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EFFECT OF SYNBIOTIC DIETARY SUPPLEMENTATION ON GROWTH, PHYSIOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN COMMON CARP (*CYPRINUS CARPIO* L.) FINGERLINGS AND ON YIELD AND PHYSIOLOGICAL PARAMETERS IN LETTUCE (*LACTUCA SATIVA* L.), CULTIVATED IN MESOCOSMOS AQUAPONIC SYSTEM

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Abstract

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The aim of the present research was to test the effect of commercial synbiotic (Bio balance®) on growth, physiological and immunological parameters in common carp fingerlings (*Cyprinus carpio* L.) and on plant productivity and physiological parameters in lettuce (*Lactuca sativa* L.) cultivated in mesocosmos aquaponic system.

The current study demonstrated for first time that the application of synbiotic as feed supplementation in extruded pellet for carp in aquaponics recirculation system could be successful. The positive effect of Bio balance® on growth rate and feed utilization in carp fingerlings cultivated in aquaponic mesocosmos was observed and average final weight and FCR in fish fed with feed supplemented with Bio balance® were higher with 9.8% and 26.1% respectively, compared to the values of these parameters for fingerlings received feed without addition of synbiotic ($P \leq 0.05$). The bactericide and phagocytosis activities and hemoglobin content in blood of carps were higher in fish from the experimental variant (S_1), compared with the values of these parameters found in fingerlings from the control variant (S_0), but the differences were not significant ($P \geq 0.05$). The lettuces from S_1 showed 2.14% higher fresh weight compared to that of lettuces from S_0, but the difference was not significant ($P \geq 0.05$). The better yield in lettuce from experimental variant S_1 where the carp feed was supplemented with synbiotic probably was result from better pH in water and the connected with this better assimilation of nutrients from the salad from S_0 variant. Fingerlings diet supplemented with Bio balance® stimulates the physiological proceses in the experimental plants. The positive effect of the synbiotics is reflected by improved leaf gas exchange parameters and nitrate reductase activity in lettuce.

Key words: aquaponics, symbiotic, common carp, lettuce, growth, immunity, physiology, photosynthesis

Introduction

Aquaponics are recirculation system, where the cultivation of hydrobionts are integrating with the production of vegetables (Nelson, 2007; Graber and Junge, 2009; Diver and Rinehart, 2010; Karimanzira et al., 2016; Diem et al., 2017; Shete et al., 2017).

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One of the main challenges connected with the cultivation of hydrobionts and plants in this innovative technology is control of diseases and pathogenic microorganisms. Their presence in the system could affect raised fish, plants and last but not least the consummator of aquaponics products. The traditional tools used in agriculture for treatment of pathogenic microogranisms could not be used in aquaponics system because they could damage the cultivated organisms. From the other side vast usage of antibiotics in aquaculture lead to development and spread of pathogenic microorganisms which possess resistance towards these terapeutants (Sørum, 2006). This increases the risk for transmission of pathogen microorganisms with antibiotic resistance from aquaculture products to humans. In 2006 the EU forbade the usage of all terapeutant antibiotics in aquaculture. This prohibition as well as the absence of promising medical treatment in aquaponics led to the necessity of the development and testing of new alternative tools in these systems. One possible solution of discussed problem could be the application of the probiotics, prebiotics, synbiotics and other functional feed additives.

The synbiotics are feed additives which combining the prebiotics and probiotics. Synergism of both constitutes which compose synbiotics are reason of their beneficial effect on hydrobionts. The combined effect of prebiotics and probiotics application is higher than the sum of effects of both constitutes if they are applying separately (Chou et al., 1991). Different studies showed the positive influence of dietary synbiotics application on survival and growth rate of hydrobionts (Rodriguez et al., 2003; Rodriguez-Estrada et al., 2009; Ai et al. 2011; Mehrabi et al., 2011) as well as on their immunological status (Ai et al., 2011; Geng et al., 2011; Ye et al., 2011).

