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EFFECT OF DIETARY SUPPLEMENTATION OF *Nile tilapia* WITH SEA BUCKTHORN AND VITAMIN E ON THE HEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES

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Abstract

The purpose of this study was to investigate the effect of dietary supplementation with sea buckthorn and vitamin E on the physiological status of Oreochromis niloticus species. The experimental design consists in a four experimental diet: V1 - control, V2 - 1% sea buckthorn/kg feed, V3 - 500 mg vitamin E/kg feed and V4 - 1% sea buckthorn+500 mg vitamin E/kg feed. The results revealed that dietary supplementation with this phytobiotic and vitamin contributed to the emergence of significant changes compared to the control variant at the level of the number of erythrocytes, erythrocyte constants (MCV, MCH, MCHC), glucose concentration and total protein. In variant V4 it was observed an increase of erythrocyte constants (MCV and MCH) and a reduction of the number of erythrocytes. Regarding to the leukocyte reaction, in V2, V3 and V4 variants was observed an improvement of the fish physiological status compared to the fish from control variant. In conclusion, the dietary supplementation with sea buckthorn and vitamin E (V4) presented a synergistic effect on the welfare status of Nile tilapia reared in a recirculating aquaculture system.

Key words*: hematological profile, Hippophae rhamnoides, leukocyte reaction, Nile tilapia, vitamin E.*

INTRODUCTION

To supply the demand for the human nutrition the aquaculture practices have become more intensively, this aspect is due to the reduction of the fish stock in the natural environment, day by day Because of the intensive growth, the risk of pathogens and disease has increased.

Other problems are represented by high production costs, diseases, stress, environmental impact, animal welfare problems and demand for organic production (Sonmez, 2017; Arslan et al., 2018; Elbesthi et al., 2020). The use of antibiotics and other chemotherapeutic agents for controlling diseases can reduce mortality and improve growth rates; however, at the same time they are quite expensive and at the same time they can cause a damage to the body (Ferguson et al., 2010).

Thus, to ensure the fish physiological status, it is sought to put more emphasis on the prevention than on the treatment of diseases.

For this reason, in order to obtain a higher production and to reduce the occurrence of diseases, nutraceuticals began to be introduced into the fish diet.

Nutraceuticals are natural bioactive or chemical compounds which have therapeutic properties on the organism welfare. So, by using nutraceuticals, an attempt is made to maintain an optimal state of health by preventing illness or reducing the symptoms/stagnation of a disease. Nutraceuticals can include phenolic substances, flavonoids, vitamins, fatty acids, carbohydrates and their derivatives, amino acids, minerals, etc. Among their mechanisms of action, we can say that they have properties as: anti-inflammatory, anti-carcinogenic, antioxidant, antimicrobial, anticholesterolemic, antihypertensive, etc. (Bem et al., 2021).

Hippophae rhamnoides L. is an ancient crop that was used as herbal medicine and as food additive for disease prevention. The increase in antioxidant activity in the case of sea buckthorn fruit administration is mainly due to the presence of large amounts of vitamin C, vitamin E, carotenoids (Ranjith et al., 2006), polyphenols (Ranjith et al., 2008), but also of some antioxidant enzymes such as the isoenzyme: superoxide dismutase (Xing et al., 2002). Withal, sea buckthorn fruits have a special effect at the physiological level, namely that of inhibiting the oxidation of low-density lipoproteins (Bao & Lou, 2006). It seems that seabuckthorn have some properties as: cholesterol reduction, role in the hemostasis process, reduction of blood pressure, glucose, anticancer, antibacterial, antiviral and radioprotective potential (Sahu et al., 2007). The introduction of sea buckthorn in the fish diet was done to observe its effect on the growth performance indicators and on the survival rate. For a good feeding efficiency, but also to obtain a quality product, it is important to obtain a fish with a fairly large body mass, in a relatively short time, in order to be able to be marketed. Antache et al., 2013, shown interesting results on lipid peroxidation, analysis which determines the freshness of fish meat, an important matter in fish quality, what corresponds to a high percentage of unsaturated fatty acids found in meat.

