The effect of diet supplemented with Proviotic® on growth, blood biochemical parameters and meat quality in rainbow trout (*Oncorhynchus mykiss*) cultivated in recirculation system

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Abstract. In 2006, the European Union banned the use of all types of therapeutic antibiotics as growth promoters in livestock farming. This lead to the need for alternative growth promoters supplements to be found. Such a possibility is the development of new nutritional strategies with the participation of probiotics. The purpose of this study was to trace the growth performance, meat quality and blood parameters of rainbow trout (*Oncorhynchus mykiss* W.) fed with additive of probiotic Proviotic®. The fish were fed with two feeds: control feed (CF) - without addition of supplement and experimental feed (EF) (with supplementation of 460 mg kg^{-1} Proviotic® in fish feed). The density of rainbow trout in recirculation system was 40 specimens per tank. The initial average weight of fish in CF variant was 13.43±2.9 g and in EF variant was 13.35±3.4 g without differences being statistically significant ($p \geq 0.05$). The continuation of experiment was 60 days. The average final weight, meat quality and blood biochemical parameters were measured at the end of experiment. Rainbow trout from the experimental group fed with supplement of Proviotic had with 5.38% higher average final weight, compared to the parameter value in trout from control variant, but without difference being statistically significant ($p \geq 0.05$). The blood biochemical parameters in experimental fish were not significantly affected by supplementation of probiotic Proviotic®. This supplement, added to extruded pellets for feeding of rainbow trout increases the quantity of crude protein and ash in fish fillets and this way is improving the quality of fish meet.
Key Words: probiotic, fish, weight gain, flesh quality.

Introduction. Aquaculture is one of the basic sectors of agriculture, which has been developing rapidly over the past few years, and today it is defined as the fastest growing food production sector in the world. It has the greatest potential to meet world wide growing demand of products from aquatic organisms (FAO 2005). The main task of aquaculture in the future will be the production of more hydrobionts per unit area compared to the natural productivity of the water basins. In order to achieve the mentioned aim, the aquafarmers will face the challenge of manage a number of problems hindering their efforts to increase the yield.

First of all, the occurrence of diseases is a result of disturbances in the relationship between fish, the environment and presence of pathogenic agents. Next, the deterioration of the environment appears, as a result of the production activity itself. The problem is also the increased morbidity due to the life activity of various etiological agents (parasites, fungi, bacteria and viruses). Over the last fifteen years, research in the aquaculture sector has therefore focused on disease studies (Bondad-Reantaso et al 2005). Although vaccines have been developed, they can not be used as a universal weapon to combat the diseases. On the other hand, the widespread and uncontrolled use of antibiotics has led to the development and spread of antimicrobial resistant pathogens (Sørum 2006). This increases the risk of transmission of resistant bacteria from aquaculture to humans and the risks associated with the introduction of these pathogens into the human body (FAO 2005). In 2006, the European Union banned the use of all types of therapeutic antibiotics as growth promoters in livestock farming (WHO 2006). This lead to the need for alternative growth promoters supplements to be found. Such a possibility is the development of new nutritional strategies with the participation of probiotics. The alternative possibility is the development of new nutritional strategies involving probiotics and other functional nutritional supplements (Denev 2008). They are distinguished by stimulating of immune system, low cost, and they are relative environmental harmless.

Probiotics are attractive organic products with wide range of actions. They include living microorganisms in their composition, the action of which is based on their ability to survive and multiply in the intestinal tract. In this way, they stimulate the intestinal microflora, which is important for the intestinal health status and the body as a whole (Uyeno et al 2015; Kogut 2018). The mechanisms by which this beneficial effect is realized are the following:

- inhibits the colonization of enteropathogenic bacterial species in the intestine, thus improving the health status of the intestinal tract (Spring et al 2000);
- improves the function of immune system, positively influencing humoral immunity and immunoglobulin status (Marinov 2004);
- they have positive influence on fermentation in intestinal microflora and energy recovery (Akhter et al 2015).

The beneficial effects of probiotics on intestinal microflora, nutrient utilization, and growth's acceleration can also be associated with changes in intestinal morphology and its impact on resistance to intestinal disease (Jafaryan et al 2010; Dawood & Koshio 2016; Kathia et al 2017).

Zhou & Li (2004) investigated the effect of different amounts of probiotics as a supplement to granulated feed and found better growth and improved immune function in common carp (*Cyprinus carpio*). Based on the analysis of the bioconversion of nutrients (nutritional coefficient) in the ration and carp growth intensity, they concluded that the optimal supplement was 0.24% in the daily ration. According to the same authors, fish immune function improves with higher probiotics (0.55%) in this species.