Optimal plant development and growth in aquaponic cropping system depends on the availability of light, water, favorable temperatures and mineral nutrients (Silva et al., 2016). Nutrition provokes variable physiological and developmental responses in the plants. They require large amount of nitrogen (N), which is a main compound of photosynthesis enzymes, pigments and products. The increase in productivity observed for many plant species is often associated with the elevation in photosynthesis capacity (Jain et al., 1999).

There are two forms of nitrogen absorbed by plants, the nitrate ion (NO^{-}_{3}) and ammonium ion (NH^{+}_{4}). Nitrate is assimilated by two reductive steps catalyzed by the enzymes NR and nitrite reductase (NiR). Nitrate reductase (NR) is one of the most important enzymes in the assimilation of exogenous nitrate (NO^{-}_{3}), play important role in amino acids biosynthesis, and regulates the protein synthesis (Campbell, 1999). The level of activity of this enzyme gives a good estimation of the nitogen status of the plant and is very offen correlated with growth and yield (Barford and Lajtha, 1992).

In cases when excessive nitrogen, and especially ammonia, are present in the aquaponics system that situation could be potentially toxic for plants. Ammonia is the main end product in the break down process of proteins in fish (Roosta, 2014). According to Alamsjah (2013) under such conditions improved plant uptake and biomass production could be achieved by input of probiotic enrichment and biofertilizer products from seaweed waste.

So far the studies exploring the effect of synbiotics when they are applied in aquaponics are still missing. The question of effect of synbiotics could be used for treatment of hydrobionts in aquaponics recirculation system on cultivated plants remains still open and unexplored.

The aim of the present research was to test the effect of commercial synbiotic (Bio balance®) on the growth, physiological and immunological parameters in common carp fingerlings (*Cyprinus carpio* L.) and on plant productivity and physiological parameters in lettuce (*Lactuca sativa* L.) cultivated in mesocosmos aquaponic system.

Materials and methods

Mesocosmos aquaponic systems

For the purpose of current trial four experimental aquaponic systems were constructed. They were built at small greenhouse (Fig. 1) with dimensions - 4/3/2 m situated at Experimenal aquaculture base – Agricultural Faculty, Trakia University, Stara Zagora, Bulgaria. Every of the experimental aquaponics system are consisted from fish tank, settling tank, moving bed biofilter and two tubes used deep water culture technology, each of them with five nets for cultivation of plants (Fig. 2). The fish tank and biofilter were aerated with a blower (Yuting®) with capacity 85l min^{-1}.

The preparation of experimental feed

The continuation of the experiment was 30 days. The experiment was conducted in two variants, each of them in two replications:

- Experimental variant (S_1) the carp fingerlings were fed with feed containing synbiotic:

- Control variant (S_0) – the carp fingerlings were fed with feed without addition of symbiotic.

The experimental feed was prepared according Telli et al. (2014) with slight modification. Synbiotic was added to oil in favorable ratio assuring concentration of 10^8 CFU/ml and then sprayed like aerosol on commercial carp feed with the size of granules 1.5 mm (28% crude protein content). The commercial synbiotic (Bio balance®-Bio care, Copenhagen,

