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THE EFFECT OF SELENIUM FERTILIZATION OF FODDER GRASSES ON THE YIELD AND SE CONTENT IN PLANTS

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Summary. Before harvesting, one-time foliar fertilization of a mixture of fodder grasses (*Lolium perenne, Phleum pratense*, *Festuca rubra*, *Festuca pratensis*) was applied with selenium in doses of 5, 10, 15 and 20 g · ha^{-1}. The grasses were gathered three times, each time weighing the green mass and determining the Se content in dry matter. Selenium in doses ≤ 20 g · ha^{-1} did not significantly increase the yield. No selenium was detected in the dry matter of plants from the control sample and plants fertilized with Se in doses of 5 and 10 g · ha^{-1}. The dose of 15 g · ha^{-1} increased the Se content to 0.08 ±0.02 in the first harvest and to 0.02 ±0.03 mg · kg DM^{-1} in the second harvest. Se in the dose of 20 g · ha^{-1} increased the content of this element to 0.18 ±0.08 in the first harvest and to 0.07 ±0.02 mg · kg DM^{-1} in the second harvest. Selenium was not detected in the third harvest. The determined Se content in any of the fertilization doses was not sufficient to meet the needs of cattle. It is proposed to increase the dose of selenium and carry out three applications (before the first mowing, after the first mowing and after the second mowing) to improve the fertilization effect.

Key words: selenium biofortification, selenium fertilization, fodder quality, Se content in fodder grasses

INTRODUCTION

Selenium is an essential micronutrient in the nutrition of farm animals. Its deficiencies contribute to significant losses in livestock production. In animals, it is present in the form of the amino acid selenocysteine in the active site of glutathione peroxidase. This enzyme is responsible for the neutralization of peroxides harmful to the cellular apparatus

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(e.g. hydrogen peroxide, organic peroxides), which arise as a side-effect of metabolic reactions [Forstrom et al. 1978, Błażejak and Milewski 2016]. Se can be also found in other proteins of key importance for the functioning of the organism – iodothyronine deiodinases, which regulate the activity of thyroid hormones, and in extracellular selenoprotein P, which transports this microelement from the liver to other organs, including the brain [Burk et al. 2003]. Selenium also reduces the toxic effect of heavy metals on the organism, binding them into complexes [Strączyk and Drobnica 2001]. Moreover, it reduces the risk of cancer [Żbikowska 1997] and it supports the immune system of animals by increasing the migration of neutrophils and stimulating B lymphocytes to proliferate and produce IgG and IgM antibodies [Hemingway 1999, Kurek et al. 2011].

When it comes to cattle, the supply of adequate amounts of selenium allows to avoid numerous diseases and metabolic disorders, e.g. white muscle disease [Divers and Peek 2011], mastitis [Hemingway 1999] or placental retention after birthing [Harrison et al. 1984]. Se deficiency is a frequent cause of livestock losses and it forces to remove animals from a herd. It also decreases the technological quality of milk. All these causes reduce the cattle productivity and the economic results of a farm.

At the same time, providing farm animals with adequate amounts of selenium is a nutritional problem. This is due to the fact that in Poland (and many other countries around the world) there are predominantly soils that are poor in this element. Plants growing on them do not accumulate a sufficient amount of selenium. Therefore, the fodder produced from these plants is also low in selenium. Piotrowska [1984] determined the average selenium content of Polish soils at 0.27 mg $\cdot$ kg^{-1}. The world average is 0,4 mg $\cdot$ kg^{-1} [Kabata-Pendias and Pendias 1979]. It is assumed that only the soil with selenium content not lower than 60 mg $\cdot$ kg^{-1}, allows for the production of plant fodder with a sufficient content of this element [Gupta and Gupta 2002]. As the majority of Polish soils do not meet this condition, the fodder crops grown there do not accumulate enough Se. It is also known that under the same environmental conditions, the ability of several plant species to accumulate selenium is different. Forage plants used for fodder production (cereals, grasses, grasses) are among the group of the least deposition of selenium in their tissues [Ellis and Salt 2003].

For the above reasons, cattle farmers are forced to supplement selenium in fodder with various feed additives (e.g. selenium yeast). Selenium licks and boluses are also used, and interventionally – injections.

