AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

The effect of thyme (*Thymus vulgaris*) and vitamin E on the *Acipenser stellatus* juvenile welfare, reared in a recirculating aquaculture system

Oana G. (Varlan) Dorojan, Victor Cristea, Mirela Creţu, Lorena Dediu, Angelica I. Docan, Marian T. Coadă

Department of Aquaculture, Environmental Sciences and Cadastre, Faculty of Food Science and Engineering, "Dunarea de Jos" University of Galaţi, Galaţi, Romania.
Corresponding author: O. G. (Varlan) Dorojan, varlanoana79@yahoo.com

Abstract. The study was conducted to evaluate the welfare status of juvenile *Acipenser stellatus*, in condition of administration of Vitamin E and thyme (*Thymus vulgaris*) in their food. The research was conducted between December 16th, 2013 and January 21st,2014, in the recirculating system of the Aquaculture, Environmental Science and Cadastre Department, University of Galati, Romania. We used four experimental groups, represented by *A. stellatus* juveniles (main initial weight 103.89±22.57 g) aged 7 months, provided from different genitors: V1 (♀2 Danube x ♂1 Aquaculture), V2 (♀1 Danube x ♂1 Danube), V3 (♀1 Danube x ♂2 Aquaculture) and V4 (♀2 Danube x ♂2 Danube. At the end of the experiment, red blood cell counts (RBC), packed cell volume (Hct), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured and analysed, with routine methods used in fish hematology. Regarding the serum's biochemical parameters the determinations were made with the help of VetTest®Chemistry Analyser, using IDEXX VetTest kits, the result interpretation being made in comparison with the reference values from the literature. No significant changes were registered ($p > 0.05$) in number of erythrocytes, hemoglobin concentration, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin variation) and MCHC (mean corpuscular hemoglobin concentration), while the packed cell volume registered significant differences ($p < 0.05$) between the four experimental variants. Also, statistical analysis showed that there are no significant differences ($p < 0.05$) between the experimental variants regarding the serum biochemical parameters including Calcium, Magnesium, Albumin, Globulins, Total protein, Glucose, Cholesterol, Triglyceride and Blood urea nitrogen. Significant differences ($p < 0.05$) were noted within the ammonia level but the obtained values are in the reference values for sturgeon.
Key Words: hematological profile, serum biochemical parameters, *Acipenser stellatus*, Recirculating Aquaculture System (RAS).

Introduction. The most reasonable and logical mitigation strategy to reduce the fishing pressure on sturgeon natural stocks would be a massive development of sturgeon aquaculture production that would help not only to compensate for the decline of fishery production, but also to satisfy the market demand to the extent that price come drastically down, thereby making illegal trade less attractive (Bronzi et al 2011).

The species *Acipenser stellatus* (Pallas, 1771) is a good candidate for Romania's aquaculture, given the results of researches conducted until now (Patriche 2001; Ceapă 2008; Dicu et al 2012). In Romania, according to CITES, stellate sturgeon catches have declined during the period 2002-2006 from 12.427 tons to 3.43 tons (72.5% in four years), aspect that makes the sturgeons aquaculture to grow with restocking programs of Danube.

The nutritional requirements for sturgeons, reared in intensive recirculating systems were not sufficiently studied (Dicu et al 2013). Recent researches had shown that the introduction of relatively small concentrations of vitamins, probiotics, prebiotics and newest phytobiotics in animal diet leads to ensuring some specific demands or

influencing in a positive way (directly or indirectly) the growth performances and the welfare status (Pop et al 2006; Denev 2008; Maurilio 2011; Kasiri et al 2011; Antache et al 2013).

Phytobiotics are natural compounds which, being incorporated into diets, lead to a optimization of animal productivity (Cristea et al 2012). Increasing number of recent studies present the positive aspects of phytobiotics administration, in diets, of different fish species, such as: the immunostimulator (Khalil et al 2009), bioproductive (El-Dakar et al 2008), antioxidant and antimicrobial effect (Abdelhamid 2010), their ability to stimulate the enzymatic equipment and to increase the nitrogen absorbtion (Al-Absawy 2010). The thyme (*Thymus vulgaris*) contains a number of important compounds, such as the phenols: thymol (44–60%) and carvacrol (2.2–4.2%) (Alcicek 2011). The therapeutic properties of thyme on fish are: antiseptic, antioxidant, digestion stimulant etc. (Coutteau et al 2011).

