Ph.D. Thesis

THE APPLICATION OF SELENIUM AND MAGNESIUM ON THE LARVAL AND FRY REARING OF THE RED DRUM *(SCIAENOPS OCELLATUS* L.*)*

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1.   Introduction, objective

The demand for fish meat is continuously growing in the world, but it is increasingly difficult for the marine and freshwater fish stocks – decreasing mostly because of overfishing – to cover this need. This situation can only be solved by the dynamically developing aquaculture, which is well demonstrated by the fact that the share of thus produced fish in the total fish production of the world is increasing from year to year. The EU, recognizing the potential of the sector, plans to significantly increase the output of its aquaculture in the coming years, to which – because of its favourable conditions – Hungary can contribute considerably. The National Aquaculture Strategic Plan of Hungary, which was approved in 2015, envisages a 25% increase of Hungarian aquaculture production by 2023. One of the solutions for this capacity increase could be the establishment of new, intensive fish farms.

Recently, there have been several attempts in European (and Hungarian) fish farming to introduce exotic fish species with high economic potential into production. Considering the significant thermal water resources of Hungary, highly saline groundwaters could be perfectly suitable for the production of warmwater marine and euryhaline fish species, which have unlimited market potential on foreign markets. The characteristics of red drum *(Sciaenops ocellatus* L.) make it ideally suited to introduction to intensive aquaculture, but its appearance in domestic production requires the development of fry rearing and feeding technologies of the species adapted to Hungarian conditions.

Based on the above, the main objective of my Ph.D research was to develop a relatively easily produced enriched live feed and feed additive with a positive effect on the health and production parameters of the fish, which would make the production of red drum safer in Hungarian conditions (among others).

During fry rearing of fish, it is extremely important to keep mortality low. A solution for this could be the selenium enrichment of live and pelleted feeds, as the trace element taken up this way can help maintain the health of the fish and significantly contribute to a favourable development of production parameters. Consequently, we were looking for an answer to the question, whether the selenium enrichment of zooplankton and the pelleted feed is possible and whether it can positively affect the production parameters of red drum larvae and fry. During the evaluation of the experiments, we also tried to determine the optimum selenium quantities for larvae and fry. As, of all microelements, selenium has the narrowest margin between essential and toxic concentrations, we also considered it important to study the toxic effect of the used selenium preparation (nano elemental selenium) during our studies.

During the experiments, we were faced with the problem that the magnesium content of the fish rearing system water did not fully correspond to the needs of red drum, and thus, the maintenance of adequate health conditions required continuous magnesium supply. Macroelement supply by dissolving crystalline magnesium chloride in the water was found to be rather costly and cumbersome, and thus, we were also interested whether the magnesium need of red drum fry could be met by Mg enrichment of the feed.

2.   Materials and methods

2.1. Nanoselenium enrichment of *Artemia* sp.

Zooplankton occurring in the nature contains significantly higher trace element contents than when it is reared artificially, and thus, in fish production, it is advisable to enrich the live feed of fish larvae with some microelements. In our experiment, we studied the enrichment of *Artemia* sp. with nano elemental selenium.

Artemia sp. eggs were incubated for 24 hours in 5-liter plastic vessels at a density of 2 g eggs per liter. During the incubation, the temperature of water, which had a salinity of 20 g/l, was continuously kept at 28 ± 1 ^{o}C, the aeration was done using airstones, the illumination was continuous. After incubation, *Artemia* nauplii were collected with a 150 μm mesh plankton net and separated from the shells and unhatched eggs. The freshly hatched brine shrimp were enriched in the nanoselenium solution during a 24-hour incubation time, with 6 treatments *(1 mg/l; 5mg/l; 10 mg/l; 50 mg/l; 100 mg/l; 500 mg/l)* in addition to the control and three replications per treatment. The total selenium content was determined using Hydride Generation Atomic Fluorescent Spectrometry (HG-AFS).

2.2. Study of nanoselenium-enriched Artemia sp. in a feeding experiment with red drum larvae

The experiment was conducted in 15 separate 40-liter aquaria (with a stocking density of 70 fish per unit) in a randomized block design, where the individual treatments *(control, 1 mg/l enrichment, 5 mg/l enrichment, 10 mg/l enrichment, 50 mg/l enrichment)* were set up in three replications. The fish were kept in 15 ppt salinity water where 1 g analytical-grade magnesium chloride was dissolved per liter in order to obtain the Ca : Mg ratio of 1 : 3, which is optimal for fish. Individual water heaters were used to keep a constant water temperature of 27 ± 0.8 ^{o}C during the experiment. The photoperiod during the study was 12 hours light / 12 hours darkness. Fish were fed live feed *ad libitum* three times a day. The duration of the experiment was 9 days, after which, sample collection for further studies was done following a 24-hour starving period.

The different production parameters were calculated on the basis of the following formulae.

- Survival (%): **S = (number harvested /number stocked) × 100;**
- Specific growth rate (% / day): **SGR = (lnW$_f$ − ln W$_i$)/ t × 100;**

where W$_f$ = body weight at harvesting (g),

$W_{i:}$ = body weight at stocking (g),

t = number of days.

The condition factor of the fish larvae was calculated according to Wakeman and Ramsey (1985).

- Condition factor: $\mathbf{K = W \times 100 / SL^3}$

where W = wet weight (g),

SL: standard length (cm).

After harvesting the fish, 5 larvae were randomly chosen from each aquarium (15 ind./treatment) for morphological studies, during which, the body weight and standard length of the fishes were individually measured.

For the study of the enzyme activity of glutathione peroxidase, 6 samples were taken from each aquarium (18 ind./treatment), which were placed into liquid nitrogen immediately after harvesting, thus stopping all vital processes. The enzyme activity of glutathione peroxidase was determined according to Sedlak and Lindsay (1968).

- $\mathbf{E\ (\mu mol/g/min.) = A_k - A_m / Mol.\ abs.\ coefficient \times 10^6 / total\ protein \times 5 \times 10^3 / t}$;

where A_k = control

A_m = sample

Molar absorption coefficient: 131000 (1/Mcm)

Total nitrogen: (g/l)

t: incubation time, 10 minutes

10^6: conversion factor between mole and micromole

5×10^3: conversion factor between the initially determined total protein concentration (g/l) and the total protein concentration (g) in the 200 µl sample used in the assay.

