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# Effect of thyme (*Thymus vulgaris*) and vitamin E on growth performance and body composition of *Acipenser stellatus* juveniles

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**Abstract**. The improvement of fish growth performance and feed efficiency by using natural feed additives, as phytobiotics, has been widely used. Thyme *(Thymus vulgaris),* a well known aromatic plant, with strong antimicrobial and antioxidant activity, is one of the most commonly phytobiotics used in aquaculture. These properties, already demonstrated in case of terrestrial animal husbandry, recommend thyme for aquaculture industry, where it could be successfully used instead of commercial antibiotics. The aim of present study is to investigate the effects of thyme (1% thyme $kg^{-1}$ feed) in association with vitamin E (500 mg $kg^{-1}$), on growth performance and body composition of different sevruga (*Acipenser stellatus*) fingerlings cohorts obtained by cross breeding of genitors with different origins (the Danube and aquaculture). The experiment was carried out in the recirculating aquaculture system (RAS) of Aquaculture, Environmental Science and Cadastre Department, "Dunărea de Jos" University of Galaţi, during a 5 weeks period. In order to emphasize the influence of the above mentioned immunostimulants on *A. stellatus* 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. The obtained results showed that thyme, combined with vitamin E, have not generated different responses, in terms of growth performance and nutrient utilization, among *A. stellatus* fingerlings obtained from wild genitors, comparing with those obtained from aquaculture genitors.
**Key Words**: phytobiotics, thyme, sevruga fingerlings, recirculating aquaculture systems (RAS).

**Introduction**. Danube sturgeons can be considered as unique values of European biodiversity, indicators of habitats and a true ecological, economic and social heritage. It is well known that natural sturgeon populations have declined drastically in the last decades of the twentieth century, many species being threatened with extinction (Ludwig et al 2009; Sandu et al 2013). Both overfishing and decline of reproductive activity due to pollution of rivers and construction of physical barriers along migration routes to spawning sites has led to the current critical state of these species (Birstein et al 1997; Onără et al 2013).

The decrease in the number of sturgeon and, as a direct consequence, the downward trend of sturgeon catches, have generated an increase of the interest related to the development of both, reproductive and fish growth under controlled technological conditions (Steffens et al 1990; Kolman 1999).

In this context, aquaculture of sturgeons can help in the conservation of declined wild populations through restocking and by providing a consistent supply without exploiting wild populations (Memis et al 2009).

Improving growth performance and feed efficiency by using natural feed aditive has been widely studied in previous scientific papers (Lee et al 2001; Ji et al 2007; Zaki et al 2012; Yılmaz et al 2012). Phytobiotics represent a wide range of bioactive compounds that can be extracted from various plant sources (Vidanarachchi et al 2005), having different benefic effects on fish such as: antioxidant, antimicrobial, anticarcinogenic, analgesic, insecticidal, antiparasitic, growth promoters, appetite

enhancement, stimulant of secretion of bile and digestive enzyme activity, hepatoprotection (Csép et al 2010, Kasiri et al 2011 ).

Thyme (*Thymus vulgaris*) is a member of Lamiaceae family. Thyme has strong antimicrobial and antioxidant activity due to its very high contents of thymol (40%), p-carvacrol (15%), cymene, eugenol and 4-allylphenol (Baranauskiene et al 2003; Lee et al 2005; Rota et al 2008), fact that made possible for this plant to be used instead of commercial antibiotics (Dorman & Deans 2000).

Nutrient supplementation in fish diets has been an economically promising method for improving the performance of different intensive fish production systems. Vitamin E is a vital and indispensable micronutrient for most animals. It is required to maintain flesh quality, immunity, the normal resistance of red blood corpuscles to haemolysis the maintenance of normal permeability of capillaries, and heart muscle (Halver 2002). In aquaculture, vitamin E is used for the fortification of feed to improve the growth, resistance to stress and disease as well as for survival of fish and shrimp (Puangkaew et al 2004; Mehrad et al 2010).

