

ACCUMULATION OF SELENIUM IN RYE PLANTS (*SECALE CEREALE* L.) AT DIFFERENT STAGES OF DEVELOPMENT AND GRAIN QUALITY DUE TO SELENATE SOIL SUPPLEMENTATION

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Abstract. The accumulation of selenium by rye at different stages of development depending on the dosages of selenium and pH of soil was investigated. The findings testify that in case of increased selenium content in the soil selenium concentration in the plant also increased. Moreover, it was revealed that in the process of vegetation total selenium content in the rye increases; however the intensity of selenium accumulation by plants decreases. The positive effect of low concentrations of selenium on biomass accumulation of rye plants was found. The total protein content in rye grain was higher by application of selenium. The results of this work show the possibilities of applying of the seleniferous fertilizers for the increasing in the content of micronutrient in the grain plants. The recommended optimal concentrations of selenium additive are 0.01-0.05 mg kg⁻¹ of soil.

Keywords: *cereals, seleniferous fertilizers, microelement, yield, proteins*

Introduction

Rye (*Secale cereale* L.) is one of the most important cereals in middle, eastern, and especially north Europe. Among the cereals, rye can be grown under extreme climatic and poor soil conditions. On a global scale rye is a minor crop, its production being about 5% that of wheat or rice (Korzun et al., 2001). However, recently, interest in this crop has increased because of its dietary value. Rye is especially rich source of phytochemicals such as phenolic acids, lignans, alkylresorcinols and benzoxazinoids (Pihlava et al., 2018).

So far scientists do not know for certain whether selenium is an essential microelement for growth and development of plants. Some researchers admit necessity of selenium for plants others believe that selenium is not an essential element for normal life history of vegetative organism. However, there are a number of facts confirming positive influence of selenium on various life processes. Thus, it has been established that selenium increases antioxidant potential and stress-resistance of plants, stimulates their growth in the conditions of oxidation stress caused by UV-radiation (Hartikainen and Xue, 1999; Xue and Hartikainen, 2000; Pennenen et al., 2002; Germ et al., 2007; Golob et al., 2018), herbicides, hypothermia (Seppänen et al., 2003; Xu et al., 2003), drought stress (Nawaz et al., 2015a; Aissa et al., 2018), plant's ageing (Xue et al., 2001; Djanaguiraman et al., 2004, 2005), salt stress (Kong et al., 2005; Diao et al., 2014; Jiang et al., 2017). There has been noted increase of yield level of some plants further to application of low concentrations of selenium (Graham et al., 2005; Thavarajah et al., 2015; Nawaz et al., 2015b). Positive effect of selenium is also

revealed in increased germinating capacity and improved survival potential of plantlets in stress conditions (hypo- and hyperthermia, hypoxia, soil salinity and acidulation etc.) In the presence of selenium lipid membrane peroxidation processes in conditions of high light intensity and low temperatures are reduced, testifying to the effect that selenium has an influence on oxidation-reduction cell status (Zhu et al., 2009).

Selenium intake by plants from soil depends on many factors, for instance, on soil pH, humus content, chemical form of selenium (Zhao et al., 2005; Hawrylak-Nowak et al., 2015). Other important factor able to influence selenium quantity in plants is plant species. There are certain selenium accumulating plants, which accumulate from 100 to several thousand mg Se per kg of dry substance, in other plants including cereals selenium content is 0.01-1.00 mg kg⁻¹ on the average (Terry et al., 2000; Wangeline et al., 2011; Pilon-Smits, 2017). Lyons et al. (2005) showed that the main reason for variability in the content of selenium in some cereals (wheat, barley, rye) is the selenium concentration in the soil, but not genotypic variation. Similar findings were also obtained in studies with three varieties of wheat (Sharma et al., 2015).

Analysis of selenium accumulation pattern is very important both for study of biochemical role of this microelement for plants and to study application potential of selenium-containing fertilizers in order to increase selenium supply of animals and humans.

Purpose of this paper was to study selenium accumulation by rye (*Secale cereale* L.) at various ontogenesis stages subject to application of selenium additive to the soil and the impact of this on the yield and grain quality.

Materials and methods

Plant cultivation

The pot experiment was conducted in Kaliningrad, Russia (54°43'N, 20°30'E), from 12 April till 17 July, 2015, in a glass covered greenhouse of the Institute for Chemistry and Biology at Immanuel Kant Baltic Federal University. The greenhouse was not heated and we followed the natural light cycle, no additional lighting was supplied. Common rye (*Secale cereale* L.) cv. Pukhovchanka was used for experiments. The rye was grown in culture pots in permanent-set soil having pH 5.4 and pH 6.6. The dimension of pot was 29 × 20 cm (height × radius) and each pot contained 10 kg of soil. Selenium was applied in the soil in the form of a sodium selenate solution (Na₂SeO₄) in concentrations 0.01 mg, 0.05 mg, 0.1 mg, 0.5 mg (in terms of 1 kg of the soil). Selenium effect on the plants was compared to control treatment (without selenium supplementation). Initial selenium content in the soil was 0.084 mg/kg. Moreover, following basic fertilizers were applied: 0.17 g of nitrogen in terms of ammonium nitrate, 0.13 g of P₂O₅ in terms of potassium hydrophosphate, 0.28 g of K₂O in terms of potassium hydrophosphate and sulfate and 0.044 of MgO in terms of magnesium sulfate per kg of the soil. Solutions of fertilizers and sodium selenate were applied in such manner so that they cannot mix with each other. First in extreme points of soil surface square basic microelements were applied, whereas sodium selenate was applied in the central point. After the soil dried out it was mixed and the rye was planted. Into each pot were planted 20 plants. During experiments the plants were watered with distilled water.

The analysis of plant were performed at three plant growth stages (GS): at tillering stage (GS 26, main shoot and 6 tillers), booting stage (GS 45, mid-boot stage: boots just

visibly swollen) and milk development stage (GS 75, medium milk) according to Zadoks' scale (Zadoks et al., 1974).

Four replications for each treatment (selenium supplements, soil pH, and stage of development) were conducted. There were 120 pots in total.

Plant analysis

Selenium was determined by atomic absorption spectrometry (Unicam M Series, Software Solaar, Thermo Scientific) with mineralization of vegetative samples through autoclave decomposition under pressure (Kurkova et al., 2008).

The extraction of reserve proteins was performed by deionized water (for albumin), 5% NaCl (for globulin), 60% ethanol (for prolamin), and 0.4% NaOH (for glutelin). Protein concentration was determined according to Bradford, using BSA as the standard (Bradford, 1976).

Statistical analysis

Statistical analysis was performed using the SigmaPlot 12.3 (Systat Software GmbH, Erkrath, Germany). The mean of four independent samples were taken to represent the result of each replicate. The results were reported as mean \pm standard deviation (SD). To identify the difference in means the one-factorial ANOVA was conducted for each factor (selenium application, growth stage, pH of soil, organs of plant) separately. Difference among means were determined by Tukey's test at a significance level of $p \leq 0.05$.