Vitamin E is among the most important nutrients influencing the fish immune system, and the supply of vitamin E in fish feed can reduce mortality and improve fish performance, while increasing specific and nonspecific immune responses (Puangkaew et al 2004; Raja et al 2008).

The analysis of blood parameters is one of the most valuable modern diagnostic tools because it has been shown that the physiological values of these parameters are species-specific and age-dependent (Anver 2004). The objective of this study was to evaluate the hematologic profile and serum biochemical parameters of different genetic combination of stellate sturgeon after administration in feed of vitamin E and thyme.

Material and Method

Experimental design. The experiment was carried out between 16th December 2013 and 21st January 2014 at the pilot recirculating system located in a laboratory of "Aquaculture, Environmental Science and Cadastre" Department, University "Dunărea de Jos " of Galati, Romania.

The system is provided with 4 rearing units with a total volume of 500 L each. The pilot recirculating grow system used for these experiments has been described, constructively speaking, also in other studies, reason for which this paper no longer contains its description (Enache et al 2011). We use four experimental groups, represented by stellate sturgeon juveniles (aged 7 months) with a mean body mass of 103.89±22.57 g, from the different genitors: V1 (♀2 Danube x ♂1 Aquaculture), V2 (♀1 Danube x ♂1 Danube), V3 (♀1 Danube x ♂2 Aquaculture) and V4 (♀2 Danube x ♂2 Danube. Fish were fed with TROCO PRE GROWER pellets, with the diameter of 2.0 mm (Table 1). The fodder was supplemented with 1% thyme kg^{-1} feed and vitamin E 500 mg kg^{-1} feed. Fish were fed three times per day (09:00, 13:00 and 17:00) with a daily ration of 2.6% of fish body weight (BW). The survival was surveyed daily.

Table 1

Nutritional composition of the TROCO PRE GROWER pellets

Parameters	*Quantity*
Protein	45%
Fat	18%
Crude fibre	1.2%
Ash	8.2%
Phosphorus	1.2%
Calcium	1.8%
Sodium	0.4%
Vitamin A	10.000 I.E
Vitamin D3	746 I.E
Vitamin E	200 mg kg^{-1}
Vitamin C (stable)	150 mg kg^{-1}
Gross energy (/kg)	21.5 MJ; 5.1 Mcal
Digestible Energy (/kg)	19.7 MJ; 4.7 Mcal
Metabolisable Energy (/kg)	17.6 MJ; 4.2 Mcal

Water quality. During the experiment, the attention was directed towards the control of the water quality parameters in order to maintain them within the rearing optimum intervals for the stellate sturgeon growth: water temperature (20.36±0.50°C), dissolved oxygen (7.98±0.20 mg L^{-1}), pH (7.74±0.06 pH units), nitrate (23.38±0.21 mg L^{-1}), nitrite (0.13±0.01 mg L^{-1}) and ammonium (0.31±0.13 mg L^{-1}).

Blood sampling and analysis. At the end of the experiment, about 1 mL of blood was taken by caudal venous puncture, from 7 fish/on each experimental unit. After sampling, a part of the blood was placed in heparinized Eppendorf tubes in advance and the other part in unheparinized tubes. Using the routine methodology of fish hematology (Blaxhall & Daisley 1973; Svobodova 2001), hematological indices were measured and analyzed. The red blood cell counts (RBCx106/µL) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian diluting solution. The hematocrit (PCV, %) was determined by duplicate using heparinised capillary tubes centrifuged for 5 minutes at 12,000 rotations $minute^{-1}$ at Hettich Haematockit 210 Centrifuge. The hemoglobin concentration in blood (Hb, g dL^{-1}) was quantitatively determined by colorimetric method with Drabkin reagent and then read at spectrophotometer Spectrocord 210 Analytikjena, at a 540 nm wavelength. Using standard formulas according to Ghergariu et al 1985, the red blood indices were calculated: the mean corpuscular volume (MCV, μm^3), the mean corpuscular hemoglobin (MCH, pg) and the mean corpuscular hemoglobin concentration (MCHC, g dL^{-1}).

Regarding the serum's biochemical parameters the determinations were made with the help of VetTest® Chemistry Analyser, using IDEXX VetTest kits for: albumin (ALB), Calcium (Ca^{2+}), Total protein (TP), Glucose (Glc), Cholesterol (CHO), Triglyceride (Trig), Magnesium (Mg^{2+}), Blood urea nitrogen (BUN), Ammonia (NH_3) and Globulins (GLOB).