Fig. 1. Experimental aquaponics systems used in trial

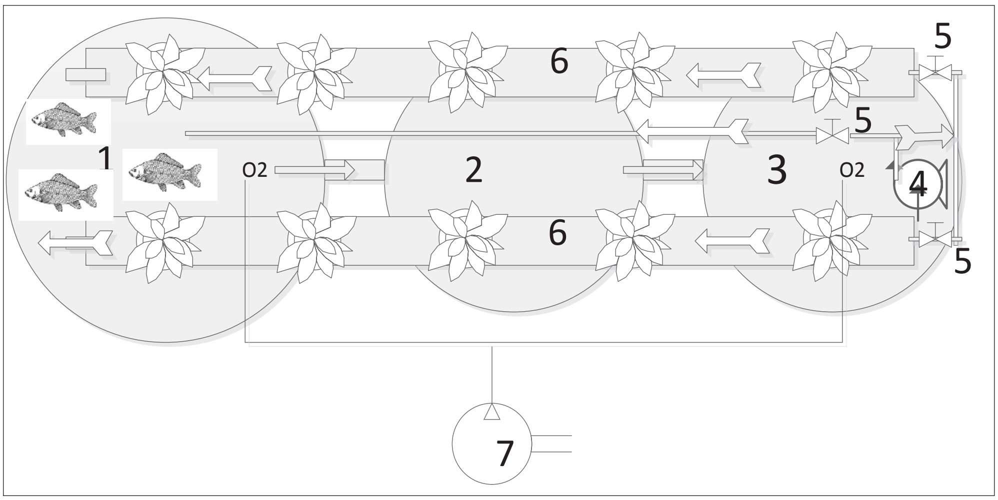

Fig. 2. Mesocosmos aquaponic system used in trial (view from above):
1) fish tank; 2) settling tank; 3) moving bed biofilter; 4) submerged pump; 5) valves; 6) tubes using deep water technology; 7) aerator.

Denmark) was used in current trial and contained the following components: *B. lactis, L. acidophilus DDS-1, S. thermophilus, B. bifidum, B. longum* and fructooligosaccharides. The sprayed with synbiotic feed (2% from daily feed ratio) was carefully mixed and dried on air at 20°C for 12 h prior the feeding of carps. The control feed was sprayed just with oil without addition of synbiotic having this way the same energy value.

Experimental fish and lettuce

The fingerlings of common carp (*Cyprinus carpio* L.) with initial weight 0.04 kg and in good health condition without visible injuries were chosen from net cages of farm situated in Studen kladenetz dam. They were transported to aquaculture base in Trakia University, Stara Zagora, Bulgaria. The stocking density which was used during the trial was 116 pcs.m^{-3}.

Forty lettuces (Zhalta krasavica variety) were chosen and transported from greenhouse situated in Plovdiv to aquaculture base in Stara Zagora at the stage of 3 leaf formation. The plants were transferred on specialized substrate (Grodan®) with the size 5 cm and afterwards the lettuce plants were planted in hydroponic pots and placed to experimental systems. The possible deficit of microelements in the experimental lettuce was avoided by foliar application of "B-essentials"® once per week according to the producer' requirements.

The hydrochemical parameters

The temperature of water (°C), conductivity (µS.cm-^{1}), pH and the oxygen content (mg.l^{-1}) was measured daily with portable meter (Hach) with the appropriate for the aim probes. The concentration of nitrate (mg.l^{-1}) in experimental systems was measured spectrophotometrically three times during the trial with the cuvette tests (Hach).

The parameters determined in common carp fingerlings during the trial

At the end of the trial the survival rate (%) of fish was determined. The fingerlings were weighed at the start and at the end of the trial. The fingerlings were not fed 14 hours prior to the measurement of weight. Specific growth rate (%.day^{-1}) and feed conversion ratio were calculated using the following equations:

$$SGR = \frac{[\ln(W1) - \ln(W0)]}{the\ number\ of\ day} x100$$

where:
SGR - specific growth rate, %.day^{-1}
W_1 - the weight of fingerlings in the end of the trial, g
W_0 - the initial weight of fingerlings, g
and

$$\text{Feed conversion ratio (FCR)} = \frac{Daily\ feed\ ratio(g)}{Average\ weight\ gain\ (g)}$$

The influence of synbiotic on condition of liver and digestive system in experimental fish were analyzed by determination of hepatosomatic and viscerosomatic indices:

Hepatosomatic index (HSI):

$$HSI = \frac{Wl}{BW} x100$$

where:
HIS - hepatosomatic index, %
Wl - weight of liver, g;
BW - body weight, g

Viscerosomatic index (VSI):

$$VSI = \frac{Wv}{BW} x100$$

where:
VSI - viscerosomatic index, %
Wv - weight of viscera, g;
BW - body weight, g

In the end of trial the experimental fish were transported to the Institute of Fisheries and Aquaculture where phagocytosis (%), bactericide activity (%) and the content of hemoglobin (g.dL^{-1}) where determined.