Results

Accumulation of selenium in rye at different stages of development

In order to study selenium accumulation by the rye the plants were grown in the soil enriched with this microelement in concentrations 0.01–0.5 mg Se kg⁻¹ of soil. Findings on selenium content in vegetative specimen were compared to the plant without selenium supplementation. Initial selenium content in the soil was 0.084 mg kg⁻¹. Selenium was identified in above-ground part of the plant at three stages of vegetation. Results of study of relation between selenium content in all plant and quantity of selenium applied in the soil at different stages of the plant ontogenesis are shown in *Figure 1*.

The findings testify that in case of increased selenium content in the soil selenium concentration in the plant also increased. Thus, after application of 0.05 mg of selenium per kg of soil, 4.74 ± 0.06 μ g of selenium was identified in the plant (at milk development stage), while after application of 0.5 mg of selenium per kg of soil selenium content in the plant was almost 16 times higher – 74 ± 2 μ g. Moreover, it was revealed that in the process of vegetation total selenium content in the rye increases, however as it is obvious on the picture intensity of selenium accumulation by plants decreases.

In order to study intensity of selenium accumulation by the rye at different ontogenesis stages total selenium content in the plant was calculated per gram of vegetating mass. Dependence of selenium concentration in the plant on selenium concentration in the soil is illustrated in *Figure 2*.

The study shows that tillering stage was characterized by maximum selenium concentration, as the plant advanced intensity of its accumulation progressively decreased. Maximum selenium concentration in the rye at tillering stage was $59.3 \pm 0.4 \mu\text{g g}^{-1}$, at booting stage – $31.7 \pm 0.3 \mu\text{g g}^{-1}$ and at milk development stage – only $11.8 \pm 0.3 \mu\text{g g}^{-1}$.

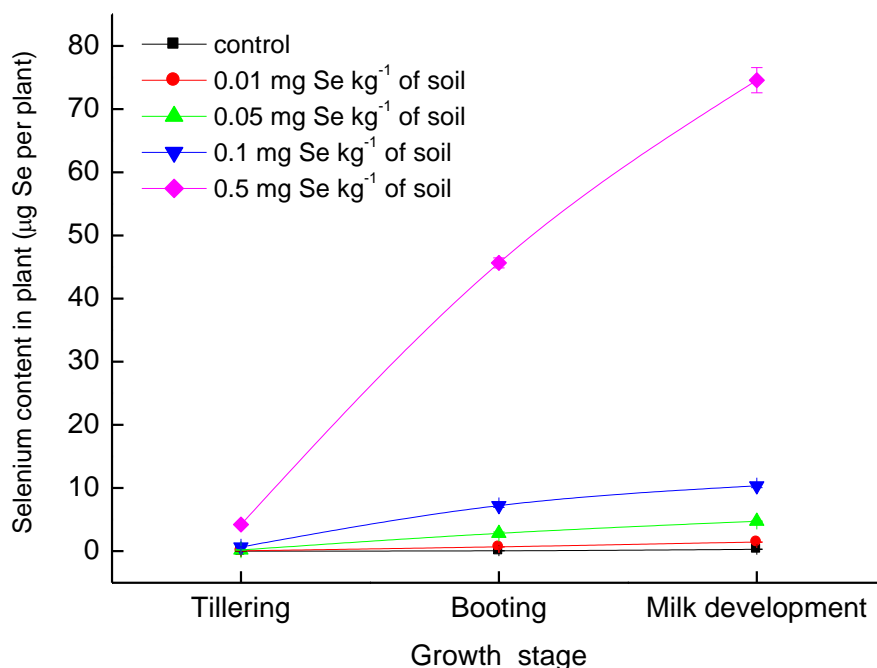


Figure 1. Selenium content in rye plants at different stages of ontogenesis in depending on the amount of selenium supplements (soil pH 6.6)

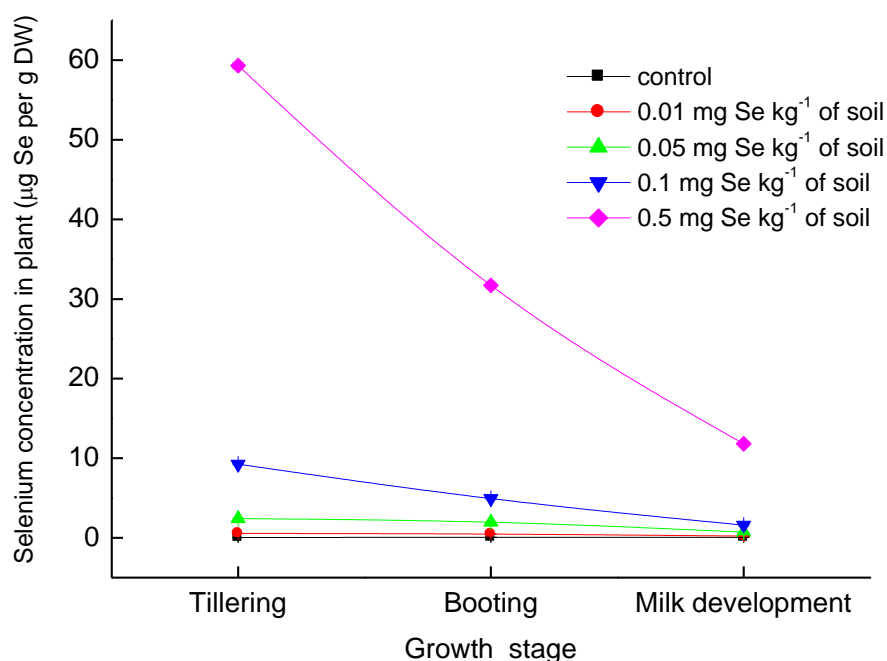


Figure 2. Selenium concentration in rye plants at different stages of ontogenesis in depending on the amount of selenium supplements (soil pH 6.6)

Influence of soil pH on the selenium accumulation in rye

As seen from the provided data, soil acidity difference being investigated influenced selenium intake by the plant. And considerable difference was obvious for the plants grown in the soil enriched with this microelement (*Table 1*). The significant higher concentration of selenium in rye plant growing on less acidic soil (pH 6.6) was determined. However, at tillering stage in plant growing on soil with pH 5.4 and without selenium supplementation the concentration of selenium was higher. At the later stages of plant development (booting and milk development), no significant difference in the selenium concentrations in plants growing on the soil with different acidity and without selenium additives was found.

At the same time, it was revealed that nature of selenium accumulation by the rye in the process of vegetation does not depend on the soil acidity: at the early stage of development – at tillering stage the microelement was received by the plant intensively, at the same time total selenium content in the plant increased.