In this experiment, in addition to administering seabuckthorn in the Nile tilapia diet, we chose to administer a vitamin, respectively vitamin E. Fish feed is usually supplemented with a certain concentration of vitamin E because it has positive effects on the immune system in fish, improving specific and non-specific immune response, reducing mortality and optimizing growth performance (Ortuno et al., 2001; Shiau & Hsu, 2002; Puangkaew et al., 2004). Vitamin E has a strong antioxidant action that protects the organism from possible changes that can lead to oxidation process of cell membranes and lipoproteins at the level of different types of tissues (Adham et al., 2000), reduces the fragility of the erythrocyte membranes and improves leukocyte functions (Kiron et al., 2004). Other studies have also shown the beneficial effect of vitamin E, administered in higher concentrations, on reproduction, disease resistance, meat quality and nutrient digestibility (Lohakare et al., 2006; Samanta et al., 2006; Galaz et al., 2010).

In fish reproduction, vitamin E administration plays a very important role. Studies have shown that vitamin E deficiency could lead to a reduction in growth performance indicators by decreasing the protein efficiency ratio, weight gain, as well as feed coefficient (Bai et al., 1998; Lee & Dabrowski et al., 2003; Huang & Lin, 2004; Paul et al., 2004; Sau et al., 2004).

Therefore, the purpose of this research was to investigate the effect of a phytobiotic, in our case sea buckthorn, vitamin E and their combination on the hematological profile, on some blood biochemical parameters and on leukocyte reaction in case of Nile tilapia.

MATERIALS AND METHODS

The research took place, for six weeks, in a pilot aquaculture recirculating system station from "Dunarea de Jos" University of Galati, Romania. The biological material used in the experiment was represented by 684 specimens of two-month-old of *Oreochromis niloticus* species with 1.81±0.01 g/specimen. The specimens were shared in twelve growth units. The experimental design involved the testing of four experimental variants. The experiment was carried out in triplicate, therefore each experimental variant received a number of three growth units. So, the experimental variants were: V1 variant - the control variant, V2 variant - fish feed was enriched with 1% sea, V3 variant - fish feed was added with 500 mg vitamin E/kg feed and V4 variant - fish feed was added with 1% sea buckthorn and 500 mg vitamin E/kg feed.

Regarding to the feeding management, the biological material was fed during the experiment with NUTRA PRO "0" granulated feed, which has a content of 54% crude protein. It should be noted that we chose a feed that was not supplemented with vitamin E. The feeding intensity was 10% of biomass per day (BW/day) for three weeks then with 5% BW/day until the end of the experiment. During the experiment, the frequency of feeding was five times per day, the food being administered manually.

During the experiment, the water temperature was kept constant with the help of twelve heaters of the 1C HEATER Indicator type (200W; 200/240V; 50-60Hz). For the growth of Nile tilapia fry under optimal conditions during the experiment, the water quality was ensured by daily monitoring of temperature (T - °C) and oxygen (DO - mg/L) using the portable oximeter Hannah HI 98186 and the pH using the WTW inoLab series device (Terminal 740). For two times per week ammonium, nitrates and nitrites concentration were measured using Merck kits and the spectrophotometer Nova 400.

The results of the water quality parameters reflect that they remained within normal range for the *Oreochromis niloticus* species throughout the duration of the experimental research (Ross, 2000; El-Sayed, 2006; DeLong et al., 2009; Peterman, 2011). The average values for OD were 6.78±0.68 mg/L; for pH 7.49±0.22 units, for water temperature 25.99±0.99˚C, the ammonium concentration was 0.21±0.11 mg/L, the nitrites concentration was 0.61±0.38 mg/L and the nitrates concentration was 159.54±62.26 mg/L.

Blood sampling and analysis

To evaluate the physiological state of the fish, were collected blood samples only at the end of the experiment because at the beginning the fish were too small. For the blood prelevation as anticoagulant was used heparin, 2-phenoxyethanol was used for the fish anesthesia and and the collection was made from the caudal vein. Some researchers reported that this anesthetic does not interfere with the analysis of blood samples (Velisek et al., 2007).

