

# Effect of Sea Buckthorn and Vitamin E on Growth Performance of *Acipenser stellatus* (Pallas 1771) Juveniles

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## Abstract

Addition of natural additives (prebiotics, probiotics, phytobiotics) in fish feed is a benefic method because it leads to improved growth performance of fish reared in intensive production systems. Sea buckthorn is one of the most commonly phytobiotics used in aquaculture because of its antioxidant, antimicrobial, immunostimulant, anti-stress, cytoprotective, liver and tissue regeneration properties. Sea buckthorn berries are rich in vitamins (B, C, E and K), flavonoids, carotenoids, tocopherols and many volatile compounds. In this context the aim of this study was to evaluate the effects of dietary vitamin E (500 mg/kg diet) and sea buckthorn on growth performance and survival of the *A. stellatus* juveniles reared in a recirculating aquaculture system. The experiment took place in the recirculating pilot system of “Dunărea de Jos” University of Galați. In order to emphasize the influence of the above mentioned immunostimulants on sevruga fingerlings with different genetic background, four experimental groups 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) have been used. At the end of the experiment no significant differences ( $p>0.05$ ) were observed in terms of final mean weight, individual growth rate, SGR, FCR, PER, between the sevruga fingerlings obtained from wild genitors, comparing with those obtained from aquaculture genitors.

**Keywords:** growth performance, recirculating aquaculture system, sea buckthorn, stellate sturgeons

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## 1. Introduction

In Romania, the first study on the growth of sturgeon in intensive recirculating systems was achieved in 2002 [1].

Studies regarding fish production (farming) obtained by recirculating systems have existed since the late 80`s, this having a tendency to rise due to the diversity of fish species grown in these systems. [2-5].

The growth of sturgeon in intensive regime requires a thorough knowledge of technological factors that may influence favorably or not their

growth and development (frequency of food administration, stocking density, feeding intensity, etc.) [6].

Improved fish growth performance increase using natural additives in fish food is a matter of increasingly studied. [7-9].

Phytobiotics contains a wide range of bioactive compounds that can be extracted from different vegetable sources [10] with different beneficial effects on the health of the fish.

Sea-buckthorn (*Hippophae rhamnoides* L.) is a species of flowering plant in the family Elaeagnaceae, native to fixed dunes and sea cliffs in Europe and Asia. Buckthorn berries are rich in vitamins (B, C, E and K), flavonoids, carotenoids, tocopherols and many volatile compounds (for example aliphatic esters, alcohols and hydrocarbons) [11, 12].

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In vitro studies have shown that the sea buckthorn fruits exhibit antioxidant, anti-microbial, immunomodulatory, anti-stress, cytoprotective, hepatoprotective and tissue regeneration properties [13]. At the same time, the fruit of sea buckthorn have a remarkable effect at physiological level inhibiting the oxidation of low density lipoproteins [14].

Since ancient times, the sea buckthorn has been used as an immunomodulatory agent in animal breeding and veterinary medicine with spectacular results; aspect that led to its introduction in the feed of animals, the main purpose being the prevention of health issues [15].

Vitamin E is an essential and required micronutrient for most animals. This vitamin presents a positive effect on the immune system of fish, improving specific and nonspecific immune response, reducing mortality and optimizing growth performance. Also, Vitamin E is a powerful antioxidant that protects the body of any changes that occur in the process of cell membranes and lipoproteins oxidation from the various tissue types [16], it reduce the fragility of red blood cell membranes and enhances circulating leukocyte functions [17]. In higher concentrations, vitamin E shows beneficial effects on reproduction, nutrient digestibility, improves resistance to stress disease and meat quality [18, 19].

In this context, the main aim of this research was to evaluate the effects of adding the sea buckthorn and vitamin E on growth performance and physiological status of sturgeon juveniles, derived from different genetic combinations lots and grown in a recirculating system.