Determination of phagocytosis activity

The phagocytosis activity was determined by the following method: One day bacterial culture (2.10^6 cells) was inactivated with temperature at 70°C for 30 min. The heparinized total blood was incubated with temperature killed cells (TKC) of *Staphylococcus aureus* (0,1 ml) at thermostat and were shacked every 5 to 10 min. Smears of the total blood were stained with Romanowsky-Giemsa solution. Phagocytosis activity was calculated by counting 100 phagocytic cells and the number of active phagocytic cells was determined.

Determination of bactericide activity

The bactericide activity was determined according Atanasova et al. (1995) and included the following stages:

The blood serum

The blood was taken directly from the hearts of experimental fish. The blood was leaved for two hours at 20°C and afterwards was centrifuged for 10 min at 3000 rpm. Received serum was used for bactericide activity determination the latest 6 hours after its receiving.

The preparation of bacterial substrate

Aeromonas hydrophila (24-hour culture) was used for determination of bactericide activity. The density of bacterial culture 25.10^6 cells.ml^{-1} was received using McFarland dilution method with physiological solution.

Bactericide activity determination

In the experimental vessels 4.5 ml of meat peptone broth, 0,5 ml blood serum and bacterial culture (25.10^6 cells.ml^{-1}) was added. Five milliliters of meat peptone bullion and bacterial culture (25.10^6 cells.ml^{-1}) was used like a control variant. The optical density was measured with spectrophotometer at ʎ=530 nm prior the samples incubation at 26°C for 24 hours. The bactericide activity determination was calculated by the following equation:

$$BA = \frac{К - E}{К} x100$$

where:
BA - bactericide activity, %
$К=К_2-К_1$

$E=E_2-E_1$

K_1 - optical density of control sample before incubation;

E_1 - optical density of experimental sample before incubation;

K_2 - optical density of control sample after incubation;

E_2 - optical density of experimental sample after incubation;

Hemoglobin content in the blood of experimental carp fingerlings

The content of hemoglobin in experimental carp was measured spectrophotometrically with an express test's Diasys diagnostic system (Germany) according the test producer' procedure.

The parameters determined in lettuce (*Lactuca sativa* L.) during the trial

Physiological parameters determined in lettuce (*Lactuca sativa *L.)

Plant growth. The fresh weight (g) of all lettuces in the end of the trial was measured for testing the influence of synbiotic on lettuces productivity.

Five randomly selected lettuce plants were measured for each analysis and the following parameters were determined:

Determination of leaf gas exchange parameters. The net photosynthesis rate, transpiration rate and stomatal conductance of the plants were measured with a portable infrared gas analyzer *LCA-4* (*Analytical Development Company Ltd,.* Hoddesdon, England), equipped with a *PLCB-4* chamber. The measurements were made in the chamber (11 cm^2 leaf area) under irradiance of 800 µmol (PAR) m^{-2} s^{-1}, temperature of 26±2 °C, external CO_2 concentration of 400 µmol mol^{-1}.

Determination of photosynthetic pigments. The photosynthetic pigments content (mg g^{-1} FW) was determined spectrophotometrically in fresh leaf tissue through extraction in 85% acetone and calculated according to Lichtenthaler (1987). The absorption was measured at 663 nm, 644 nm and 440 nm, for chlorophyll *a*, *b* and carotenoids, respectively.

Determination of nitrate reductase activity. The nitrate reductase activity (NR, EC 1.6.6.1) in leaves was determined spectrophotometrically by the method of Kaiser and Lewis (1984). The concentration of nitrite was calculated by drawing a calibration curve of nitrite. The enzymatic activity was expressed as µg NO_2^- g^{-1} h^{-1}.