The determination of the accumulated selenium content was done using the Hydrid Generation Atomic Fluorescence Spectrometry (HG-AFS) method.

2.3. Study on the feeding of nursed fry of red drum with nanoselenium-enriched pelleted feed

All commercially available fish feeds contain approximately the same quantities of selenium, while the selenium demand of individual species is different.

The positive effect of nanoselenium on red drum fry was also studied. A commercially available feed with 42 % protein and 22 % fat content was used to prepare the experimental feed. Based on analytical measurements, its original selenium content was 0.46 mg/kg in the form of organic selenium (selenomethionine). The feed was ground to powder and enriched

using nanoselenium suspension. After homogenisation and addition of 5% water, it was pelleted once again using a pelleting machine with a 2.5 mm sieve matrix. The red drum used in the experiment were stocked into a larval and fry rearing recirculating system with a water volume of 1.7 m^3 (18 round tanks with a volume of 70 liters each) one week before the beginning of the experiment for acclimatization purposes. 540 fish with average weight of 3.5 ± 0.1 g were stocked (30 fish/tank). In addition to the control group, 5 treatments *(1 mg/kg Se, 1.5 mg/kg Se, 2.5 mg/kg Se, 5.5 mg/kg Se, 10.5 mg/kg Se)* were set up in three replications. During the 8-week experiment, the temperature of the 5 ppt salinity water was continuously kept at 25 ± 1°C with the help of electric heaters placed into the buffer tank. Continuous aeration was done using a central air blower, through airstones. The photoperiod was 12 hours light / 12 hours darkness. Feeding was done *ad libitum*, 4 times a day, feed consumption was continually registered in order to be able to calculate the feed conversion ratios.

In order to accurately monitor the growth, each fish was measured to the nearest 0.1 g on a digital scale once a week, mortalities were registered daily.

Production parameters were calculated on the basis of the following formulae:

- Survival (%): **S = (number harvested /number stocked) × 100**

- Feed conversion ratio (g/g): **FCR = F / (W_f − W_0)**

where F = total weight of the feed (g)

W_0 = initial body weight (g)

W_f = final body weight (g).

- Specific growth rate (% / day): **SGR = (lnW_f − ln W_i)/ t × 100**;

- where W_f = body weight at harvesting (g),
- W_i = body weight at stocking (g),
- t = number of days.

- Weight gain (%): **WG = (W_f − W_0) / W_0 × 100**

where: W_f = body weight at harvesting (g)

W_0: body weight at stocking.

For the selenium analysis, 4 fish were taken from each aquarium after a 24-hour starving period applied in order to empty the intestines of fish fry. Then the liver, eyes and fillet of the fish were removed as they were studied separately. The samples were stored deep-frozen (< -30 °C) until the beginning of the digestion.

The determination of the total selenium content was done using the Hydrid Generation Atomic Fluorescence Spectrometry (HG-AFS) method.

For the determination of the free fatty acid content, 3 samples were taken from each tank, which were stored deep-frozen ($< -30\ ^oC$) until the analyses. During the studies, the total fat content was determined by extraction, while free fatty acids were analysed using the liquid chromatography (HPLC) method.

2.4. Study of a magnesium-enriched pelleted feed in a feeding experiment with red drum fry

The water used in the studies did not contain sufficient magnesium for the red drum (Ca^{2+}: 150.02 mg/l, Mg^{2+}:147.7 mg/l), and therefore, its supplementation through feeding was attempted in our experiment. The basic feed mix was prepared on the basis of an own formula by homogenization of several ingredients, then it was pelleted in a flat die pelleting machine on a 2.5 mm matrix. The analysis of the element composition of the basic mix determined that 1 kg of the control feed contained 254.73±14.21 mg magnesium, which was further referred to as 250 mg for ease of calculation. Magnesium chloride hexahydrate ($MgCl_2 \times 6H_2O$) was used to enrich the feed with magnesium. The following treatments were set up in three replications: *100, 200, 300, 400 mg Mg/kg*.

The experimental stock was placed into a larval and fry rearing recirculating system with a water volume of 1.7 m^3 (15 round tanks with a volume of 70 liters each) one week before the beginning of the experiment for acclimatization purposes. 225 fish with average weight of 14.56 ± 0.30 g were stocked (15 fish/tank). During the 8-week experiment, the temperature of the water was continuously kept at $25 ± 1^oC$ with the help of electric heaters placed into the tanks, the salinity was set at 5 ppt. Continuous aeration was done using a central air blower, through airstones. The photoperiod was 12 hours light / 12 hours darkness. Feeding was done *ad libitum*, 4 times a day, feed consumption and mortalities were continually registered.

The studied parameters (K factor, S, SGR, FCR, WG) were determined using the calculation methods shown above. FCR was calculated on a weekly basis from the feeds consumed, taking into account the mortalities. When calculating the feed conversion ratio, the biomass was corrected with the weight of the dead fish each week because of the high mortalities found in certain treatments (Li-Robison, 2012).

For the element analysis 3 fish were randomly chosen from each tank. Their backbone and fillet were removed and their magnesium content was determined using the ICP-MS method.

2.5. Statistical evaluation of the data

Statistical evaluation of the data was performed using the programs *Microsoft Excel 2013* and *SPPS for Windows 20.0*. The results were evaluated by using one-way analysis of variance. For determining the relationships between the studied factors, Pearson's correlations were calculated and regression analysis calculations were also carried out.

3.   Results

3.1. Nanoselenium enrichment of Artemia sp.

At the 500 mg/l dosage, all brine shrimp died by the end of the 24-hour enrichment period, as selenium was already obviously toxic to *Artemia* at this concentration, and therefore, this treatment was not applied further. The significant effect of Se enrichment was excellently demonstrated by analysis of variance, as the trace element concentrations accumulated in the zooplankton showed statistically significant differences ($p<0.05$) between all treatments. It was interesting to observe that the highest accumulation of the element was found in the 50 mg/l treatment, while a significant decrease was experienced in the highest enriching treatment (100 mg/l). Most probably, the 100 mg/l concentration, similarly to the 500 mg/l treatment, was toxic to the zooplankton but the effect was not strong enough to kill the brine shrimp during the 24-hour enrichment period. Still, their activity decreased, and therefore, the expected selenium accumulation did not take place.