Table 1. Selenium concentration in rye plants ($\mu\text{g g}^{-1}$) in depending of soil acidity and selenium addition concentration

Stage of ontogenesis	Selenium addition concentration mg Se kg ⁻¹ soil	pH 5.4	pH 6.6
Tillering	0	0.042±0.002 ^a	0.031±0.002 ^b
	0.01	0.58±0.03 ^a	0.55±0.02 ^a
	0.05	2.2±0.1 ^b	2.41±0.07 ^a
	0.1	7.4±0.1 ^b	9.24±0.08 ^a
	0.5	56.8±0.7 ^b	59.3±0.4 ^a
Booting	0	0.048±0.004 ^a	0.051±0.003 ^a
	0.01	0.42±0.01 ^b	0.47±0.03 ^a
	0.05	1.81±0.04 ^b	1.97±0.04 ^a
	0.1	4.38±0.05 ^b	4.94±0.08 ^a
	0.5	24.9±0.1 ^b	31.7±0.3 ^a
Milk development	0	0.046±0.002 ^a	0.047±0.001 ^a
	0.01	0.167±0.005 ^b	0.212±0.004 ^a
	0.05	0.65±0.02 ^b	0.71±0.01 ^a
	0.1	1.42±0.07 ^b	1.6±0.1 ^a
	0.5	10.4±0.6 ^b	11.8±0.3 ^a

Means within rows followed by different lower-case letters stand for significance different at $p \leq 0.05$

Selenium distribution in different organs of the rye plant

Study of selenium distribution in different organs of the rye plant testified that selenium content in the grain was higher than in the stem (*Table 2*). It was particularly evident in case of low selenium content in the soil; in case of higher selenium additives in the soil difference between its content in the stem and in the ear became not so evident. Thus, there was no significant difference between the content of selenium in the stem and grain of plants grown with the addition 0.05 mg selenium per kg soil and by soil acidity of 5.4.

Table 2. Selenium content in grain and stem (μg per plant) of rye plants depending on selenium supplements concentration and pH of the soil

Soil pH	Selenium addition concentration mg Se kg^{-1} soil	Grain	Stem
5.4	0	0.21±0.01 ^a	0.12±0.01 ^b
	0.01	0.75±0.08 ^a	0.42±0.04 ^b
	0.05	2.7±0.1 ^a	1.59±0.09 ^b
	0.1	6.4±0.5 ^a	3.4±0.5 ^b
	0.5	35.4±1.3 ^a	33.1±1.7 ^a
6.6	0	0.18±0.04 ^a	0.11±0.01 ^b
	0.01	0.82±0.05 ^a	0.52±0.03 ^b
	0.05	2.93±0.03 ^a	1.75±0.04 ^b
	0.1	6.1±0.2 ^a	4.5±0.2 ^b
	0.5	38.3±1.2 ^a	35.1±0.6 ^b

Means within rows followed by different lower-case letters stand for significance different at $p \leq 0.05$

Effect of soil applied selenium concentration on biomass accumulation of rye

This paper studies effect of applied selenium soil addition on accumulation of biomass by the rye plants at various ontogenesis stages (*Table 3*). The findings show that optimal for the rye plant is selenium additive in concentration of 0.01-0.05 mg kg^{-1} of soil (soil pH is 6.6). Under these conditions at all stages of development the plants built up maximum biomass both compared to the reference specimen and to the specimen with higher selenium concentrations. At booting stage positive effect of selenium additive in the form of sodium selenate was fixed for all plants irrespective of concentration of selenium applied in the soil. In the case of soil pH 5.4 selenium effect on the plant was more toxic. Positive effect was fixed at tillering stage after application of 0.01 mg Se kg^{-1} of soil and at booting stage after application of 0.05-0.1 mg Se per kg of soil, whereas at milk development stage selenium effect was purely toxic irrespective of concentration.

Table 3. Influence of selenium concentration in soil on the biomass (g) of rye plants at different ontogenesis stages

Stage of ontogenesis	Soil pH	Selenium addition concentration, mg Se kg^{-1} soil				
		0	0.01	0.05	0.1	0.5
Tillering	5.4	0.074±0.004 ^a	0.081±0.002 ^a	0.075±0.004 ^a	0.077±0.005 ^a	0.073±0.004 ^a
	6.6	0.072±0.005 ^b	0.083±0.005 ^a	0.078±0.003 ^{ab}	0.072±0.001 ^b	0.071±0.001 ^b
Booting	5.4	1.39±0.024 ^b	1.42±0.016 ^b	1.47±0.018 ^a	1.48±0.024 ^a	1.38±0.020 ^b
	6.6	1.22±0.038 ^b	1.44±0.031 ^a	1.43±0.018 ^a	1.46±0.046 ^a	1.44±0.036 ^a
Milk development	5.4	6.73±0.029 ^a	6.54±0.049 ^b	6.42±0.065 ^c	6.28±0.057 ^d	6.24±0.048 ^d
	6.6	6.41±0.080 ^{bc}	6.79±0.047 ^a	6.68±0.069 ^a	6.47±0.049 ^b	6.32±0.022 ^c

Means within rows followed by different lower-case letters stand for significance different at $p \leq 0.05$

Moreover, we studied selenium effect on accumulation of biomass separately by the ear and stem of the rye (*Table 4*). Application of lower concentrations of selenium at

soil pH 6.6 promoted increase of biomass both of the stem and of the ear. At this, for the ear stimulating effect of selenium was fixed for all its concentrations, while maximum ear mass was fixed after application of 0.05 mg Se kg⁻¹ of soil. In more acid soil we revealed mass decrease both for the ear and the stem after enriching the soil with selenium.

Table 4. Influence of selenium concentration in soil on the biomass (g) of different organs of rye plants at milk development stage

Plant part	Soil pH	Selenium addition concentration, mg Se kg ⁻¹ soil				
		0	0.01	0.05	0.1	0.5
Stem	5.4	2.67±0.042 ^a	2.61±0.044 ^{ac}	2.63±0.023 ^{ab}	2.54±0.031 ^c	2.59±0.031 ^{bc}
	6.6	2.62±0.034 ^b	2.84±0.041 ^a	2.54±0.030 ^c	2.53±0.022 ^c	2.41±0.021 ^d
Ear	5.4	4.02±0.030 ^a	3.90±0.030 ^b	3.83±0.053 ^{bc}	3.75±0.065 ^{cd}	3.67±0.040 ^d
	6.6	3.79±0.049 ^c	3.96±0.039 ^b	4.14±0.044 ^a	3.87±0.052 ^{bc}	3.84±0.034 ^c

Means within rows followed by different lower-case letters stand for significance different at $p \leq 0.5$

Influence of selenium on quantitative composition of reserve protein fractions in rye

Quantitative composition of reserve protein fractions was depending on selenium supply concentration to the soil (Table 5). The total protein content was higher by application of selenium. However, only the contents of globulin and glutelin in the rye grains increased with increasing Se fertilization. For albumin maximum of content was shown for low selenium concentrations (control and 0.01 mg kg⁻¹). Prolamin concentrations were not affected by selenium concentration in soil.

