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THE SYNERGISTIC EFFECT OF TECHNOMOS® PREBIOTIC AND BETAPLUS® PROBIOTIC ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF NILE TILAPIA JUVENILES (*OREOCHROMIS NILOTICUS*, LINNAEUS, 1758)

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Abstract

This study aimed to evaluate the effect of additives used in tilapia feed and represented by TechnoMos® prebiotic (Saccharomyces cerevisiae) and BetaPlus® probiotic (Bacillus subtilis and Bacillus licheniformis) on the growth and biochemical composition of biological material. The analysis of the results obtained on the principal growth indicators shows a positive correlation between the applied feeding regime and the biomass gain. In the first experimental stage, there was an upward trend of biomass gain depending on the feeding regime applied, obtaining values between 1439 ± 18.29 g in the control variant (V0) and 1774.61 ± 47.65 g in the experimental variant with synbiotic (V3). In the second experimental stage, the biomass gain obtained varied from 3286 ± 15.05 g in the control variant to 4078.52 ± 69.84 g in the synbiotic variant. Biochemical analysis of tilapia muscle tissue showed an increase in the content of proteins, lipids in biomass of the samples fed with the probiotic Betaplus® correlated with the increase in biomass weight, compared to the control variant. In conclusion, the analysis of growth indicators shows the beneficial effect of symbiotic variant through their synergistic action and the influence of probiotic applied on improving biomass gain and biochemical composition.

Key words: *biochemical composition, growth, prebiotic, probiotic, tilapia.*

INTRODUCTION

The Nile Tilapia ranks second in the world in terms of importance as an aquatic animal due to its high demand, fast growth, and reasonable prices (El Asely et al., 2020a, 2020b). Often a single type of food additive can be used to feed fish, but using a mixture with several strains than one type appears to be more effective (Dawood et al., 2015; Yilmaz et al., 2019; Yılmaz et al., 2020).

Probiotics and prebiotics for fish are commercially available in powder form. They are generally added directly to fish ponds or mixed with feed ingredients. In any case, these approaches can drastically reduce the survival rates of probiotic cells. Incorporation of probiotics and prebiotics into pelleted fish feed could guarantee more than fish would get those functional ingredients with synergic effects. It was reported that the fingerlings fed with mixed probiotic pellets had a higher percentage of weight gain and specific growth rate, while the feed conversion rate was lower than the mixed probiotic fodder (Sivakumar et al., 2020).

The genus *Bacillus* is most studied as a host-associated probiotic for a wide variety of aquatic animals. The introduction of probiotics into aquaculture began with the advent of their use of scientific and sustainable technologies such as recirculating aquaculture systems, zero-water aquaculture production systems, and biofloc technologies.

Bacillus sp. are rich in exogenous enzymes such as lipase, protease, amylase, phytase, chitinases, cellulases, and β-1,3-glucanases, which help break down nutrients and improve digestion (Wang, 2007; Liu et al., 2009; Wu et al., 2012; Soltani et al., 2019).

The mixture of *Bacillus subtilis* and *Bacillus licheniformis* has shown significant benefits in aquaculture, including improved growth performance, immunomodulation, and survival rate (Abarike et al., 2018).

The application of probiotics in RAS has improved water quality, feeding efficiency, and the growth of various crop organisms (Rurangwa & Verdegen, 2014). Addition of *Bacillus subtilis* to the batch sequencing reactor (SBR) of RAS has increased the efficiency of sludge treatment (Lu et al., 2012). Another study performed in RAS with dietary supplements of *Bacillus subtilis* improved growth performance, immunity, and genetically modified tilapia disease resistance (Zhu et al., 2019).

Prebiotics are used as indigestible food ingredients metabolized by bacteria or probiotics that promote health (Ringo et al., 2016). Prebiotics reduce fish mortality caused by the invasion of pathogens and intensify the biological responses of the host. The use of mannan oligosaccharides (MOS), the insulin that is obtained from the yeast cell wall, *Saccharomyces cerevisiae*, and prebiotics such as fructooligosaccharides (FOS) are immunostimulators that improve the immune response and disease resistance to fish (Carbone & Faggio, 2016).

Synbiotics are products that contain both probiotics and prebiotics (Akrami et al., 2015). The use of symbiotics in aquaculture is considered recent, and studies indicate positive effects on the host, related to enzymatic digestion, production of acetic, lactic, and butyric acids (products of prebiotic fermentation by probiotic bacteria), and activation of the innate immune system. (Huynh et al., 2017; Ringø and Song, 2016).

The positive effect of using two or more feed additives results in three modes, namely: additivity, synergism, or potentiation (Meseguer and Cerezuela, 2011). Thus, the action of the probiotic bacteria have used may be increased by prebiotics due to the contribution of this component to the metabolism and activation of the growth of these bacteria (Akhter et al., 2015).