Table 1: Effect of the treatments on the Se accumulation by *Artemia* sp. at the end of the 24-hour enrichment period.

Treatment (mg/l)	Se (mg/kg dry weight)		SD
0	0.0002 [a]	±	0.000
1	0.670 [b]	±	0.002
5	3.788 [c]	±	0.055
10	6.340 [d]	±	0.037
50	26.914 [e]	±	0.153
100	4.740 [f]	±	0.066
500	-		-

Significant differences were observed between the results marked with different letters in the table columns ($p<0.05$)

3.2. Study of nanoselenium-enriched Artemia sp. in a feeding experiment with red drum larvae

Feeding of *Artemia* sp. with increasing nanoselenium concentrations improved the viability of larvae, even though only the 10 mg/l treatment differed significantly from the control group. Here, 69 % survival was experienced. Nanoselenium applied at concentrations higher than 10 mg/l already had negative effects on the health of the larvae. The nanoselenium enrichment of the live feed had favourable effects on the weight gain as well. The average body weight was 0.095 g in the control group. Up to the 5 mg/l concentration (average weight: 0.140 g), body weights showed an increasing trend, but a statistically significant difference was only

found between the control group and the 5 mg/l treatment. A considerable decrease was experienced in the 50 mg/l group, and thus, it can be stated that this concentration was toxic to the larvae.

The body length of the fish larvae ranged between 14.6 mm (50 mg/l) and 18.9 mm (5 mg/l), depending on the treatment. These two values differed significantly. In terms of body length – similarly to body weight – the 5 mg/l and the 50 mg/l treatments were found to be the best and the worst, respectively. The latter is explained by the toxic effect of the excessive selenium uptake. The selenium enrichment of live feeds improved the specific growth rate (SGR) of red drum larvae. The most favourable SGR (24.52 %) was experienced in the 5 mg/l treatment, which was found to be significantly better than the value obtained for the control group (20.22 %). Application of Se dosages above 5 mg/l considerably decreased the SGR, the 50 mg/l treatment already affected the larvae negatively (15.77 %).
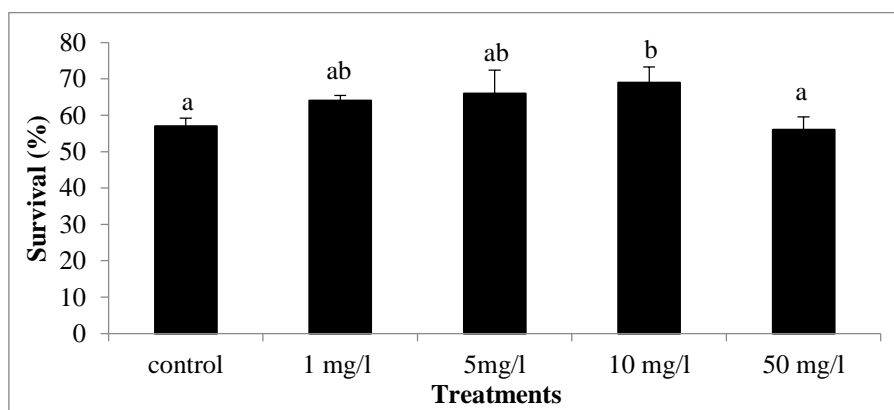

Figure 1: Survival of red drum *(Sciaenops ocellatus)* larvae

There is significant difference between the results marked with different letters (Tukey HSD, p <0,05)

Table 2: Effect of nanoselenium-enriched live feeds on growth indices of red drum
(Sciaenops ocellatus) larvae

Treatments	Weight (W) (g)	SD		Standard length (SL) (mm)	SD		K factor	SD		SGR (%)	SD	
control	0.095 [ab]	±	0.005	16.7 [ab]	±	0.5	2.03 [NS]	±	0.29	20.22 [a]	±	0.64
1 mg/l	0.117 [a]	±	0.007	17.3 [ab]	±	0.1	2.30 [NS]	±	0.20	22.29 [ab]	±	0.86
5 mg/l	0.140 [a]	±	0.018	18.9 [a]	±	0.8	2.07 [NS]	±	0.12	24.52 [b]	±	1.56
10 mg/l	0.106 [ab]	±	0.034	17.0 [ab]	±	2.1	2.12 [NS]	±	0.11	22.34 [ab]	±	1.98
50 mg/l	0.058 [b]	±	0.006	14.6 [b]	±	0.2	1.78 [NS]	±	0.26	15.77 [e]	±	1.24

There is significant difference between the results marked with different letters within the same column (Tukey HSD. p <0.05); NS = not significant

Values of the condition factor (K) exhibited a trend similar to the previous two indices, but the positive or negative effect of the selenium enrichment of the feed could not be proven statistically. We did not find statistically significant differences among the treatments in the enzyme activity of glutathione peroxidase either.
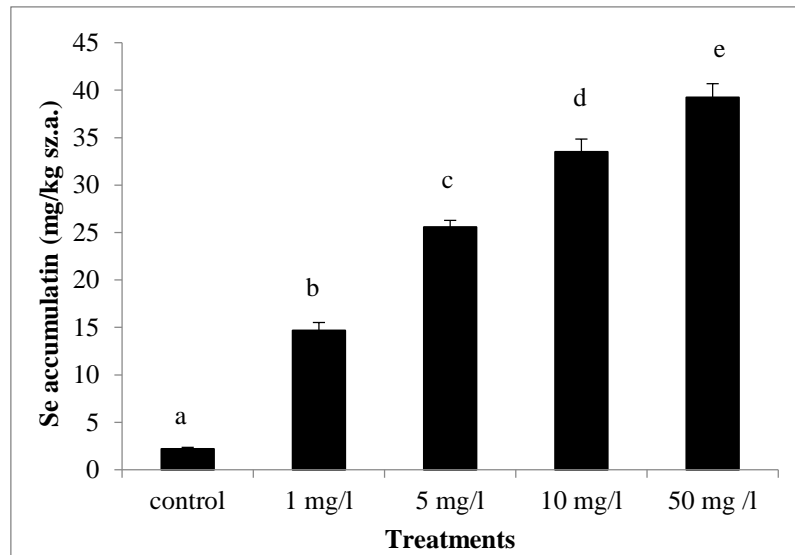

Figure 2: Selenium accumulation of red drum *(Sciaenops ocellatus)* larvae

There is significant difference between the results marked with different letters (Tukey HSD p <0.05)

The selenium accumulation by the larvae ranged between 2.19 *(control)* and 39.24 mg/kg *(50 mg Se/l)*. The differences between all treatments were significant, which demonstrated that the selenium uptake by red drum larvae from the nanoselenium-enriched live food was successful.