Statistical analysis. The hematological parameters of the four experimental groups were expressed by mean and standard deviation (M±SD). Differences in hematological parameters between the experimental variants were processed statistically with Microsoft Office 2010 using ANOVA test.

Results and Discussion

Blood hematological parameters. The effects on hematological parameters of vitamin E and thyme were showed in Figure 1. The result showed that there was no significant difference in the number of erythrocytes ($p = 0.15$) and hemoglobin concentration ($p = 0.06$) in the four experimental variants, values being situated in the normal intervals for sturgeons (Knowles et al 2006; Dicu et al 2013). However, higher values of the number of red cells were found in the case of V2 variant (♀1 Danube x ♂1 Danube). Also, the concentration of hemoglobin was higher in case of V2 and V4 were fish were obtained from Danube genitors.

Regarding the hematocrit values, significant changes were registered between the four experimental variants ($p=0.007$), the obtained values being below the reference values reported by other authors for sturgeons (26-46%) (Knowles et al 2006; Docan et al 2011; Dicu et al 2013). Generally, fish hematocrit varies depending on physiology, health and activity of the fish and large fluctuations indicate, according to some authors (Farrell 2011) stressful conditions or even chronic stress appearance. In our study, the lower quantity of hematocrit can be the sign of anemia or dehydratation of the stellate sturgeon organism.

Lower hematocrit values were reported by other authors (Poston et al 1976), when brook trout (*Salvelinus fontinalis*) fry was administrated a high dose of vitamin E (5000 mg kg^{-1}). Also, Baker & Davies (1996), reported in the case of African catfish (*Clarias gariepinus*) fed with a high α-tocopheryl acetate dose (500 mg kg^{-1} dry feed) a lower hematocrit than fish fed with the basal diet.

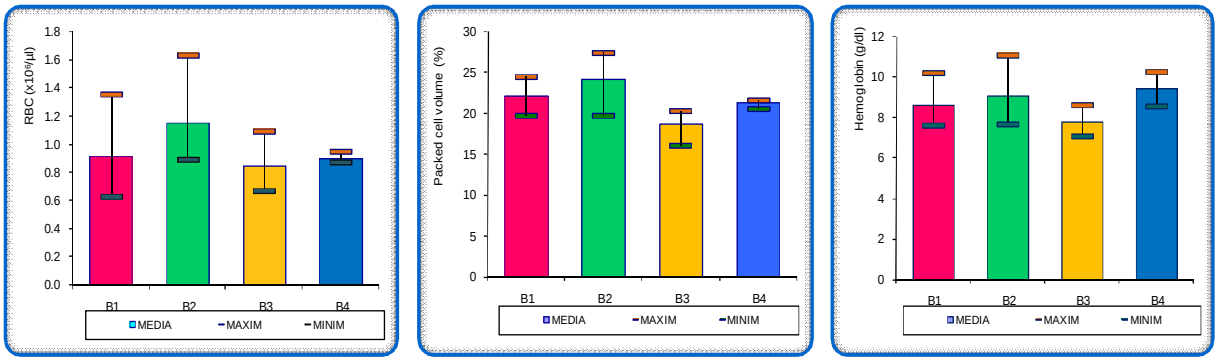

Figure 1. Mean, minimum and maximum values of RBCc erythrocytes, hematocrit and hemoglobin of *A. stellatus.*

Based on the obtained values of the hematological indices we calculate the erythrocytes constants (MCV, MCH, and MCHC) of stellate sturgeon (Figure 2). Their diagnostic value is very important because they help to detect the presence of some physiological lesions in the process of hemoglobin formation and offers information on the size, shape and hemoglobin quantity in erythrocytes (Ghergariu et al 1985).