The present study aims to investigate the synergistic effects of supplementation of a probiotic *(Bacillus subtilis* and *Bacillus licheniformis*) and the prebiotic (yeast extract *Saccharomyces cerevisiae,* MOS, β-1,3-glucans) on growth and biochemical composition muscle tissue of the juvenile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Experimental design

The study was carried out during an experiment organized in two stages, using the pilot recirculating system within the Department of Foood Science, Food Engineering, Biotechnology, and Aquaculture, University "Dunarea de Jos" of Galați. The experimental recirculating aquaculture system used is provided with 12 growth units (glass growth units with a thickness of 10 mm) with a volume of 0.132 m^3 each (36 × 37.5 × 98 cm), described by Mocanu et al. (2011). Removal of residual solids from the growth units resulting from metabolism has been performed employing a pressure sand filter, while a biological filtration unit was used to control the concentration of ammoniacal nitrogen produced by the culture biomass - trickling filter. The sterilization and disinfection of the water on the essential supply water supply circuit of the growth units was performed on the Tetra Quiet UV-C 35000 sterilization, with a power of 36 W. These water remediation steps is performed using three pumps type *DAB A 80 180 XM* (flow rate: Q = 0.6 - 8.7 m^3/h). To ensure the required dissolved oxygen (DO) has been using a compressor *Fiap Air Active 10000* type with a power of 100 W, pressure 0.042 MPa, which introduces an airflow of 8400 l/h.

The biological material (Nile tilapia) was obtained after reproduction of the mature tilapia, previously grown in the existing recirculating aquaculture system within the department. This biological material was at that time in the juvenile stage and had three months in the first stage and five months in the second experimental stage. Its sorting was performed and a homogeneous batch of fish was retained and distributed randomly in the 12 growth units of the recirculating aquaculture system.

The present study was carried out in two experimental stages, and four experimental variants have been established in triplicate, namely: Control variant (V_0) - without probiotics and prebiotics; Probiotic variant (V_1) - with BetaPlus® probiotics – 1%×BW; Prebiotic variant (V_2) - with TechnoMos® prebiotics – 1%×BW; Synbiotic variant (V_3) - with BetaPlus® probiotics and TechnoMos® prebiotics - 1:1% × BW.

For the first stage was used 960 fish, with an average weight of 1.52 g/fish, and in the second stage 360 fish with an average weight of 73.96 g/fish. During the first experimental stage (40 days), the fish were fed with commercial extruded feed ALLER FUTURA EX, with a protein content of 64% and 12%, the diameter of the granules being between 0.5-1 mm, at a feeding frequency of three meals per day (Table 1). In the second experimental stage (50 days), the fish were fed with ALLER SILVER, with a content of 45% crude protein and 20% lipids, the diameter of the pellets being 2 mm. Feeding intensity was calculated, and feeding frequency was twice daily. The biochemical composition of the feed were shown in Table 1.

Table 1. Biochemical composition of fodder pellets used.

Biochemical composition	ALLER FUTURA EX	ALLER SILVER
Crude protein	64%	45%
Fat	12%	20%
Crude cellulose	0.5%	2%
Crude ash	11%	8.1%
Phosphor	1.5%	1%
NFE	5%	16%
Digestible energy	5035/21 kcal kg^{-1}	5035/21 kcal kg^{-1}
Vitamin A	10000 UI kg^{-1}	10000 UI kg^{-1}
Vitamin D3	1000 UI kg^{-1}	1000 UI kg^{-1}
Vitamin E	400 UI kg^{-1}	200 UI kg^{-1}
Vitamin C	180 UI kg^{-1}	150 UI kg^{-1}
Cystine	0.4%	0.6%
Lysine	2.5%	2.4%
Methionine	0.50%	0.75%

During the experimental period, the daily administered ratio was updated every ten days and, the specific quantities of the two feed additives applied had been calculated, (TechnoMos® prebiotic and BetaPlus® probiotic) and incorporated into feed. TechnoMos® prebiotic is an extract from selected yeast, obtained from *Saccharomyces cerevisiae*, rich in mannan oligosaccharides and beta-glucans (β-1,3-glucans). The probiotic BetaPlus® consists of BioPlus® *2B* and betaine (nitrogenous substance), the concentration being 1 × 10^{12} CFU/kg feed and betaine - 936 000 mg/kg. BioPlus® *2B* is a 1: 1 mixture of *Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750). These prebiotics and probiotics used are supplied by the company Biochem from Lohne, Germany through the Romanian subsidiary Biochem Animal Health and Nutrition affiliated to the one from Lohne and located in Cluj-Napoca, Romania. The prebiotic and probiotic embedding protocol was described by Bocioc E. (2011).

The water quality parameters in the RAS were monitored using the following equipment: the dissolved oxygen concentration was measured with the Hanna HI 98186 oximeter; the pH was measured with the pH meter WTW, model 340; the nitrogen compounds concentrations were measured using the Spectroquant NOVA 400 portable spectrophotometer, using compatible kits from Merck.

Technological indicators assessment

At the end of the each experimental stage, the following technological indicators were calculated:

- Individual Weight Gain (IWG) = Final Weight (Wt)–Initial Weight (W0) (g);
- Weight Gain (W) = Final Weight (Wt) – Initial Weight (W0) (g);
- Relative Weight Gain (RWG %) = (Wt–W0) × 100/Wt;
- Fulton condition factor (K) =$100 \times W/L^3$.

Somatic measurements were made at the end of each experimental stage at all fish. Total length (TL) and body weight (BW) for each variant were used to determine the relationship W=a×Lb, where "a" is the intercept (the initial growth coefficient), and "b" is the allometric coefficient (Ricker, 1975). The coefficient of variation (CV, %) was calculated as the ratio of the standard deviation to the mean of weight to have a measure of fish dispersion.