Correlation analysis was also performed between the treatments and individual measured parameters, which showed a moderate positive correlation *(r=0.578)* between the body weight and survival of the fish larvae, i.e. the bigger body weight resulted in better health and, therefored in better survival. SGR was in positive correlation with all studied parameters. Growth rate was in close correlation with the survival *(r=0.782)*, body weight *(r=0.863)* and body length *(r=0.792)* of the fish larvae. SGR had a moderate correlation with the condition factor *(r=0.610)* and the enzyme activity of glutathione peroxidase *(r=0.532)*. There was a close positive correlation *(r=0.765)* between the quantities of selenium accumulated in the *Artemia* and in the larvae, which is an excellent proof of the incorporation of this trace element from the brine shrimp into the body of the larvae.

During the evaluation of the experiment results, we were also looking for an answer to the question whether the optimum quantity of nanoselenium for red drum larvae could be

determined from the available data. This was calculated using regression analysis. Based on the regression of trend functions, a very close correlation was found between the studied parameters and selenium application (body weight: $R^2=0.9789$; standard length: $R^2=0.9911$; SGR: $R^2=0.9694$). The Se quantity where the function culminates was calculated by derivating from the trend function formulae. This maximum was 5.25 mg/l for body weight, 5.22 mg/l for body length and 5.69 mg/l for SGR. The regression of the trend functions showed a close correlation between survival and selenium treatments ($R^2=0.8646$).
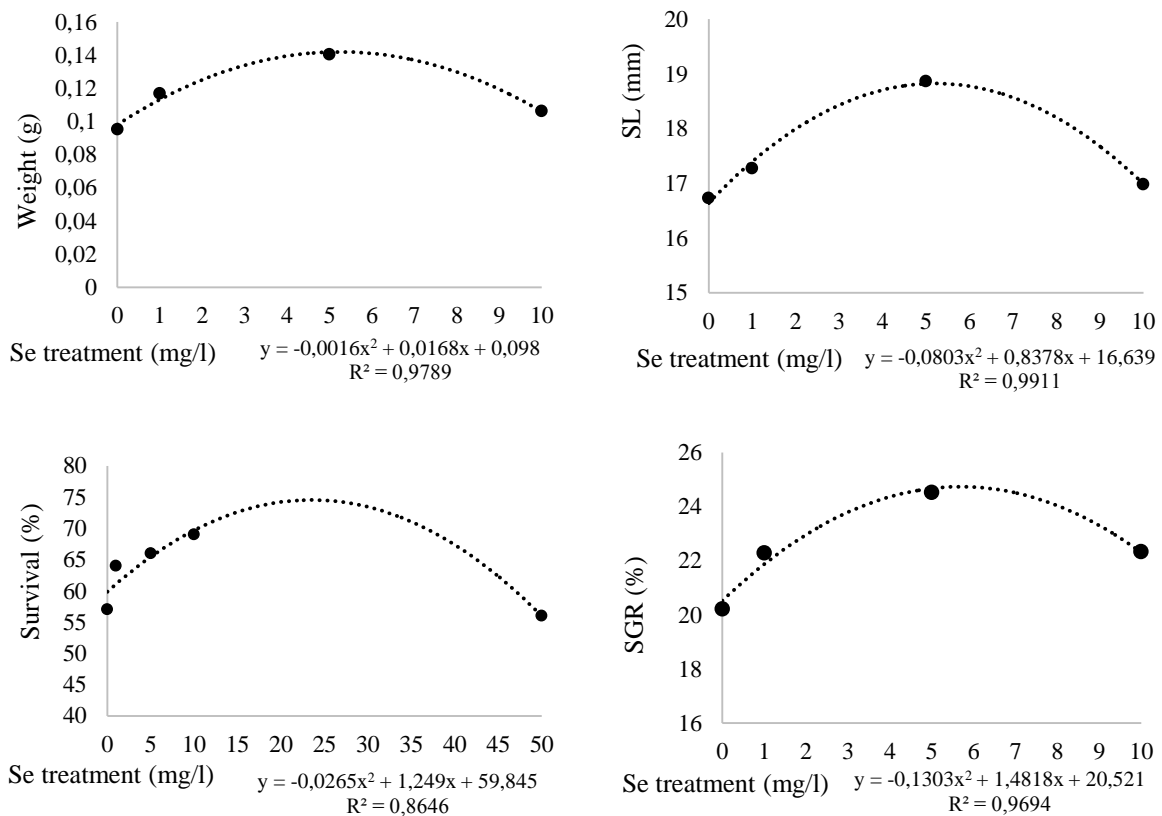

Figure 3: Determination of the selenium optimum of larvae

3.3. Study on the feeding of nursed fry of red drum with nanoselenium-enriched pelleted feed

The average body weight of the fish was significantly lower in the 10.5 mg/kg treatment than in the control group, which was probably caused by the toxic effect of the selenium. During the study of the fry biomass, too, a significantly lower value was obtained in the 10.5 mg Se/kg treatment (589 g), while the biomasses in other treatments were similar to the control group (722-767 g). As a result of nanoselenium supplementation, survival was significantly better in the 1, 1.5, 2.5 and 5.5 mg/kg treatments than in the control group (90 ± 0 %) and the 10.5 mg/kg group (85 ± 2 %), while there were no significant differences between the other

groups. Cannibalism or considerable size variability were not experienced in any of the treatments.