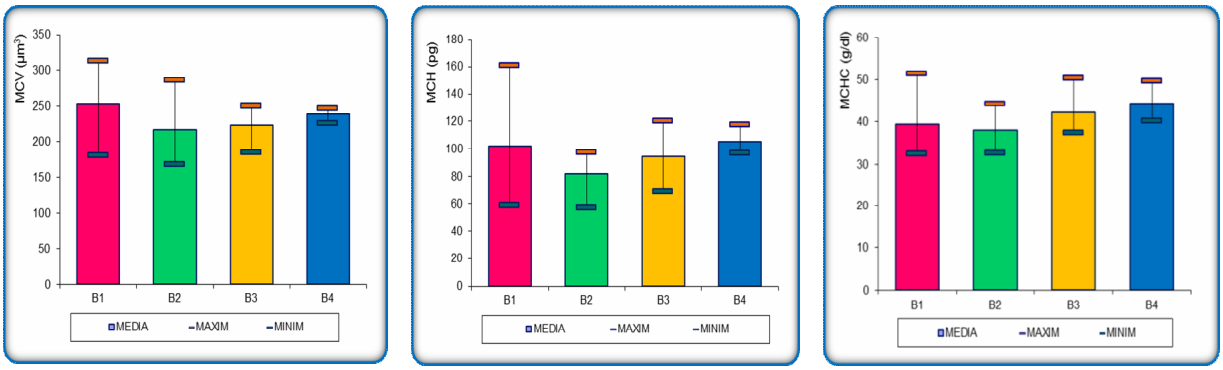

Figure 2. Mean, minimum and maximum values of MCV, MCH and MCHC of *A. stellatus.*

By analysing the data values regarding red blood constants (MCV, MCH and MCHC), we can notice that no significant differences ($p > 0.05$) were recorded between the four experimental variants, values being similar with those reported by others authors (Knowles et al 2006; Docan et al 2011; Dicu et al 2013).

The serum's levels of measured parameters of stellate sturgeon are presented in Table 2. Blood biochemistry is the most economical and authentic tool to assess the health status of the fish. Generally, the blood profile of any fish can change with the fish species, age, cycle of sexual maturity and health conditions (Shahsavani et al 2010a; Shahsavani et al 2010b; Yousefisn et al 2011). Also, blood chemistry parameters among fish species may be affected by sampling technique, analyses methods, water quality and diet (Sakamoto et al 2001).

The present study revealed no significant differences ($p > 0.05$) in the serum levels of Ca^{2+}, Alb, Glob, TP, Glu, Chol, Trig, Mg^{2+} and BUN, while the level of NH_3 registered significant differences ($p > 0.05$) between the four experimental variants.

Electrolytes (Ca^{2+} and Mg^{2+}) have multiple physiological roles in the body. The extra- and intracellular distribution of these ions is essential for the control of osmotic equilibrium and the cellular hydro-ionic, as well as for maintaining membrane permeability (Patriche et al 2011c). In our study Ca levels varied between 7.16-7.56 mg dL^{-1}, without significant differences between the four experimental variants ($p = 0.21$), values being similar with those reported in the literature (Table 3) (Patriche et al 2010; Patriche et al 2011b).

Table 2

Biochemical measurements of stellate sturgeon's blood serum after feeding with vitamin E and thyme

Parameter	Experimental variants			
	V1	V2	V3	V4
Ca^{2+} (mg dL^{-1})	7.167±0.231	7.433±0.231	7.567±0.252	7.400±0.100
Mg^{2+} (mg dL^{-1})	1.423±0.143	1.483±0.078	1.543±0.142	1.510±0.044
ALB (g dL^{-1})	0.067±0.058	0.033±0.058	0.167±0.058	0.067±0.115
GLOB (g dL^{-1})	1.900±0.200	1.867±0.058	2.167±0.115	2.000±0.173
TP (g dL^{-1})	1.967±0.252	1.900±0.100	2.333±0.153	2.067±0.252
GLU (mg dL^{-1})	44.00±2.00	52.67±6.65	57.67±11.01	45.00±1.73
CHOL (mg dL^{-1})	99.667±2.082	102.667±2.887	115.667±23.438	104.333±9.504
TRIG (mg dL^{-1})	375.000±0.000	363.667±19.630	375.000±0.000	375.000±0.000
BUN (mg dL^{-1})	2.333±0.577	2.333±0.577	2.333±1.155	3.333±0.577
NH_3 (umol L^{-1})	227.667±63.509	187.000±43.715	115.667±9.452	142.667±7.638