The analysis of hematological parameters involves the determination of erythtocytes number (RBCc - x 10^6cell/µL), hemoglobine concentration (Hb - g/dL), hematocrit (Ht - %) and of some biochemical parameter such as concentration of total protein (TP - g/dL) and glucose (GLU - mg/dL). For the red blood cell counting was used Vulpian reactive (solution to achieve the dilution) and the Neubauer hemocytometer. For the hematocrit determination we used heparinized hematocrit capillary tube and a micro hematocrit centrifuge (Haematokrit 24 - Hettich). The centrifugation time was for 5 minutes at 12000 rpm. For the determination of hemoglobin concentration were used Drabkin reagent and SPECORD 210 Analytikjena spectrophotometer. These analyzes were carried out according to Blaxhall (1973). Based on these indices, the erythrocyte constants (mean corpuscular volume - μm^3, mean corpuscular hemoglobin - pg and mean corpuscular hemoglobin concentration - g/dL) were calculated (Ghergariu et al., 1985; Svobodova, 2001).

The concentration of glucose and total protein serum were made with a colorimetric methods and the readings were done at 635 nm (GLU), respectively at 546 nm (TP). For the GLU determination was used the o-toluidine reagent and the Biuret method was used for the TP concentration.

Leukocyte reaction

Relative and absolute number of the white blood cells determination were made by means of the blood smears which were microscopically examined using the Zeizz Axio Imager microscop. The smears staining was done by the MayGrünwald Giemsa panoptic method (MGG) (Mogodan et al., 2020). The identification of the type of leukocytes it was made according to Svobodova et al. (1991) description.

Statistical analysis

The results were statistically analyzed using descriptive statistics and ANOVA test. The results are showing as as mean ± standard deviation. Programs used were SPSS Statistics 17.0 and Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

Assessment of physiological state through blood analysis shows us information about fist metabolism, we mean to the oxygen transport capacity and oxygen consumption, disease potential, immune status, the degree of stress, nutritional status etc. (Witeska et al., 2022) From this reason determination of

hematological parameters represent a useful tool for fish welfare assessment (Grant, 2015; Fazio, 2019).

Following the analysis of hematological parameters, the following aspects were noted:

Regarding to the erythrocytes number was registered a significant increase in variant V2 ($p<0.05$) in which feed was supplemented with sea buckthorn (Table 1). Thus, compared to the count obtained in the V2 variant, it was observed a reduction with 6.30% in the V1 variant; 21.59% in the V3 variant and with 30.31% in the V4 variant. Nevertheless, obtained results falls within the range of 0.950-2.835 RBCc $x10^6$ cell/µL (Table 1). Although significant differences recorded between the variants, proving that the diet administered to cultured biomass significantly influenced the number of erythrocytes. However, the obtained values fell within the optimal range described for the *Oreochromis niloticus* species, between 0.7 and 2.8 RBCc x 10^6 cell/µL blood (Bittencourt et al., 2003).

Table 1 The results for the hematological parameters obtained in the experimental variants

Experimental variant	Hematological parameter (Average±SD)					
	RBCc ($x10^6$/µL)	Ht (%)	Hb (g/dL)	MCV (µm^3)	MCH (pg)	MCHC (g/dL)
V1	2.096±0.29	32.50±2.39	10.26±1.05	158.27±24.76	49.68±7.21	31.65±3.09
V2	2.237±0.21	30.41±3.78	10.86±0.68	137.20±20.76	49.01±5.29	36.27±5.29
V3	1.754±0.35	32.39±2.27	10.07±0.46	191.84±38.06	59.67±11.27	31.25±2.50
V4	1.559±0.28	32.06±2.76	10.80±0.87	212.70±44.73	71.65±14.60	33.77±1.82

Note: V1 - control variant; V2 - 1% Hippophae rhamnoides; V3 - 500mg vitamin E/ kg, V4 - 1% Hippophae rhamnoides + 500 mg vitamin E per kg feed; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration.

The hematocrit recorded values between 21.00% and 36.00%. However they did not registered significant changes ($p>0.05$) between the experimental variants. Comparing the results with the values obtained in control variant (V1), in variant V2 was recorded a reduction with 6.43% in hematocrit, in the variant V4 with 1.35% and in the variant V3 with 0.34%. It must be specified that the obtained results regarding to the hematocrit are within optimal limits for tilapia, 15%-45% (Bittencourt et al., 2003) (Table 1).