The specific growth rate (SGR) showed a decreasing trend as a result of increasing selenium supplementation dosages. This value was 3.43 % in the 10.5 mg/kg treatment, which was significantly worse than the value calculated for the control group (3.7 %). The feed conversion ratio (FCR) of the fry was very favourable during the experiment, its values ranged between 0.77 – 0.90 g/g depending on the treatment. The FCR calculated for the 10.5 mg/kg concentration was significantly different from that of the control group – and all other treatments –, which suggested toxicity of the applied selenium dosage. The best FCR was found in the 1.5 and 2.5 mg/kg groups. However, the feeding of nanoselenium-enriched feed did not significantly affect the weight gain (WG) of the fry. The selenium content of the rearing water was continually monitored during the experiment but it did not change measurably, and thus, it can be stated that there was no cross-contamination between the individual treatments, i.e. the obtained results were due to the different selenium content of the feed.
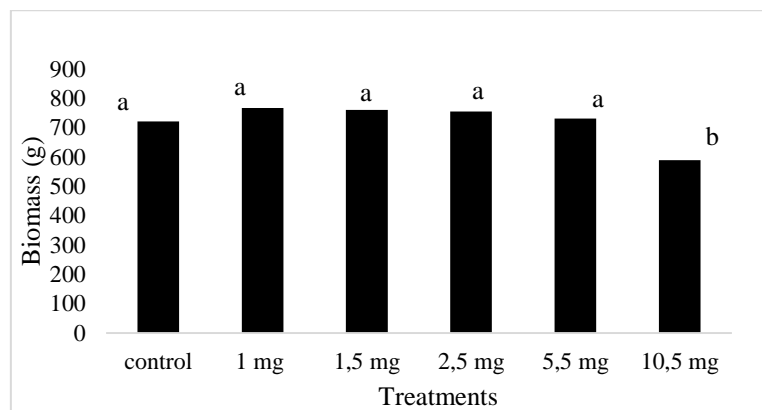

Figure 4: Effect of nanoselenium supplementation on red drum biomass

There is significant difference between the results marked with different letters (Tukey HSD, p <0.05)

Table 3: Study of the growth, feed utilization indices and the survival

Treatments	SGR (%)	SD	FCR (g/g)	SD	WG (%)	SD	S (%)	SD
control	3.70 [a]	±0.16	0.80 [a]	±0.04	665 [NS]	±66	90 [a]	±0
1 mg Se/kg	3.64 [ab]	±0.09	0.79 [a]	±0.06	638 [NS]	±36	98 [b]	±2
1.5 mg Se/kg	3.67 [ab]	±0.08	0.77 [a]	±0.04	653 [NS]	±35	97 [b]	±5
2.5 mg Se/kg	3.65 [ab]	±0.07	0.77 [a]	±0.01	645 [NS]	±28	98 [b]	±2
5.5 mg Se/kg	3.63 [ab]	±0.12	0.82 [a]	±0.01	635 [NS]	±50	97 [b]	±5
10.5 mg Se/kg	3.43 [b]	±0.22	0.90 [b]	±0.08	559 [NS]	±80	85 [a]	±2

There is significant difference between the results marked with different letters within the same column (Tukey HSD, p <0.05) NS= not significant

We also wanted to know how much selenium accumulated in the different organs of the fry, and therefore, we studied the liver, flesh and eye of the fish. Selenium was not stored uniformly in fish organs, the highest levels were measured in the liver (3.38 to 14.37 mg/kg depending on the treatment), significantly less was measured in the eye, while the lowest quantities of the microelement were accumulated in the fish flesh. Probably only the selenium that could not be stored in other organs was found here. The liver, as an important organ of storage, stored 171 % and 356 % more selenium than the fillet in the control group and the 10.5 mg Se/kg treatment, respectively. The values measured in the eye showed a trend similar to that in the liver but the quantity of trace element stored there was significantly lower, ranging between 2.09 and 7.91 mg/kg. In the fillet, the highest selenium levels were measured in the 10.5 mg/kg group; these were significantly higher than the values measured for the other treatments.
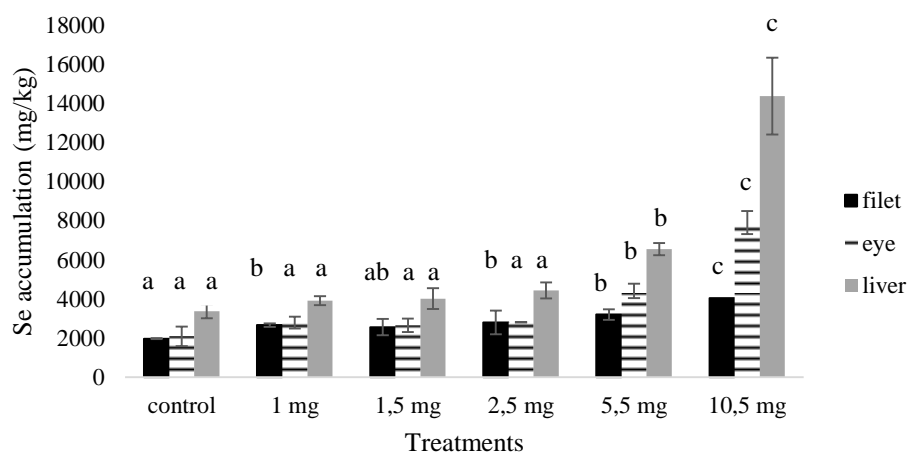

Figure 5: Selenium accumulation in different organs of the red drum resulting from the treatments

Significant differences between the bars belonging to the same data set are indicated by different letters (Tukey HSD, p <0.05)

Free fatty acids, similarly to the selenium, take part in the antioxidant system of the organism, and therefore, at the end of the experiment, we studied whether the free fatty acid levels had changed in the fish as a result of the individual treatments. Significantly higher values than in the control group (72 mg/100 g) were measured in all treatments, the highest free fatty acid level (308.1 mg/100 g) was found in the 1 mg/kg group. The amount of ω3 and ω6 fatty acids

also increased and selenium supplementation also changed the ratio of ω3 and ω6 fatty acids: while their share was nearly equal in the control group. 71.5-74.4 % of the total free fatty acids consisted of ω3 fatty acids in this treatment.
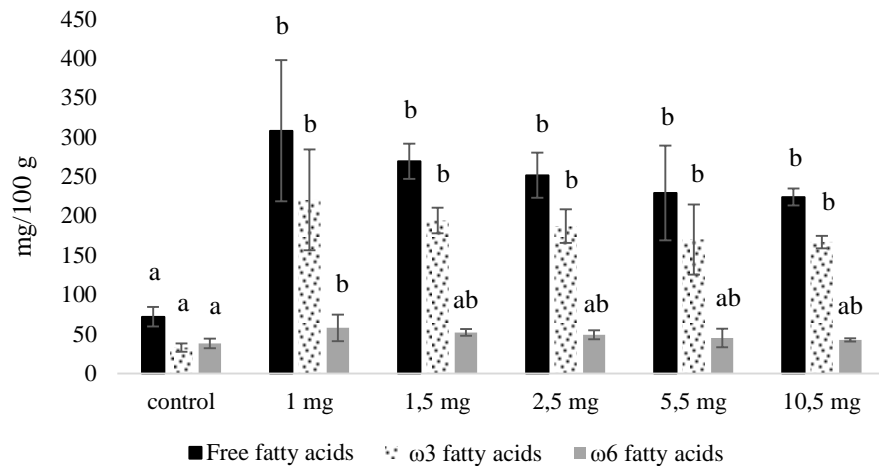

