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EFFECT OF ROSEMARY, SEA BUCKTHORN AND GINGER AS FEED ADDITIVE ON HEMATOLOGICAL PROFILE AND SOME BIOCHEMICAL PARAMETERS OF *Oreochromis niloticus* SPECIES

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Abstract

In the present study, three phytobiotics were added to the basal diet of Oreochromis niloticus reared in a recirculating aquaculture system. Therefore, was investigated the influence of phytobiotics on hematological profile, some blood biochemical indices and leukocyte reaction. The experiment was carried out during 12 weeks. The experimental variants were: V1 - control; V2 - 1% Rosmarinus officinalis/kg feed; V3 - 1% Hippophae rhamnoides/kg feed and V4 - 1% Zingiber officinale/kg feed. The results of some parameter of hematological profile showed that supplemented diet with sea buckthorn and ginger exhibited significantly ($p<0.05$) lowest values of red blood cells count, white blood cells count, absolute number of lymphocytes and neutrophils, relative number of neutrophils. Regarding to blood biochemical analysis was observed a significant ($P<0.05$) reduction of plasma cortisol concentration and a slight decrease ($P>0.05$) of glucose concentration in V3 variant. Also, only the sea buckthorn (V3) and ginger (V4) showed an immunomodulatory effect during the experiment because they determined the intensifying the lysozyme activity. In conclusion, dietary supplementation with sea buckthorn and ginger reduced the technological stress and improved the immunity of Nile tilapia.

Key words*: hematological profile, lysozyme activity, Nile tilapia, phytobiotics, recirculating aquaculture system.*

INTRODUCTION

The use of dietary additives in fish farms is one of the methods commonly used to improve weight gain, feed efficiency, and/or disease resistance in cultured fish (Akrami et al., 2015). Recently, the immunostimulants, due to increased resistance to infectious diseases by enhancing both specific and nonspecific defence mechanisms of fish and animals (Harikrishnan et al., 2010), are used for fish disease control because they offer an alternative and a cost-prohibitive or even limited efficacy to currently existing drugs, chemicals and antibiotics (Sakai, 1999). They are improving the immune status of the fish by enhancing the lysozyme, leukocyte reaction and the values of haematological profile parameters.

The latest studies showed that a large number of medicinal chinese herbs were used as immunostimulants such as *Viscum album*, *Urtica dioica* and *Zingiber officinale* (Dugenci et al., 2003), *Radix astragalin* and *R. angelicae* (Jian and Wu, 2004), *Astragalus radix* and *Scutellaria radix* (Yin et al., 2006), *Achyranthes aspera* (Rao et al., 2006), *Eclipta alba* (Christybaptita et al., 2007), *Rosmarinus officinale* (Xie et al., 2008), *A. radix* and *Ganoderma lucidum* (Yin et al., 2009) who reported an improvement in the innate immunity of fishes.

The most widely used medicinal herbs worldwide is a rosemary (*Rosmarinus*

officinalis), due to its good antioxidant activity (Caillet et al., 2007). The most significant feature of the antioxidant activity of rosemary is the association between diterpenes and radical scavenging activity (Nieto et al., 2018). The biological activities of this plant are mainly related to the phenolic and the volatile constituents (Arranz et al., 2015) such as carnosol, carnosic acid and rosmarinic acid present in the extract of rosemary and a-pinene, bornyl acetate, camphor and eucalyptol present in the essential oil of this plant (Arranz et al., 2015). Minor components may have a potential influence on the biological activity due to the possibility of synergistic effect among their components (Hussain et al., 2010).

Rosemary extract has been tested as a feed additive in mammals, and has been demonstrated to have strong antidiabetic (Al-Jamal and Alqadi, 2011), hepatoprotective (Cui et al., 2012), choleretic (Romo Vaquero et al., 2012) and antiadipogenic (Gaya et al., 2013) effects. However, to our knowledge, only one study revealed some general effects on the physiological condition of the fish as reduced hepato-somatic index and increased spleen-somatic index and bile-somatic index (Yilmaz et al., 2013).

Several studies confirmed that the oral administration of rosemary leaf powder could enhance growth production, improve antioxidant status and immunological parameters, and alleviate the adverse effects of high stocking density stress on common carp fingerlings (Yousefi et al., 2019). Additionally, fish fed a diet supplemented with rosemary (*Rosmarinus officinalis*) showed significantly improving growth rates and feed efficiency compared with those in the control group at Nile tilapia, *Oreochromis niloticus* (L.) (Hassan et al., 2018). Moreover, dietary supplementation with 0.5% rosemary significantly enhanced innate immunity and antioxidant status of *O. niloticus* fed aflatoxin B1 contaminated diet (Naiel et al., 2019). Also, Jiang et al. (2011) found that rosemary extract revealed great antibacterial activity against Gram-positive and Gram-negative stained bacteria. Besides these positive effects, rosemary-supplemented sea bass (*Dicentrarchus labrax*) diet did not affect kidney function indicators and liver enzymes (Yılmaz et al., 2013).