Regarding to the hemoglobin concentration, a significant reduction was recorded ($p<0.05$) in variant in which vitamin E was included in fish feed (V3). Thus, compared to the V3 variant, was observed an increase in hemoglobine concentration with 1.89% in V1 variant (control), 7.25% in the V4 variant, respectively with 7.85% in the V2 variant. The values recorded in our research are within the reference range for the species *Oreochromis niloticus*, for hemoglobin concentration is between 6.58 g/ dL and 15.98 g/dL (Bittencourt et al., 2003) a fact that demonstrates that the results obtained (in range between 7.45 and 12.32 g/dL) are normal for tilapia, although significant differences were recorded between the experimental variants (Table 1).

Concerning to the mean erythrocyte volume significant differences were recorded between variants ($p<0.05$), minimum average value was obtained in the V2 variant (1% sea buckthorn), and the maximum value in the variant in which sea buckthorn and vitamin E were administered (V4) (Table 1). If we refer to the average value obtained in the control variant (V1), there was observed a reduction in the average erythrocyte volume with 13.31% in the V2 variant and an increase in it with 21.21% in the V3 variant, respectively with 34.39% in the V4 variant. The MCV concentration obtained in our experiment were between 87.50 µm^3 and 357.90 µm^3, these being within the reference range for Nile tilapia describde by Bittencourt et al. (2003) (12.36 µm^3 and 528.57 µm^3).

Also, significant differences ($p<0.05$) between variants were obtained in case of mean erythrocytar hemoglobin (MCH). Similar to the MCV concentration, the lowest concentration was recorded in the variant in which the feed was added with sea buckthorn (V2) and the maximum value in the V4 variant (Table 1). By comparison with V1 variant, the MCH

concentration was reduced with 1.34% in the V2 variant and increased with 20.11% in the V3 variant, respectively with 44.22% in the V4 variant. The obtained results (between 49.01 pg and 71.65 pg, Table 1), fell within the range for the Nile tilapia species, described by Hamid et al. (2013), between 5 and 80.4 pg.

In case of MCHC concentration, a significant increase was observed in V2 variant ($p<0.05$) (Table 1), compared to the other experimental variants. Thus, the MCHC concentration increased with 7.70% compared to the V4 variant, with 14.91% compared to the V1 variant, respectively with 16.38% compared to the V3 variant. The mean erythrocyte hemoglobin concentration values fell within optimal range described for the *Oreochromis niloticus* species by Bittencourt et al., (2003), respectively between 19.84-87.73 g/dL, due to the fact that the minimum value was 23.29 g/dL, and the maximum value recorded was 52.97 g/dL.

Based on the results obtained in the V2 variant, we can state the fact that the reason why the highest value of the number of erythrocytes and hemoglobin concentration, respectively the lowest value of hematocrit which was recorded, is represented by the high vitamin C content of sea buckthorn fruits (Ranjith et al., 2006). Same results were recorded at the *Arapaima gigas* species fed with a feed supplemented with vitamin C (Menezes et al., 2006).

Following the analysis of hematological parameters and erythrocyte constants, it was noted that the dietary supplementation with *Hippophae rhamnoides*, vitamin E and with *Hippophae rhamnoides* in combination with vitamin E contributed to the emergence of significant changes as against to the control (V1) at the level of the red blood cells count, hemoglobin concentration, MCV concentration, MCH and MCHC. Dietary supplementation with vitamin E in combination with sea buckthorn contributed to the increase of erythrocyte constants (MCV and MCH) and to the reduction in red blood cells count.

For the determination of the relative and absolute number of leukocytes, lymphocytes (small and large), monocytes, granulocytes and the absolute number of platelets, the microscopic analysis of a number of 72 blood smears was used.

The leukogram of the fish taken in the study indicated only small lymphocytes (Lm), large lymphocytes (LM), monocytes (M) and neutrophil granulocytes (N). The leukograms obtained for each experimental variant, at the end of the experiment, are presented in figure 1. During the experiment a series of changes appeared at the level of the leukogram depending on the supplementation of Nile tilapia with *Hippophae rhamnoides*, vitamin E and combination between this phytobiotic and vitamin compared to the control variant.