According to various authors who worked in this field, probiotics added to the diet of different hydrobionts species increase body weight, survival, improve the bioconversion of nutrients in the diet and immune system of the organisms (Nonaka et al 1981a; Nonaka et al 1981b; Matsuo & Miyazono 1993; Nakamura et al 1996; Raa 1996; Verlhac et al 1996; Galeotti 1998; Spring et al 2000; Paulsen et al 2001; Fritts & Waldroup 2003; Hooge et al 2003; Rodriguez et al 2003; Standen et al 2016; Gobi et al 2018).

All this showed the permanently increasing role of these substances for the future development of aquaculture and reason why new products should be developed and tested for the need of this sector.

The purpose of this study was to trace the growth performance, meat quality and blood parameters of rainbow trout (*Oncorhynchus mykiss* W.), fed with additive of probiotic Proviotic®.

Material and Method

The experimental trouts. The continuation of trial was 60 days and was conducted in period October-December 2017. The 160 healthy without visible injuries rainbow trouts were chosen and transported from trout farm in Tvyrdica to experimental aquaculture base situated in Trakia University (Stara Zagora). The fish were split in the two experimental groups, each of them in two replications, with 40 fish/tank:

- experimental feed (EF) - the fish were fed with feed supplemented with probiotic Proviotic ®;
- control feed (CF) - the fish were fed with feed without supplementation.

The average initial weight of trouts from two experimental groups were:

- EF: 13.35±3.4 g;
- CF: 13.43±2.9 g.

The differences in the initial weight of experimental fish were not significant ($p \geq 0.05$). The continuation of the trial was 60 days.

Recirculation aquaculture system (RAS). The recirculation system consisted of tanks for cultivation of fish, two mechanical filters (settling tanks), moving bed biofilter and pump sections (Figure 1). The useful volume of fish tanks was 0.8 m^3. The flow rate in RAS was maintained at 25 L min^{-1}. The bottoms of fish tanks as well as the bottoms of filter's tanks were cleaned by syphoning every day removing this way 20% of total volume of RAS. The same volume of fresh water was added daily for compensation of water's lost during the cleaning process and evaporation.

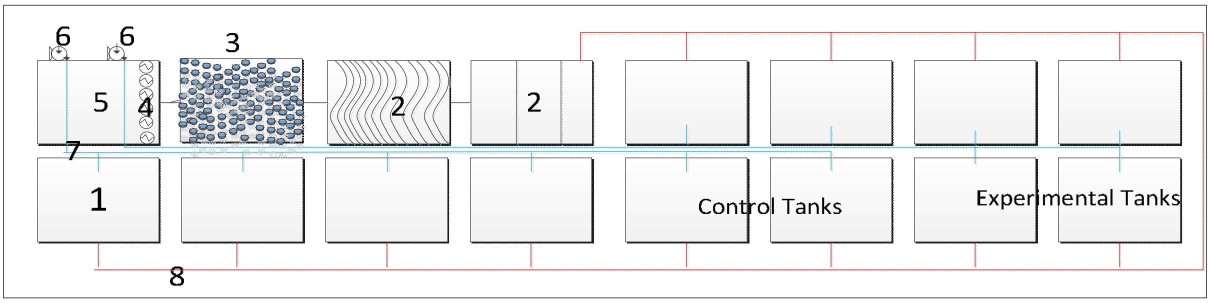

Figure 1. Recirculation aquaculture system used in trial: 1) fish tanks; 2) mechanical filters; 3) moving bed biofilter; 4) heating elements; 5) pump tank; 6) pumps; 7) inlet water; 8) outlet water.

Fish feed. The experimental fish were fed with a commercial extruded feed produced by the "Aqua-Garant" with the size of pellets 2 mm. The content of commercial trout's feed used in a current trial is shown in Table 1. The probiotic Proviotic® (250 mg) (Genesis laboratory) was added to the granules at a concentration of 460 mg kg^{-1} fish feed. The Proviotic® consisted from bacteria *Lactobacillus bulgaricus* isolated from snowdrop. The sunflower oil at quantity of 1 mL was also added to every 10 g of experimental feed (EF) and the components were very well mixed. The same amount of sunflower oil was added to the control feed (CF) and the granules and oil were also mixed. Afterwards, both tested feed were left for 12 hours at an air temperature of 20°C and were used for the feeding of experimental fish. The daily feed ratio was maintained at 2.8% from the fish biomass. The feed was supplied manually four times a day.