Biochemical composition of tissue

Proteins were determined with Gerhardt equipment by using the Kjeldahl method, fats were determined by Soxhlet solvent extraction method (petroleum ether) with Raypa extraction equipment, the dry matter was determined by heating at a temperature of 105±2ºC using Sterilizer Esac and ash was evaluated by calcification at temperatures of 550±20°C, in a Nabertherm furnace.

Statistical analysis

Data were analyzed using SPSS 21 for Windows. Results regarding fish growth performance and the biochemical composition of the tissue were expressed by average and standard deviation (Average±SD).

Kolmogorov-Smirnov tests determined the normality of the data used for analysis. Oneway

ANOVA and Duncan's multiple range tests were used to compare the differences between the experimental groups. Significance was determined at $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Water quality

Oreochromis niloticus (tilapia) is a thermophilic species, the water temperature was the main parameter that had to be kept in the optimal range. In the first experimental stage, the temperature of the water varied between 27.00-28.80°C, with an average value of 27.82±0.48°C, and in the second experimental stage, the temperature remained the same, with a variation between 27.20-28.90°C and an average of 28.34±0.44°C (Figure 1).

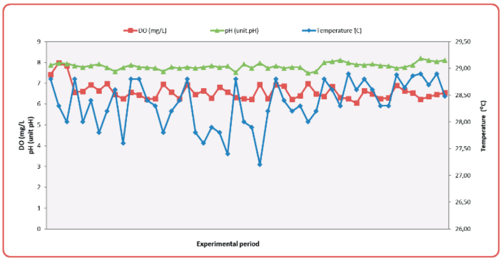

Figure 1. Temperature, dissolved oxygen, and pH during the experimental period

During the experimental period, dissolved oxygen was maintained between 6.25-7.98 mg/L, with a mean value of 7.28±0.48 mg/L in the first stage and a range between 6.05-7.98 mg/L, with a mean of 6.59±0.38 mg/L in the second stage (Figure 1). The pH values recorded during the experimental period varied between 7.45-7.97 pH units, with an average of 7.77±0.12 pH units in the first stage and a range between 7.48-8.20 pH units, with a mean of 7.83±0.15 pH units in the second experimental stage (Figure 1).

Nitrites ($N\text{-}NO_2^-$) recorded during the experimental period remained in the range of 0.03-0.09 mg/L, and nitrates ($N\text{-}NO_3^-$) indicated values between 18.4-21.4 mg/L. Ammonium ion (NH_4^+) values ranged from 0.02-0.06 mg/L. All the registered values of nitrogen compounds have shown in Figure 2.

In the current experimental period, the water quality parameters were kept within the optimal range of *Oreochromis niloticus*, as evidenced by the fact that the water conditioning equipment was able to treat and reuse the water efficiently, achieving a daily water exchange in the proportion of 10-15%.

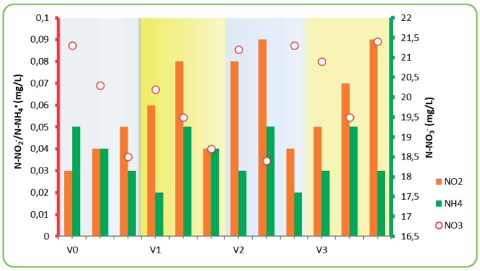

Figure 2. Nitrogen compounds during the experimental period

Water quality indicators during the experimental period had maintained in the optimal range of the species (Nile tilapia), except for nitrate values, which due to the low water exchange showed a minimum exceedance of the optimal interval of the crop species.

Fish growth

The study on the main determination of the synergistic effect of TechnoMos® prebiotic and BetaPlus® probiotic on the growth indicators of juveniles tilapia (*Oreochromis niloticus*) was carried out in two experimental stages. At the beginning of the experimental period, the average individual biomass for the four experimental groups did not show significant differences ($p>0.05$). The distribution of the groups in terms of body mass variability showed normal distribution, an aspect also highlighted by the Kolmogorov-Smirnov test ($p>0.05$). Table 2 summarizes the technological indicators calculated for the first experimental stage.

During the experimental trial, periodically weighing had performed (at an interval of 10 days) to update the administered ratio and, respectively, the quantities of prebiotics and probiotics applied in the feed. In Figure 3 is presented the evolution of fish biomass during the first experimental stage. From Figure 3, it can be noticed the ascending trend of the variants in which the synbiotic was applied, compared to the control variant. There is a more pronounced increase in the last period of the stage, especially in the case of the experimental variant (V_3) where the TechnoMos® prebiotic and the BetaPlus® probiotic were administered in a ratio of 1:1.

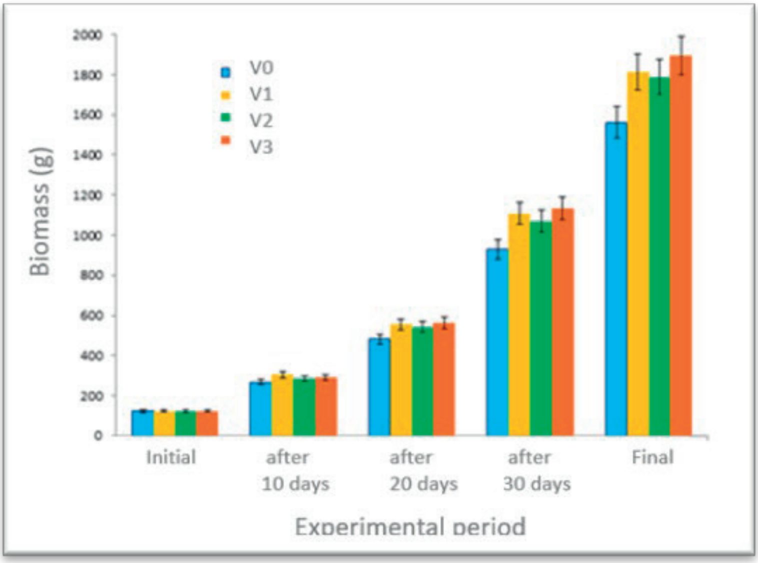

Figure 3. Evolution of biomass during the first experimental stage

Table 2. Technological indicators of the juveniles tilapia in the first experimental stage