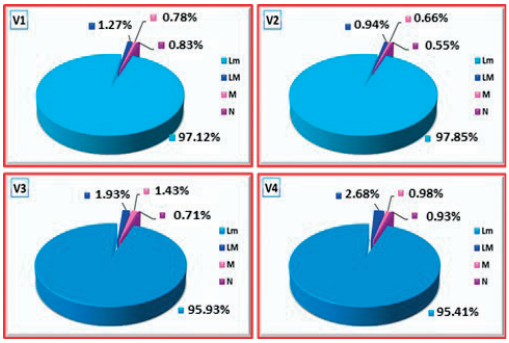

Figure 1. Relative number of leukocytes of Nile tilapia from each variant

Note: Lm - small lymphocytes, LM - large lymphocytes, M - Monocytes, N - neutrophil granulocytes.

In case of small lymphocytes (Lm), a significant decrease ($p<0.05$) of them was registered in the V4 variant (95.41±0.79%) compared to the other experimental variants. In comparison with V1, there was registered a reduction with 1.23% in V3 variant and with 1.76% in V4 variant and an increase in small lymphocytes (%) with 0.75% in V2 variant. The highest value was recorded in the V2 variant.

Unlike little lymphocytes (Lm - %), the highest value of the large lymphocytes (LM - %) registered a significant increase ($p<0.05$) in the V4 variant in which sea buckthorn with vitamin E was administered (2.68±0.37% - Figure 1). Thus, in case of large lymphocytes, we can state that in the V4 variant was registered an increase with 111.02%, and with 51.97% in the V3 variant in comparison with V1 variant. In the V2 variant was noted a reduction with 25.98% in comparison with V1 variant (Figure 1).

Regarding to the monocytes (%), a significant increase ($p<0.05$) was found in the variant in which the diet was supplemented with vitamin E (V3 variant). If we refer to the results registered

in V1 variant we can say that there was a reduction of monocytes (%) with 15.38% in V2 variant and an increase of them with 25.64% in the V4 variant and with 83.33% in V3 variant.
But, in case of relative number of neutrophil granulocytes (%) insignificant differences ($p>0.05$) were recorded between the variants. The highest value was registered in V4 variant (0.93±0.36%) and the lowest in V2 variant (0.55±0.15%) (Figure 1).
In order to present in detail the changes in the leukocyte reaction, the absolute number of leukocyte and platelets were determined. In this sense, the results can be seen in the Table 2.

Table 2. Absolute leukocytes number obtained in the experimental variants

Experimental variant	Absolute leukocytes number (x10^3 cell./µL)					
	Leukocytes	Lymphocytes		Monocytes	Neutrophil granulocytes	Platelets
		small	large			
V1	418.96±68.81	406.38±64.19	5.52±2.46	3.40±2.09	3.66±2.52	59.72±28.71
V2	115.14±32.00	112.76±31.60	1.05±0.49	0.71±0.25	0.62±0.18	6.93±3.14
V3	81.45±16.58	78.36±16.46	1.58±0.43	1.16±0.55	0.54±0.14	11.76±4.77
V4	60.81±6.11	58.03±5.94	1.62±0.24	0.60±0.15	0.56±0.20	5.63±1.55

Note: V1 - control variant; V2 - 1% Hippophae rhamnoides; V3 - 500mg vitamin E/ kg, V4 - 1% Hippophae rhamnoides + 500 mg vitamin E per kg feed.