At the same time, sea buckthorn (*Hippophae rhamnoides* L.) is a versatile food and nutraceutical crop with various applications, from con-trolling soil erosion to being a source of horse fodder, nutritious foods, drugs, and skin-care products (Kumar et al., 2011). It should be noted that all parts of sea-buckthorn are a rich source of bioactive components, their highest concentration being found in fruit (vitamins A, C, E, K, carotenoids, organic acids, minerals etc.) (Yang & Kallio, 2002). Moreover, the berries are rich in carotenoids, such as zeaxanthin, beta β-carotene, β-cryptoxanthin, litein, lycopene and γ-carotene (Anderson et al., 2009). However, all parts of rΓthis wonder plant are considered to be a good source of a large number of bioactive compounds, including carotenoids, tocopherols, sterols, flavonoids, lipids, vitamins, tannins, minerals, etc. which contribute to its wide usage as a natural anti-oxidant (Kumar et al., 2011).

Sea-buckthorn was used as an immune-modulator agent in case of animal breeding and veterinary medicine, the outcomes being spectacular, fact that led to the administration of sea buckthorn in animal feeds in order to prevent some health problems (Morar, 2003).

In fish diets, *Hippophae rhamnoides* it is used successfully for improving disease resistance and growth performance (Todoran, 2015).

Ginger (*Zingiber officinale*) is widely used around the world in food as a spice. Ginger is generally considered as a safe herbal medicine; contains alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fiber, carbohydrate, vitamins, carotenoids and minerals; natural antioxidants as gingerols, shogaols and zingerone; essential oils which has potent anti-inflammatory effects and oleoresin (Jahanjoo et al., 2018). Previously, studies have indicated that ginger is effective for the controling of a range of bacterial, fungal and parasitic conditions (Chrubasik et al., 2005). In different fish, it has been demonstrated that ginger administration significantly increased growth performance, different immune responses, and resistance to against different pathogenic bacteria (Ahmadifar et al., 2019).

Thus, in order to evaluate the welfare status of the fish it is taken into account the determination of the haematological parameters, some biochemical blood parameters and immunological tests.
Therefore, the present study aims to investigate the efficiency of some herbal as feed additive on haematological changes and leukocyte reaction in case of *Oreochromis niloticus* reared in a recirculating aquaculture system.

MATERIALS AND METHODS

Experimental design
This experiment was carried out in the research laboratory of the Department of Food Science, Food Engineering, Biotechnology and Aquaculture, from "Dunarea de Jos" University, Galati. The recirculating system was described in our precedent paper (Antache et al., 2013). The design of this system consists in four rearing units, with a volume of $1m^3$ each, and a series of water quality conditioning units (Cristea et al., 2002). The experiment lasted 12 weeks. In this research the biological material consisted in a total number of 168 individuals of Nile tilapia, with an initial average weight of 359.57 ± 61.44 g/fish that were randomly distributed in four rearing units. The experimental variants were organized as follows: V1 - control, V2 - 1% rosemary (*Rosmarinus officinalis*)/kg feed, V3 - 1% sea buckthorn (*Hippophae rhamnoides*)/kg feed and V4 - 1% ginger (*Zingiber officinale*)/kg feed. These phytobiotics were purchased from a Plafar market, like dried plants, after which they were grounded and used as powder.
The additivation of fish feed with phytobiotics was achieved using an aqueous solution of gelatine with 2% concentration. The feed was sprayed, mixed and then dried at 25ºC. Fish were fed with SOPROFISH pelleted feed, with 38% crude protein and 7% crude fat. The feed biochemical composition was related by Antache et al. (2013). Fish were fed four times per day with a daily ration of 2% from fish body weight. At the end of the experiment the individual average weight was 597.18 ± 113.48 g/fish in V1, 589.33 ± 90.42 g/fish in V2, 620.20 ± 84.40 g/fish in V3 and 616.93 ± 103.64 g/fish in V4.
Blood sampling and analysis
Blood sampling has been carried out at the beginning (V0) and at the ending of experimental period. Before to start the sampling method, fish were anesthetized with 2-phenoxyethanol (8 mL/40 L of water for 5 minutes) in order to reduce handling stress. Was sampling 4 mL of blood at 7 fish, by caudal venous puncture using heparin as anticoagulant, from each growth unit. For each sample were used two Eppendorf tubes. So, for haematology analyses was added anticoagulant in Eppendorf tubes and for biochemical analyses was not added anticoagulant. Blood analysis was performed by method used in fish haematology described by Blaxhall and Daisley (1973). This analysis consisted in determination of red blood cells count (RBCc, x 10^6 cells/mm^3), hemoglobin (Hb, g/dl) and hematocrit (PVC, %).
The erythrocyte number was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer. The hematocrit was performed by duplicate using capillary tubes and a micro hematocrit centrifuge. The hemoglobin concentrations were measured spectrofotometrically with SPECORD 210 Analytikjena at λ-540 nm, using Drabkin reagent. Then, using standard formulas described by Ghergariu et al. (1985) and Svobodova (2001) were calculated the erythrocyte constants: mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dl).
Regarding to blood biochemical parameters have been analysed the glucose concentration (GLU - mg/dL), cortisol concentration (ng/mL), total protein (TP - g/dL and lysozyme activity (LYS Units/mL). To obtain blood serum, the blood without anticoagulant was centrifuged 10 minutes, at 3500 rotation/min. Determination of glucose, total protein and lysozyme activity from serum was performed spectrophotometric using the spectrophotometer SPECORD 210 Analytikjena. Dosage of glucose was made by colorimetric method with o-toluidine, readings were made at 635 nm wavelength. Total protein from serum were determined by Biuret method, the readings was done at a 546 nm wavelength. Lysozyme activity was measured, from serum, based on the turbiometric assay, Enzymatic

