

The effect of dietary supplementation with Seabuckthorn (*Hippophae rhamnoides*) and Spirulina (*Spirulina platensis*) on the growth performance of some sturgeon hybrids

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Abstract. In order to reduce the dependence of aquaculture on antibiotics, plants can be considered as an effective alternative way to control bacterial and viral infections. Furthermore, using herbal plants in fish diets leads to improve growth performance, feed efficiency, digestion, survival, and health. Thus, a feeding trial was performed to assess the effect of spirulina and seabuckthorn on growth performance of two hybrid sturgeons species obtained by cross breeding of genitors with different origins (the Danube and aquaculture). Plants were added at 1.0 %/kg feed in the fish diet for 36 days. The experimental variants were: hybrid sturgeons provided from crossing ♂ *Huso huso* from the Danube with ♀ *Acipenser ruthenus* from aquaculture, feed with sea buckthorn 1%/kg feed (V1) and spirulina 1%/kg feed (V2) and hybrid sturgeons provided from crossing ♂ *H. huso* from Danube with ♀ Bester from aquaculture, feed with sea buckthorn 1%/kg feed (V3) and spirulina 1%/kg feed (V4). At the end of the experiment, we can conclude that the best results were obtained in the case of hybrid sturgeon coming from the crossing of ♂ *H. huso* from the Danube with ♀ Bester from aquaculture (V4), feed with spirulina (1%/kg feed).

Key Words: feed additives, weight gain, sturgeons, recirculating aquaculture systems.

Introduction. Intensive aquaculture practices consist in growing fish in high stocking densities, which can have significant adverse effects on fish welfare, growth performance and feed efficiency (Adeyemo 2014; Bahrami et al 2015). Unfortunately, fish producers use a lot of antibiotics and chemicals to prevent and control diseases (Harikrishnan et al 2011). However, the use of antibiotics in fish farming is forbidden in many countries, therefore lately, many researchers have focused on improving fish growth performance and immunity by supplementing diets with feed additives, such medicinal herbs, and algae.

Generally, the primary effects of medicinal plants are to improve feed efficiency and/or daily gain (Adekunle & Oladoye 2015), but also can be a good alternative to replace antibiotics and chemicals to prevent and control diseases (Harikrishnan et al 2010; Vaseeharan & Thaya 2014; Syahidah et al 2015).

Spirulina (*Spirulina platensis*) is a photosynthetic, filamentous, blue–green microalgae being a rich source of vitamins, essential amino acids, minerals, essential fatty acids (γ -linolenic acid), and antioxidant pigments such as carotenoids and phycocyanin (Seyidoglu et al 2017). As feed additives in fish feed, *S. platensis* improves growth, feed efficiency, carcass quality, and physiological response to stress and disease of fish (Mustafa & Nakagawa 1995; Shima 2016). Several studies have been conducted to investigate the effects of *S. platensis* on growth performance, survival rate and immune responses at fish such as, tilapia *Oreochromis niloticus* (Takeuchi et al 2002), common carp *Cyprinus carpio* (Csépi et al 2010; Nasreen 2014), rainbow trout

Oncorhynchus mykiss (Mahdi et al 2013), sturgeon *Acipenser transmontanus* (Palmevegiano et al 2008), etc.

Sea-buckthorn (*Hippophae rhamnoides*) is a plant rich in vitamins (vitamin C, vitamin E, and carotenoids like β -carotene and lycopene B2) and secondary plant metabolites like flavonoids (quercetin and kaempferol) are abundant in fruit pulp and seeds (Kagliwal et al 2012). In fish diets, *H. rhamnoides* it is used successfully for improving disease resistance and growth performance (Todoran 2015).

The main objective of the present study was to investigate the effects of *H. rhamnoides* and *S. platensis* on survival and growth performance of two hybrid sturgeons diets.

Material and Method

Experimental design. Feeding experiment of this research was conducted during 36 days in a recirculating aquaculture system provided with four rearing units (500 L each) and equipments for water quality conditioning. 196 sturgeon hybrids which were obtained by cross breeding of genitors with different origins (Danube and Aquaculture) at Horia sturgeon station, situated in Tulcea County, Romania. Fish were randomly distributed in order to create four experimental variants. The experimental groups were: hybrid sturgeons (aged 5 months) provided from crossing ♂ *Huso huso* from the Danube with ♀ *Acipenser ruthenus* from aquaculture, feed with *H. rhamnoides* 1%/kg feed (V1) and *S. platensis* 1%/kg feed (V2) and hybrid sturgeons (aged 5 months) provided from crossing ♂ *H. huso* from the Danube with ♀ Bester from aquaculture, feed with *H. rhamnoides* 1%/kg feed (V3) and *S. platensis* 1%/kg feed (V4). Fish were fed with extruded pellets, with the diameter of 2.0 mm (Table 1). Fish feeding was fulfilled by hand, three times per day (9⁰⁰; 13⁰⁰; 17⁰⁰) at a rate of 1% of total fish biomass.