Results and discussion

Hydrochemical parameters in mesocosmos systems

The values of hydrochemical parameters which were measured during the trial could be seen in Table 1. The values of water temperature, pH, oxygen, conductivity and nitrate were favorable for raised fingerlings according Regulation 4 (2000) and were without statistical differences ($P \geq 0.05$) between both experimental variants except for pH ($P \leq 0.05$). Masser et al., (1999) reported that the nitrification in aquaculture biofilters is distinguished with the highest efficiency when pH varied between 7.0 and 8.0. Hochmuth (1991) recommend that pH ranges for hydroponic systems should be between 5.5 and 6.5. The increasing of pH value leads to decrease of nutrients' solubility and some micronutrients deficiency in cultivated plants (Tyson et al., 2004). On this base it could be concluded that pH is favorable for plants cultivated in aquaponics system if it ranged from neutral to slightly acid reactions. The favorable pH in S_1 probably improved the nutrients assimilation from lettuce plants and explained the lower concentration of nitrates in the water of aquaponics systems from S_1 variants compared with the nitrate concentration found for S_0 (Table 1).

The influence of synbiotic dietary supplementation on growth, immunology and physiology in carp fingerlings cultivated in aquaponics system

During the trial the survival of carp fingerlings was 100% in every of control and experimental variants.

The average final weight in fingerlings fed with feed contained synbiotic was with 9.8% higher than this found for carps from the control group (Fig. 3-A) ($P \leq 0.05$). The average values of specific growth rate in carps from S_1 and S_0 were 1.04%.day^{-1} and 0.77%.day^{-1} respectively and difference was statistically significant ($P \leq 0.05$). Better FCR was found also for carps from experimental group S_1 – 1.5 against 2.03 for the fish from experimental group S_0 ($P \leq 0.05$). The positive effect of synbiotic on growth and feed utilization in carp fingerlings is in agreement with the study made from

Table 1
Average of values of hydrochemical parameters during the trial

Hydrochemical parameter	S_1	S_0
Temperature /°C/	23.3±1.7	23.2±1.8
pH	7.9±0.2*	8.1±0.13
Conductivity /µS.cm-^{1}/	218.06±25.9	224.7±11.1
Oxygen /mg.l^{-1}/	6.9±0.7	6.8±0.8
Nitrate /mg.l^{-1}/	3.4±1.1	4.9±2.1

* Asterisk (*) denotes a significant different at P<0.05

Dehaghani et al. (2015), where Biomin IMBO synbiotic was used and significantly enhanced the growth parameters and feed utilization. Different studies demonstrated that lactic acid bacteria and other probiotics could increase the growth and utilization of received feed in hydrobionts (Lara-Flores et al., 2003; Suzer et al., 2008; Sun et al., 2011). Dehaghani et