Technological indicators	Control (V_0)	Probiotic (V_1)	Prebiotic (V_2)	Synbiotic (V_3)
The initial number of fish	80	80	80	80
The final number of fish	80	80	80	80
Survival rate (%)	100	100	100	100
Initial biomass (g)	121.80±0.32	122.21±0.29	121.70±0.23	122.48±0.62
Initial biomass (kg m^{-3})	0.92±0.00	0.92±0.00	0.93±0.00	0.93±0.00
Final biomass (g)	1561±18.51	1813.26±28.52	1788.58±80.29	1897.09±48.23
Final biomass (kg m^{-3})	11.83±0.14	13.55±0.61	13.74±0.22	14.34±0.37
Weight gain (g)	1439±18.29	1691.05±28.71	1666.88±18.29	1774.61±47.65
Weight gain (kg m-^{3})	10.90±0.14	1691.05±28.71	1666.88±18.29	1774.61±47.65
Initial weight (g fish$^{-1)}$	1.52±0.01	1.53±0.03	1.52±0.02	1.53±0.01
Final weight (g fish^{-1})	19.52±0.23	22.67±0.36	22.36±1.00	23.71±0,.0
Individual weight gain (g)	17.99±0.23	21.14±0.36	20.48±1.00	22.18±0.60
Relative Weight Gain (%)	92.20±0.08	93.19±0.29	93.26±0.12	93.54±0.13

Note: Data are presented as triplicate mean ± SD.

Table 3. Technological indicators of the juveniles tilapia in the second experimental stage

Technological indicators	Control (V_0)	Probiotic (V_1)	Prebiotic (V_2)	Synbiotic (V_3)
The initial number of fish	30	30	30	30
The final number of fish	30	30	30	30
Survival rate (%)	100	100	100	100
Initial biomass (g)	1924±5.93	2320.68±8.66	2221.74±31.34	2408.82±8.71
Initial biomass (kg m^{-3})	14.58±0.04	17.58±0.07	16.83±0.24	18.25±0.07
Final biomass (g)	5210±120.47	6120±208.22	5731.33±198.35	6487.33±62.14
Final biomass (kg m^{-3})	39.47±0.91	46.36±1.58	43.42±1.50	49.15±0.47
Weight gain (g)	3286±115.05	3799.32±205.47	3509.59±181.31	4078.52±69.84
Weight gain (kg m-^{3})	24.89±0.87	28.78±1.56	26.59±1.37	30.90±0.53
Initial weight (g fish$^{-1)}$	64±8.08	77.36±9.5	74.06±10,20	80.29±8.22
Final weight (g fish^{-1})	173.67±4.02	204±6.94	191.04±6,61	216.24±2.07
Individual weight gain (g)	109.52±3.83	126.67±6.85	116.99±6.04	135.95±2.33
Relative Weight Gain (%)	63.05±0,76	62.05±1.26	61.21±1.08	62.87±0,48
Fulton condition factor (K)	1.83±0.03	1.83±0.01	1.83±0.02	1.85±0.02

Note: Data are presented as triplicate mean ± SD.

Some researchers have reported that certain probiotics administered in feed did not have any effect on growth. Thus, the non-viable yeast *Saccharomyces cerevisiae* (Marzouk et al., 2008), *Pseudomonas* spp. (El-Rhman et al., 2009), *Enterococcus faecium* (Biomate SF-20®), *Bacillus subtilis* + *Bacillus licheniformis* (Bioplus 2B®), viable yeasts of *Saccharomyces cerevisiae* under the trade name Levucell SB 20® (Shelby et al., 2006) have shown that these probiotics do not influence the growth of tilapia. Table 3 shows the technological indicators calculated for the second experimental stage by processing the initial and final obtained data.
Although at the end of the first experimental stage, the statistical analysis did not show significant differences between the body mass corresponding to the four experimental variants (ANOVA, $p>0.05$), after this period of maintaining the same experimental conditions, a significant increase of fish in the group with synbiotic (V_3) was noticed. At the beginning of the second experimental stage, the post hoc analysis, Duncan, revealed four subsets of values, corresponding to each experimental variant, and the average individual mass in the four experimental variants was 80.29±8.22 g for the variant with symbiotic, 77.36±9.5 g, for the probiotic variant, 74.06±10.20 g for the prebiotic variant, respectively 64±8.08 g for the control variant. Post-hoc testing had performed after Levene pretesting which confirmed the homogeneity of the variance of values ($p>0.05$).