The main aim of the present study was to evaluate the effect of dietary supplementation with thyme and vitamin E on growth performance, feed conversion, feed utilization and whole body composition of four genetic combination (Danube and aquaculture) of *Acipenser stellatus* (Pallas, 1771) reared in controlled conditions, in a recirculating aquaculture system (RAS).

## Material and Method

***Experimental design***. The current study took place at the pilot station of Aquaculture, Environmental Science and Cadastre Department of Food Science and Engineering Faculty, Galati, during 16$^{th}$ December 2013 and 21$^{st}$ January 2014, using 4 rearing units with a volume of 500 L each. It must be mentioned that the recirculating system construction particularities have been described in the previous study of Enache et al (2011).

The biological material used in the present experiment consists in 7 months old *A. stellatus* juveniles, with a mean body mass of 103.89±22.57 g, provided from Kaviar House farm, Tulcea region. Fish were obtained by artificial reproduction. Four experimental groups from different genitors have been used: (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). All fish were fed with a commercial diet containing 45% protein and 18% lipids (Table 1). The fodder was supplemented with 1% thyme kg feed$^{-1}$ and Vitamin E 500 mg kg feed$^{-1}$. The applied feeding ratio was 2.6% from body weight (BW), administered in three meals per day, according with the following program: 09:00, 13:00 and 17:00.

Table 1

Biochemical composition of the TROCO PRE GROWER pellets

| *Parameters* | *Quantity* |
|---|---|
| Protein | 45% |
| Fat | 18% |
| Crude fibre | 1.2% |
| Ash | 8.2% |
| Phosphor | 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***. The main water physico-chemical parameters were analysed. For temperature and dissolved oxygen (DO) daily determination an oxygen meter WTW Multi 3410 was used. WTW model 340 pH-meter was used for determining the pH values. Nitrogen compounds ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$) were monitored once a week by using a Spectroquant Nova 400 type spectrophotometer, compatible with Merk kits.

***Proximate analysis of fish meat***. In order to determine the biochemical composition of *A. stellatus* meat, biological material samples were taken both in the initial and final stage of the experiment. The proximate composition of fish fillets was analyzed using the AOAC (2000) method. Chemical composition of meat crude protein was analysed according to the Kjeldahl method (N x 6.25) and crude lipids were determinated by Soxhlet method, using petroleum ether as a solvent. Dry matter was determined by drying the samples at 105±2ºC using Sterilizer Esac and ash was evaluated by calcification at temperatures of 550±20°C in a Nabertherm furnace.

***Calculations***. After the experimental period fish were weighed individually (W±1 g) and their growth performance was calculated: Weight Gain (W) = Final Weight ($Wt_1$) - Initial Weight ($Wt_0$) (g), Food Conversion Ratio (FCR) = Total feed (F)/Total weight gain (W) (g $g^{-1}$), Specific Growth Rate (SGR (%Body weight $day^{-1}$)) = $[(Ln\ Wt_1 – Ln\ Wt_0)/t] \times 100$, Fullton coefficient ($F = w/Lt^b$ where W–final weight, Lt-final total length average, b-allometric coefficient, experimentally determined), coefficient of variation (%) (CV) = (standard deviation of weight distribution/average of weight distribution)x100.

Also, both at the beginning and at the end of the experiment, biometric measurements were made individually (±1 mm) for each fish apart, for determining total length Lt - total length; Lf - fork length; Lr - length of rostrum; H - height.

The main indicators used for the evaluation of biochemical meat compositions were as follows: Protein efficiency ratio (PER) = Total weight gain (W)/amount of protein fed (g); Protein utilization efficiency (PUE) = 100 ($Wt_1$ x Pf– $Wt_0$ x Pi)/( F x amount of protein fed) (%), where: Pf = final body protein (%), Pi = initial body protein (%); Retained protein (RP) = $Wt_1$ x Pf –$Wt_0$ x Pi; Retained lipids (RL) = final individual weight x Lf – initial individual weight, where: Lf = final body lipids (%), Li = initial body lipids (%).