Table 1

Nutritional composition of the extruded 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

Water quality. Temperature, oxygen content, and pH were monitored daily and nitrogen compounds weekly. For temperature and dissolved oxygen (DO) an oxygen meter WTW Multi 3410 was used and for pH, a WTW model 340 pH-meter was used. Nitrogen compounds were determined using a Spectroquant Nova 400 type spectrophotometer, compatible with Merk kits.

The average and standard deviation (\pm SD) of water quality parameters monitored throughout the study were: water temperature ($22.1 \pm 1.16^\circ\text{C}$), dissolved oxygen ($6.95 \pm 1.11 \text{ mg L}^{-1}$), pH (7.90 ± 0.1), nitrate ($23.9 \pm 8.4 \text{ mg L}^{-1}$), nitrites ($0.01 \pm 0.04 \text{ mg L}^{-1}$) and ammonia ($0.15 \pm 0.06 \text{ mg L}^{-1}$), the values being in the optimal range for sturgeons growing (Mims et al 2002).

Fish performance. At the beginning and at the end of the experiment, the fishes from each rearing unit were individually weighed ($W \pm 1$ g) and measured (± 1 mm) for determining total weight -W and total length- Lt.

At the end of the experimental period the following growth and feed utilization indices were calculated:

$$\text{Weight Gain (W)} = \text{Final Weight (Wt)} - \text{Initial Weight (W0)} \text{ (g)},$$

Where Wt and W0 represent final and initial body weights of fish;

$$\text{Specific growth rate (SGR)} (\% \text{ d}^{-1}) = 100 \times (\ln \text{Wt} - \ln \text{W0}) / t$$

Where t represents the duration of the feeding trial;

$$\text{Food Conversion Ratio (FCR)} = \text{dry weight of feed (g)} / \text{wet weight gain by fish (g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{Total weight gain (W)} / \text{amount of protein from feed (g)}$$

Statistical analysis. Data was analyzed using SPSS program Version 21. t-test and one-way ANOVA and Duncan's multiple range tests were used to compare the differences between the experimental groups. Statistical differences between variables were tested using ANOVA ($p = 0.05$).

Results and Discussion. The use of plants in the fish diet is becoming an increasingly accepted idea among aquaculturists to the detriment of chemical additives or antibiotics. However, their use in fish diets is still limited, this being accomplished only at experimental scale.

In our study, administration of *S. platensis* and *H. rhamnoides* in the sturgeon diet did not significantly affect the growth performance. At the beginning of the experiment statistical analysis of body weight revealed no significant differences between the experimental groups (t-test, $p > 0.05$; $p = 0.53$ (V1 and V2), $p > 0,05$ respectively; $p = 0.98$ (V3 and V4)).

The mean weight of fish in V1 was 144.86 ± 33.019 g, 141.41 ± 27.15 g in V2, 194.63 ± 68.26 g in V3 and 194.34 ± 58.98 g in V4 (Figure 1). At the end of the experiment, the mean body weight of fish recorded the following values: V1 – 212.25 ± 43.97 ; V2 – 209.29 ± 47.45 , V3 – 308.03 ± 93.30 and V4 - 328.48 ± 93.69 , with significant differences ($p < 0.05$) between the two hybrids, but with no significant differences ($p > 0.05$) between the hybrids feed with *H. rhamnoides* and *S. platensis* (Figure 1).

Initial weight, final weight, weight gain, specific growth rate and survival rate of the sturgeons hybrids is presented in Table 2. The individual weight gain, SGR and FCR of the sturgeons registered almost similar values between groups fed with *H. rhamnoides* and *S. platensis*, with slightly higher values for hybrids provided from V3 and V4 variants. The individual weight gain was 113.40 g/fish in V3 and 134.14 in V4, while in V1 and V2 was around 67 g/fish. Also, the best values of SGR were recorded in V4 and V3 (1.46 % BW/day, 1.28 % BW/day respectively). Higher PER values and low FCR values registered in the case of V3 and V4 variants indicates an efficient use of nutrients for body growth of these surgeons hybrids.