Table 3

Reference interval of the serum parameters in some sturgeons

Parameter	Measure unit	*Acipenser guldenstaedti 1 year (Patriche et al 2010)*	*Huso huso 1 year (Patriche et al 2011b)*	*Acipenser stellatus, 1 year (Patriche et al 2011a)*	*Acipenser brevirostrum (Knowles et al 2006)*
TP	mg dL^{-1}	2.6±0.5	2.15±0.47	2-3.5	2.7-5.3
BUN	mg dL^{-1}	14.4±1	4.5±0.7	-	-
Ca^{2+}	mg dL^{-1}	8.1±0.5	7.65±0.5	-	6.6-12.1
Mg^{2+}	mg dL^{-1}	1.6±0.2	1.8±0.2	-	1.6-2.3
Chol	mg dL^{-1}	96±20	62±13.8	-	42-133
Trig	mg dL^{-1}	362±30	244±30	-	-
Alb	g dL^{-1}	-	-	29.7	0.8-1.7

Regarding the Mg level from plasma this recorded slightly lower values (1.42+1.54 mg dL^{-1}) than the normal limits reported by literature (Patriche et al 2010; Patriche et al 2011b).

Albumin and globulin are two important parts of total protein (TP), and changes in these parameters affect the level of TP. Albumin and globulin concentration are commonly used for evaluating the effect of nutrients on fish immunity. Albumin in fish blood performs the transportation of lipids and helps in the general metabolism of fish (Andreeva 1999). The values obtained for albumin were lower than those reported by Patriche (2008) for stellate sturgeon, ranging between 0.03-0.16 g dL^{-1}, without significant differences ($p = 0.24$) between the experimental variants.

Globulins in blood samples recorded values between 1.86 and 2.16 g dL^{-1}, values beeing slightly lower than the admitted limits for sturgeon (3.63-4.5 g dL^{-1}) (Mitranescu et al 2010).

Total serum protein represented the most important indicator of the nutritional state of the organism and of the fish health condition, being influenced by fish species, age, sex, water temperature, quantity and quality of feed (Patriche 2008). Serum protein values for stellate sturgeon, obtained in current study are slightly lower than others from the literature (Patriche et al 2011b). So, serum proteins registered values between 1.90 and 2.33 g dL^{-1}, the differences between the variants being statistically insignificant ($p = 0.11$). Some authors reported that the concentrations of total protein, albumin and globulin in plasma represent indicators of liver function and therefore the decrease of serum protein could be attributed to renal excretion or impaired protein synthesis, or due to liver hypofunction or disorder (Bernet et al 2001; Kori-Siakpere 2006). The lower level of TP in case of V2 and V4 variants can be explained by the fact that stellate sturgeon

provided from Danube genitors is less adapted to supplementary feeding affecting their liver conditions.

For fish, blood glucose presents large variations depending on a number of factors such as diet, size, age and nutritional and reproductive status, water temperature and others. After Patriche (2008) blood glucose registered values between 40-90 mg dL^{-1}. In our study, glucose recorded similar values with those reported by (Patriche 2008) (44.00±12 mg dL^{-1} in V1, respectively 57.67±11.01 mg dL^{-1} in V3), without significant differences between the experimental variants (p = 0.09).

Triglycerides are used to evaluate nutritional status, lipid metabolism, and its high concentrations may occur with nephritic syndrome or glycogen storage disease (Yang & Chen 2003). The most important function of triglyceride is to store and provide cellular energy. In our study, triglycerides recorded values between 363.66 and 375 mg dL^{-1}, being similar to others from the literature (Patriche et al 2010; Patriche et al 2011b), reported for sturgeons.

Cholesterol is an important structural component of cell membranes and the outer layer of plasma lipoproteins. In our study, cholesterol recorded average values of 99.66±2.08 mg dL^{-1} in V1, respectively 115.66±23.43 mg dL^{-1} in V3, with no significant differences between the experimental variants (p = 0.48), according to the interval reference for sturgeon (Patriche et al 2010; Patriche et al 2011b; Knowles et al 2006) .

Blood urea nitrogen (BUN) resulted from the proteins catabolism. The blood urea nitrogen concentration is an important indicator for providing information functional from liver and kidneys (Patriche 2011b). In our study BUN registered values between 2.33 and 3.33 mg dL^{-1}.

Regarding the ammonia level from blood plasma, significant differences were registered between the experimental variants (p = 0.03). Ammonia values varied between 115.66±9.45 in V4 and 227.66±63.50 umol L^{-1} in V1, being within the reference values for sturgeons (Mitranescu et al 2010).