Figure 6: Total free fatty acid content and quantity of ω3 - and ω6 fatty acids within the free fatty acids

Significant differences between the bars belonging to the same data set are indicated by different letters (Tukey HSD, p <0.05)

In order to determine the optimum selenium quantity required during the fry rearing of red drum, regression analysis was done for some production parameters. Polynomial functions were fitted to the values calculated in the experiments and the optimum selenium level according to the given parameter was obtained at the culmination of the trend curve. Fry survival was significantly better than the control up to the 5.5 mg/kg treatment. Based on the regression of the trend function, there was a very close correlation between the survival rate and the selenium enrichment of the feed $(R^2 = 0.8696)$.

The Se quantity where the function reaches its maximum was calculated from the equation. In case of survival, this value was at 4.2 mg/kg. Accordingly, higher selenium content of the feed already had negative effect on fry survival.

The nanoselenium enrichment of the feed had a positive effect on feed utilization, but the FCR value calculated for the 10.5 mg/kg dosage was significantly worse. The best feed conversion was found in the 1.5 and 2.5 mg/kg groups, but the polynomial function allowed us to exactly determine the optimum selenium content of the feed. The lowest FCR was calculated for the 1.8 mg/kg concentration, higher trace element contents resulted in worse FCR values.

Biomass values were higher than the control up to the 5.5 mg/kg treatment, but the 10.5 mg Se/kg dosage was found to be toxic also in this parameter. Based on the regression of the trend function, there was a very close correlation between the biomass and selenium application *(R² = 0.9727)*, the equation showed that the biomass was the highest at 2.8 mg/kg selenium enrichment of the feed.
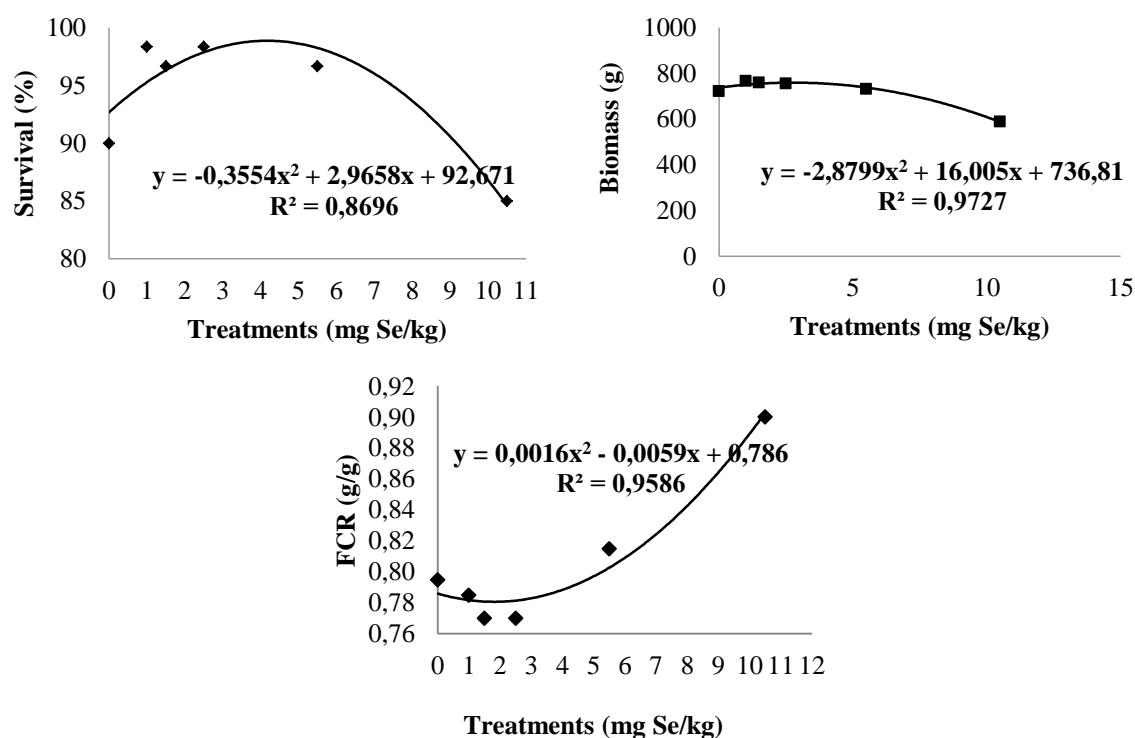

Figure 7: Determining the optimum selenium dosage for fry

The correlation analysis showed moderate negative to close negative correlation between the Se accumulated in the liver, fillet and eye, on the one hand, and the weight gain, on the other. Likewise, a medium negative correlation was also found between the survival and the Se content of the liver (r = − 0.685) and the eye (r = − 0.648).

Table 4: Study of the relation between the production parameters of the fry and selenium accumulated in the individual organs using the Pearson correlation

Studied parameters	Se content of liver	Se content of eye	Se content of fillet
Weight gain	-0.652(**)	-0.704(**)	-0.690(**)
Biomass	-0.800(**)	-0.811(**)	-0.599(**)
Survival	-0.685(**)	-0.648(**)	-0.295 (NS)
FCR	0.761(**)	0.797(**)	0.574(*)
SGR	-0.671(**)	-0.710(**)	-0.692(**)
WG	-0.662(**)	-0.703(**)	-0.695(**)

Se content of liver		0.988(**)	0.886(**)
Se content of eye			0.898(**)

(**) The correlation is significant on level SD $_{1\%}$

(*) The correlation is significant on level SD 5%

(NS) Not significant

Moderate positive to close positive correlations were found between the FCR and the Se content of the organs, i.e. the higher was the accumulation of the trace element, the worse was the feed conversion. The specific growth rate (SGR) of the fish was also in moderate to strong correlation with the Se accumulated in the different organs, the sign of the correlation was negative in all cases. A similar trend was experienced with WG (between the Se content of the liver and the WG: r = − 0.662; between the Se content of the eye and the WG: r = − 0.703; between the Se content of the fillet and the WG: r = − 0.695).