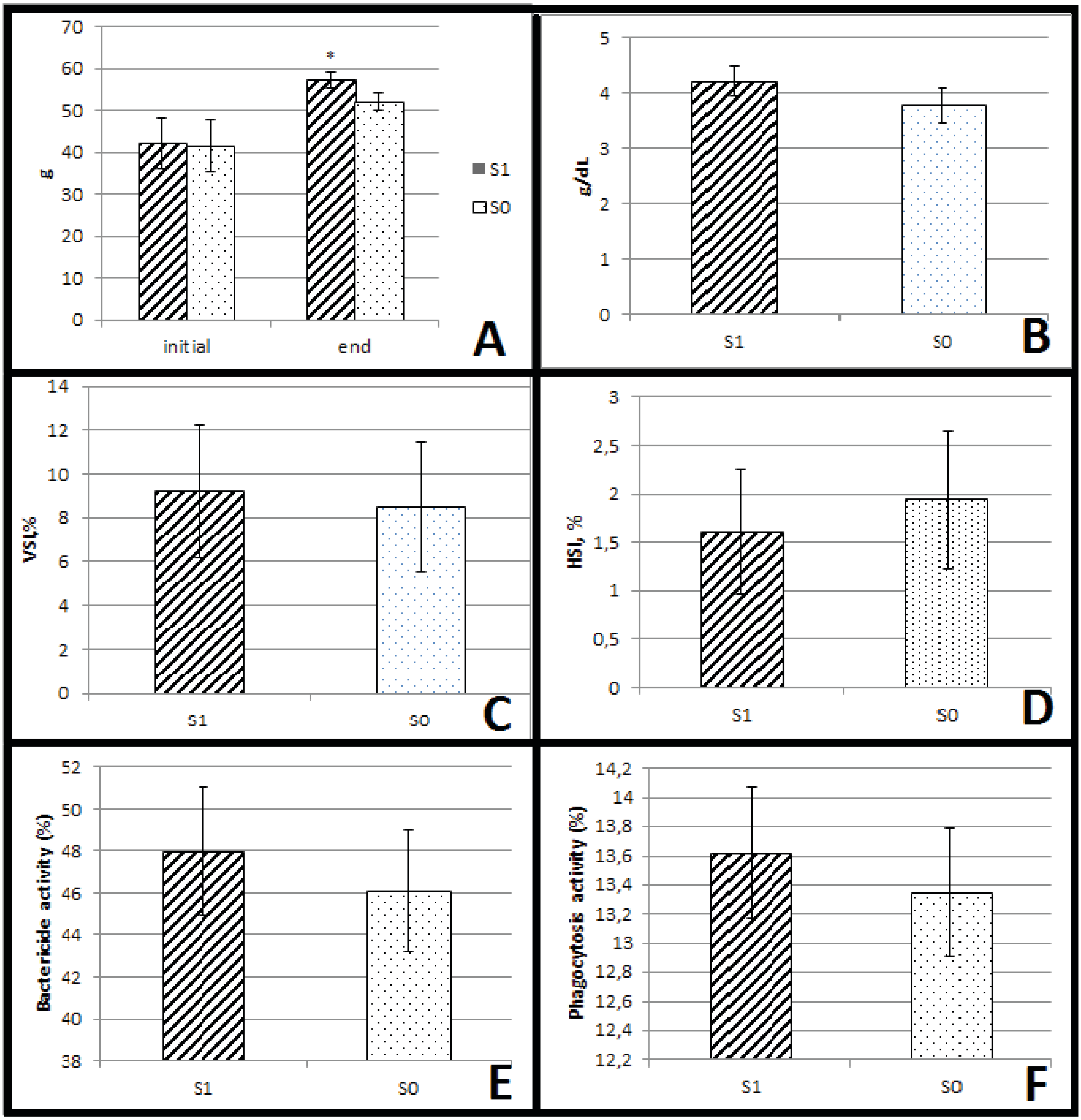

Fig. 3. Investigated parameters in carp fingerlings during the trial:
A) The initial and final weight in carp' fingerlings (g); B) The content of hemoglobin in the blood of experimental fingerlings ($g.dL^{-1}$); C) viscerosomatic index (VSI) in fingerlings (%); D) hepatosomatic index (HSI) in fingerlings (%); E) bactericide activity (%) in fingerlings; F) phagocytosis activity (%) in fingerlings; (S_1) the carp fingerlings were fed with feed contained synbiotic Biobalans; (S_0) – the carp fingerlings were fed with feed without addition of symbiotic. Asterisk (*) denotes a significant different at P<0.05

al. (2015) stated that the higher growth in carp fingerlings is a result from improved digestive enzyme activity from synbiotic' application which leads to better efficiency in the digestive system of fish.

Viscerosomatic and hepatosomatic indices were shown on Fig. 3C and Fig. 3D. The fingerlings fed with feed supplemented with synbiotic Bio balance® showed higher viscerosomatic index and lower hepatosomatic index in comparison with these values found for carps fed with feed without supplementation of synbiotic and differences were respectively 0.72% for the first index and 0.33% for the second index. The received data for viscerosomatic (VSI) and hepatosomatic indices (HSI) were not showed significant differences for these parameters in fingerlings from different experimental groups ($P \geq 0.05$). Asadi Rad et al. (2012) found that VSI was significantly higher in nile tilapia (*O. niloticus*) when the fish was fed with the feed supplemented with 1.0 g yeast kg^{-1} diet brewer's yeast, *S. cerevisiae,* the HSI was not affected by probiotics supplementation in the same study. Azevedo et al. (2016) received similar to the present research's results and the HSI and VSI values were not affected when the diet of Nile tilapia (*O. niloticus*) was supplemented with mannan oligosaccharide (MOS), yeast *Saccharomyces cerevisiae*), probiotic (*Bacillus subtilis* - BS, C-3102 strain) and their combination.

