, E.R., A.M.A.-S. Goda & M.A. Kabir Chowdury. 2006. Effect of dietary probiotic Biogen supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia Oreochromis niloticus (L.). Aquacult. Res., 37: 1473-1480.

[20] Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry 226, 497–509.

[21] Fuller, R (1989). Probiotics in man and animals. J. Appl. Bacteriol., 66, 365–78.

[22] Giatsis, C., Sipkema, D., Ramiro-Garcia, J., Bacanu, G.M., Abernathy, J., Verreth, J., Smidt, H., and Verdegem M. (2016). Probiotic legacy effects on gut microbial assembly in tilapia larvae. Sci Rep 6, 33965. https://doi.org/10.1038/srep33965

[23] Giri, S. S., Sukumaran, V., & Oviya, M. (2013). Potential probiotic Lactobacillus plantarum VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, Labeo rohita. Fish & Shellfish Immunology, 34, 660–666. https://doi.org/10.1016/j.fsi.2012.12.008

[24] Gobi, N., Vaseeharan, B., Chen, J. C., Rekha, R., Vijayakumar, S., Anjugam, M., & Iswarya, A. (2018). Dietary supplementation of probiotic Bacillus licheniformis Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against Aeromonas hydrophila in tilapia Oreochromis mossambicus. Fish & Shellfish Immunology, 74, 501–508. https://doi.org/10.1016/j.fsi.2017.12.066

[25] Gordon, R.E., Haynes, W.C., Pang, C.H.N., Smith, N.R., (1973). The Genus Bacillus. US Department of Agriculture Handbook, vol. 427, 1973.

[26] Guidoli, M.G., Mendoza, J.A., Falcón, S.L, Boehringer, S.I., Sánchez, S., Macías, MEFN. (2018). Autochthonous probiotic mixture improves biometrical parameters of larvae of Piaractus mesopotamicus (Caracidae, Characiforme, Teleostei). Ciência Rural, Santa Maria, 48:07, e20170764.

[27] Hai, N. V. (2015). The use of probiotics in aquaculture. Journal of Applied Microbiology, 119(4), 917–935. https://doi.org/10.1111/jam.12886

[28] Hassaan M. S., Soltan, M. A., Jarmołowicz, S. and Abdo, H. S. (2018). Combined effects of dietary malic acid and Bacillus subtilis on growth, gut microbiota and blood parameters of Nile tilapia (Oreochromis niloticus). Aquaculture Nutrition; 24(1): 83-93.

[29] Irshath, A.A., Rajan, A.P., Vimal, S., Prabhakaran, V.-S., Ganesan, R. (2023). Bacterial Pathogenesis in Various Fish Diseases: Recent Advances and Specific Challenges in Vaccine Development. Vaccines, 11, 470. https://doi.org/10.3390/vaccines11020470

[30] Lawal M.O., Aderolu A.Z., Adewumi G.A. and Mudiaga A. (2019). Growth, nutrient utilization, haematology and biochemical parameters of African catfish (Clarias gariepinus, Burchell, 1822) fed with varying levels of Bacillus subtilis. Agrosearch, 19(1): 13-27 https://dx.doi.org/10.4314/agrosh.v19i1.2 13

[31] Liu, Y., Gibson, G.R., and Walton, G.E. (2016). An in vitro approach to study effects of prebiotics and probiotics on the faecal microbiota and selected immune parameters relevant to the elderly. PLoS One 11 (9), e0162604.

[32] Maas RM, Verdegem M.C.J., Debnath S, Marchal L., Schrama J.W. (2021). Effect of enzymes (phytase and xylanase), probiotics (B. amyloliquefaciens) and their combination on growth performance and nutrient utilisation in Nile tilapia. Aquaculture 533: 736226

[33] Mehbood, A., Khan, N., Atiq, U., Iqbal, K.J., Tayyab, R., Batool, S.S., Batool, H.S., Amjad, S., & Tanveer, M. (2017). Effect of fenugreek as a feed additive on the growth, body composition and apparent nutrients digestibility of striped catfish Pangasius hypophthalmus fry. Pakistan J. Zool., 49, 2037-2042