Table 5. Influence of selenium in soil (pH 6.6) on quantitative composition of protein fractions in the grain of rye plants

Selenium addition concentration, mg Se kg ⁻¹ soil	Total protein content, %	Reserve proteins, % of total protein content			
		Albumins	Globulins	Prolamins	Glutelins
0	12.4±0.4 ^b	24.7±0.7 ^{ab}	19.2±0.2 ^d	25.4±0.4 ^a	16.5±0.2 ^d
0.01	13.9±0.6 ^a	25.3±0.4 ^a	19.8±0.6 ^d	24.7±0.7 ^{ab}	17.8±0.6 ^c
0.05	14.3±0.3 ^a	23.9±0.9 ^b	21.6±0.6 ^c	25.1±0.8 ^a	18.2±0.4 ^c
0.1	13.8±0.3 ^a	21.8±0.6 ^c	23.4±0.4 ^b	23.4±0.6 ^b	19.7±0.8 ^b
0.5	13.6±0.5 ^a	20.1±0.4 ^d	25.9±0.8 ^a	24.2±0.9 ^{ab}	23.4±0.7 ^a

Means within rows followed by different lower-case letters stand for significance different at $p \leq 0.05$

Discussion

The study of selenium accumulation by the rye plants at different growth stages in depending on selenium soil application in concentrations 0.01 – 0.5 mg Se kg⁻¹ was conducted. There are four main methods for selenium application to plants: (1) adding Se to the soil; (2) foliar or fruit spraying; (3) soaking seeds in a Se solution before sowing; and (4) hydroponic cultivation with a nutrient solution containing Se (Puccinelli et al., 2017). The first two methods are most widely used due to their efficient and convenient. Despite a number of advantages of foliar processing, it is important to take into account the structural and chemical characteristics of the plant

leaves. In the case of cereals, it is very difficult to produce true foliar processing, some fertilizer is deposited on the soil and extracted by the plant from there. In addition, the application of selenium into the soil before planting, allows us to estimate its accumulation into the plant at different stages of development.

The findings on selenium accumulation by the rye plants display in general the picture typical for cereals, when some elements (especially those participating in synthesis of labile organic compounds) are absorbed by plants at early ontogenesis stages with a rate exceeding dry substance accumulation. Later, after completion of tillering stage stem starts its intensive growth, which leads to sharp decrease of relative content of these elements in dry substance as a result of “dilution” effect.

The one reason of increased intensity of selenium accumulation by the rye in ontogenesis in the course of our experiment was probably exhaustion of the soil in the culture pot as a consequence of absorption of most part of selenium from the soil at initial stages of the plant development. However, as computation shows selenium content in the soil after tillering stage decreased by 1-2%, while selenium concentration per gram of dry vegetating material decreased by 20%. Therefore, the reason of decreased intensity of selenium accumulation by the plants in vegetation process could not be only soil exhaustion with this microelement.

High selenium content in vegetating material at tillering stage was probably associated first of all with physiological functions of selenium. At tillering stage metabolic processes in nurslings are active resulting in intensive generation peroxides of hydrogen and organic molecules, by-products of many biochemical reactions. Hypothetically selenium microelement in plants, same as in mammal organisms, is an essential component of antioxidant system contributing to cell protection against destructive effect of active forms of oxygen. Antioxidant action of selenium may be explained by its influence on activity of glutathione-peroxidase – ferment having foremost significance in protection of plant cell from continuously generated hydroperoxides (Durán et al., 2016; Wu et al., 2017). Thus, intensive selenium accumulation by rye plants at tillering stage testifies to important role of this microelement for normal growth of rye especially at early stages of the plant development.

Assimilation of selenium by the plant is influenced by the physicochemical factors of the soil, such as redox status, pH and microbiological activity (Mehdi et al., 2013). In the present study the rye plant growing on less acidity soil accumulated more selenium. Increase of selenium content in the plants grown in less acid soil can be explained, on the one hand, by the fact that in the soil of high acidity anionic adsorption increases therefore most part of selenium is in absorbed condition, on the other hand, in case of low soil pH, hexavalent selenium is more prone to restorative process, and consequently in acid soil it is mostly in the form of selenite-ion which is not easily accessible for the plants. Lack of trustworthy difference in selenium intake by the plants depending on soil acidity in the case of small concentrations of the microelement is most probably conditioned by the fact that absorption and restorative processes insignificantly influence total pool of bioavailable selenium in the soil.

The study indicated that selenium predominantly accumulates in the grain, in comparison with the stem of rye. This result conforms to literary facts received earlier for winter wheat (Schulz et al., 1998). Selenium concentration in the grain was conditioned, most of all, by its physiological role in the plant, in particular, by selenium ability to substitute sulfur in most important amino acids (Sors et al., 2005; Reynolds et

al., 2017; El Mehdawi, 2018). Moreover, predominant selenium accumulation in the ear may indirectly evidence necessity of this microelement. Such result is also of practical importance and should be taken into account when enriching cereals with selenium in order to increase selenium supply of animals and humans.

One of the peculiar features of selenium microelement is its dual nature, i.e. possible manifestation of both antioxidant and pro-oxidant features. Basic factor conditioning one or another selenium action is its concentration.

Positive effect of selenium on growth processes of the rye may be connected with its antioxidant features, i.e. ability to liquidate excessively accumulated free radicals. Thereupon excessive quantities of selenium, on the contrary, lead to intensification of generation processes of active oxygen forms. Some authors attribute positive effect of selenium on plant growth processes to its influence on hormonal status, in particular, under effect of sodium selenite increase of indoleacetic acid and decrease of abscisic acid were observed (Wang et al., 2018).

Negative effect of high concentrations of selenium on accumulation of biomass by the rye plants may also be attributed to substantial disturbance of the cell amino acid balance. Rye pertains to the plants of the 3rd group, i.e. plants not accumulating selenium, for which excessive substitution of cysteine or methionine in proteins by selenium-cysteine or selenium-methionine is particularly typical. At soil pH 5.4 selenium effect on the plant was more toxic. Toxic effect of selenium for the plants grown on more acid soil may be attributed to the fact that at this pH selenate-ion is more prone to restorative processes and may transform to selenite-ion form being more toxic for plants.

The influence of selenium on protein metabolism has been shown in this work. The content of the total protein was higher by the addition of selenium, in particular by increasing the proportion of globulins and glutelin. Earlier a number of authors also noted that the biological role of selenium in plants can be associated with its effect on the metabolism of nitrogen (Poluboyarinov and Golubkina, 2015; Reis et al., 2018).

Conclusions

The research shows that selenium is more intensively accumulated by the rye plants at initial stage of development, it is mainly concentrated in the plant's ear, and in small concentrations selenium exerts stimulating effect on biomass accumulation. Thus, the findings certify to the important biological importance of selenium for growth and development of plants. However, mechanisms of protection effect of selenium in vegetative organisms remain unclarified. One of possible mechanisms of such effect is selenium antioxidant features which were proved in respect with humans and animals.