Based on the above, the direct effect of the trace element quantities accumulated in individual fish organs on the production parameters of the fry was proven. The results showed that the selenium demand of the nursed fry was mostly satisfied by the original selenium content of the manufactured feed.

3.4. Study of a magnesium-enriched pelleted feed in a feeding experiment with red drum fry

The highest average body weight was found in the 200 mg/kg treatment (42.06 ± 8.15 g), while the lowest was measured in the fry of the 400 mg/kg group (31.53 ± 7.90 g); however, there was no detectable statistically significant difference even between these two treatments. There were no significant differences among the weekly body weight measurements, either. The average weight measured in the individual treatments was relatively the same even at the end of Week 8. The study of the condition factor (K) of the fish did not reveal significant differences among the treatments, either; however, the K factors calculated for all groups (with the exception of the 400 mg/kg treatment) were higher than in the control group (2.36). The condition factor was the most favorable (2.76) in the fish of the 200 mg/kg treatment, but it was not statistically different from the rest. No significant differences were found in the specific growth rate (SGR) of the individual groups, either (1.34-1.70 %/day).

The SGR of the fry grew from the control group to the 200 mg/kg treatment, then a decreasing trend was experienced in the 300 and 400 mg/kg treatments. The values of the feed conversion ratio (FCR) ranged between 2.29 ± 0.20 g/g and 3.54 ± 0.09 g/g depending on the

treatment. The values of the 200, 300 and 400 mg/kg treatments were significantly better than those of the control group and the 100 mg/kg group.
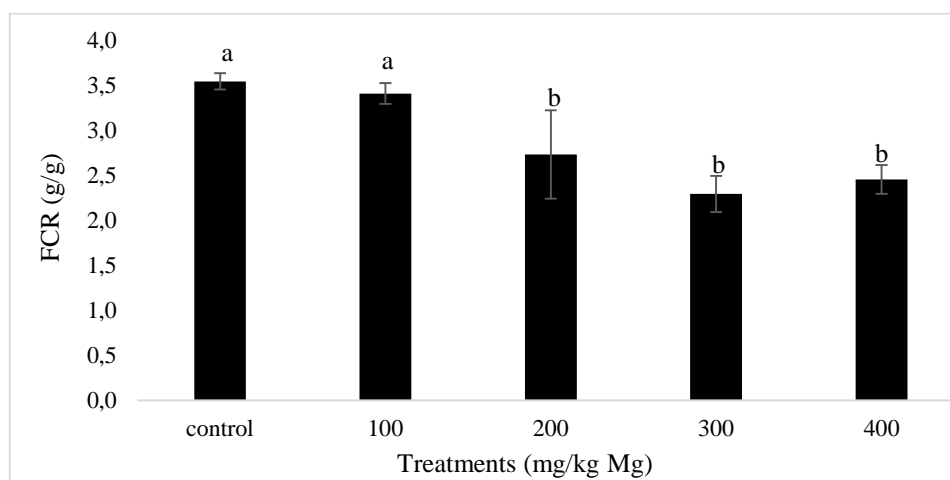

Figure 8: Effect of the treatments on the feed conversion of red drum

There is significant difference between the results marked with different letters (Tukey HSD, $p<0.05$)

The feed conversion ratio was the best (2.29 ± 0.20 g/g) in the 300 mg/kg group. It is important to note that the feed used in the experiment was prepared by us and its nutrient content was considerably worse than in commercially available professional feeds. This explains why the feed conversion ratio was worse than the $0.9 - 1.5$ values „customary" for such experiments. Studying the survival of the fish, it was found that the survival in all treatments was better than in the control group but this result could not be validated statistically. The number of harvested fish was the highest (73 ± 13 %) in the 300 mg/kg treatment, which was significantly higher than in the control group ($47 \pm 18\%$). The weight gain (WG) of the fish increased from the control group to the 200 mg/kg treatment, then decreased. The highest and lowest values were obtained in the 200 mg/kg treatment (191 ± 55 %) and in the 400 mg/kg treatment (115 ± 54 %), respectively. However, the differences in this parameter between the treatments were not statistically significant, either.

At the end of the experiment, 3 fish were collected from each tank for analysis of the element composition.

Table 5: Calcium and magnesium content of the studied fish (mg/kg d.w.)

Treatment	Ca flesh	SD	Mg flesh	SD	Ca bone	SD	Mg bone	SD
control	6083 [a]	±1624	2815 [a]	±878	123789 [a]	±4957	1957 [a]	±50
100	3676 [ab]	±238	2486 [a]	±172	86922 [ab]	±9077	1493 [a]	±144
200	2869 [b]	±647	2119 [a]	±37	65530 [b]	±5165	1238 [a]	±112
300	2449 [b]	±1091	2654 [a]	±22	89306 [ab]	±17482	1662 [a]	±344

| 400 | 2415 [b] | ±481 | 2996 [a] | ±847 | 106182 [ab] | ±39902 | 1770 [a] | ±561 |

There is significant difference between the results marked with different letters within the same column (Tukey HSD, $p<0.05$)

The study of fish fillets showed that the calcium content of fish flesh decreased with the increase of the magnesium content of the feed. No significant differences were found in the magnesium content of the flesh. The calcium content of the fish bones was significantly lower in all treatments than in the control group, while the magnesium content of the bones did not change significantly.

4. Novel scientific results

1. It was determined during my experiments than freshly hatched *Artemia* sp. can successfully accumulate nanoselenium in a 24-hour enrichment period. The increase of its concentration in the zooplankton was parallel to the enrichment concentration until the toxicity threshold. My results also proved that nano elemental selenium – albeit in dependence from the concentration – can have a toxic effect (> 50 mg/l) on *Artemia* sp. nauplii. On the basis of the results, it can be stated that the live feed enrichment technology presented in the thesis can improve the nutritional value of *Artemia* sp.