Table 1

Nutritional content of commercial feed used in the trial /according to fish feed producer "Aqua-Garant"

Ingredients	*Unit*	*Content*
Crude protein	%	45
Fibers	%	1.5
Fat	%	16
Phosphorus	%	1.05
Vit. A	Thousands IU	10
Vit. D3	IU	1500
Vit. E	mg	200
Digestive energy	MJ kg^{-1}	18.5

Investigated parameters

Hydrochemical analysis. The daily measurement of water parameters (temperature (°C), pH, dissolved oxygen (mg L^{-1}) and electrical conductivity (μS cm^{-1})) were conducted with portable meter HQ30D (Hach Lange), connected with probes for oxygen, conductivity and pH measurement. The ammonium and phosphate were measured once per week according to BSS 3587-79 and EN 6878-1.

Growth parameters in experimental fish. The mortality cases in experimental tanks were recorded during the trial and survival (%) was calculated using the following formulae:

Survival (%) = (final number of fish / initial number of fish) x100

The experimental fish were weighed at the technical balance with accuracy 0.01 g at the start of trial. The average final weights (g) were measured at the end of experiment and specific growth rate (SGR) (%body wt gain day^{-1}) and FCR were calculated with the following equations:

SGR (% body wt gain day^{-1}) = [Ln final weight (g)-Ln initial weight(g)]/number of days x 100;

FCR = fed feed (g) / weight gain of fish (g)

Blood biochemical parameters in experimental fish. The blood was taken from the hearts of the examined fish (6 specimens per variant) with disposable sterile plastic syringes (3 mL) with a needle. Heparin sodium (1%) was used as an anticoagulant. The blood was centrifuged at 3000 rpm for separating the plasma. Afterward the biochemical parameters (glucose (GLU) (mmol L^{-1}), urea (UREA) (mmol L^{-1}), total protein (TP) (g L^{-1}), albumin (ALB) (g L^{-1}), alanine aminotransferase (ALAT) (U L^{-1}), aspartate aminotransferase (ASAT) U L^{-1}), the content of calcium (Ca) (mmol L^{-1}), phosphorus (P) (mmol L^{-1}), magnesium (Mg) (mmol L^{-1}), triglyceride (TG) (mmol L^{-1}) and cholesterol (mmol L^{-1})) in blood plasma, were examined by the colorimetric method with blood analyzer (Mindray SC – 120).

Meat quality in experimental fish. At the end of the trial the fish fillets from the back side of 6 fish specimens per variant were obtained and homogenized. The received muscles homogenates were used for analysis of moisture, crude protein, fat and ash in Central research laboratory (Faculty of Agriculture, Trakia University) according to the following methods:

- moisture (%) and dry matter (%) - Bulgarian State Standard (BSS)11374-86;
- crude protein content (%) - BSS-ISO 5983, Kjeldahl method on Kjeltec 8400, FOSS, Sweden;
- fat content (%) - BSS-ISO 6492, Soxhlet extraction method, using Soxtec 2050, FOSS, Sweden;
- crude ash content (%) - BSS 11374-86.

Statistical analysis of data. The data was analyzed statistically with ANOVA single factor STATISTICA 6.0 software (StatSoft Inc 2002). Results were considered significantly different at the level of $p < 0.05$.

Results and Discussion. The minimal and maximal measured water temperatures during the trial were respectively 15.9°C and 20°C. They were similar and without the differences between experimental tanks to being statistically proven (Figure 2) ($p \geq 0.05$). The maximal specific growth in rainbow trout was gained at 17.2°C (Hokanson et al 1977). The average values of electrical conductivity in EF and CF variants were close and the differences were not significant (Figure 2) ($p \geq 0.05$). Water pH in tanks with CF variant was higher with 0.3% than this found for EF variant but difference was not significant (Figure 2) ($p \geq 0.05$). The values of this parameter were in the optimal range for trout species, which should vary between 7 and 8 according to Zaykov & Staykov (2013). Oxygen content in both experimental variants was similar and close to optimal value for this fish species. According to Svobodova et al (1993) the value of oxygen should be between 8 and 10 mg L^{-1} for salmoninds. The average value of ammonium from the control was lower with 14.2% compared with its value in experimental variants, but the difference was not significant (Figure 2) ($p \geq 0.05$). The presence of ammonia in water is resulting in toxic effect in all vertebrates causing convulsions, coma and death (Randall & Tsui 2002). According to Francis-Floyd et al (2009) ammonia in the water occurs in two forms: ionized ammonia (NH_4^+) and un-ionized ammonia, NH_3^0. The unionized ammonia is more toxic for hydrobionts than ionized ammonia, because NH_4^+ does not easily cross fish gills and is less bioavailable than unionized form. The average values of phosphate in both tested variants were similar and the difference between them without being statistically proven (Figure 2) ($p \geq 0.05$). Dalmin et al (2001) reported that supplementation of *Bacillus* sp. in feed for *Penaeus monodon* improved water quality in cultivation vessels. The conducted from us trial did not show the influence of supplemented in diet probiotic on water quality.