## 2. Materials and methods

*Experimental design.* The experiment took place between 29th January -30th March 2014, at the pilot recirculating system located in a laboratory of Aquaculture, Environmental Science and Cadastre Department, University “Dunărea de Jos” of Galati. The recirculating system is provided with 4 rearing units with a volume of 500 l each and the conditioning units of the water quality.

The biological material used for the experiment was represented by sevruga juveniles from 8 month provided from Kaviar House, from Tulcea.

We use four experimental groups, represented by *A. stellatus* juveniles (aged 8 months) 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. The fodder was supplemented with 1% sea buckthorn/kg feed and Vitamin E 500 mg/kg 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).

After 60 experimental days the fish were weighed and the growth performance of the fish were calculated:

- Weight Gain (W)=Final Weight (Wt)-Initial Weight (W0) (g)
- Food Conversion Ratio (FCR)=Total feed (F)/Total weight gain (W) (g/g)
- Specific Growth Rate (SGR)=100 x (ln Wt-ln W0)/t (% BW/d)
- Protein efficiency ratio (PER)=Total weight gain (W)/amount of protein fed (g)

## 3. Results and discussion

*Water quality.* During this experiment, an important objective was to assess the continuous quality of culture medium.

Water temperature ( $20.48 \pm 0.05^\circ\text{C}$ ), dissolved oxygen ( $7.8 \pm 0.12$  mg/l) and pH ( $7.84 \pm 0.06$  units. pH) showed constant values, without significant differences between the four experimental variants ( $p < 0.05$ ). Also, the compounds of nitrogen were maintained in the optimum gap to increase sturgeon, medium values of nitrite, ammonium and nitrate being  $0.14 \pm 0.02$  mg/l  $0.39 \pm 0.11$  mg/l,  $22.70 \pm 1.47$  mg/l.

*The growth performance.* Both at the beginning and at the final moment of the experiment, the material was weighed and measured the values recorded being statistically analyzed. Statistical analysis of biometric parameters: W (weight average individual), Lt (total length), Lf (fork length), Lr (rostrum length), H (maximum height) showed significant differences ( $p > 0.05$ ) between four experimental groups both at the beginning and at the final moment of the experiment (Table 1).

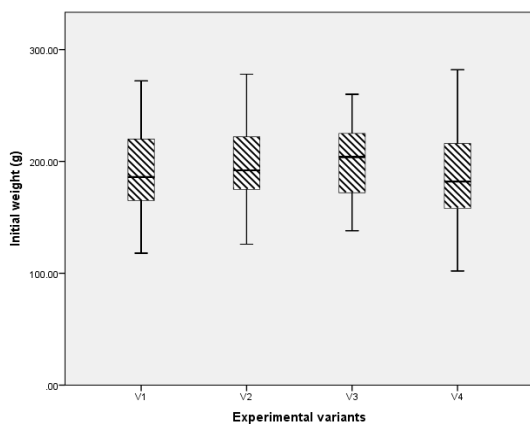
**Table 1.** Somatic characteristics' at the start and in the end of the experiment

Experimental period	Experimental variants	W (g)	Lt (cm)	Lf (cm)	Lr (cm)	H (cm)
Initial moment	V1	191.81±36.90	44.91±2.80	36.97±2.36	6.81±0.47	3.72±0.26
	V2	193.31±33.62	45.55±2.24	37.646±2.40	6.8±0.41	3.89±0.23
	V3	199.18±35.83	45.46±3.06	37.23±2.40	6.95±0.57	3.89±0.29
	V4	186.24±39.18	44.73±3.12	36.75±2.53	6.89±0.42	3.77±0.36
Final moment	V1	301.77±80.15	53.78±4.53	44.39±3.82	8.01±0.63	4.40±0.49
	V2	305.09±59.67	53.76±3.20	44.29±2.66	7.86±0.46	4.46±0.38
	V3	329.25±71.13	54.70±4.08	44.96±3.42	7.96±0.72	4.52±0.44
	V4	287.46±83.51	52.98±4.73	43.49±3.74	7.78±0.56	4.49±0.54