[34] Merrifield DL, Dimitroglou A, Bradley G, Baker RTM, Davies SJ (2010b) Probiotic applications for rainbow trout (Oncorhynchus mykiss Walbaum) I. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. Aquaculture Nutrition 16: 504–510

[35] Mohsen Abdel-Tawwab M., Khattab, Y.A.E., Ahmad M.H.& Shalaby A.M..E. (2006). Compensatory Growth, Feed Utilization, Whole-Body Composition, and Hematological Changes in Starved Juvenile Nile Tilapia, Oreochromis niloticus (L.), Journal of Applied Aquaculture, 18:3, 17-36, DOI: 10.1300/J028v18n03_02

[36] Nwanna, L.C. and Tope-Jegede H. (2017) Effects of dietary Lactobicillus plantarum on the growth, carcass quality, and immune response of African catfish (Clarias gariepinus) challenged with Salmonella typhi, Journal of Applied Aquaculture, 29:1, 62-80

[37] Oroji, E., Mehrgan M.S., *, Islami H.R., Sharifpour I. (2021). Dietary effect of Ziziphora clinopodioides extract on zootechnical performance, immune response, and disease resistance against Yersinia ruckeri in Oncorhynchus mykiss. Aquaculture Reports. 20:100827.

[38] Ozorio ROA., Kopecka-Pilarczyk, J., Peixoto, M.J, Lochmann, R., Santos, R.J., Santos, G., Weber, B., Calheiros, J., Ferraz-Arruda, L., Vaz-Pires, P., & Goncalves J.F.M., (2015). Dietary probiotic supplementation in juvenile rainbow trout (Oncorhynchus mykiss) reared under cage culture production: effects on growth, fish welfare, flesh quality and intestinal microbiota. Aquaculture Research, 1–16

[39] Putra, I., Rusliadi, R., Fauzi, M., Tang, U.M. and Muchlisin, Z.A. 2017. Growth performance and feed utilization of African catfish Clarias gariepinus fed a commercial diet and reared in the biofloc system enhanced with probiotic. F1000 Research, 6: 1545

[40] Rodigues, ML., Damasceno, DZ., Gomes, RLM., Sosa, BDS., Moro, E.B., Boscolo, W.R., Bittencourt F., Signor, A. (2020). Probiotic effects (Bacillus cereus and Bacillus subtilis) on growth and physiological parameters of silver catfish (Rhamdia quelen). Aquaculture Nutrition, 27(2) 454-467.

[41] Saenz de Rodriganez, M.A., Diaz-Rosales P., Chabrillion, M., Smidt, H., Arijo, S., Leon-Rubio, J.M., Alarcon, F.J., Balebona, M.C., Morinigo, M.A., Cara, J.B and Moyano, F.J (2009). Effect of dietary administration of probiotics on growth and intestine functionality of juvenile Senegalese sole (Solea senegalensis, Kaup 1858). Aquaculture Nutrition, 15, 177-185

[42] Shadrack, R.S., I. Manabu, and S. Yokoyama. 2021. Efficacy of single and mix probiotic bacteria strain on growth indices, physiological condition and bio-chemical composition of juvenile amberjack (Seriola dumerili). Aquaculture Reports (20) 100753.

[43] Silva, V.V., Salomão, R.A.S., Mareco, E.A., Pai, M.D., Santos, V.B (2020). Probiotic additive affects muscle growth of Nile tilapia (Oreochromisniloticus). Aquaculture Research, 52(5): 2061-2069. https://doi.org/10.1111/are.15057

[44] Sundberg, L-R., Ketola, T., Laanto, E., Kinnula, H., Bamford, J.K.H, Penttinen, R., Mappes J. (2016). Intensive aquaculture selects for increased virulence and interference competition in bacteria. Proc. R. Soc. B 283, 20153069. http://dx.doi.org/10.1098/rspb.2015.3069

[45] Susmita Das S., Mondal K., Kumar pal A., Sengupta C. (2021). Evaluation of the probiotic potential of Streptomyces antibioticus and Bacillus cereus on growth performance of freshwater catfish Heteropneustes fossilis. Aquaculture Reports 20: 100752