The absolute number of leukocytes it fell within the range of 45.56-526.65 x10^3 leukocytes/µL. The values obtained in the V1 variant are significantly higher ($p<0.05$) by comparison with the results registered in the V2, V3 and V4 experimental variants (Table 2). Thus, the values recorded in the control variant (V1) are higher than the optimal interval described for the Nile tilapia by Hrubec et al. (2000), respectively between 21.56 and 154.69 x10^3 leukocytes/µL.
In case of small lymphocytes a significant increase ($p<0.05$) was observed in V1 variant by comparison with the other experimental variants (Table 2). So, a reduction was observed with 72.25% in V2 variant, 80.72% in V3 variant and with 85.72% in V4 variant (Table 2). It must be specified that the results obtained in control variant - V1 are not in the interval for the *Oreochromis niloticus* species, 6.78-136.39x10^3 small lymphocytes/µL (Hrubec et al., 2000), these being much larger.
The results of absolute number of large lymphocytes registered significant differences ($p<0.05$) between V1, V2, V3 and V4 variants. The highest value was registered in V1 variant and the lowest value in V2 variant. Absolute number of large lymphocytes was recorded in the range of 0.45-10.53 x 10^3 large lymphocytes/µL. But this time the values obtained in the control variant (V1) fall within the reference range: 2.85-30.83 x 10^3 large lymphocytes/µL (Hrubec et al., 2000). However, compared to the average value recorded in the control variant, a reduction in the absolute number of large lymphocytes was recorded with 80.98% in the V2 variant, with 71.38% in V3 variant and with 70.65% in V4 variant (Table 2).
Regarding to the absolute number of monocytes, in the V4 variant was recorded the lowest value, this being statistically significant ($p<0.05$). Thus, by comparison with the control variant, the absolute number of monocytes was decreased with 79.12% in V2 variant, with 65.88% in V3 variant and with 82.35% in the V4 variant (Table 2). However, the obtained results fall within the range described by the Hrubec et al., (2000), (0.40-4.29 x 10^3 monocytes/µL).
In case of neutrophil granulocytes the absolute number recorded an increase V1 variant ($p<0.05$) compared to the other experimental variants. However, the obtained results are found in the reference interval for this species, between 0.56-9.87 x 10^3 neutrophils/µL (Hrubec et al., 2000). It should be specified that the mean value recorded in the V3 variant is below the lower limit of the reference range. By comparison with V1 variant the reduction was with 83.06% in the V2 variant, with 85.25% in the V3 variant and with 84.70% in the V4 variant (Table 2).
Regarding to the absolute platelet count the lowest number was obtained in V4 variant and the highest number in the V1 variant (Table 2). Between the variants were registered significant differences ($p<0.05$). If the results obtained in V1 variant falls within the range for the platelet

count (25.06 x10^3 cells/µL and 85.24 x10^3 cells/µL) described by Hrubec et al., 2000, the values recorded in V2, V3 and V4 variants are in the range described by Tavares-Dias and Oliveira (2009) (2.00-78.90 x10^3 cell/µL).

The research showed that under the influence of stress, due to the increase in corticosteroid hormones (cortisol, catecholamines), platelets count registered an increase and the coagulation time recorded a reduction (Docan, 2010).

The significant increase in the leukocytes, respectively lymphocytes, from the circulating blood in V1 variant (control) signifies the appearance of a strong immunomodulatory effect which may be due, among other things, to the presence of a stress factor (Martins et al., 2002). However, research has shown that the absolute number of leukocytes is much higher in juveniles than in adults (Hrubec et al., 2000).

In case of monocytes, absolute number is normal because it was obtained in the reference range described by the literature, but also because some researchers reported that monocytes represent a percentage of 10% of the leukocytes count (Table 2).

Evaluation of the leukocyte count by the absolute number of cellular elements highlighted the fact that the addition of feed from the V2, V3 and V4 variants led to the improvement of the fish compared to the variant in which the feed was not supplemented (V1).

In fish, the stress response is characterized in particular by the stimulation of the hypothalamus, which contributes to the activation of the neuroendocrine system and subsequently to the appearance of some metabolic and physiological changes (Lowe & Davison, 2005).

In terms of blood biochemistry parameters the concentration of glucose and total proteins were analyzed, in this research.

Comparing the results obtained in the case of glucose concentration a significant reduction ($p<0.05$) of it were registered in V2, V3 and V4 variants compared to V1 variant (control) (Figure 2). Thus, there was a reduction with 14.16% in V4 variant, with 18.99% in V3 variant and 22.82% in V2 variant compared to V1 variant. The obtained results fell within the interval of 59.81-142.38 mg/dL, but Bittencourt et al. (2003) reported for *Oreochromis niloticus* a reference range between 22.7-107.0 mg/dL. The values recorded in the control variant (V1) do not fall within the reference range, being more higher.