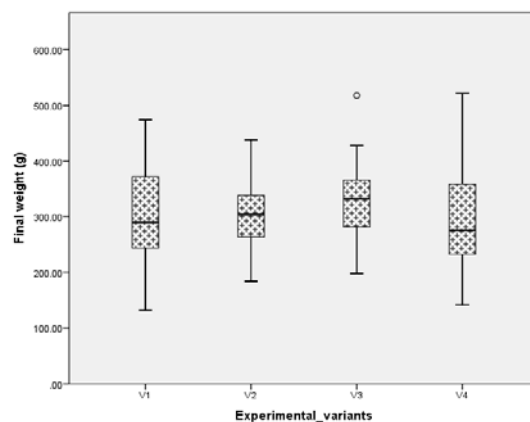
W- weight average individual; Lt-total length; Lf-fork length; Lr-rostrum length; H-maximum height.

Initial mean weight and total length of juvenile sturgeons used in the experiment was not

significantly different for each experimental group ( $p>0.05$ ;  $p=0.51$ ;  $p=0.47$ ) (Figure 1 and Figure 2).



**Figure 1.** The variation of the average individual weight–median, minimum, maximum values and quartiles registered at the initial moment of the experiment



**Figure 2.** The variation of the average individual weight–median, minimum, maximum values and quartiles registered at the end of the experiment

Thus, regarding the variables of the body weight and total length, the statistical testing showed a normal distribution for all groups, as evidenced by

using Levene's test ( $p>0.05$ ) and the coefficient of variation (Table 2).

**Table 2.** The coefficient of variation for the variables body weight and the total length recorded in the four experimental groups

Variable		CV (%)			
		V1	V2	V3	V4
Weight	Initial	19.24	17.4	17.99	21.04
	Final	26.56	19.56	21.6	29.05
Total length	Initial	6.25	4.89	6.73	6.98
	Final	8.43	5.96	7.45	8.93

At the end of the experimental period, the mean individual weight for the four experimental groups was 301.77±80.15 g for the lot V1, 305.09±59.67 g for the lot V2, 329.25±71.13 g for the lot V3 and 287.46±83.51g for lot V4, without significant

differences ( $p>0.05$ ;  $p=0.134$ ).

Lots distribution regarding variable body mass showed lots with normal distribution, as demonstrated using Levene test ( $p>0.05$ ;  $p=0.086$ ) and histograms (Figure 3).

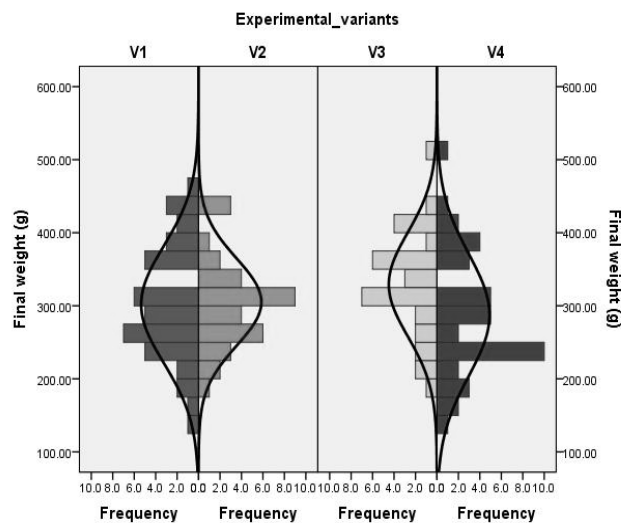


Figure 3. Mass classes' histogram

After analyzing the coefficient of variation (CV) for the variable body mass, emphasizing heterogeneity was observed for lots V1, V3 and V4, where CV increased by approximately 38.05%, 20.07% and 38.07%, while in the case of V2 homogeneity of the batch has been preserved, CV showing an increase of only 12.41% from baseline (Table 1).