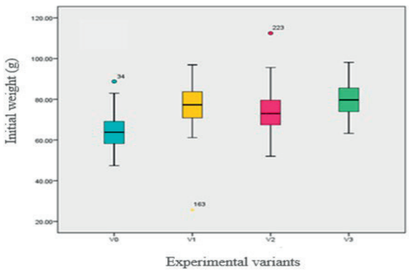

Figure 4. The variation of the initial body masses - boxplot (median, minimum, maximum values and quartiles) in the four experimental variants

Also, at the end of the second experimental stage, the statistical analysis showed significant differences between the four experimental groups (ANOVA, $p<0.05$), the Duncan test highlighting the same four growth subgroups, as at the beginning of the stage. Thus, the highest average body mass was also obtained in variant V_3 (216.24±2.07 g), followed by variant V_1 (204±6.94 g) and V_2 (191.04±6.61 g) while in the control variant (V_0) the body weight was 173.67±4.02 g (Figures 4 and 5).

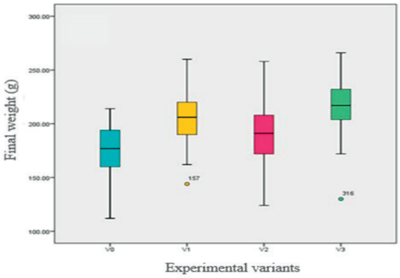

Figure 5. The variation of the final body masses - boxplot (median, minimum, maximum values and quartiles) in the four experimental variants

The coefficient of variation calculated for the individual mass of the initial groups registered values between 10.24-13.77%, an aspect that suggests the homogeneity of the lots (Table 4). From the analysis of the coefficient of variation at the end of the experimental stage, it has observed the increase of the homogeneity of the batch in the variant with probiotic, where a coefficient of variation of 10.68% was obtained, while in the control variant, CV increased by approximately 6.90 %, an aspect that underlines the appearance of heterogeneity. The determination of the correlation between total length (cm) and body mass (g) was made based on data obtained from biometrics performed at the beginning and end of the second experimental stage for fish in each variant. Thus, the data obtained from the biometric measurements were processed to determine the growth equations. Growth estimation was performed using power regression: $W = a \times Lt^b$. The correlations between length and body mass for each experimental variant are shown in Figures 6 and 7.
Analysing the values of the coefficient "b" negative allometry was observed in the case of all experimental variants respectively the increase of the body mass was achieved faster than the increase of the total length. A better value of the allometric coefficient was obtained in the synbiotic variant (V_3), where the growth also highlighted higher values.

Table 4. Coefficient of variation (CV %) for the experimental groups at the beginning and the end of the second experimental stage

Experimental variant		**V_0**	**V_1**	**V_2**	**V_3**
Initial coefficient of variation (%)	Lean body mass	12.55	10.51	13.72	10.17
	Total length	5.04	3.86	5.25	3.83
Final coefficient of variation (%)	Lean body mass	13.45	10.4	13.43	10.57
	Total length	5.34	3.78	5.58	3.92

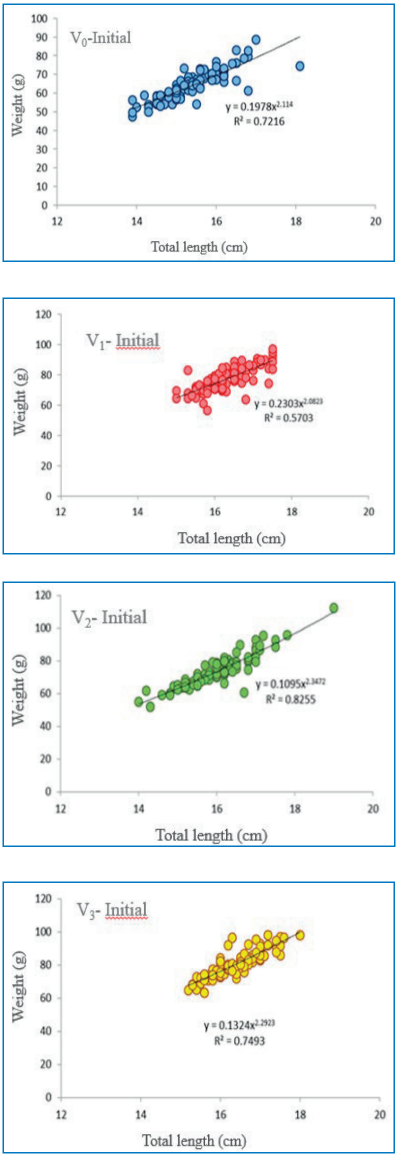

Figure 6. Length-Body mass correlation at the beginning of the second stage in the four experimental variants

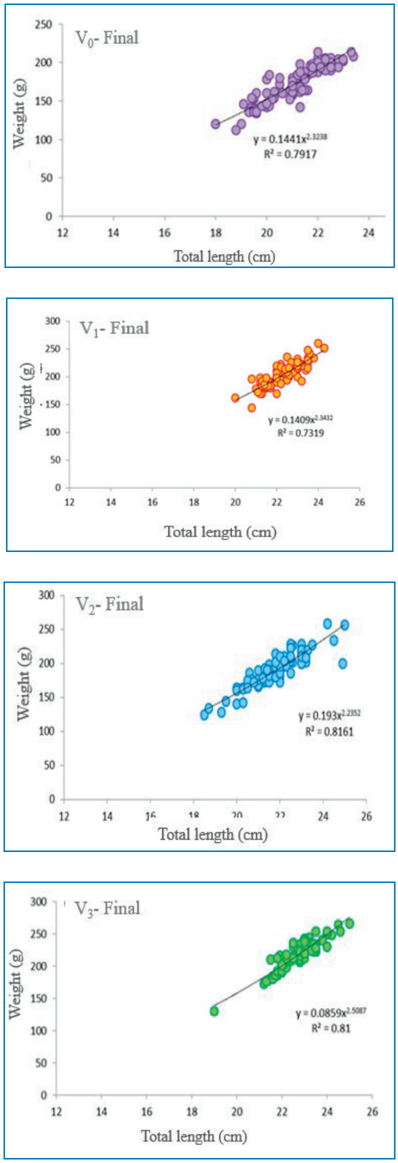

Figure 7. Length-Body mass correlation at the end of the second stage in the four experimental variants