In addition, this study identified the optimum concentration of selenium supplements (0.01-0.05 mg Se kg⁻¹ soil). By these concentrations not only increase of studied plants biomass, but also achieved the necessary level of selenium (100 µg g⁻¹) in cereals, which guarantees the security of this trace element humans and animals were observed. Thus, the data allow more fully uncover the function of this trace element in plants, and thus create the basis for the use of selenium-containing fertilizers is not only to increase the availability of selenium for humans and animals, but also to increase the productivity of crop production.

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APPENDIX

One way analysis of variance

Data source: Tillering_0

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	0.0420	0.00200	0.001000
pH 6.6	4	0	0.0310	0.00200	0.001000

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.000242	0.000242	60.500	<0.001
Residual	6	0.0000240	0.00000400		
Total	7	0.000266			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5. vs. pH 6.6	0.0110	2	11.000	<0.001	Yes

One way analysis of variance

Data source: Tillering_0.01

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	0.580	0.0300	0.0150
pH 6.6	4	0	0.550	0.0200	0.01000

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.00180	0.00180	2.769	0.147
Residual	6	0.00390	0.000650		
Total	7	0.00570			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.147).

Power of performed test with alpha = 0.050: 0.196

The power of the performed test (0.196) is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One way analysis of variance

Data source: Tillering_0.05

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	2.200	0.1000	0.0500
pH 6.6	4	0	2.410	0.0700	0.0350

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.0882	0.0882	11.839	0.014
Residual	6	0.0447	0.00745		
Total	7	0.133			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.014).

Power of performed test with alpha = 0.050: 0.790

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	0.210	2	4.866	0.014	Yes

One way analysis of variance

Data source: Tillering_0.1

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	7.400	0.1000	0.0500
pH 6.6	4	0	9.240	0.0800	0.0400

Source of Variation	DF	SS	MS	F	P
Between Groups	1	6.771	6.771	825.756	<0.001
Residual	6	0.0492	0.00820		
Total	7	6.820			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	1.840	2	40.639	<0.001	Yes

One way analysis of variance

Data source: Tillering_0.5

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	56.800	0.700	0.350
pH 6.6	4	0	59.300	0.400	0.200

Source of Variation	DF	SS	MS	F	P
Between Groups	1	12.500	12.500	38.462	<0.001
Residual	6	1.950	0.325		
Total	7	14.450			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 0.999

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	2.500	2	8.771	0.001	Yes

One way analysis of variance

Data source: Booting_0

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	0.0480	0.00400	0.00200
pH 6.6	4	0	0.0510	0.00300	0.00150

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.0000180	0.0000180	1.440	0.275
Residual	6	0.0000750	0.0000125		
Total	7	0.0000930			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.275).

Power of performed test with alpha = 0.050: 0.084

The power of the performed test (0.084) is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One way analysis of variance

Data source: Booting_0.01

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	0.420	0.01000	0.00500
pH 6.6	4	0	0.470	0.0300	0.0150

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.00500	0.00500	10.000	0.020
Residual	6	0.00300	0.000500		
Total	7	0.00800			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.020).

Power of performed test with alpha = 0.050: 0.712

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	0.0500	2	4.472	0.020	Yes

One way analysis of variance

Data source: Booting_0.05

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	1.810	0.0400	0.0200
pH 6.6	4	0	1.970	0.0400	0.0200

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.0512	0.0512	32.000	0.001
Residual	6	0.00960	0.00160		
Total	7	0.0608			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.001).

Power of performed test with alpha = 0.050: 0.997

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6		0.160	2	8.000	0.002

One way analysis of variance

Data source: Booting_0.1

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	4.380	0.0500	0.0250
pH 6.6	4	0	4.940	0.0800	0.0400

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.627	0.627	140.944	<0.001
Residual	6	0.0267	0.00445		
Total	7	0.654			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	0.560	2	16.790	<0.001	Yes

One way analysis of variance

Data source: Booting_0.5

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	24.900	0.1000	0.0500
pH 6.6	4	0	31.700	0.300	0.150

Source of Variation	DF	SS	MS	F	P
Between Groups	1	92.480	92.480	1849.600	<0.001
Residual	6	0.300	0.0500		
Total	7	92.780			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	6.800	2	60.821	<0.001	Yes

One way analysis of variance

Data source: Milk development_0

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	0.0460	0.00200	0.001000
pH 6.6	4	0	0.0470	0.001000	0.000500

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.00000200	0.00000200	0.800	0.406
Residual	6	0.0000150	0.00000250		
Total	7	0.0000170			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.406).

Power of performed test with alpha = 0.050: 0.049

The power of the performed test (0.049) is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One way analysis of variance

Data source: Milk development_0.01

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	0.167	0.00500	0.00250
pH 6.6	4	0	0.212	0.00400	0.00200

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.00405	0.00405	197.561	<0.001
Residual	6	0.000123	0.0000205		
Total	7	0.00417			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	0.0450	2	19.878	<0.001	Yes

One way analysis of variance

Data source: Milk development_0.05

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	0.650	0.0200	0.01000
pH 6.6	4	0	0.710	0.01000	0.00500

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.00720	0.00720	28.800	0.002
Residual	6	0.00150	0.000250		
Total	7	0.00870			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = 0.002$).

Power of performed test with $\alpha = 0.050$: 0.993

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	0.0600	2	7.589	0.002	Yes

One way analysis of variance

Data source: Milk development_0.1

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	1.420	0.0700	0.0350
pH 6.6	4	0	1.600	0.1000	0.0500

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.0648	0.0648	8.698	0.026
Residual	6	0.0447	0.00745		
Total	7	0.110			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.026).

Power of performed test with alpha = 0.050: 0.643

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	0.180	2	4.171	0.026	Yes

One way analysis of variance

Data source: Milk development_0.5

Group Name	N	Missing	Mean	Std Dev	SEM
pH 5.4	4	0	10.400	0.600	0.300
pH 6.6	4	0	11.800	0.300	0.150

Source of Variation	DF	SS	MS	F	P
Between Groups	1	3.920	3.920	17.422	0.006
Residual	6	1.350	0.225		
Total	7	5.270			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.006).

Power of performed test with alpha = 0.050: 0.927

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
pH 5.4 vs. pH 6.6	1.400	2	5.903	0.006	Yes

One way analysis of variance

Data source: Organ_5.4_0

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	0.210	0.01000	0.00500
Stem	4	0	0.120	0.01000	0.00500

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.0162	0.0162	162.000	<0.001
Residual	6	0.000600	0.0001000		
Total	7	0.0168			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem	0.0900	2	18.000	<0.001	Yes

One way analysis of variance

Data source: Organ_5.4_0.01

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	0.750	0.0800	0.0400
Stem	4	0	0.420	0.0400	0.0200

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.218	0.218	54.450	<0.001
Residual	6	0.0240	0.00400		
Total	7	0.242			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem		0.330	2	10.436	<0.001

One way analysis of variance

Data source: Organ_5.4_0.05

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	2.700	0.1000	0.0500
Stem	4	0	1.590	0.0900	0.0450

Source of Variation	DF	SS	MS	F	P
Between Groups	1	2.464	2.464	272.287	<0.001
Residual	6	0.0543	0.00905		
Total	7	2.519			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem	1.110	2	23.336	<0.001	Yes

One way analysis of variance

Data source: Organ_5.4_0.1

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	6.400	0.500	0.250
Stem	4	0	3.400	0.500	0.250

Source of Variation	DF	SS	MS	F	P
Between Groups	1	18.000	18.000	72.000	<0.001
Residual	6	1.500	0.250		
Total	7	19.500			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem	3.000	2	12.000	<0.001	Yes

One way analysis of variance

Data source: Organ_5.4_0.5

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	35.400	1.300	0.650
Stem	4	0	33.100	1.700	0.850

Source of Variation	DF	SS	MS	F	P
Between Groups	1	10.580	10.580	4.620	0.075
Residual	6	13.740	2.290		
Total	7	24.320			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference ($P = 0.075$).