***Statistical data processing***. Data was analyzed using SPSS version 17. The Kolmogorov Smirnov test was used to test the normal distribution of the data. 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

***Water quality***. During the experiment, the physico-chemical parameters of technological water were situated within the normal range for sturgeon growth. Water temperature (20.36±0.50°C), dissolved oxygen (7.98±0.20 mg $L^{-1}$) and pH (7.74±0.06 pH units) had a relatively constant trend, with no significant differences between the four experimental variants ($p > 0.05$). Regarding the dynamics of water nitrogen compounds, directly related with fish metabolism and nitrification products, those were maintained within the optimal range among the experimental period, the average values of nitrate, nitrite and ammonium concentrations being as follows: 23.38±0.21 mg $L^{-1}$, 0.13±0.01 mg $L^{-1}$, 0.31±0.13 mg $L^{-1}$.

***Growth performance***. In Table 2 are presented the initial biomass biometric characteristics. At the beginning of the experiment no significant differences (Anova, p = 0.918) were registered between the four groups. The weight (W) homogeneity of the tested groups was verified and confirmed with the help of the Levene test (p = 0.748).

After 36 days, when comparing the individual final weight and the other biometric measurements that we made, no significant differences ($p > 0.05$) between the experimental variants were observed (Table 2).

Table 2

Somatic characteristics of experimental biological material

| *Experimental period* | *Exp. variants* | *W (g)* | *Lt (cm)* | *Lf (cm)* | *Lr (cm)* | *H (cm)* |
|---|---|---|---|---|---|---|
| Initial | V1 | 102.45±20.65 | 36.06±2.44 | 29.62±2.02 | 5.56±0.43 | 3.16±0.21 |
| | V2 | 104.72±23.80 | 36.07±2.40 | 29.76±1.91 | 5.57±0.37 | 3.26±0.29 |
| | V3 | 105.57±22.66 | 42.06±35.4 | 29.97±2.15 | 5.57±0.49 | 3.24±0.36 |
| | V4 | 102.81±23.18 | 36.04±2.56 | 29.79±2.36 | 5.65±0.37 | 3.25±0.40 |
| Final | V1 | 190.50±36.17 | 44.63±2.85 | 36.48±2.47 | 7.03±0.49 | 3.78±0.31 |
| | V2 | 201.02±30.14 | 45.08±2.09 | 36.87±1.79 | 6.98±0.37 | 3.83±0.25 |
| | V3 | 198.42±37.46 | 44.98±2.89 | 36.59±2.38 | 7.00±0.56 | 3.87±0.26 |
| | V4 | 188.33±38.26 | 44.58±3.11 | 36.41±2.33 | 6.97±0.38 | 3.78±0.41 |

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

Mean body weight of fish recorded the following values: 190.50±36.17 g in V1, 201.02±30.14 g in V2, 198.42±37.46 g in V3 and respectively, 188.33±38.26 g in V4, without significant differences between the experimental variants (Anova; $p = 0.341$). Also, regarding the final length, no significant differences were registered between the four experimental groups (Anova; $p = 0.818$).

At the beginning of the experimental period, the variation coefficient for weight (CV) registered a value of 20.16% in V1, 22.73% in V2, 21.47% in V3, 22.55% in V4 respectively, while at the end of the experiment, it decreased to 18.88% in V3, 18.99% in V1, 20.32% in V4, 14.99% in V2 respectively. This is also emphasized by the histograms (Figure 1) which reveals pronounced heterogeneity at the end of the experiment in the case of V2 and V4 variants. Also, the CV for length presented a decrease tendency comparing with the initial moment (6.79% in V1, 6.66% in V2, 8.74% in V3, 7.12% in V4 to 6.40% in V1, 6.66% in V2, 6.44% in V3, 6.98% in V4) fact that concludes an accentuation of heterogeneity among experimental lots (Figure 2).