The technological indicators of juvenile sturgeon growth are presented in Table 3. From primary analysis of these indicators a superior technological performance can be seen at V3 variant. Thus, the best individual spore growth was recorded for the lot V3 (109.95 g/ex), followed by lot V2 (111.77 g/ex), respectively V1 and V4 lots (109.95 g/ex; 101.22 g/ex).

Table 3. The synthetic table regarding the technological performance indicators for the sevruga after the administration of Vitamin E and sea buckthorn in feed

Parameter	V1	V2	V3	V4
Initial biomass (kg)	8248	6766	6374	7636
Initial number of fish	43	35	32	41
Mean individual weight (g/ex)	191.81	193.31	199.19	186.24
Final biomass (kg)	12976	10678	10536	11786
Final number of fish	43	35	32	41
Mean final weight (g/ex)	301.77	305.09	329.25	287.46
Individual weight gain (g/ex)	109.95	111.77	130.06	101.22
Total weight gain (g)	4728	3912	4162	4150
Specific growth rate (SGR) (%/day)	0.73	0.74	0.81	0.70
Feed conversion ratio (FCR) (g/g)	1.57	1.56	1.38	1.66
Protein efficiency ratio (PER) (g/g)	1.55	1.57	1.77	1.47

Also in terms of the specific growth rate, the best values were recorded for the lot V3 (0.81% BW/day), while the lots V4, V1 and V2 have obtained almost the same values (0.70% BW/day; 0.73% BW/day; 0.74% BW/d). Subunit values of SGR showed a slower growth rate due to the fish age, a similar situation being reported for *A. baeri*. [20-22].

Feed conversion factor (FCR) varies inversely with protein efficiency ratio (PER), recording

higher values also in the experimental V3 (FCR-1.56 g/g; PER 1.77 g/g).

Correlations between length and mean individual weight for each experimental variant are shown in Figure 4.

Analyzing the allometric coefficient "b", it shows a negative allometric scaling regarding the experimental groups V2 and V3, where length growth disadvantage the body mass growth, while regarding the experimental groups V1 and V4, the

value of "b" indicates a positive allometric scaling (3,189 respectively 3,103), aspect that shows a better condition of sturgeon specimens which proceed from female ♀2Danube.

Also, the analysis of determination coefficient  $R^2$ , showed a strong correlation between body weight and total length concerning V1 and V4 lots where the determination coefficient reached a value of 0.9428 and respectively 0.9322.