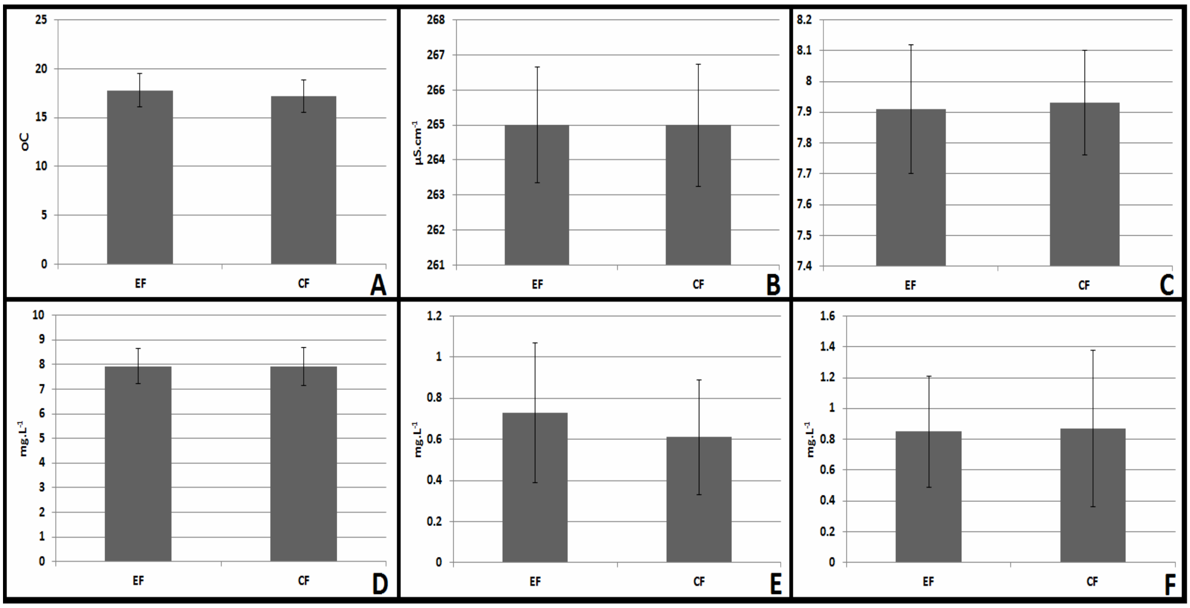

Figure 2. Average values in hydrochemical parameters during the trial: A. temperature; B. conductivity; C. pH; D. dissolved oxygen; E. ammonium; F. phosphate (EF = experimental feed; CF = control feed).

The survival rate in trout from both tested variants was 100%. The average values of final weight and SGR in fish from EF were higher respectively with 5.38% and 5.31% compared with the values found in CF, but the differences were not significant (Table 2) ($p \geq 0.05$). The better FCR was found in rainbow trout fed with feed containing Proviotic ® and it was with 8.3% lower than this found for trout from control variant, but the difference was not significant (Table 2) ($p \geq 0.05$). Our results are in confirmation with results received from study made from other researchers (Metailler & Hollocou 1993; Gildberg et al 1995, 1997) who did not find the effect on the growth rate of juveniles in seabass (*Dicentrarchus labrax*), Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*) when their food was supplemented with lactic acid bacteria but are controversial to study made in common carp where feed supplementation of diet improve the fish growth (Noh et al 1994). Growth performance in trout fed with multi-strain probiotic bacterial additives (*Bacillus* sp., *Pediococcus* sp., *Enterococcus* sp., *Lactobacillus* sp.) at concentration of 0.2% from dietary ratio was not affected by dietary probiotic supplementation (Ozorio et al 2016).