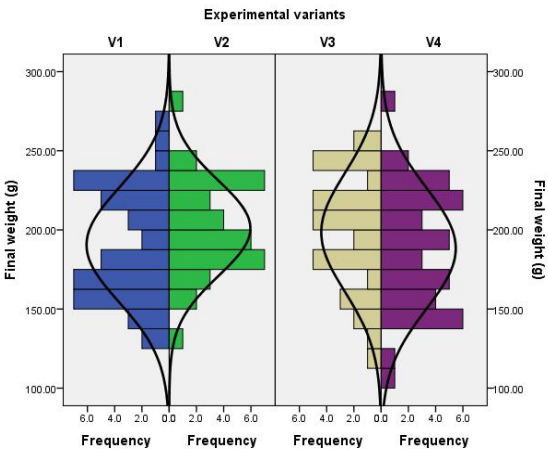


Figure 1. Weight classes histogram.

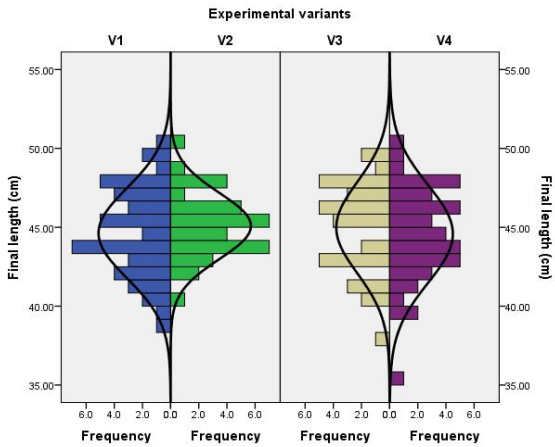


Figure 2. Length classes histogram.

During the experimental period no mortalities were recorded, fact similar in all experimental variants. Data related with growth performance of *A. stellatus* are presented in Table 3.

Table 3

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

| *Growth performance* | *Experimental variants* | | | |
|---|---|---|---|---|
| | *$V_1$* | *$V_2$* | *$V_3$* | *$V_4$* |
| Number of fish | 44 | 36 | 33 | 42 |
| Initial biomass (g) | 4508 | 3770 | 3484 | 4318 |
| Mean individual weight (g fish$^{-1}$) | 102.40 | 104.72 | 105.58 | 102.81 |
| Initial stocking density (kg/m$^3$) | 9.01 | 7.54 | 6.96 | 8.63 |
| Final numbers of fish | 44 | 36 | 33 | 42 |
| Final biomass (g) | 8382 | 7237 | 6548 | 7910 |
| Mean final fish weight (g fish$^{-1}$) | 190.5 | 201.03 | 198.42 | 188.33 |
| Final stocking density (kg/m$^3$) | 16.76 | 14.47 | 13.09 | 15.82 |
| Individual weight gain (g) | 88.05 | 96.31 | 92.85 | 85.52 |
| Total weight gain (g) | 3874 | 3467 | 3064 | 3592 |
| Specific growth rate (SGR) (% day$^{-1}$) | 1.72 | 1.81 | 1.75 | 1.68 |
| Daily growth rate (g kg$^{-1}$ day$^{-1}$) | 2.45 | 2.68 | 2.58 | 2.38 |
| Feed conversion ratio (FCR) (g $^{-1}$g) | 1.09 | 1.01 | 1.06 | 1.13 |
| Protein efficiency ratio (PER) (g g$^{-1}$) | 2.00 | 2.14 | 2.04 | 1.93 |

Analysis of the technological indicators emphasized the fact that the administration of Vitamin E and thyme in feed has no significant effect on growth performance of *A. stellatus* juveniles.

Thus, it can be noticed that the main technological indicators registered almost identical values in all four experimental groups. However, the best values of FCR, SGR and PER were obtained in V2 and V3 groups, where *A. stellatus* was obtained from the same female (♀$_1$ D) genitors, from Danube.