Bactericide activity in fingerlings from S_1 was higher with 3.9% (Fig. 3E and Fig. 3D) compared to the value of this parameter in carps from S_0, but the difference was not significant ($P \geq 0.05$). The carps fed with feed contained synbiotic also showed higher phagocytosis activity compared to carps from control group and the difference in this parameter was 7.4% (Fig. 3E and Fig. 3D), but without being statistically proven ($P \geq 0.05$). The received from current study results are controversial to the results received from Hoseinifar et al. (2015) which found that immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) are improved when synbiotic was administrated to fish feed. Not significant interaction between dietary *B. subtilis* and FOS on immune response and disease resistance of large yellow croaker was observed when they were used in combination for supplementation of feed for this fish species (Ai et al., 2011). Cerezuela et al. (2011) showed that the effect of synbiotic on fish immunity could highly varied from beneficial to no effect and depends from the type of synbiotic, fish species, dose, time of application, etc.

The content of hemoglobin in fingerlings from S_1 was higher with 10.6% compared to the value of this parameter found for carps from S_0 (Fig.3B), without the difference being statistically significant ($P \geq 0.05$). This result is in confirmation of the studies made from Hassaan et al. (2014) where the fish fed with synbiotic supplemented feed showed the higher Hb level compared with the value received from experimental variant where the fish were fed with feed without supplementation of synbiotic.

The influence of synbiotic Bio balance® application in mesocosmos aquaponics system on productivity and physiology in lettuce (Lactuca sativa L.)

It could be seen on Fig. 4 that the lettuces from S_1 showed with 2.14% higher fresh weight compared with the weight of lettuces from S_0. The better yield in lettuce from experimental variant supplemented with synbiotic probably was result from better pH in water and connected with this better assimilation of nutrients from the salad from S_0 variant. Other possible explanation of higher weight in lettuce from S_1 compared with this found for S_0 could be the growth promoting effect of some of bacteria which constituted the tested synbiotic. Different studies showed that lactic acid bacteria has potential growth promoting effect on different plants (Murthy et al., 2012; Takei et al., 2008). The idea for growth promoting effect of Bio balance® on the experimental lettuce in current study is too speculative because the differences in the growth between plants from both experimental groups were not statistically significant ($P \geq 0.05$) and this effect could not be proved for the moment.

Results presented in Table 2 show changes in leaf gas exchange parameters of plants grown in aquaponics with different feeding regime of carp fingerlings. The photosynthetic CO_2 assimilation (P_N) in plants from experimental variant supplemented with synbiotic was significantly higher (with 34%) compared to the control plants. In the same experimental variant transpiration rate (E) in plants increases with 45% compared to control. Stomata are known to play important role in the control of CO_2 supply to mesophil plant cells. Sim-

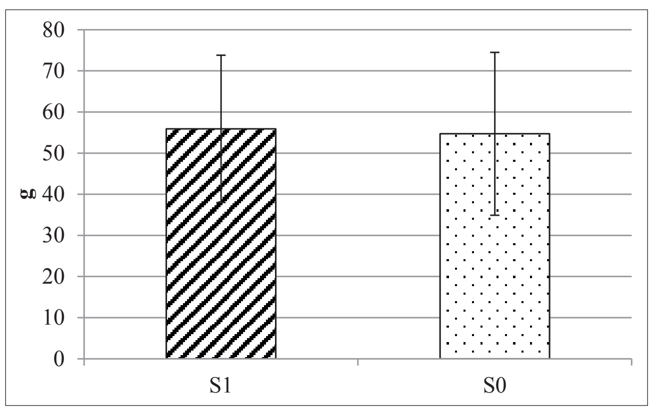

Fig. 4. The fresh weight in lettuce (*Lactuca sativa* L.) in the end of trial

ilar effect was observed in stomatal conductance of experimental plants supplemented with synbiotic that is not statistically significant.

The data presented in Table 3 proves the applied treatment does not tend to change the content of all pigments in plants from both experimental variants.