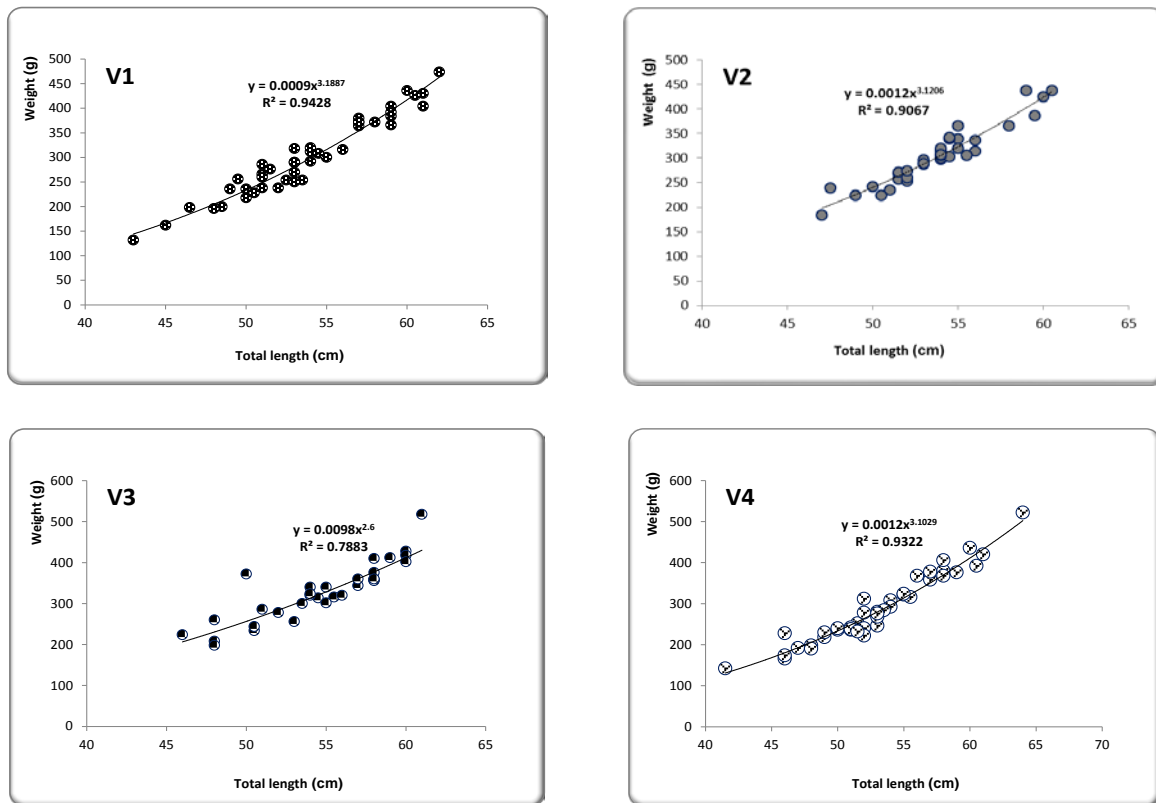


Figure 4. Length-Weight regression at the end of the experimental period

## Conclusions

The beneficial effects of juvenile sturgeon dietary supplementation with sea buckthorn and vitamin E were marked out in this study, the technological indicators highlighting higher growth performances in all four experimental variants.

## Acknowledgements

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## References

1. Cristea, V., Grecu, I., Ceapă, C., Talpeș, M., Roșu, A., Research about *Acipenser stellatus* growth in superintensive system with aquarium units, Internațional Symposium „Eurofood”, 2003, 815-834.
2. Martinsa, C. I. M., Edinga, E. H., Verdegema, M. C. J., Heinsbroeka, L. T. N., Schneiderc, O., Blanchetond, J. P., Roque d'Orbcasteld, E. and Verreth, J. A. J., New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability, *Aquacultural Engineering*, 2010, 43(3), 83-93
3. Rosenthal, H., Recirculation systems in western europe. World Symposium on Aquaculture in Heated Effluents and Recirculation System, Stavanger, Institut für Kuesten-und Binnfischerei, Bundesforschungsanstalt Hamburg, BRD, 1980.
4. Verreth, J. A. J., Eding, E. H., European Farming Industry of African Catfish (*Clarias gariepinus*) Facts and Figures. *J. World Aquacult. Soc.*, 1993, 24, 6-13