For determining the relative robustness of experimental plots, length-weight regressions were used (Figure 3), which revealed the exponent "b" as allometric coefficient.

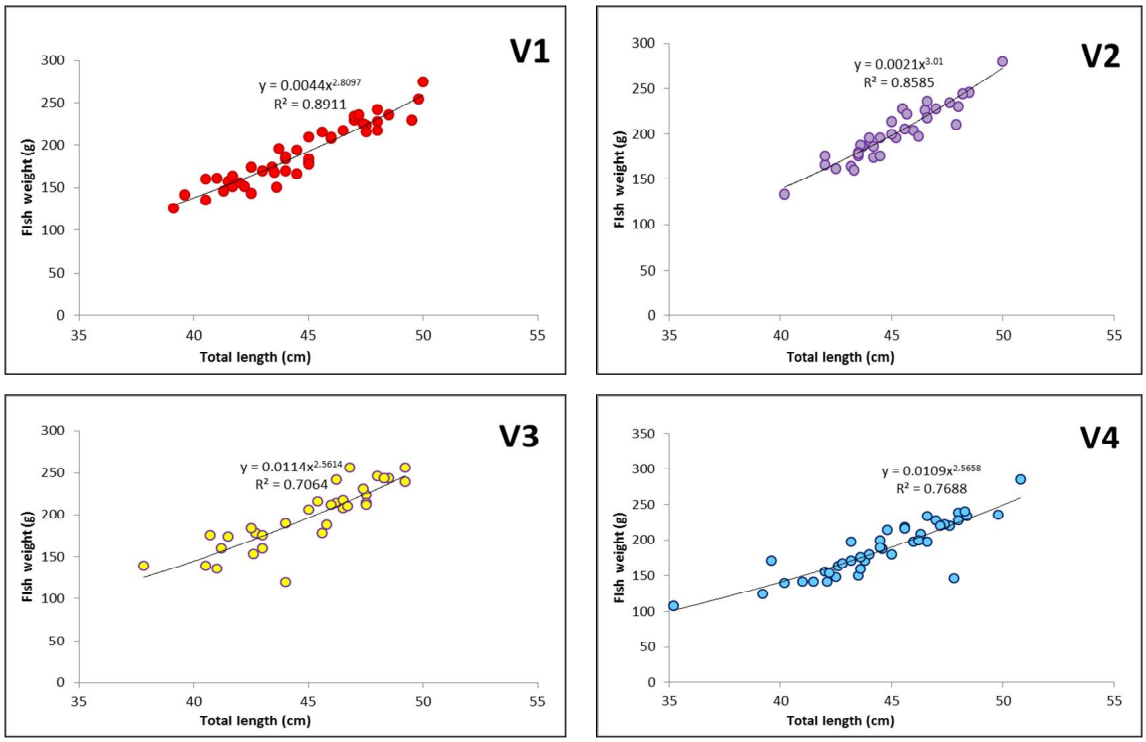


Figure 3. Length-weight regression of all four experimental variants, at the end of the experiment.

By analyzing the allometric coefficients, it can be observed a negative allometry (b < 3) for V1, V3, V4 groups, while in V2 the biological material condition status registered a positive allometry (b = 3.01), fact confirmed also by the technological indicators obtained.

Considering the $r^2$ results, a good correlation between total length and body weight was observed, fact that indicates the proportion on which the obtained weight gain can be attributed to the increase of length. By analysing the allometric coefficient, it can be seen a negative allometric scaling (b < 3) for V1, V3 and V4 experimental variants, while in case of V2, a positive allometric scaling (b = 3.01) is observed. This reveals a better condition for *A. stellatus* juveliles reared in V2, comparing with other experimental variants, fact also confirmed by the technological indicators values, higher in case of this experimental variant.