Conclusions. The current findings can provide a helpful reference for evaluating the health, nutritional status, physiological status of *A. stellatus* provided from aquaculture and Danube genitors.

The studies on determining the dietary vitamin requirements of sturgeon are scarce. Our results showed that supplementation of stellate sturgeon diet with thyme and a high dose of Vitamin E (700 mg kg^{-1}) had no influence on some of hematological and biochemical parameters. Although the fish provided from aquaculture genitors presented a better physiological state it can be said that the stellate sturgeon welfare was influenced by the addition of vitamin E and thyme in the diet or by the genetic heritage. However, further studies are still necessary to evaluate the beneficial role of vitamin E and thyme in fish fed and how they affect the physiological status of fish.

Acknowledgements. The data presented in the paper are the result of the scientific work framed into PN II 116/2012 AQUASTUR research project "The genetic evaluation and monitoring of molecular and biotechnological factors that influence the growth performance of Danube sturgeons species, reared in intensive recirculating systems" and the authors thank for the support.

References

Abdelhamid H. M. B., 2010 Physiology and nutritional studies on improving growth of Nile tilapia (*0. niloticus*) fry using some medicinal plants as a feed additives. MSc. Thesis, University of Kafr El-Sheikh, Egypt.

Al-Absawy A. N. M., 2010 Nutritional requirements for Nile tilapia, *0. niloticus*, cultured in El-Max research station with special references to their growth and feeding habits. MSc. Thesis, Faculty of Science Al-Azhar University, Cairo, Egypt.

Alcicek Z., 2011 The effects of thyme (*Thymus vulgaris* L.) oil concentration on liquid-smoked vacuum-packed rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) fillets during chilled storage. Food Chemistry 128(3):683-688.

Andreeva A. M., 1999 Structural and functional organization of the blood albumin system in fish. Vopr Ikhtiol 39:825-832.
Antache A., Cristea V., Dediu L., Grecu I., Docan A., Mocanu (Cretu) M., Plăcintă (Ion) S., Coadă M. T., 2013 The influence of some phytobiotics on oxidative stress at *Oreochromis niloticus* grown in an intensive recirculating aquaculture system. Lucrări Ştiinţifice–Seria Zootehnie Iasi 59:253-257.
Anver C. E., 2004 Blood chemistry (electrolytes, lipoprotein and enzymes) values of black scorpion fish (*Scorpaena porcus*, 1758) in the Dardanelles, Turkey. Journal of Biological Science 4:716-719.
Baker R. T. M., Davies S. J., 1996 Changes in tissue u-tocopherol status and degree of lipid peroxidation with varyng o-tocopheryl acetate inclusion in diets for African catfish. Aquaculture Nutrition 2:71-79.
Bernet D., Schmidt H., Wahli T., Burkhardt-Holm P., 2001 Effluent from a sewage treatment works causes changes in serum chemistry of brown trout (*Salmo trutta* L.). Ecotoxicology and Environmental Safety 48:140-147.
Blaxhall P. C., Daisley K. W., 1973 Routine hematological methods for use fish blood. Journal of Fish Biology 5(6):771-781.
Bronzi P., Rosenthal H., Gessner J., 2011 Global sturgeon aquaculture production: an overview. Journal of Applied Ichthyology 27:169-175.
Ceapă C., 2008 Sturgeon meat market - key issue for sturgeon aquaculture success. 2nd Annual Conference on Sturgeon Farming - Warsaw, Poland. Available at: http://www.acadian-sturgeon.com/uploads/Cornel_Ceapa_-_Sturgeon_Meat_Market_-_Warsaw_2008_small.pdf.
Cristea V., Antache A., Grecu I., Docan A., Dediu L., Mocanu (Cretu) M., 2012 The use of phytobiotics in aquaculture. Lucrări Ştiinţifice–Seria Zootehnie Iasi 57:250-255.
Coutteau P., Ceulemans S., Van Alexander H., 2011 Botanical extracts improve productivity and economics in aquaculture. NUTRIAD International, Belgium. Available at: https://www.was.org/documents/MeetingPresentations/AA2011/AA2011_0443.pdf.
Denev S. A., 2008 Ecological alternatives of antibiotic growth promoters in the animal husbandry and aquaculture. DSc. Thesis, Department of Biochemistry Microbiology, Trakia University, Stara Zagora, Bulgaria, 294 pp.
Dicu M. D., Cristea V., Dediu L., Mocanu (Creţu) M., 2012 Preliminary results about growth performance and food conversion ratio of *Acipenser stellatus* juvenils fed with different dietary protein levels. Animal Science and Biotechnologies 45(2):37-42.
Dicu (Stroe) M. D., Cristea V., Docan A., Grecu I. R., Dediu L., Coadă M. T., 2013 The influence of feeding frequency on the hematological profile of *A. stellatus* (Pallas 1771), reared in a recirculating aquaculture system. Lucrări Ştiinţifice–Seria Zootehnie Iasi 59:242-246.
Docan A., Dediu L., Cristea V., 2011 Effect of feeding with different dietary protein level on hematological indices of juvenile Siberian sturgeon, *Acipenser baeri* reared under recirculating systems condition AACL Bioflux 4(2):180-186.
El-Dakar A. Y., Hassanien G. D., Gad S. S., Sakri S. E., 2008 Use of dried basil leaves as a feeding attractant for hybrid tilapia, *0. niloticus x 0. auraus*, fingerlings. Mediterranean Aquaculture Journal 1(1):35-44.
Enache I., Cristea V., Ionescu T., Ion S., 2011 The influence of stocking density on the growth of common carp, *Cyprinus carpio*, in a recirculating aquaculture system. AACL Bioflux 4(2):146-153.
Farrell A. P., 2011 Encyclopedia of fish physiology. Elsevier, ISBN 0123745454, 9780123745453, 2272 pp.
Ghergariu S., Pop A., Kadar L., 1985 [Veterinary clinical laboratory guide]. Ceres Publishing House", Bucharest, pp 82-90 [in Romanian].
Kasiri M., Farahi A., Sudagar M., 2011 Effects of supplemented diets by levamisole and *Echinacea purpurea* extract on growth and reproductive parameters in angelfish (*Pterophyllum scalare*). AACL Bioflux 4(1):46-51.