Numerous researchers have reported that feeding tilapia with probiotics has led to improved growth. Tilapia fed on *Saccharomyces cerevisiae* (Lara-Flores et al., 2003, 2010), *Bacillus subtilis* + *Saccharomyces cerevisiae* (Marzouk et al., 2008, Lara-Flores et al., 2003, 2010), *Bacillus subtilus, Lactobacillus plantarum, Bacillus subtilis* + *Lactobacillus plantarum* (Essa et al., 2010), commercial mixtures of probiotics such as Biogen® (Ghazalah et al., 2010; El-Haroun et al., 2006; Mehrim, 2009), and Premalac® (Ghazalah et al., 2010) have been shown to contribute to growth improvement.

The use of both *Bacillus subtilis*, *Bacillus licheniformis*, and the combination showed a significant improvement in tilapia growth. The capacity of bacteria *Bacillus* sp. to contribute to growth improvement is dose-dependent (Elsabagh et al., 2018).

The biochemical composition of muscle tissue

Evaluation of the synergistic effect of Technomos® prebiotic and Betaplus® probiotic in the administered feed on the retention of nutrients in fish meat involved a comparative analysis of the biochemical composition of the muscle tissue of the Nile tilapia at the end of each experimental stage. Following the biochemical analyses performed at the end of the first stage, in the four experimental variants, the statistical analysis did not show significant differences ($p>0.05$) for the moisture, lipids and ash content. Regarding the percentage of protein content, there were significant differences between the variants ($p<0.05$). Thus, the experimental variant with probiotic registered significant differences compared to the control variant ($p<0.05$), respectively, compared to the prebiotic variant $p<0.05$), but without significant differences in the synbiotic variant (V_3).

The results obtained have correlated with data from the literature where similar effects have been reported (Hasan, 2007). The average values of the biochemical parameters of the fish meat obtained at the end of the first experimental stage in the four variants are represented in Figure 8.

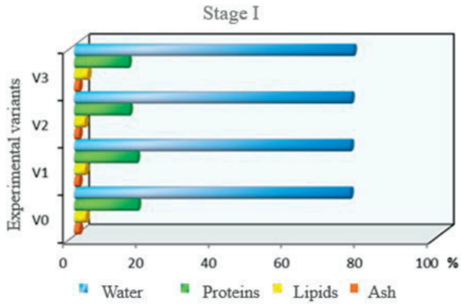

Figure 8. Comparative evolution regarding the biochemical composition of the tilapia muscle at the end of the first experimental stage

The biochemical analyses of the muscle tissue performed at the end of the second stage show significant differences ($p<0.05$) between the experimental variants in terms of the percentage content of water ($p<0.05$), proteins ($p<0.05$) and dry matter ($p<0.05$). The water content (water) of the muscles indicates significant differences in the samples collected from the probiotic variant (74.96%) compared to the control variant (74.9%), respectively with the variant where prebiotic was applied (75.31%) ($p<0.05$), but insignificant with the variant in which synbiotic was administrated (76.11%).

The content of total protein in the muscle tissue of the control variant (V_0) and the probiotic variant (V_1) showed significant differences with as well as between the control and the prebiotic variant (V_2), but no significant differences in the variant with synbiotic (V_3).

The average values obtained for lipids are following the water values from the analysed samples, noting an increase in the case of the probiotic variant (V_1), but without significant differences with the other experimental variants ($p>0.05$). Similar results that indicated the beneficial effects of incorporating oils from different sources in the diet of common carp and are related to the values obtained by Manjappa et al. (2002), in the experiment performed on carp (*Cyprinus carpio*) with an individual weight of 2.13–2.21 g, over 120 days, fed diets in which the concentration of lipids varied. The biochemical parameters of the muscle tissue analysed in the second experimental stage are shown in Figure 9.

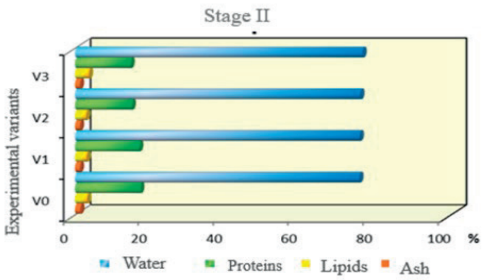

Figure 9. Comparative evolution regarding the biochemical composition of the tilapia muscle at the end of the second experimental stage

The results regarding the biochemical composition showed at higher quality of the muscular tissue in the variant with the probiotic Betaplus®, aspect emphasized by decreasing of the water content respectively increasing of the lipids and proteins content in the body mass compared to the control variant and the other experimental variants. The incorporation of oils in feed diets administered to carp has led to a decrease in water content in favor of increasing the concentration of lipids and proteins in body mass, as weight gain (Mocanu et al., 2020).