Significant effects have been reported after administration of *S. platensis* on growth performance of *A. baeri* by Palmegiano et al (2005). After the inclusion of *S. platensis* in the proportion of 50%, the author obtained an improvement of growth performance, feed efficiency and the highest protein efficiency. The author recommends on the future introduction of *S. platensis* into fish food as a good opportunity to partial substitute fish meal.

Regarding the administration of *H. rhamnoides* in fish diets, there are very few studies made, generally with good results in terms of their growth performance (Antache et al 2013; Dorojan et al 2014; Todoran 2015) or welfare and disease resistance (Csépi et al 2010).

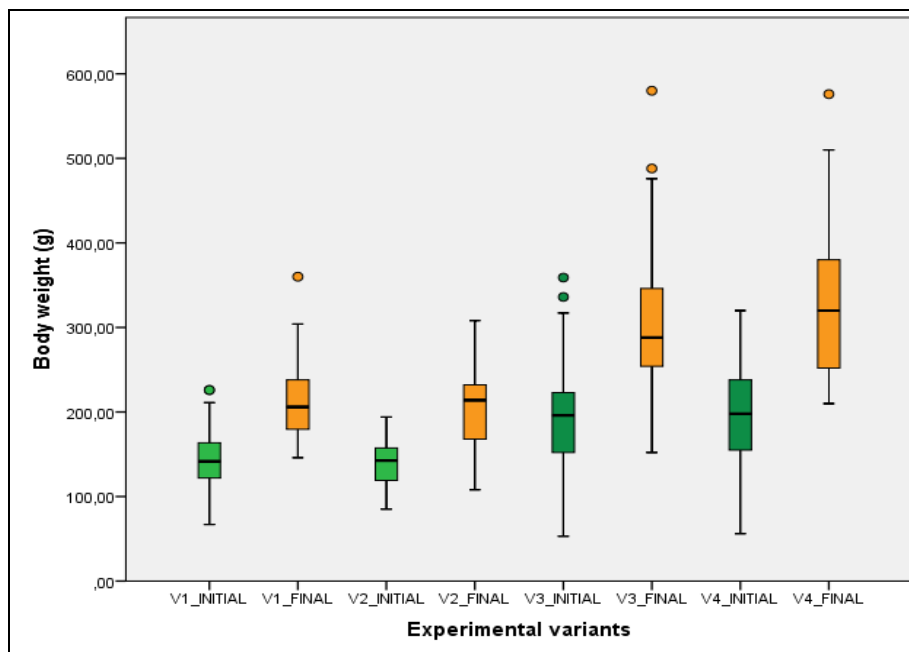


Figure 1. The variation of the average individual weight – median, minimum, maximum values and quartiles registered at the beginning and at the end of the experiment.

Table 2

Technological performance indicators, obtained at the end of the experimental period

Growth performance	Experimental variants			
	V1	V2	V3	V4
Initial biomass (g)	8,691.6	8,485	7396	7,385
Number of fish	60	60	38	38
Mean individual weight (g fish ⁻¹)	144.86	141.42	194.63	194.34
Final biomass (g)	11,673.91	11302	10,165	10,840
Final number of fish	55	54	33	33
Mean final fish weight (g fish ⁻¹)	212.25	209.30	308.03	328.48
Individual weight gain (g) (g/ex)	67.39	67.88	113.40	134.14
Total weight gain (g)	2,982.31	2817	2,769	3,455
Specific growth rate (% BW/day)	1.06	1.09	1.28	1.46
Feed conversion ratio (FCR) (g ⁻¹ g)	0.90	0.91	0.76	0.63
Protein efficiency ratio (g g ⁻¹)	2.42	2.38	2.87	3.43
Survival (%)	91.67	90.00	86.84	86.84

The determination of the correlation between the total length (TL) and body weight (W) was carried out with the data obtained from the biometric measurements from the beginning and from the end of the experiment.

The analysis of the body-length regression revealed a higher coefficient of determination ($R^2 = 0.93$) for the hybrid sturgeon from V4, where, at the end of the experiment, were registered the best values of feeding efficiency indicators. An increase in the regression coefficient was also observed in the case of the hybrid from the V2 variant were also were administrated *S. platensis* (Figure 2). Also, the value of the regression coefficient "b" registered a better condition of the sturgeon hybrid from the variants V4 and V2 (3.01 and 2.97), body mass growth is directly proportional to length gain.