Activity of Lysozyme Protocol (Sigma, EC 3.2.1.17). For this test was prepared a substrate, in 66 mM Potassium Phosphate Buffer, with 6.24 pH at 25°C, a volume of 0.01% (w/v) suspension of *Micrococcus lysodeikticus* (Sigma, M3770). Lyophilised powder of chicken egg white lysozyme (Sigma, L6876) was used as standard. One unit of lysozyme activity was defined as a reduction in absorbancy of 0.001/min, at a 450 nm wavelength. Serum cortisol determination was performed using the kit: NovaTec Cortisol-DNOV001 based on competitive immunoenzymatic colorimetric method for quantitative determination of cortisol in human serum or plasma. Absorption was read at 450 nm using an ELISA microwell plate reader.

The leukocytar reaction was obtained by microscopic examination of 200 leukocytes on blood smears (in dublicate for each fish), using Zeizz Axio Imager microscop and immersion objective (10 oc. x 100 ob.). Blood smears were immediately dried, fixed with methanol and then colored with May-Grünwald Giemsa panoptic method (MGG). The type of leukocytes were determined based on identification characters listed by Svobodova et al. (1991). Absolute number of circulating blood leukocytes and thrombocytes was determined in comparison with 1000 erythrocytes counted on haemocytometer, per blood volume unit.

Statistical analysis

The results, of haematological and biochemical parameters, of the experimental groups were statistically analysed using descriptive statistics and ANOVA test. Programs used were Microsoft Excel 2010 and SPSS Statistics 17.0. The results were presented as mean ± standard deviation.

RESULTS AND DISCUSSIONS

The determination of the haematological profile represent an important analysis in the detection of the nutritional deficiencies which can cause anaemia, appeared as a result of the significant reduction of hematocrit values and hemoglobin concentrations. From this reason, in fish, the determination of the hematological parameters represent a useful tool that can be used to monitor the changes at the physiological and pathological level (Kori-Siakpere et al., 2005).

The values of hematological parameters obtained at the beginning (V0) and at the end of the experiment (V1 - control, V2 - 1% rosemary/kg feed, V3 - 1% sea buckthorn/kg feed and V4 - 1% ginger/kg feed), are presented in the Figures 1-6.

Thus, the changes that appeared during the experimental research, following the administration of rosemary, sea buckthorn and ginger, at the level of the haematological picture are presented below.

At the end of the experiment the ***red blood cells count (RBCc)*** registered a significant reduction ($p < 0.05$) in case of V3 variant in which was administered sea buckthorn as phytobiotic (Figure 1). However, compared to V1 variant, there was a reduction in the number of erythrocytes in all variants in which phytobiotics were administered (by 31.56% in V3 variant; 11.65% in V4 variant; respectively by 0.99% in V2 variant). It should be noted that the values obtained in the case of our research were registered in the range of 1.08-2.44 x 10^6 erythr./µL blood, these values being within the optimal limits for the *Oreochromis niloticus* species, respectively between 0.7 and 2.8 x 10^6 erythr./ µL blood (Bittencourt et al., 2003).