Power of performed test with $\alpha = 0.050$: 0.353

The power of the performed test (0.353) is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One way analysis of variance

Data source: Organ_6.6_0

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	0.180	0.0400	0.0200
Stem	4	0	0.110	0.01000	0.00500

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.00980	0.00980	11.529	0.015
Residual	6	0.00510	0.000850		
Total	7	0.0149			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = 0.015$).

Power of performed test with $\alpha = 0.050$: 0.778

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem	0.0700	2	4.802	0.015	Yes

One way analysis of variance

Data source: Organ_6.6_0.01

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	0.820	0.0500	0.0250
Stem	4	0	0.520	0.0300	0.0150

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.180	0.180	105.882	<0.001
Residual	6	0.0102	0.00170		
Total	7	0.190			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem		0.300	2	14.552	<0.001

One way analysis of variance

Data source: Organ_6.6_0.05

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	2.930	0.0300	0.0150
Stem	4	0	1.750	0.0400	0.0200

Source of Variation	DF	SS	MS	F	P
Between Groups	1	2.785	2.785	2227.840	<0.001
Residual	6	0.00750	0.00125		
Total	7	2.792			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem		1.180	2	66.751	<0.001

One way analysis of variance

Data source: Organ_6.6_0.1

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	6.100	0.200	0.1000
Stem	4	0	4.500	0.200	0.1000

Source of Variation	DF	SS	MS	F	P
Between Groups	1	5.120	5.120	128.000	<0.001
Residual	6	0.240	0.0400		
Total	7	5.360			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem	1.600	2	16.000	<0.001	Yes

One way analysis of variance

Data source: Organ_6.6_0.5

Group Name	N	Missing	Mean	Std Dev	SEM
Grain	4	0	38.300	1.200	0.600
Stem	4	0	35.100	0.600	0.300

Source of Variation	DF	SS	MS	F	P
Between Groups	1	20.480	20.480	22.756	0.003
Residual	6	5.400	0.900		
Total	7	25.880			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = 0.003$).

Power of performed test with $\alpha = 0.050$: 0.975

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Grain vs. Stem		3.200	2	6.746	0.003

One way analysis of variance

Data source: Tillering_5.4

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	0.0740	0.00400	0.00200
0.01	4	0	0.0810	0.00200	0.001000
0.05	4	0	0.0750	0.00400	0.00200
0.1	4	0	0.0770	0.00500	0.00250
0.5	4	0	0.0730	0.00400	0.00200

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.000160	0.0000400	2.597	0.079
Residual	15	0.000231	0.0000154		
Total	19	0.000391			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.079).

Power of performed test with alpha = 0.050: 0.371

The power of the performed test (0.371) is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One way analysis of variance

Data source: Tillering_6.6

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	0.0720	0.00500	0.00250
0.01	4	0	0.0830	0.00500	0.00250
0.05	4	0	0.0780	0.00300	0.00150
0.1	4	0	0.0720	0.001000	0.000500
0.5	4	0	0.0710	0.001000	0.000500

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.000427	0.000107	8.754	<0.001
Residual	15	0.000183	0.0000122		
Total	19	0.000610			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.983

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.01 vs. 0.5	0.0120	5	6.871	0.002	Yes
0.01 vs. 0.1	0.0110	5	6.299	0.004	Yes
0.01 vs. 0	0.0110	5	6.299	0.004	Yes
0.01 vs. 0.05	0.00500	5	2.863	0.302	No
0.05 vs. 0.5	0.00700	5	4.008	0.080	No
0.05 vs. 0.1	0.00600	5	3.436	0.161	Do Not Test
0.05 vs. 0	0.00600	5	3.436	0.161	Do Not Test
0 vs. 0.5	0.001000	5	0.573	0.994	Do Not Test
0 vs. 0.1	0.000	5	0.000	1.000	Do Not Test
0.1 vs. 0.5	0.001000	5	0.573	0.994	Do Not Test

A result of “Do Not Test” occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One way analysis of variance

Data source: Booting_5.4

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	1.390	0.0240	0.0120
0.01	4	0	1.420	0.0160	0.00800
0.05	4	0	1.470	0.0180	0.00900
0.1	4	0	1.480	0.0240	0.0120
0.5	4	0	1.380	0.0200	0.01000

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.0331	0.00828	19.418	<0.001
Residual	15	0.00640	0.000426		
Total	19	0.0395			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.1 vs. 0.5	0.100	5	9.685	<0.001	Yes
0.1 vs. 0	0.0900	5	8.717	<0.001	Yes
0.1 vs. 0.01	0.0600	5	5.811	0.007	Yes
0.1 vs. 0.05	0.01000	5	0.969	0.957	No

0.05 vs. 0.5	0.0900	5	8.717	<0.001	Yes
0.05 vs. 0	0.0800	5	7.748	<0.001	Yes
0.05 vs. 0.01	0.0500	5	4.843	0.027	Yes
0.01 vs. 0.5	0.0400	5	3.874	0.094	No
0.01 vs. 0	0.0300	5	2.906	0.289	Do Not Test
0 vs. 0.5	0.01000	5	0.969	0.957	Do Not Test

A result of “Do Not Test” occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One way analysis of variance

Data source: Booting_6.6

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	1.220	0.0380	0.0190
0.01	4	0	1.440	0.0310	0.0155
0.05	4	0	1.430	0.0180	0.00900
0.1	4	0	1.460	0.0460	0.0230
0.5	4	0	1.440	0.0360	0.0180

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.160	0.0401	32.633	<0.001
Residual	15	0.0184	0.00123		
Total	19	0.179			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with alpha = 0.050: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.1 vs. 0	0.240	5	13.696	<0.001	Yes
0.1 vs. 0.05	0.0300	5	1.712	0.746	No
0.1 vs. 0.5	0.0200	5	1.141	0.924	Do Not Test
0.01 vs. 0.01	0.0200	5	1.141	0.924	Do Not Test
0.01 vs. 0	0.220	5	12.555	<0.001	Yes
0.01 vs. 0.05	0.01000	5	0.571	0.994	Do Not Test
0.01 vs. 0.5	0.000	5	0.000	1.000	Do Not Test
0.5 vs. 0	0.220	5	12.555	<0.001	Yes
0.5 vs. 0.05	0.01000	5	0.571	0.994	Do Not Test
0.05 vs. 0	0.210	5	11.984	<0.001	Yes