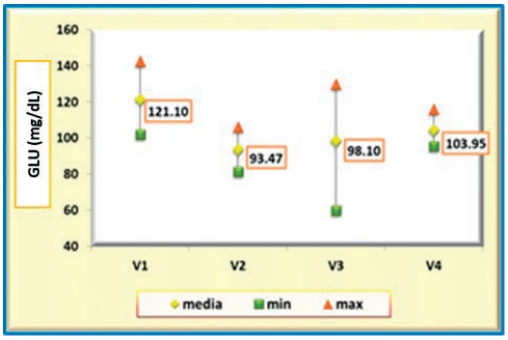

Figure 2. Glucose concentration in case of Nile tilapia from each experimental variant

Sharif (2012) reported that the administration of vitamin C in Nile tilapia feed contributed to a significant reduction in glucose concentration compared to variants in which the feed was not supplemented with vitamin C. It is proven once again by this research the therapeutic value of administration sea buckthorn in fish, precisely because of the high content of vitamin C. In the V2 variant (sea buckthorn) the lowest glucose value was recorded.

In case of total protein concentration the highest value was registered in V3 variant ($p<0.05$) (Figure 3) and by comparison with the other experimental variants were registered a significant differences.

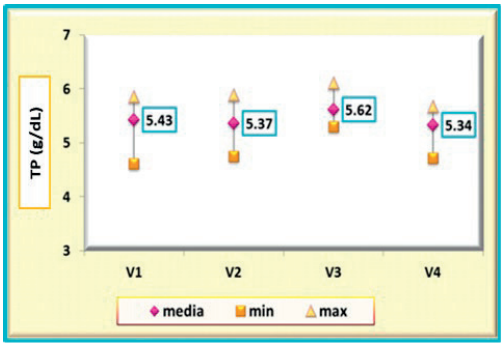

Figure 3. Total protein concentration for the Nile tilapia from each experimental variant.

By comparison with the control variant (V1) there was a decrease of total proteins with 1.11% in the V2 variant and 1.66% in the V4 variant, respectively an increase with 3.50% in V3

variant. The values of total protein concentration fell within the range of 4.62 - 6.11 g/dL and the optimal interval described by Hrubec et al. (2000) was between 2.9 and 6.6 g/dL for Nile tilapia.
Studies have shown that the administration of vitamin C in the Nile tilapia diet led to lower total serum protein concentrations compared to the variants in which vitamin C was not administered (Sharif, 2012), same values were obtained in V2 variants (1% *Hippophae rhamnoides*/kg feed), respectively in V4 variant in which the feed was supplemented with 1% *Hippophae rhamnoides* and 500 mg vitamin E/kg feed.

CONCLUSIONS

In conclusions assessment of hematological parameters and erythrocyte constants highlighted the fact that supplementing the diet with the phytobiotic *Hippophae rhamnoides* , vitamin E and with the phytobiotic *Hippophae rhamnoides* in combination with vitamin E contributed to the appearance of changes in a significant mode ($p<0.05$) in comparison with the control (V1) in case of the number of erythrocytes, hemoglobin concentration and erythrocytar constants (MCV, MCH and MCHC). Addition of food with vitamin E and sea buckthorn (V4) contributed to the increase of erythrocyte constants (VEM and HEM) and reduction of the number of erythrocytes. The biochemical analysis of the blood indicated an increase the glucose concentration ($p<0.05$) in the V1 variant (control) in which the feed was not added a phytobiotic or vitamin. The values obtained are much higher than those presented in the literature. Among variants V2, V3 and V4, the lowest value of glucose concentration was recorded in V2 variant (1% *Hippophae rhamnoides*) (93.47±7.48 mg/dL).
Regarding to the leukocyte count analysis by the absolute number of cellular elements noted that administration of 1% *Hippophae rhamnoides*, 500 mg vitamin E and 1% *Hippophae rhamnoides* in combination with 500 mg vitamin E in *Oreochromis niloticus* diet led to the improvement of the Nile tilapia welfare compared to the variant in which the feed was not added.

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