The ***hematocrit*** registered a slowly increase ($P>005$) at the end of the experiment compared to the initial moment (Figure 2), but between the experimental variants there were no significant differences ($P>0.05$). Thus, the PVC values increased with 4.93% in V1 variant, 3.52% in V4 variant; 2.82% in V2 variant and 0.70% in V3 variant. During the experiment PVC (%) values ranged from 23 to 33%. The average values obtained are recorded in the reference ranges reported in the literature for the *Oreochromis niloticus* species (23-41%, Tavares Dias and Faustino, 1998; 15-45%, Bittencourt et al., 2003).

At the end of the experiment, from the four experimental variants, the lowest ***haemoglobin concentratio***n was also obtained in the variant in which sea buckthorn was administered (V3 variant - 9.67 ± 1.21 g/dL) (Figure 3). The increase in haemoglobin concentrations obtained at the end of the experiment was significant ($P<0.05$) compared to the value of haemoglobin concentration obtained at the

initial moment (V0). The decrease of the haemoglobin concentration in V3variant shows that the administration of sea buckthorn had a positive effect on the physiological state of the biological material throughout the experimental period. However, compared to the control variant (V1), the physiological state of the biological material from the variants in which sea buckthorn was administered, but also from the other two variants (V2 and V4) is an optimal one. During the experiment, the minimum value of the haemoglobin concentration was 5.5 g/dL, and the maximum value was 11.81 g/dL, these values delimiting the interval in which all the values of the haemoglobin concentration were included. The values obtained are found in the optimal range for Nile tilapia: 5.4-12.7 g/dL (Tavares Dias and Faustino, 1998) and 6.58-15.98 g/dL (Bittencourt et al., 2003).

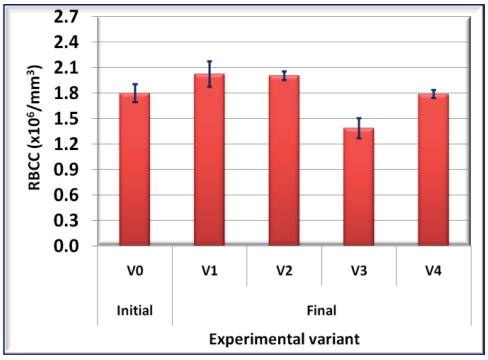

Figure 1. Changes in erythrocytes number (RBCc) of different experimental groups

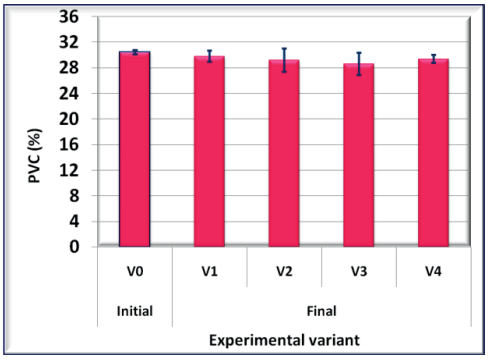

Figure 2. Changes in hematocrit (PVC) of different experimental variants during the experiment

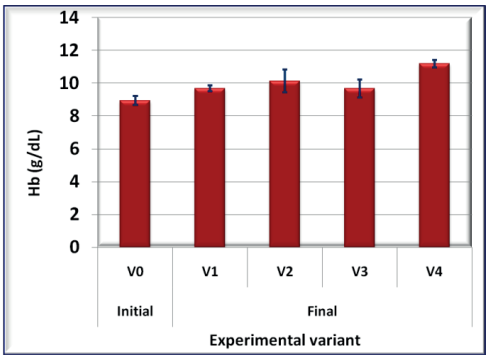

Figure 3. Changes in hemoglobine concentration (Hb) of different experimental groups

Regarding to the erythrocytar constants the ***mean corpuscular volume (MCV)***, in the variant in which sea buckthorn was administered (V3), the MCV concentration increased significantly ($P<0.05$) compared to the control variant (V1), also compared to the value obtained at the beginning of the experiment (V0) (Figure 4). The MCV concentration increased in V3 variant with 43.76% compared to V2 variant; with 40.46% compared to V1 variant; respectively with 29.26% compared to V4 variant.

Also in case of the mean of MCV concentration, the same trend was observed in the occurrence of significant differences as in the case of the number of erythrocytes (RBCc), only that the values recorded in the case of MCV concentration are indirectly proportional. Hamid et al. (2013) showed that the average of MCV can reach a maximum value, but at the same time an optimal value, of 214 μm^3 (Hamid et al., 2013).