A result of “Do Not Test” occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One way analysis of variance

Data source: Milk development_5.4

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	6.730	0.0290	0.0145
0.01	4	0	6.540	0.0490	0.0245
0.05	4	0	6.420	0.0650	0.0325
0.1	4	0	6.280	0.0570	0.0285
0.5	4	0	6.240	0.0480	0.0240

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.640	0.160	61.475	<0.001
Residual	15	0.0391	0.00260		
Total	19	0.679			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0 vs. 0.5	0.490	5	19.205	<0.001	Yes
0 vs. 0.1	0.450	5	17.637	<0.001	Yes
0 vs. 0.05	0.310	5	12.150	<0.001	Yes
0 vs. 0.01	0.190	5	7.447	<0.001	Yes
0.01 vs. 0.5	0.300	5	11.758	<0.001	Yes
0.01 vs. 0.1	0.260	5	10.190	<0.001	Yes
0.01 vs. 0.05	0.120	5	4.703	0.032	Yes
0.05 vs. 0.5	0.180	5	7.055	0.001	Yes
0.05 vs. 0.1	0.140	5	5.487	0.011	Yes
0.1 vs. 0.5	0.0400	5	1.568	0.800	No

One way analysis of variance

Data source: Milk development_6.6

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	6.410	0.0800	0.0400
0.01	4	0	6.790	0.0470	0.0235
0.05	4	0	6.680	0.0690	0.0345
0.1	4	0	6.470	0.0490	0.0245
0.5	4	0	6.320	0.0220	0.0110

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.608	0.152	46.792	<0.001
Residual	15	0.0488	0.00325		
Total	19	0.657			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with alpha = 0.050: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.01 vs. 0.5	0.470	5	16.486	<0.001	Yes
0.01 vs. 0	0.380	5	13.329	<0.001	Yes
0.01 vs. 0.1	0.320	5	11.225	<0.001	Yes
0.01 vs. 0.05	0.110	5	3.858	0.096	No
0.05 vs. 0.5	0.360	5	12.628	<0.001	Yes
0.05 vs. 0	0.270	5	9.471	<0.001	Yes
0.05 vs. 0.01	0.210	5	7.366	<0.001	Yes
0.01 vs. 0.5	0.150	5	5.262	0.015	Yes
0.01 vs. 0	0.0600	5	2.105	0.585	No
0 vs. 0.5	0.0900	5	3.157	0.221	No

One way analysis of variance

Data source: Stem_5.4

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	2.670	0.0420	0.0210
0.01	4	0	2.610	0.0440	0.0220
0.05	4	0	2.630	0.0230	0.0115
0.1	4	0	2.540	0.0310	0.0155
0.5	4	0	2.590	0.0310	0.0155

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.0371	0.00928	7.543	0.002
Residual	15	0.0185	0.00123		
Total	19	0.0556			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = 0.002$).

Power of performed test with $\alpha = 0.050$: 0.959

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0 vs. 0.1	0.130	5	7.413	<0.001	Yes
0 vs. 0.5	0.0800	5	4.562	0.039	Yes
0 vs. 0.01	0.0600	5	3.421	0.164	No
0 vs. 0.05	0.0400	5	2.281	0.512	Do Not Test
0.05 vs. 0.1	0.0900	5	5.132	0.018	Yes
0.05 vs. 0.5	0.0400	5	2.281	0.512	No
0.05 vs. 0.01	0.0200	5	1.140	0.925	Do Not Test
0.01 vs. 0.1	0.0700	5	3.992	0.081	No
0.01 vs. 0.5	0.0200	5	1.140	0.925	Do Not Test
0.5 vs. 0.1	0.0500	5	2.851	0.305	Do Not Test

A result of “Do Not Test” occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One way analysis of variance

Data source: Stem_6.6

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	2.620	0.0340	0.0170
0.01	4	0	2.840	0.0410	0.0205
0.05	4	0	2.540	0.0300	0.0150
0.1	4	0	2.530	0.0220	0.0110
0.5	4	0	2.410	0.0210	0.0105

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.408	0.102	109.266	<0.001
Residual	15	0.0140	0.000932		
Total	19	0.422			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.01 vs. 0.5	0.430	5	28.164	<0.001	Yes
0.01 vs. 0.1	0.310	5	20.304	<0.001	Yes
0.01 vs. 0.05	0.300	5	19.649	<0.001	Yes
0.01 vs. 0	0.220	5	14.410	<0.001	Yes
0 vs. 0.5	0.210	5	13.755	<0.001	Yes
0 vs. 0.1	0.0900	5	5.895	0.006	Yes
0 vs. 0.05	0.0800	5	5.240	0.016	Yes
0.05 vs. 0.5	0.130	5	8.515	<0.001	Yes
0.05 vs. 0.1	0.0100	5	0.655	0.990	No
0.1 vs. 0.5	0.120	5	7.860	<0.001	Yes

One way analysis of variance

Data source: Grain_5.4

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	4.020	0.0300	0.0150
0.01	4	0	3.900	0.0300	0.0150
0.05	4	0	3.830	0.0530	0.0265
0.1	4	0	3.750	0.0650	0.0325
0.5	4	0	3.670	0.0400	0.0200

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.292	0.0729	34.943	<0.001
Residual	15	0.0313	0.00209		
Total	19	0.323			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0 vs. 0.5	0.350	5	15.323	<0.001	Yes
0 vs. 0.1	0.270	5	11.821	<0.001	Yes
0 vs. 0.05	0.190	5	8.318	<0.001	Yes
0 vs. 0.01	0.120	5	5.254	0.015	Yes

0.01 vs. 0.5	0.230	5	10.070	<0.001	Yes
0.01 vs. 0.1	0.150	5	6.567	0.003	Yes
0.01 vs. 0.05	0.0700	5	3.065	0.244	No
0.05 vs. 0.5	0.160	5	7.005	0.001	Yes
0.05 vs. 0.1	0.0800	5	3.503	0.149	No
0.1 vs. 0.5	0.0800	5	3.503	0.149	No

One way analysis of variance

Data source: Grain_6.6

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	3.790	0.0490	0.0245
0.01	4	0	3.960	0.0390	0.0195
0.05	4	0	4.140	0.0440	0.0220
0.1	4	0	3.870	0.0520	0.0260
0.5	4	0	3.840	0.0340	0.0170