Khalil F. F., Farrag F. H., Mehrim A. I., 2009 Using *Marjorana hortensis* against contamination of mono-sex Nile tilapia, *0. niloticus* diets by lead oxide. Abbassa International journal for Aquaculture. ISSN 1687-7683, special issue for Global Fisheries Research Conference, Cairo International Convention Center, 24-26 October 2009, pp. 407-428.
Kori-Siakpere O., Ake J. E. G., Avworo U. M., 2006 Sublethal effects of cadmium on some selected hematological parameters of Heteroclarias (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). International Journal of Zoological Research 2:77-83.
Knowles S., Hrubec T. C., Smith S. A., Bakal R. S., 2006 Hematology and plasma chemistry reference intervals for cultured shortnose sturgeon (*Acipenser brevirostrum*). Veterinary Clinical Pathology 35(4):434-440.
Maurilio L. F., 2011 Review - the use of probiotic in aquaculture: an overview. International Research Journal of Microbiology 2(12):471-478.
Mitranescu E., Fumaris F., Tudor L., Crasnojan A., Orasanu A., Mitranescu D., Simion V., 2010 The blood biochemical profile as an objective welfare indicator in a sturgeon farm. Bulletin UASVM, Veterinary Medicine 67(1):133-136.
Patriche N., 2001 [Sevruga, biology and artificial reproduction]. Ceres Publishing House, Bucharest, pp. 55-136 [in Romanian].
Patriche T., 2008 [Fish immunity]. Didactic and Pedagogical Publishing House, ISBN: 978-973-30-2070-7 [in Romanian].
Patriche T., Patriche N., Bocioc E., 2010 Serum biochemical parameters of juvenile stages of the Ossetra sturgeon *Acipenser güldenstaedti* (Brandt, 1833). Bulletin UASVM Animal Science and Biotechnologies 67(1-2):300-303.
Patriche T., Patriche N., Bocioc E., 2011a Determination of some normal serum parameters in juvenile Sevruga sturgeons *Acipenser stellatus* (Pallas, 1771). Archiva Zootechnica 14(1):49-54.
Patriche T., Patriche N., Bocioc E., Coadă M. T., 2011b Normal serum biochemical parameters of juvenile stages the beluga sturgeon *Huso huso* (Linnaeus, 1578). International Sturgeon Conference, 30 March - 1 April, Tulcea – Romania.
Patriche T., Patriche N., Bocioc E., Coadă M. T., 2011c Serum biochemical parameters of farmed carp (*Cyprinus carpio*). AACL Bioflux 4(2):137-140.
Pop M., Halga P., Avarvarei T., 2006 [Animal nutrition and feeding]. Vol. 1-2¬3. Tipo Moldova Publishing House, Iasi [in Romanian].
Poston H. A., Combs G. F., Leibovitz L., 1976 Vitamin E and selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): gross, histological and biochemical deficiency signs. Journal of Nutrition 106:892-904.
Puangkaew J., Kiron V., Somamoto T., Okamoto N., Satoh S., Takeuchi T., Watanabe T., 2004 Non-specific immune response of rainbow trout (*Onchorhynchus mykiss* Walbaum) in relation to different status of vitamin E and highly unsaturated fatty acids. Fish and Shellfish Immunology 16:25-39.
Raja J., Iyyadurai V., Kunchitham S., 2008 Effect of dietary vitamin E on growth, fecundity, and leukocyte count in goldfish (*Carassius auratus*). Israeli Journal of Aquaculture BAMIDGEH 60(2):121-127.
Sakamoto K., Lewbart G. A., Smith T. M., 2001 Blood chemistry values of juvenile red pacu (*Piaractus brachypomus*). Veterinary Clinical Pathology 30:50-52.
Shahsavani D., Kazerani H. R., Kaveh S., Gholipour-Kanani H., 2010a Determination of some normal serum parameters in starry sturgeon (*Acipenser stellatus* Pallas, 1771) during spring season. Comparative Clinical Pathology 19:57-61.
Shahsavani D., Mohri M., Gholipour-Kanani H., 2010b Determination of normal values of some blood serum enzymes in *Acipenser stellatus* Pallas. Fish Physiology and Biochemistry 36:39-43.
Svobodova Z., 2001 Stress in fishes (a review). Bull VURH Vodnany 4:169-191.
Yousefisn M., Sheikholeslami A., Kor Davood M., 2011 Serum biochemical parameter of male, immature and female Persian sturgeon (*Acipenser persicus*). Australian Journal of Basic and Applied Sciences 5(5):476.