Concerning to ***mean corpuscular hemoglobin (MCH)***, at the end of the experiment, there was a significant increase ($P<0.05$) in the variants in which were administered phytobiotics compared to the control variant (V1) and compared to the initial value (V0). Compared to the control (V1), the MCH concentration increased with 3.02% in V2 variant, 27.24% in V4 variant and 48.01% in V3 variant (Figure 5). This is correlated with the hemoglobin concentration, because even in the case of hemoglobin the highest values were recorded at the end of the experiment.

The results obtained during the experiment are in the range of 41.11-109.20 pg. In the literature, at the Nile tilapia, a mean range

between 5 pg and 80.4 pg has been reported for MCH concentration (Hamid et al., 2013), but average values between 50.2 pg and 65.4 pg have also been reported (Goda, 2008).

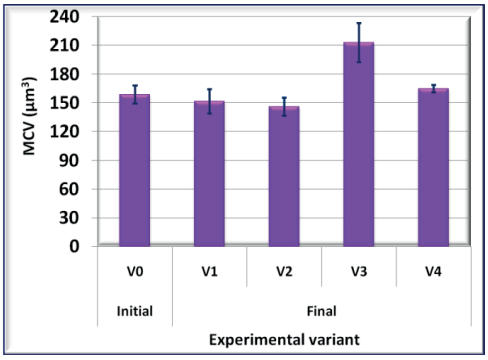

Figure 4. Changes in MCV concentration of different experimental variants during the experiment

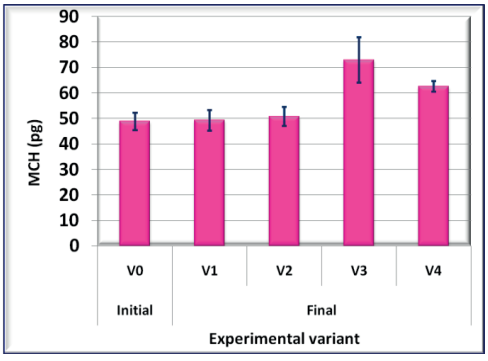

Figure 5. Changes in MCH concentration of different experimental variants during the experiment

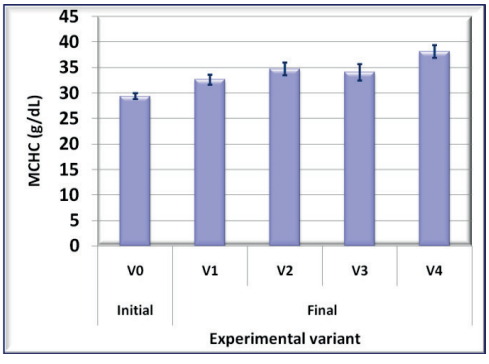

Figure 6. Changes in MCHC concentration of different experimental variants during the experiment

Due to the increase in hemoglobin concentration, as in the case of MCH concentration, there was a significant increase ($p < 0.05$) in ***mean corpuscular hemoglobin concentration (MCHC)*** at the initial momement (V0). However, there were no significant differences between the experimental variants ($P > 0.05$). The highest mean of the MCHC concentration was recorded in the variant in which was administered ginger (V4 - 38.16 ± 2.78 g/dL) (Figure 6). Regarding to the mean of the MCHC concentration, the values ranged from 25.18 to 42.50 g/dL. The values obtained fall within the reference range for the *Oreochromis niloticus* species, respectively 19.84 g/dL and 87.73 g/dL (Bittencourt et al., 2003).

At the end of the experiment, it was observed that with increasing of hemoglobin concentration, the average of MCH concentration and the average of MCHC concentration also increased in the variants in which rosemary (V2) and ginger (V4) were administered. The MCH concentration recorded a high value in the variant in which sea buckthorn was administered due to the small number of red blood cells in this variant.

In order to evaluate the fish welfare status, besides the analysis of the hematological profile, the determination of some biochemical indicators of the blood is also used.

Jawad et al. (2004), showed that the biochemical parameters of blood plasma can vary from species to species, being influenced by both biotic and abiotic factors, such as water quality, temperature, season, age, sex and last but not least by the food composition. However, the biochemical parameters of the blood can be used as biomarkers, due to their sensitivity and the fact that they are less variable (glucose, cortisol, total proteins) (Owolabi, 2011).

Cortisol and glucose are the most common stress indicators in fish, which elevate during stress and increase energy expenditure (Abdel-Tawwab, 2012; Ghelichpour et al., 2018).

In terms of ***glucose concentration***, at the end of the experiment, there were no significant differences between the experimental variants ($P > 0.05$). The results obtained in the variants in which the diet was supplemented with phytobiotics were close to the value obtained in the control variant (V1) (Figure 7). However, the values recorded at the end of the experiment were significantly lower ($P < 0.05$)

than the value obtained at the initial of the experiment (V0). In this case, it can be seen that the administration of sea buckthorn led to a lower concentration of glucose compared to the variants in which rosemary and ginger were administered. The average of the glucose concentration are registered within the normal limits for the *Oreochromis niloticus* species, respectively 22.7-107.0 mg/dL (Bittencourt et al., 2003). Though, Hamid Ahmed et al. (2013) extended the glucose range from 33.3 mg/dL to 250 mg/dL in Nile tilapia.