***Meat biochemical composition analysis***. Table 4 presents the results obtained on the biochemical composition of *A. stellatus* juvenile, both at the beginning and at the end of the experimental period.

A statistically significant increase of protein content (p = 0.000465) can be observed in all four experimental groups. The highest values were recorded in case of group V2 (15.60±0.69%), while group V3 registered the lowest value of protein (14.45±0.23%).

The water content of *A. stellatus* meat registered a significant decrease (p = 0.000569), comparing final values, registered at the end of the experiment, with the initial values, registered at the beginning of the experimental period. No statistically significant differences were found between the experimental variants (p > 0.05) in terms of meat crude protein content, lipids and also ash, while by analyzing meat water content, significant differences among the four experimental groups were obvious (p = 0.0051).

Table 4

The biochemical composition of *A. stellatus* meat

| *Composition %* | *Initial* | *Final* | | | |
|---|---|---|---|---|---|
| | | *V1* | *V2* | *V3* | *V4* |
| Ash | 1.10±0.11 | 1.10±0.01[ac] | 0.99±0.01[ac] | 1.06±0.01[ac] | 1.20±0.23[ac] |
| Lipids | 1.70±0.47 | 4.51±1.37[ac] | 3.49±1.14[ac] | 5.50±0.77[ac] | 2.58±0.02[ac] |
| Proteins | 12.07±0.9 | 15.55±0.38[ad] | 15.60±0.69[ad] | 14.45±0.23[ad] | 15.37±0.88[ad] |
| Water | 84.93±0.21 | 70.91±1.54[bd] | 70.94±0.15[bd] | 76.54±0.73[bd] | 75.44±0.06[bd] |
| Water/Protein | 7.05±0.51 | 4.56±0.21 | 4.55±0.21 | 5.30±0.14 | 4.92±0.29 |
| Protein utilization efficiency-PUE | - | 8.14±0.77 | 8.62±1,02 | 7.26±0.21 | 7.72±1.21 |

a - not significant differences between the experimental variants, p > 0.05; b - significant differences between the experimental variants, p < 0.05; c - not significant differences between the experimental variants, p > 0.05; d - significant differences between the experimental variants, p < 0.05.

In order to assess the nutritional value of *A. stellatus* juveniles meat, the percentage of water (A) and fish muscle tissue protein content (P) were calculated as a ratio (A/P). Because this report is not influenced by the fat content, is considered the most valuable chemical indicator for evaluating the quality of meat protein (Mocanu 2011).

Thus, by analysing the A/P ratio, it can be stated that a higher protein value was observed in case of V2 (4.54) and V1 (4.55) variants, compared with V4 (4.90) and V3 (5.29).

In order to assess the nutrient retention efficiency of *A. stellatus* juvenile, the following main indicators were calculated: PUE, PER, retained protein (RP) and retained lipids (RL).

The data presented in Table 4 reveal that in case of V2 experimental variant it can be observed a high meat protein and fat accumulation degree. Also, the evolution of PER and PUE emphasize the fact that protein registered a better valorification in V2 variant, where the highest value of total biomass was registered (201.03 g individual$^{-1}$), as the end of experimental period.

In conclusion, taking into consideration the obtained results, it can be stated that the use of thyme in a concentration of 1% and vitamin E (500 mg kg feed$^{-1}$) as dietary supplements for growing *A. stellatus*, has led to an improvement of meat biochemical quality composition, due to a significant decrease in the percentage of water, respectively increasing the percentage of protein, fact that reveals a better meat nutritional value.

**Conclusions**. Although, no significant changes in fish growth and feed utilisation were observed between the four groups, it is recommended that an adequate vitamin E and thyme dietary supplementation to be applied for rearing *A. stellatus*, in order to achieve a better meat quality level. Thus, based on the obtained results, it can be recommended the use of thyme in combination with Vitamin E (500 mg kg feed$^{-1}$) in *A. stellatus* diet, in order to obtain positive results on growth performance, feed conversion, nutrient utilization and protein efficiency.

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