CONCLUSIONS

The present study showed that the supplementation of feed administered with synbiotic has a beneficial effect on growth indicators by the synergistic action of the combination of prebiotic and probiotics, simultaneous the influence of the probiotics applied on the weight gain. Biochemical analysis of tilapia muscle tissue showed an increase in the content of proteins, lipids in the body mass of the samples fed with the probiotic Betaplus® correlated with the increase in body mass, compared to the control variant. In conclusion, the probiotic Betaplus® used as a feed additive both simple and combined with the prebiotic Technomos® indicates a synergistic effect on growth and influences the biochemical composition of muscle tissue.

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REFERENCES

Abarikea, E.D., Caia, J., Lua, Y., Yua, H., Chend, L., Jichang, J., Jufen, T., Liang, J., & Kuebutornyea, F.K.A. (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 and Shellfish Immunology, 82,* 229–238.

Akhter, N., Wu, B., Memon, A.M., & Mohsin, M. (2015). Probiotics and prebiotics associated with aquaculture: A review. *Fish Shellfish Immunol*., 45, 733–741.

Akrami, R., Nasri-Tajan, M., Jahedi, A., Jahedi, M., Razeghi Mansour, M., & Jafarpour, S.A. (2015). Effects of dietary synbiotic on growth, survival, lactobacillus bacterial count, blood indices and immunity of beluga (*Huso huso* Linnaeus, 1754) juvenile. *Aquac. Nutr*., http://dx.doi.org/10.1111/anu.12219

Bocioc, E. (2011). *Research on the use of probiotics in industrial recirculating aquaculture systems*, Thesis from University "Dunarea de Jos" of Galați.

Carbone, D., & Faggio, C. (2016) Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of *Sparusaurata* and *Dicentrarchuslabrax*. *Fish & Shellfish Immunology*, 54, 172-178.

Dawood, M.A.O., Koshio, S., Ishikawa, M., & Yokoyama, S. (2015). Interaction effects of dietary supplementation of heat-killed *Lactobacillus plantarum* and *β-glucan* on growth performance, digestibility and immune response of juvenile red sea bream, *Pagrus major*. *Fish Shellfish Immunol*., 45, 33–42.

Ghazalah, A.A., Ali, H.M., Gehad, E.A., Hammouda, Y.A., & Abo-State, H.A. (2010). Effect of probiotics on performance and nutrients digestibility of Nile tilapia (*Oreochromis niloticus*) fed low protein diets. *Nature and Sci*., 8, 46-53.

El Asely, A., Amin, A., Abd El-Naby, A.S., Samir, F., El-Ashram, A., & Dawood, M.A.O. (2020a). Ziziphus mauritiana supplementation of Nile tilapia (*Oreochromis niloticus*) diet for improvement of immune response to *Aeromonas hydrophila* infection. *Fish Physiol. Biochem*. https://doi.org/10.1007/s10695-020-00812-w

El Asely, A.M., Reda, R.M., Salah, A.S., Mahmoud, M.A., & Dawood, M.A.O. (2020b). Overall performances of Nile tilapia (*Oreochromis niloticus*) associated with using vegetable oil sources under suboptimal temperature. *Aquac. Nutr*., 1-10.

El-Haroun, R.E., Goda, A.M., & Chowdhury, M.A.K. (2006). Effect of dietary probiotic biogens supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). *Aquac Res*., 37, 1473–1480.

El-Rhman, A., Khattab, A.M., & Shalaby, Y.A. (2009). *Micrococcus luteus* and *Pseudomonas species* as probiotics for promoting the growth performance and health of Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.*, 27, 175–180.

Elsabagh, M., Mohamed, R., Moustafa, E.M., Hamza, A., Farrag, F., Decamp, O., Dawood, M.A.O., & Eltholth, M. (2018). Assessing the impact of Bacillus strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, *Oreochromis niloticus*. *Aquac. Nutr*., 1–10. https://doi.org/ 10.1111/anu.12797.

Essa, M.A., El-Serafy, S.S., El-Ezabi, M.M., Daboor, S.M., Esmael, N.A. et al. (2010). Effect of different dietary probiotics on growth, feed utilization and digestive enzymes activities of Nile tilapia, *Oreochromis niloticus*. *J Arabian Aquacult Soc*., 5, 143-161.

Hasan, Y.H.A. (2007). *Physiological Effects of Some Additives on Growth, Blood Constituents and Immunity in NILE Tilapia (Oreochromis niloticus).* THESIS Animal and Poultry Production Department, Faculty of Agriculture Assiut University.

Huynh, T.G., Shiu, Y.L., Nguyen, T.P., Truong, Q.P., Chen, J.C., & Liu, C.H. (2017). Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. *Fish Shellfish Immunol*., 64, 367–382. https://doi.org/10.1016/j.fsi.2017.03.035

Lara-Flores, M., Olvera-Novoa, M.A., Guzman-Me´ndez, B.E., & Lo´pez-Madrid, W. (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 216, 193–201.

Lara-Flores, M., Olivera-Castillo, L., & Olvera-Novoa, M.A. (2010). Effect of the inclusion of a bacterial mix (*Streptococcus faecium and Lactobacillus acidophilus*), and the yeast *(Saccharomyces cerevisiae*) on growth, feed utilization and intestinal enzymatic activity of Nile tilapia (*Oreochromis niloticus*). *Internat J Fish Aquaculture*, 2, 93-101.