2. My results allow to conclude that feeding selenium-enriched *Artemia* sp. can have a positive effect on the production indices of red drum (growth, SGR, survival), and thus, can clearly be applied with success in the fry rearing of red drum. Based on the results of the experiment, the optimum selenium content of the live feed for fry rearing of red drum was found to be between 3.94 and 4.30 mg/kg (on a dry weight basis). I proved that excessive nanoselenium administration was toxic to red drum larvae (50 mg/kg treatment; 27 mg/kg Se in *Artemia* dry weight).

3. The analytical results proved that red drum larvae could accumulate selenium from *Artemia* sp. in an almost directly proportional way in relation to the treatment.

4. The performed experiment proved that nanoselenium enrichment of the pelleted feed used in red drum fry rearing improved fish survival but did not significantly affect other production indices (FCR, SGR, WG). This was probably caused by the fact that the 0.46 mg/kg selenium content of the commercial feed satisfied most of the selenium requirement of red drum larvae. Taking into account the results obtained in the experiment, the toxic effect of nanoselenium administered in higher dosages (10.5 mg/kg) could be proven.

5. Analytical results showed that higher dosages of selenium application resulted in accumulation of most trace elements in the fish liver, but the eye of the fish was also an important storage organ. Based on the above, it can be stated that nanoselenium was not stored uniformly in the different fish organs, the accumulation in eyes are also significant.

6. I determined that nanoselenium-enriched feeds significantly increased the level of free fatty acids, in particular, fatty acids of the ω3 group, in the body of red drum, which could be an explanation for the excessive selenium uptake.

7. As a result of my research, it was determined that the optimum selenium content of *Artemia* sp. for the fry rearing of red drum was higher than the 0.25–0.7 mg/kg (d.w.) Se generally recommended for the fry of marine fishes, if the least toxic nano elemental selenium was used for the enrichment of brine shrimp.

8. I concluded during the research that the magnesium requirement of red drum fry reared in moderately hard water cannot be satisfied with the magnesium provided with the feed. In addition to values of the production parameters (S, WG, SGR), this is also corroborated by the magnesium and calcium levels measured from the flesh and bones of the fish.

5. Results applicable in the practice

1. Red drum is a euryhaline fish species of the coastal zone of Central America, whose current production is associated with marine water. It is clearly considered a new species of Hungarian aquaculture. However, as a result of the significant geothermal water resources of Hungary, its production – according to the diversification – may be successful in Hungarian conditions as well. This study contributes to making safer the larvae- and fry rearing of the red drum, but further research is required to develop the complex production technology – in addition to taking into account the economic aspects and market conditions.

2. In the 2014-2020 programming period, funding for introduction of new fish species into production is available in several measures *(2.1, 2.2, 2.4)* of the Fisheries Operational Programme of Hungary (hereinafter: MAHOP). Taking into account the special focus of MAHOP on supporting investments, in particular, establishment of fish production systems using innovative technologies, funding may also be available for developing the infrastructural background for introduction of the species into the aquaculture of Hungary.

3. Red drum is not reproduced in Hungary because of the lack of marine water, and thus, seed supply cannot be solved from own sources. Therefore, for the lack of suitable technology, producers have to import fry from abroad, which is expensive. The transportation cost can be reduced by buying the material for fattening in the smallest possible size (larvae or fry) as this allows transporting more fish per unit volume of water. Larvae need to be fed with live feed for at least 4 to 8 days after the transportation. It is a well-known fact that the microelement content of zooplankton living in the sea is much higher than that of artificially reared zooplankton, which is also valid for the selenium content of *Artemia* sp. This may result in worse survival and slower development of the fish.

4. This was proven by the experimental results presented in the thesis, as the results of feeding selenium-enriched feeds were not only positive in the larval stage, but the favourable effect of selenium enrichment of the feed was also proven in nursed fry.

5. The enrichment technology of live and artificial fish feeds presented in the thesis is simple, it does not require significant material investment or time from farmers, but its application can make red drum rearing safer in the life stages most critical in terms of mortality and development. Changing of the production parameters to more favourable

does not only mean safer fish rearing for farmers, but also saves costs (reduced mortality, better feed utilization, i.e. using less feed).

6. Red drum kept in freshwater is sensitive to the ion composition of water, and thus, according to our experiences, adequate ion composition of the rearing water is not only important for avoiding the osmotic stress after the transportation (Fehér, 2014), but it is important to maintain it throughout the entire rearing cycle. In our experiment, it was studied whether the magnesium requirement of red drum could be satisfied by adding the macro element to the feed instead of dissolving it in water. No conclusive result was obtained but the trends in the studied parameters suggested that magnesium enrichment of the feed may favourably affect the production parameters of red drum in an environment where the magnesium content of water is relatively low or the magnesium-calcium ratio is not adequate. Continuous adding of magnesium to water is very unfeasible on fish farms where fish is produced in hundreds of cubic meters of water. If the magnesium requirement of the fish can partly be satisfied by increasing the magnesium content of the feed, the producer may significantly decrease the costs.

7. Based on the above, it can be stated that the experiments performed during my PhD research contain several elements that, when applied in practice, may contribute to the introduction of red drum into Hungarian aquaculture.

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List of publications related to the dissertation

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1. Csorvási, É., Fehér, M., **Juhász, P.**, Stündl, L., Bársony, P.: Bioaktív takarmány-kiegészítők hatása intenzíven nevelt pontyivadék (Cyprinus carpio) termelési paramétereire.
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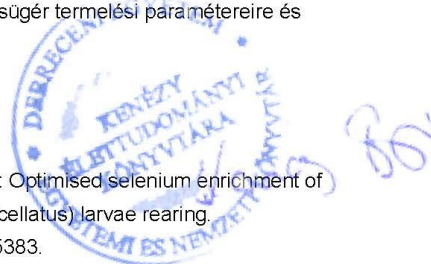
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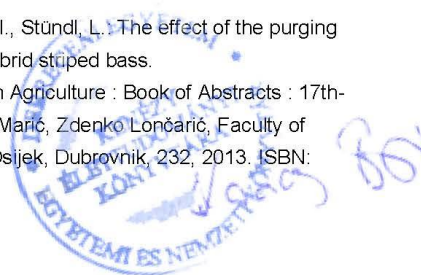
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Total IF of journals (all publications): 0,506
Total IF of journals (publications related to the dissertation): 0,506

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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