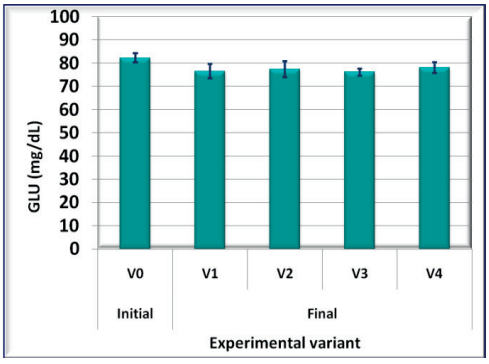

Figure 7. Changes in glucose concentration (GLU) of different experimental variants during the experiment

Regarding to ***cortisol concentration***, although the mean values varied, there were no significant differences (P>0.05) between the experimental variants (Figure 8). The lowest cortisol concentrations were recorded in the variant in which sea buckthorn was administered (V3 - 366.26 ± 94.29 ng/mL). The low value of cortisol concentration obtained in variant V3 can be associated with the large amount of antioxidants present in sea buckthorn. Apines-Amar et al. (2013) showed that ginger administration significantly reduced plasma cortisol levels in *Epinephelus fuscoguttatus*. Also, demonstrated that ginger is a strong anti-stress agent, the ginger-treated fish had significantly lower cortisol levels compared to the ascorbic acid-treated ones, a well-known anti-stress agent in fish (Jalali et al., 2010; Barros et al., 2014). Apines-Amar et al. (2013) found lower plasma cortisol levels in the ginger-treated *E. fuscoguttatus*, which was accompanied by higher growth performance. Similar results were also obtained in *O. mykiss* fed with lycopene or cineole supplemented diets and reared under high stocking density (Taheri Mirghaed et al., 2018). This explains in our case the reduction of cortisol in the variant in which sea buckthorn was administered (V3).

In case of the ***total proteins***, a reduction with 8.07 percent was observed in the variant in which the diet was supplemented with 1% *Rosmarinus officinalis*, and an increase by 3.64% and 6.65% in the variants in which the fish diet was supplemented with 1% *Hippophae rhamnoides*, respectively 1% *Zingiber officinale*, compared to the variant in which were not administered phytobiotics (V1) (Figure 9). However, no significant differences were obtained between the experimental variants (P>0.05) (Figure 9).

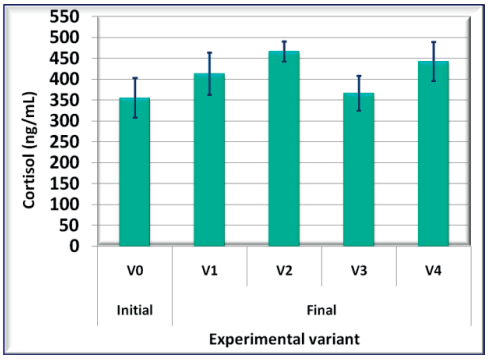

Figure 8. Changes in cortisol concentration of different experimental variants during the experiment

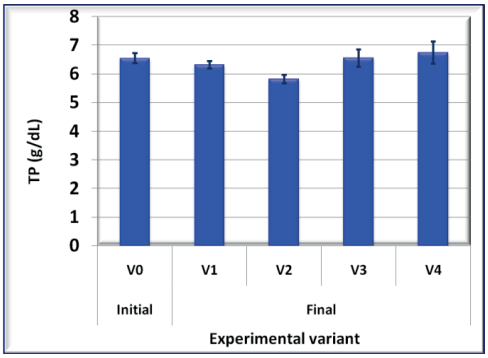

Figure 9. Changes in total protein concentration (TP) of different experimental variants during the experiment

At the end of the experiment, an intensification of ***lysozyme activity*** was observed in variant V3 (10.37 ± 1.39 U/mL) (Figure 10).

Thus, there was an increase in lysozyme activity by 10.08% and 11.46% in V3 variant, respectively in V4 variant, and a decrease by

4.25% in lysozyme activity in V2 variant compared to lysozyme activity recorded in the control variant (V1), but the differences were statistically insignificant (P>0.05).