5. Martins, C. I. M., Eding E. H., Schneider O., Rasmussen R., Olesen B., Plesner, L., Verreth, J. A. J., Recirculation Aquaculture Systems in Europe. CONSENSUS. Oostende, Belgium, Consensus working Group, European Aquaculture Society, 2005, 31.
6. (Varlan) Dorojan, O. G., Cristea, V., (Plăcintă) Ion, S., Petrea, S. M., The influence of some phytobiotics (thyme, sea buckthorn) on growth performance of stellate sturgeon (*A. stellatus*, Pallas, 1771) in an industrial recirculating aquaculture system; Bioengineering of animal resources Symposium; Animal Science and Biotechnologies, 2014, 47 (1), 205-210.
7. Lee, M. H., Lee, H. J., and Ryu, P. D., Publichealth risks: Chemical and antibiotic residues. Rev.Asi.Aust.J. Anim.Sci., 2001, 14, 402-413.
8. Seung-Cheol, J., Takaoka, O., Jeong, G. S., Lee, S. W., Ishimaru, K., Seoka, M., Takii, K., Dietary medicinal herbs improve growth and some non-specific immunity of red sea bream *Pagrus major*, Fisheries Science, 2007, 73, 63–69.
9. Antache, A., Cristea, V. Grecu, I., Dediu, L., Docan, A., (Cretu) Mocanu, M., Ion Placintă, S., and Coadă, M. T., The Influence of some Phytobiotics On Oxidative Stress At *Oreochromis Niloticus* Grown in an Intensive Recirculating Aquaculture System. Scientific papers Animal Science, 2013, 59, 253-257.
10. Vidanaratchi, J. K., Mikkelsen, L. L., Sims, I., Iji, P. A., and Choct, M., Phytobiotics: alternatives to antibiotic growth promoters in monogastric animal feeds, Recent Advances in Animal Nutrition in Australia, 2005,15.
11. Ranjith, A, Kumar, K. S, Venugopalan, V. V, Arumughan, C., Sawhney, RC, Singh, V., Fatty acids, tocals, and carotenoids in pulp oil of three sea buckthorn species (*Hippophae rhamnoides*, *H. salicifolia*, and *H. tibetana*) grown in the Indian Himalayas. J Am Oil Chem Soc., 2006, 83, 359-364.
12. Vodnar, D. C, Socaciu, C. Green tea increases the survival yield of Bifidobacteria in simulated gastrointestinal environment and during refrigerated conditions. Chem Cent J., 2012, 6, 61.
13. Gupta, A., Kumar, R., Pal, K., Banerjee, P. K., Sawhney, R. C., A preclinical study of the effects of sea buckthorn (*Hippophae rhamnoides* L.) leaf extract on cutaneous wound healing in albino rats, Int. J. Low. Extrem. Wounds, 2005, 4 (2), 88–92.
14. Bao, M., Lou, Y., Flavonoids from seabuckthorn protect endothelial cells (EA.hy926) from oxidized low-density lipoprotein induced injuries via regulation of LOX-1 and eNOS expression, J Cardiovasc Pharmacol., 2006, 48(1), 834-841.
15. Morar R., Eridiarom, Erisol, Polivitarom or Herbal Alternatives, Editura Todesco, Cluj-Napoca, 2003. In Romanian
16. Adham, K. G., Hashem, H. O., Abu-Shabana, M. B., Kame, A. H., Vitamin C deficiency in the catfish (*Clarias gariepinus*), Aquacult Nut., 2000, 6, 129–139.
17. Kiron, V., Puangkaew, J., Ishizka, K., Satoh, S., Watanabe, T., Antioxidant status and nonspecific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with three lipid sources, Aquaculture, 2004, 234, 361-379.
18. Bahareh Mehrad, and Mohammad Sudagar, Dietary vitamin E requirement, fish performance and reproduction of guppy (*Poecilia reticulata*), AACL Bioflux, 2010, 3(3). <http://www.bioflux.com.ro/aacl>.
19. Puangkaew, J., V. Kiron, T. Somamoto, N. Okamoto, S. Satoh, T. Takeuchi, and T. Watanabe, Non-specific immune response of rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) in relation to different status of vitamin E and highly unsaturated fatty acids. Fish Shellfish Immun, 2004, 16, 25-39.
20. Zare, R., Bahmani, M., Yavari, V., Kazimi, R., Pasha, H., Pourdehghan, I. M., Fazeli, N., Yooneszade, B. and Nateghi, S. A., The effects of rearing density on growthperfor mance and food conversion ratio of siberian sturgeon (*Acipenser baeri* Brandt), Asian Fisheries Science, 2009, 22, 107-115
21. Medale, F. and Kaushik, S. J., Energy utilization by farmed Siberian sturgeon (*Acipenser baeri*) from 3 age classes, Actes du Premier Colloque International Sur L'esturgeon, Bordeaux. CEMAGREF, 1989, 13-23
22. Köksal, G., Rad, F. and Kindir, M., Growth Performance and Fed Conversion Efficiency of Sturgeon Juveniles (*Acipenser baerii*) Reared in Concrete Raceways, Turk J Vet Anim Sci, 2000, 435–442.