Liu, C.H., Chiu, C.S., Ho, P.L., & Wang, S.W. (2009). Improvement in the growth performance of white shrimp, *Litopenaeus vannamei,* by a protease-producing probiotic, Bacillus subtilis E20, from natto. *J Appl Microbiol*., 107, 1031–1141.

Lu, L., Tan, H., Luo, G., & Liang, W. (2012) The effects of *Bacillus subtilis* on nitrogen recycling from aquaculture solid waste using heterotrophic nitrogen assimilation in sequencing batch reactors. *Bioresour Technol.*, 124, 180–185.

Marzouk, M.S., Moustafa, M.M., & Mohamed, N.M. (2008). The influence of some probiotics on the growth performance and intestinal microbial flora of Oreochromis niloticus. *Proceedings of 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt*, 1059-1071.

Manjappa, K., Perar, K., & Barlaya, G. (2002). Growth performance of common carp, Cyprinus carpio fed varying lipid levels through low protein diet, with a note on carcass composition and digestive enzyme activity. *Acta Ichthyologica et Piscatoria*, XXXII, Fasc. 2.

Mehrim, A.I. (2009). Effect of dietary supplementation of Biogen® (Commercial Probiotic) on mono-sex Nile tilapia Oreochromis niloticus under different stocking densities. *J Fish Aquat Sci*., 4, 261–273.

Meseguer, J., & Cerezuela, R. (2011). Current Knowledge in Synbiotic Use for Fish Aquaculture: A Review. *J. Aquac. Res. Dev.*, s1, 1–7. https://doi.org/10.4172/2155-546.S1-008

Mocanu, C.M., Cristea, V., Dediu, L., Dicu, D., Docan, A., & Ionescu, T. (2011). The influence of different stocking densities on growth performances of *Oncorhynchus mykiss* (Walbaum, 1792) in a recirculating aquaculture system. Iași, *Volumul de Lucrări Ştiinţifice-Seria Zootehnie,* 56(16), 326– 331.

Mocanu, E., Patriche, N., Tenciu, M., Savin, V., & Popa, M.D. (2020). Stimulating disease resistance for common Carp (*Cyprinus carpio*) reared in recirculating system, by utilising feeding diets supplemented with fatty acids. *Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie*, 74, http://www.uaiasi.ro/firaa/Pdf/Pdf_Vol_74/Elena_Mocanu.pdf

Ringø, E., Olsen, R.E., Gifstad, T.Ø., Dalmo, R.A., Amlund, H., Hemre, G.I., et al. (2016). Prebiotics in aquaculture: a review. *Aquaculture Nutrition, 16*(2), 117-136.

Ringø, E., & Song, S.K. (2016). Application of dietary supplements (synbiotics and probiotics in combination with plant products and β-glucans) in aquaculture. *Aquac. Nutr*., 22, 4–24.

Ricker, W.E. (1975). Computation and interpretation of biological statistics of the fish population. *Bulletin of the Fisheries Research Board of Canada*, 191, 1–382.

Rurangwa, E., & Verdegem, M.C.J. (2014). Microorganisms in recirculating aquaculture systems and their management. *Rev Aquac*., 7, 117–130.

Shelby, R., Lim, C., Yildirim-Aksoy, M., & Delaney, M.A. (2006) Effects of probiotic diet supplements on disease resistance and immune response of young Nile Tilapia, *Oreochromis niloticus*. *J Appl Aquac*., 18, 49–60.

Sivakumar, K., Rama, M.S., Janani, R., Muthupriya, P., & Magesh, R. (2020). Effect of probiotic dietary on growth performances and feed utilization of *Cyprinus carpio* fingerlings. *Bull Pure Appl Sci Zool., 39*(2), 463.

Soltani, M., Ghosh, K., Hoseinifar, S.H., Kumar, V., Lymbery, A.J., Roy, S., & Ringø, E. (2019). Genus *Bacillus,* promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. *Rev Fish Sci Aquac*., 27, 331–379.

Wang, Y (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture*, 269, 259–264.

Wu, Z.X., Feng, X., Xie, L.L., Peng, X.Y., Yuan, J., & Chen, X.X. (2012). Effect of *Bacillus subtilis Ch9* for grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), on growth performance, digestive enzyme activities and intestinal microflora. *J Appl Ichthyol*., 28, 721–727.

Zhu, C., Yu, L., Liu, W., Jiang, M., He, S., Yi, G., Wen, H., & Liang, X. (2019). Dietary supplementation with *Bacillus subtilis* LT3-1 enhance the growth,immunity and disease resistance against *Streptococcus* *agalactiae* infection in genetically improved farmed tilapia, *Oreochromis niloticus*. *Aquac Nutr*., 25, 1241–1249.

Yilmaz, S., Yigit, N.Ç.S.E.M., & Çelik, E.Ş. (2019). Combined effects of dietary *Bacillus subtilis* and Trans-cinnamic acid on growth performance, whole body compositions, digestive enzymes and intestinal bacteria in Rainbow trout (*Oncorhynchus mykiss*). *J. Zoolog. Syst. Evol. Res*., 1.

Yılmaz, S., Ergun, S., Yigit, M., & Çelik, E.Ş. (2020). Effect of combination of dietary *Bacillus subtilis* and trans-cinnamic acid on innate immune responses and resistance of rainbow trout, *Oncorhynchus mykiss* to *Yersinia ruckeri*. *Aquac. Res*., 51, 441–454.