In recent years, it has been proposed to simplify animal nutrition by increasing the selenium content in plant feed materials. This can be done by agrotechnical biofortification, i.e. by soil and/or foliar fertilization with this element. This method allows to eliminate or significantly reduce the need to use feed additives.

An additional benefit of biofortification of plants into selenium could be an increase in their yield. So far, there is no information on selenium fulfilling any necessary physiological role in the organisms of forage plants. Plants take it from the soil and incorporate it into organic compounds due to its similarity to sulfur (the same ion channels and enzymes are used for this purpose) [Anderson 1993]. However, it has been suggested that the presence of Se improves the plants' ability to neutralize reactive oxygen species, reducing their susceptibility to environmental stresses [Feng et al. 2013]. Low-dose selenium fertilization has a positive effect on the growth and development of some plant species, such as lettuce [Ramos et al. 2010]. If the yield-generating effect of selenium was proven beyond any doubt, we could speak of it as an element beneficial in the nutrition of crops, which is not classified as micro- or macroelement, but supplementing it allows to improve the condition of plants and their yield.

Numerous studies document that fertilization of Se is an effective way to increase its content in the yield of fodder plants, for example in the above-ground parts of maize [Płaczek 2012], in wheat and rye grain [Eurola et al. 2002], in buckwheat seeds [Jiang et al. 2015] or in fescue [Valle et al. 2002]. There are no similar studies for fodder grass mixtures grown in Polish conditions, mowed three times during the season**.** It is not known whether their fertilization is effective and how long its effect is (the increase in selenium content after a single application is visible only in the first harvest, or it also persists in the second and third). There is also a need to answer the question whether the selenium fertilization increases the yield of fodder grasses?

The aim of the study was to determine the effect of one-time foliar fertilization of a fodder grass mixture with selenium in doses of 5, 10, 15, 20 g · ha^{-1} on the content of this element in plants of three harvests. The determined content of selenium in the harvested grass mass was assessed in terms of cattle nutrition. The second aim was to determine the effect of selenium biofortification on the yield of grasses.

MATERIALS AND METHODS

The experiment was carried out in 2019 (April–October) in Poland, in the village Dąbrowa in the Wieluń district (Łódzkie Voivodeship), on class IVa arable land (light loamy sand), on the site after potato cultivation. Before setting up the experiment, soil samples were taken and analyzed at the Research Institute of Horticulture in Skierniewice. Soil test results: salinity 0.32 g KCl · kg^{-1}; pH with 1M KCI 6.45 (potentiometric determination); soil organic matter 1.87%; total carbon 10.9 g · kg^{-1}; total nitrogen 1.1 g · kg^{-1} (Dumas method); available phosphorus 146 mg · kg^{-1}; available potassium 104 mg · kg^{-1} (Egner-Riehm method); available magnesium 57.4 mg · kg^{-1} (Schachtschabel method); total sulfur 197 mg · kg^{-1}; total selenium 0.16 mg · kg^{-1} (the Inductively Coupled Plasma Optical Emission Spectrometry method – ICP-OES); sulphate sulfur (SO_4^{2-}) 56.9 mg · kg^{-1} (ICP-OES after extraction in a solution of 0.25 mol · l^{-1} acetic acid and ammonium acetate). Therefore, the selenium content in the soil is much lower than the threshold of 60 mg Se · kg^{-1} allowing for the production of a feed that is rich enough in this element, given by Gupta and Gupta [2002].

Table 1 presents the meteorological data for the Wieluń district in several months of the field research and calculated values of the Sielianinov hydrothermal coefficient (*k*) [Selyaninov 1928 according to Kulik et al. 2016]. Basing on the value of *k*, several months were assigned to the humidity classes according to the interpretation of Skowera and Puła [2004]. On this basis, it was found that: in April and June the conditions were very dry, in August – dry, in July and September – quite dry, in October – optimal, and in March and May – wet.