At the end of the experiment, all the fish were weighed and measured and the recorded values were statistically analyzed. The biometric parameters statistical analysis: W (weight), H (maximum height), TL (total length) and FL (fork length) shows

insignificant differences ($p > 0.05$) between the control groups of the same variant and significant differences ($p \leq 0.05$) between the two experimental variants, the biometric measurements mean values are presented in Table 2.

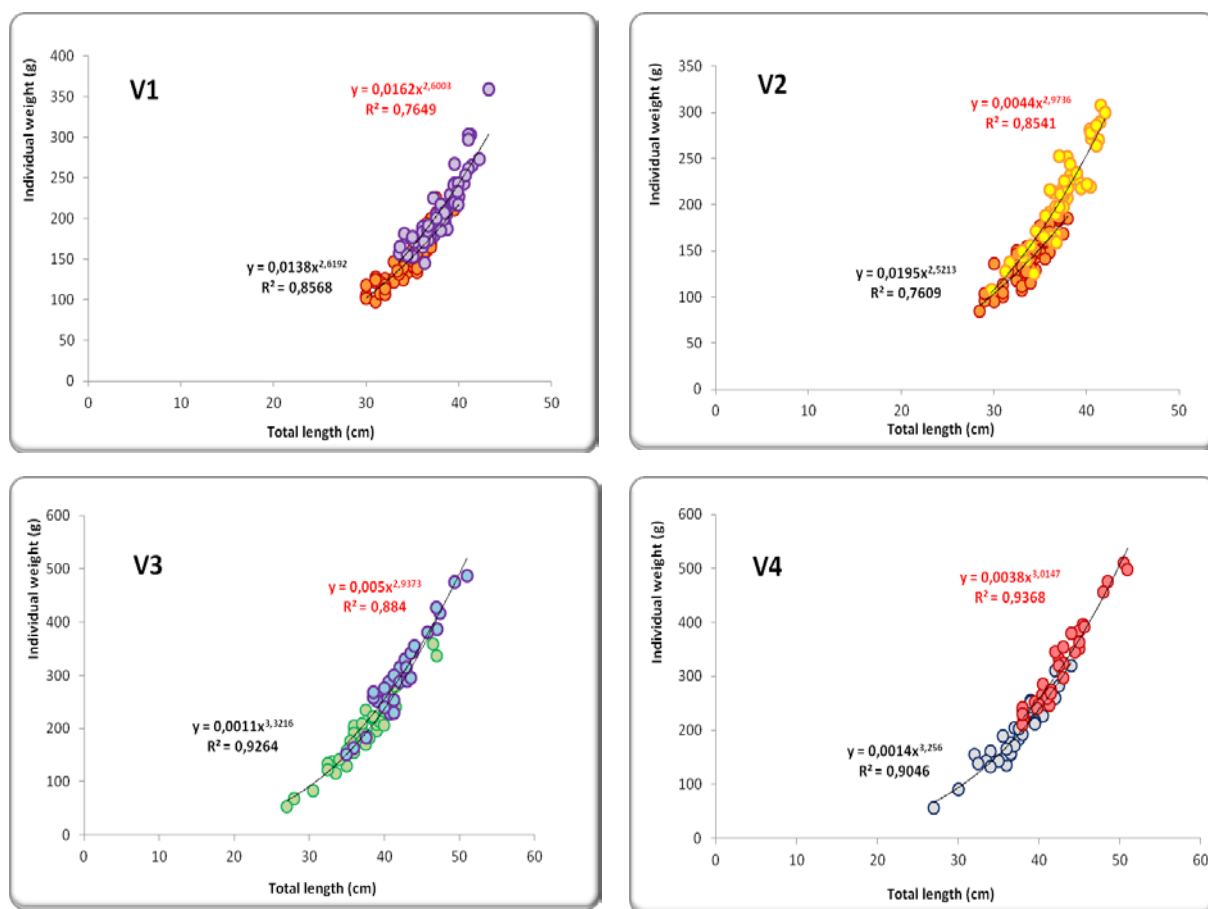


Figure 2. Length-weight regression of all experimental variants, at the initial moment and the end of the experiment.

Conclusions. Fish growth is a complex process and can be influenced by many variables such as fish species, nutrients present in the feed and growing conditions. The results of our study showed that supplementing sturgeon food with *S. platensis* and *H. rhamnoides* could improve fish growth performance and feed utilization. However, further studies are required in order to evaluate their beneficial properties and to clarify the action mechanism of these herbs on the fish growth and welfare.

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