Table 2

Growth parameters in rainbow trout (*O. mykiss*) during the trial

Growth parameters	$\bar{x} \pm SD$	
	CF	*EF*
Initial weight (g)	13.43±2.90	13.35±3.40
Final weight (g)	43.90±11.20	46.40±11.80
SGR (% body weight gain day^{-1})	1.96±0.60	2.07±0.57
FCR	1.56±0.85	1.43±0.75

The differences in average values of blood biochemical parameters in both variants did not show statistical differences (Table 3) ($p \geq 0.05$). The average values of following blood parameters: urea (UREA), total protein (TP), albumin (ALB), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG), cholesterol (CHO) in fish fed with supplement of probiotic were lower respectively with 30%, 3.96%, 9.17%, 28.4%, 12.6%, 14.1% and 31.6% compared with the values in these parameters measured in blood of fish from the control variant, but the differences were not significant (Table 3) ($p \geq 0.05$). The mineral content (Ca, P and Mg) in blood of fish from CF and EF were similar and differences in content of minerals in both tested variants were not significantly different (Table 3) ($p \geq 0.05$). Our data differed from the data received from Panigrahi et al (2010) who found that supplementation with *Lactobacillus rhamnosus* or the same bacteria in heat-killed or freeze-dried form of feed for rainbow trout affect blood parameters. When the fish were fed with probiotic supplemented feed the plasma cholesterol significantly increased and the heat-killed probiotic fed group registered significantly high values of triglycerides, alkaline phosphatase activity, and plasma protein compared to the control diet fed groups. The received from us results for alanine transaminase (ALT), alkaline phosphatase (ALP) showed the same tendency found from Kamgar & Ghane (2014) when the feed for rainbow trout were supplemented with *Bacillus subtilis*, but the average value of total protein (TP), albumin (ALB) levels in their study were higher than this found for the control group. Similarly to our study lower triglyceride and cholesterol content in blood were found in chickens when they were fed with supplementation of *Lactobacillus* culture in their diet (Kalavathy et al 2003).

Table 3

Blood parameters in rainbow trout (*O. mykiss*) during the trial

Blood parameters	*Unit*	*n*	$\bar{x} \pm SD$	
			CF	*EF*
GLU	mmol L^{-1}	6	4.86±1.02	5.85±3.05
UREA	mmol L^{-1}	6	1.0±0.31	0.7±0.14
TP	g L^{-1}	6	40.4±3.21	38.8±2.63
ALB	g L^{-1}	6	20.7±1.65	18.8±3.15
ALT	U L^{-1}	6	11.4±1.44	8.16±6.90
ALP	U L^{-1}	6	603.6±245.00	527.16±230.02
Ca	mmol L^{-1}	6	2.27±1.48	2.015±0.61
P	mmol L^{-1}	6	5.92±1.38	4.28±1.31
Mg	mmol L^{-1}	6	0.72±0.52	0.71±0.23
TG	mmol L^{-1}	6	1.91±0.26	1.64±0.31
CHO	mmol L^{-1}	6	7.9±2.08	5.4±1.13

The average values of meat quality parameters (moisture, dry mater and fat) in fish from control group were similar to this found in fish from EF groups (Table 4) ($p \geq 0.05$). Interestingly the average crude protein content in fish from experimental group was higher with 1.59% compared with the content in this parameter found for fish fed with control feed (Table 4) ($p < 0.05$). Expectedly the same tendency was found for the ash content and its quantity in samples from fish from EF was higher with compared with the average value of this parameter measured for the fish from CF. Our current study was in confirmation of research made by Abdelhamid et al (2009) who found that the increasing of the probiotic level increased fish carcass protein.

Table 4

Meat quality parameters of experimental fish (%)

Parameters	*n*	$\bar{x} \pm SD$	
		CF	*EF*
Moisture	6	75.3±0.70	74.8±0.42
Dry matter	6	24.7±0.70	25.1±0.42
Crude protein	6	24.7±0.71	25.1±0.42*
Fat	6	4.45±0.81	4.04±0.64
Ash	6	1.66±0.03	1.75±0.03*

* shows significant statistical differences ($p < 0.05$).

Conclusions. The probiotic Proviotic® added to feed for feeding of rainbow trout increases the quantity of crude protein and ash in fish fillets and this way is improving the quality of fish meet but not significantly affect the growth and the blood biochemical parameters in experimental trouts.

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