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.303	0.0758	39.000	<0.001
Residual	15	0.0292	0.00194		
Total	19	0.332			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.05 vs. 0	0.350	5	15.878	<0.001	Yes
0.05 vs. 0.5	0.300	5	13.610	<0.001	Yes
0.05 vs. 0.1	0.270	5	12.249	<0.001	Yes
0.05 vs. 0.01	0.180	5	8.166	<0.001	Yes
0.01 vs. 0	0.170	5	7.712	<0.001	Yes
0.01 vs. 0.5	0.120	5	5.444	0.012	Yes
0.01 vs. 0.1	0.0900	5	4.083	0.072	No
0.1 vs. 0	0.0800	5	3.629	0.128	No
0.1 vs. 0.5	0.0300	5	1.361	0.868	Do Not Test
0.5 vs. 0	0.0500	5	2.268	0.517	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a

procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One way analysis of variance

Data source: Total protein content

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	12.400	0.400	0.200
0.01	4	0	13.900	0.600	0.300
0.05	4	0	14.300	0.300	0.150
0.1	4	0	13.800	0.300	0.150
0.5	4	0	13.600	0.500	0.250

Source of Variation	DF	SS	MS	F	P
Between Groups	4	8.240	2.060	10.842	<0.001
Residual	15	2.850	0.190		
Total	19	11.090			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with alpha = 0.050: 0.997

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.05 vs. 0	1.900	5	8.718	<0.001	Yes
0.05 vs. 0.5	0.700	5	3.212	0.208	No
0.05 vs. 0.1	0.500	5	2.294	0.507	Do Not Test
0.05 vs. 0.01	0.400	5	1.835	0.697	Do Not Test
0.01 vs. 0	1.500	5	6.882	0.002	Yes
0.01 vs. 0.5	0.300	5	1.376	0.863	Do Not Test
0.01 vs. 0.1	0.1000	5	0.459	0.997	Do Not Test
0.1 vs. 0	1.400	5	6.424	0.003	Yes
0.1 vs. 0.5	0.200	5	0.918	0.964	Do Not Test
0.5 vs. 0	1.200	5	5.506	0.011	Yes

A result of “Do Not Test” occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One way analysis of variance

Data source: Albumins

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	24.700	0.700	0.350
0.01	4	0	25.300	0.400	0.200
0.05	4	0	23.900	0.900	0.450
0.1	4	0	21.800	0.600	0.300
0.5	4	0	20.100	0.400	0.200

Source of Variation	DF	SS	MS	F	P
Between Groups	4	74.848	18.712	47.253	<0.001
Residual	15	5.940	0.396		
Total	19	80.788			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with alpha = 0.050: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.01 vs. 0.5	5.200	5	16.527	<0.001	Yes
0.01 vs. 0.1	3.500	5	11.124	<0.001	Yes
0.01 vs. 0.05	1.400	5	4.449	0.045	Yes
0.01 vs. 0	0.600	5	1.907	0.667	No
0 vs. 0.5	4.600	5	14.620	<0.001	Yes
0 vs. 0.1	2.900	5	9.217	<0.001	Yes
0 vs. 0.05	0.800	5	2.543	0.410	No
0.05 vs. 0.5	3.800	5	12.077	<0.001	Yes
0.05 vs. 0.1	2.100	5	6.674	0.002	Yes
0.1 vs. 0.5	1.700	5	5.403	0.012	Yes

One way analysis of variance

Data source: Globulins

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	19.200	0.200	0.1000
0.01	4	0	19.800	0.600	0.300
0.05	4	0	21.600	0.600	0.300
0.1	4	0	23.400	0.400	0.200
0.5	4	0	25.900	0.800	0.400

Source of Variation	DF	SS	MS	F	P
Between Groups	4	120.032	30.008	96.179	<0.001
Residual	15	4.680	0.312		
Total	19	124.712			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.5 vs. 0	6.700	5	23.990	<0.001	Yes
0.5 vs. 0.01	6.100	5	21.842	<0.001	Yes
0.5 vs. 0.05	4.300	5	15.396	<0.001	Yes
0.05 vs. 0.1	2.500	5	8.951	<0.001	Yes
0.1 vs. 0	4.200	5	15.038	<0.001	Yes
0.1 vs. 0.01	3.600	5	12.890	<0.001	Yes
0.1 vs. 0.05	1.800	5	6.445	0.003	Yes
0.05 vs. 0	2.400	5	8.593	<0.001	Yes
0.05 vs. 0.01	1.800	5	6.445	0.003	Yes
0.01 vs. 0	0.600	5	2.148	0.567	No

One way analysis of variance

Data source: Prolamins

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	25.400	0.400	0.200
0.01	4	0	24.700	0.700	0.350
0.05	4	0	25.100	0.800	0.400
0.1	4	0	23.400	0.600	0.300
0.5	4	0	24.200	0.900	0.450

Source of Variation	DF	SS	MS	F	P
Between Groups	4	9.968	2.492	5.065	0.009
Residual	15	7.380	0.492		
Total	19	17.348			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = 0.009$).

Power of performed test with $\alpha = 0.050$: 0.802

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0 vs. 0.1	2.000	5	5.703	0.008	Yes
0 vs. 0.5	1.200	5	3.422	0.163	No
0 vs. 0.01	0.700	5	1.996	0.630	Do Not Test
0 vs. 0.05	0.300	5	0.855	0.972	Do Not Test
0.05 vs. 0.1	1.700	5	4.847	0.026	Yes
0.05 vs. 0.5	0.900	5	2.566	0.401	Do Not Test
0.05 vs. 0.01	0.400	5	1.141	0.925	Do Not Test
0.01 vs. 0.1	1.300	5	3.707	0.116	No
0.01 vs. 0.5	0.500	5	1.426	0.848	Do Not Test
0.5 vs. 0.1	0.800	5	2.281	0.512	Do Not Test

A result of “Do Not Test” occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

One Way Analysis of Variance

Data source: Glutelins

Group Name	N	Missing	Mean	Std Dev	SEM
0	4	0	16.500	0.200	0.1000
0.01	4	0	17.800	0.600	0.300
0.05	4	0	18.200	0.400	0.200
0.1	4	0	19.700	0.800	0.400
0.5	4	0	23.400	0.700	0.350

Source of Variation	DF	SS	MS	F	P
Between Groups	4	112.432	28.108	83.160	<0.001
Residual	15	5.070	0.338		
Total	19	117.502			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P = <0.001$).

Power of performed test with $\alpha = 0.050$: 1.000

All pairwise multiple comparison procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
0.5 vs. 0	6.900	5	23.737	<0.001	Yes
0.5 vs. 0.01	5.600	5	19.265	<0.001	Yes
0.5 vs. 0.05	5.200	5	17.889	<0.001	Yes
0.5 vs. 0.1	3.700	5	12.728	<0.001	Yes

0.1 vs. 0	3.200	5	11.008	<0.001	Yes
0.1 vs. 0.01	1.900	5	6.536	0.003	Yes
0.1 vs. 0.05	1.500	5	5.160	0.017	Yes
0.05 vs. 0	1.700	5	5.848	0.007	Yes
0.05 vs. 0.01	0.400	5	1.376	0.863	No
0.01 vs. 0	1.300	5	4.472	0.044	Yes