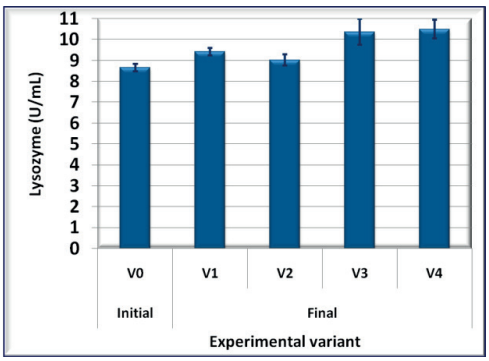

Figure 10. Changes in lysozyme activity (Lys) of different experimental variants during the experiment

From the analysis of lysozyme activity, an improvement of immunity can be observed in the variant in which sea buckthorn and ginger were administered, fact for which we can say that these phytobiotics have an immunomodulatory action.

The analysis of blood smears from a quantitative and qualitative point of view can give us the necessary information in order to study innate/native immunity. Leukocytes are the body's first line of immune defence (Cristea et al., 2012), so they play a very important role in defending the body if it is attacked by various pathogens.

The relative number of the leukocytes are presented in Table 1.

Table 1. Variation in the relative number of leukocytes at Nile tilapia during the experiment

Experimental variant		Relative number of leukocytes (%)			
		Lymphocytes		Monocytes	Neutrophilic granulocytes
		small	large		
V0 (initial)		96.42±1.92	1.23±0.86	0.58±0.18	1.78±0.89
Final	V1	95.53±0.78	0.79±0.22	1.09±0.34	2.58±0.54
	V2	96.44±0.93	1.03±0.41	1.12±0.20	1.40±0.46
	V3	96.81±0.30	0.58±0.19	1.07±0.20	1.54±0.39
	V4	95.71±0.61	0.80±0.22	1.69±0.46	1.79±0.22

Small lymphocytes (%). At the end of the experiment, the highest value of the relative number of small lymphocytes was recorded in the variant in which sea buckthorn was administered (V3 - 96.81 ± 0.30%), but which was insignificantly higher than in the other experimental variants (P>0.05). However, the initial relative number was significantly higher (P<0.05) than the relative number obtained in the control variant at the end of the experiment (Table 1).

Large lymphocytes (%). It was found that after 12 weeks the administration of phytobiotics did not influence the relative number of large lymphocytes. Thus, the highest value was recorded in variant V2 (1.03 ± 0.41%) and the lowest in V3 (0.58 ± 0.19%), the differences being insignificant (P>0.05). Compared to the initial value, was obtained a significant difference (p<0.05)

Monocytes (%). An insignificant increase (P>0.05) of the mean monocytes value was obtained in variant V4 (1.69 ± 0.46%) compared to the other experimental variants. The lowest average value was obtained in the variant in which sea buckthorn was administered (V3 - 1.07 ± 0.20%). The mean values increased significantly (p<0.05) compared to the value obtained at the initial moment (V0) (Table 1).

Neutrophil granulocytes (%). At the end of the experiment, a significant increase (P<0.05) of the relative number of neutrophils was observed compared to the average number obtained in the variants in which phytobiotics were administered. Therefore, the average values decreased by 45.74% in V2 variant, by 40.31% in V3 variant; respectively by 30.62% in the V4 variant.

The aspects indicated by the absolute number of leukocytes are presented in Table 2.

The absolute number of leukocytes. In the experiment, significant differences were observed between the experimental variants

(P<0.05). The average value obtained in variant V2 is significantly (P<0.05) higher than the initial value. This aspect can be observed in Table 2. During the experiment, the values obtained were in the range 43.93-132.71 x 1000 leukocytes/mm^3. The values obtained are included in the reference range described in the literature for tilapia, respectively 21.559-154.690 x 1000 leukocytes/mm^3 (Hrubec et al., 2000).

The absolute number of small lymphocytes. Because from all the leukocytes, the small lymphocytes are dominated by the changes that occurred during the experiment, they had approximately the same tendency as in the case of the absolute number of leukocytes. The absolute number of small lymphocytes recorded the highest average value also in the variant in which rosemary was administered, being preceded by the average value from variant V1, V3 and V4. Thus, significant differences were noticed between the experimental variants (P<0.05). If we report the results obtained to the initial mean value, we find a significant increase (P<0.05) of the mean values at the end of the experiment (Table 2).

The absolute number of small lymphocytes showed during the experimental research a minimum value of 45.42 x 1000 small lymphocytes/mm^3 and a maximum value of 128.90 x 1000 small lymphocytes/mm^3, this falling within the gap described in the literature (Hrubec et al., 2000).