Yang J. L., Chen H. C., 2003 Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. Zoological Studies 42:455-461.
*** http://www.cites.org/eng. Accessed September, 2013.

Received: 02 February 2015. Accepted: 12 April 2015. Published online: 17 April 2015.
Authors:
Oana-Georgiana (Varlan) Dorojan, Department of Aquaculture, Environmental Sciences and Cadastre, Faculty of Food Science and Engineering 111, Domnească Street 800201, "Dunărea de Jos" University of Galaţi, Galaţi, Romania, e-mail: varlanoana79@yahoo.com
Victor Cristea, Department of Aquaculture, Environmental Sciences and Cadastre, Faculty of Food Science and Engineering 111, Domnească Street 800201, "Dunărea de Jos" University of Galaţi, Galaţi, Romania, e-mail: v.cristea@ugal.ro
Mirela Creţu, Department of Aquaculture, Environmental Sciences and Cadastre, Faculty of Food Science and Engineering 111, Domnească Street 800201, "Dunărea de Jos" University of Galaţi, Galaţi, Romania, e-mail: mirela.cretu@ugal.ro
Lorena Dediu, Department of Aquaculture, Environmental Sciences and Cadastre, Faculty of Food Science and Engineering 111, Domnească Street 800201, "Dunărea de Jos" University of Galaţi, Galaţi, Romania, e-mail: lorena.dediu@ugal.ro
Angelica Ionelia Docan, Department of Aquaculture, Environmental Sciences and Cadastre, Faculty of Food Science and Engineering 111, Domnească Street 800201, "Dunărea de Jos" University of Galaţi, Galaţi, Romania, e-mail: Angelica.Docan@ugal.ro
Marian Tiberiu Coadă, Department of Aquaculture, Environmental Sciences and Cadastre, Faculty of Food Science and Engineering 111, Domnească Street 800201, "Dunărea de Jos" University of Galaţi, Galaţi, Romania, e-mail: marian.coada@ugal.ro

How to cite this article:
Dorojan (Varlan) O. G., Cristea V., Creţu M., Dediu L., Docan A. I., Coadă M. T., 2015 The effect of thyme (*Thymus vulgaris*) and vitamin E on the *Acipenser stellatus* juvenile welfare, reared in a recirculating aquaculture system. AACL Bioflux 8(2):150-158.