The results presented in Figure 5 show significant increase in the activity of nitrate reductase enzyme in lettuce from S1. Its value is 26% above then this found for the variant without synbiotic addition (S_0). The higher NR activity in the plants from the systems where carps feed was supplemented with synbiotic could be explained with their ability to translocate and utilize better nitrogen.

The study we made showed that application of synbiotic in aquaponics systems could be beneficial for cultivated fish and plants. The application of Bio balance® synbiotic positively affects the pH in the system, growth and FCR in carp fingerlings, net photosynthetic rate**,** transpiration rate and activity of nitrate reductase in lettuces cultivated in mesocosmos aquaponics system. Further studies should be done connected with the application of different synbiotics, different doses and the way of application.

Table 2
Leaf gas exchange in lettuce (*Lactuca staiva* L.) cultivated in aquaponics: P_N – Net photosynthetic rate [μmol (CO_2) m-2 s-1]; E – Transpiration rate [mmol (H_2O) m^{-2} s^{-1}]; g_s – stomata conductance [mol m^{-2} s^{-1}]

Parameters	S_1	S_0
P_N	12.81±2.27*	9.54±1.12
E	1.44±0.29*	0.99±0.11
g_s	0.10±0.03	0.09±0.01

Table 3
Content of photosynthetic pigments in lettuce (*Lactuca staiva* L.) cultivated in aquaponics: Chl. a – chlorophyll a; Chl. b – chlorophyll b; Car – carotenoids (mg g^{-1} FW)

Parameters	S_1	S_0
Chlorophyll a	0.74±0.05	0.79±0.05
Chlorophyll b	0.29±0.02	0.26±0.02
Carotenoids	0.31±0.03	0.33±0.02

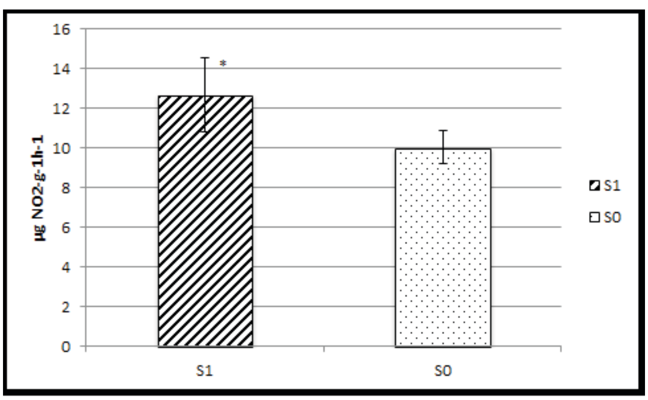

Fig. 5. The activity of enzyme nitrate reductase in lettuce (*Lactuca staiva* L.) cultivated in aquaponics.
Asterisk (*) denotes a significant different at P<0.05

Conclusions

The present study demonstrated for first time that the application of synbiotic as feed supplementation for carp extruded pellet in aquaponics recirculation system could be successful. The positive effect of Bio balance® on growth rate and feed utilization in carp fingerlings cultivated in aquaponic mesocosmos was observed and average final weight and FCR in fish fed with feed supplemented with Bio balance® were higher with 9.8% and 26.1% respectively, compared with the values of these parameters found for fingerlings received feed without addition of synbiotic (P≤0.05). The bactericide and phagocytosis activities and hemoglobin content in blood of carps were higher in fish from S_1, compared to the values of these parameters in fingerlings from S_0, but the differences were not significant (P≥0.05). The lettuces from S_1 showed with 2.14% higher fresh weight compared with this one of lettuces from S_0, but the difference was not significant (P≥0.05). The better yield in lettuce from experimental variant S_1 where the carp feed was supplemented with synbiotic probably was result from better pH in water and connected with this better assimilation of nutrients from the salad of S_0 variant. Fingerlings diet supplemented with Bio balance® stimulates the physiological proceses in the experimental plants. The positive effect of the synbiotics is reflected by improved leaf gas exchange parameters and nitrate reductase activity in lettuce.

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