Table 2. Variation in the absolute number of leukocytes and platelets at Nile tilapia during the experiment

Experimental variant		Absolut number (x 1000 cel./mm^3)					
		Leukocytes	Lymphocytes		Monocytes	Neutrophilic granulocytes	Platelets
			small	large			
V0 (Initial)		62.16±3.86	59.96±3.41	0.71±2.22	0.45±0.17	1.04±0.83	22.51±9.21
Final	V1	86.83±19.60	83.06±19.26	0.83±0.27	0.90±0.25	2.17±0.35	32.00±6.65
	V2	108.97±14.16	105.20±14.29	1.20±0.22	1.20±0.12	1.48±0.34	28.78±8.91
	V3	74.32±18.38	72.00±18.00	0.53±0.23	0.77±0.15	1.14±0.32	25.51±6.20
	V4	63.61±9.42	60.88±9.04	0.67±0.21	1.08±0.32	1.12±0.07	23.57±7.44

The absolute number of large lymphocytes registered a significant increase (p<0.05) also in the variant in which rosemary was administered (V2 - 1.20 ± 0.22 x 1000 cell/mm^3). In our experiment, the absolute number of large lymphocytes ranged from 0.275 to 1.632 x 1000 cell/mm^3. The obtained results are not found in the limits described for tilapia, respectively 2.852-30,833 x 1000 large lymphocytes/mm^3 (Hrubec et al., 2000).

The absolute number of monocytes. There were no significant differences between the experimental variants (P>0.05). The mean values obtained at the end of the experiment were significantly higher (P<0.05) than the value obtained at the beginning of the experiment (V0). The lowest average value of the absolute number of monocytes was obtained in the variant in which sea buckthorn was administered (V3 - 0.77 ± 0.15 x 1000 monocytes/mm^3). The results recorded by us are found in the reference range for the absolute number of monocytes for tilapia ranging between 0.511 and 1.550 x 1000 monocytes/mm^3 (Hrubec et al., 2000). The variation of the absolute number of monocytes is presented in Table 2.

The absolute number of neutrophils. At the end of the experiment, a significant reduction (P<0.05) of the number of neutrophils was observed in the variants in which phytobiotics were administered compared to the control variant (V1). The lowest values were recorded in the variant in which sea buckthorn (V3) and ginger (V4) were administered. The mean values of the absolute number of neutrophils, obtained during the experiment, fall within the reference range for tilapia, 0.557-9.873 x 1000 neutrophils/mm^3 (Hrubec et al., 2000).

Absolute number of platelets. At the end of the experiment, was observed a reduction tendency, but insignificantly (P>0.05), of the average values in the variants in which phytobiotics were administered compared to

the control variant. Therefore, the average values decreased by 26.34% in V4 variant, by 20.28% in V3 variant; respectively by 10.06% in the V2 variant compared to control variant (Table 2). Recent studies have shown that platelets are involved in homeostasis process and play a defending role in organism, it is produced, in Teleostean fish in the spleen and kidneys (Tavares-Dias and Oliveira, 2009).

The increase in the number of leukocytes at the end of the experiment, especially in the variant in which rosemary was administered, can be corroborated with the increase in antibody production, which led to a good state of comfort, so tilapia specimens are less affected by stressor or being less susceptible to disease. Tawwab et al. (2010) obtained similar results when administered green tea (*Camelia sinensis*) to *Oreochromis niloticus*.

In the variant in which sea buckthorn was administered, it was observed that at the end of the experiment the number of monocytes was lower than in the other experimental variants, this aspect shows the absence of foreign substances in the blood circulation (Bocioc, 2011).

CONCLUSIONS

Following the analysis of the haematological parameters, we can notice an improvement in the variants in which phytobiotics were administered, their positive action was observed especially in the variants in which sea buckthorn and ginger were administered.

Following the biochemical analysis of the blood we can say that the supplementation of the Nile tilapia diet with 1% *Hippophae rhamnoides*, respectively 1% *Zingiber officinale* led to the end of the experimental period, after the obtained results, to the reduction of technological stress and to the improvement of immunity. Although the administration of phytobiotics did not contribute to significant changes in cortisol and total protein throughout the experimental period, the administration of sea buckthorn led to a lower concentration of glucose compared to variants in which rosemary and ginger were administered. At the same time, only sea buckthorn and ginger showed an immunomodulatory effect during the experiment due to the intensification of lysozyme activity.

After the analysis of the relative number of leukocytes (%) we can conclude that at the end of the experiment the administration of phytobiotics contributed to obtaining significant changes ($P<0.05$) compared to the control variant at the level of neutrophil granulocytes, and compared to the initial mean value at the level of the relative number of small lymphocytes and monocytes.

In conclusion, dietary supplementation with sea buckthorn and ginger has reduced the technological stress and improved the immunity of *Oreochromis niloticus* species.

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