Fertilization was applied in the following doses: pre-sowing 60 kg N · ha^{-1} in the form of ammonium sulphate, 50 kg K · ha^{-1} in the form of potassium chloride, 17.5 kg P · ha^{-1}

Table 1. Meteorological conditions in the Wieluń district in period March–October 2019

Tabela 1. Warunki meteorologiczne w powiecie wieluńskim w okresie marzec–październik 2019 roku

	Month Miesiąc							
	March Marzec	April Kwiecień	May Maj	June Czerwiec	July Lipiec	August Sierpień	Septembee Wrzesień	October Październik
Average daily air temperature Średnia dobowa temperatura powietrza [°C]	5.9	9.8	13.2	22	19	20.1	14	10.4
Accumulated total precipitation Suma opadów atmosferycznych [mm]	43.8	14.4	84.8	34.9	61.3	60.1	50.2	51.7
Selyaninov's hydrothermal coefficient (HTC) Współczynnik Sielianinowa (*k*)	2.38	0.49	2.07	0.53	1.04	0.96	1.19	1.6

in the form of granulated superphosphate. Additionally, after the first and the second harvest, 30 kg N · ha^{-1} in the form of urea and 25 kg K · ha^{-1} in the form of potassium chloride were applied. In total, 120 kg N, 100 kg K and 17.5 kg P per ha were used.

In the available area, 15 experimental plots with a surface area of 20 m^2 each (5 × 4 m) were distributed, at a distance of at least 1 m between plots. A pasture mixture of fodder grasses containing 55% perennial ryegrass (*Lolium perenne*), 25% timothy (*Phleum pratense*), 10% red fescue (*Festuca rubra*) and 10% meadow fescue (*Festuca pratensis*) were sown. The sowing rate was as recommended by the producer: 40 kg · ha^{-1}.

Se fertilization doses were determined on the basis of the literature [Valle et al. 2002] on 5, 10, 15 and 20 g Se · ha^{-1}. No Se fertilization was used in control samples. Each research sample and control sample were performed thrice, which gives a total of 15 trials. The doses were randomly assigned to the plots to limit the impact of local variability of soil conditions on the experiment result.

Selenium in a safe and bioavailable form of sodium selenate [Zayed et al. 1998, Eurola et al. 2002, Cartes et al. 2005, Hawkesford and Zhao 2007, Płaczek 2012] was applied once as a foliar treatment, 40 days before the first harvest. A foliar treatment was undertaken because the Se fertilization doses were microscopic and it was impossible to apply them in the form of a soil-applied fertilizer.

The grasses were cut three times at a height of 5 cm at the beginning of heading. The swath from each plot was weighed and converted into green matter yield per hectare. A representative sample of the mass was taken from each plot and then dried at room temperature to a constant mass. The content of selenium in plant material was determined at the Lubuski Centre for Innovation and Agrotechnical Implementation in Sulechów by the ICP-OES method; the determination limit: 0.005 mg · l^{-1}.

Statistical methods

The results were analyzed using the two-way ANOVA. When testing the hypotheses, the significance level α ≤0.05 was adopted. Microsoft Excel spreadsheet was used for the calculations.

RESULTS AND DISCUSSION

The yields of green mass of fodder grasses per hectare are presented in Table 2. Statistical analysis showed a significant difference between the mean yield of green forage in the 1st, 2nd and 3rd harvest**.** It was also found that in the same harvest, the mean values for different doses of the fertilizer did not differ significantly. There is no interaction between the average yields for the 1st, 2nd and 3rd swaths and the dose of the selenium applied.

Table 2. The effect of fertilization with different doses of selenium on the yield of green mass of fodder grasses in three harvests (average of three repetitions ±standard deviation, SD)

Tabela 2. Wpływ nawożenia zróżnicowanymi dawkami selenu na plon zielonej masy traw pastewnych w trzech pokosach (średnia z trzech powtórzeń ±odchylenie standardowe, SD

Dose of selenium Dawka selenu [g · ha^{-1}]	Yield of green mass Plon zielonki [Mg · ha^{-1}]		
	Harvest No. 1 Pokos 1.	Harvest No. 2 Pokos 2.	Harvest No. 3 Pokos 3.
0	3.34 ±0.56	2.74 ±0.59	6.33 ±0.77
5	3.79 ±0.94	2.86 ±0.62	6.80 ±0.34
10	3.72 ±0.17	3.08 ±0.25	5.81 ±1.88
15	3.55 ±0.65	2.95 ±0.53	5.93 ±1.15
20	3.71 ±0.65	3.08 ±0.59	7.39 ±1.63

It should be assumed that the differences between the average green mass yields of succeeding harvests (e.g. more than one and a half times higher yield in the 3rd harvest than in the 1st harvest or more than two times higher yield in the 3rd harvest than in the 2nd harvest) are the sole effect of variability of hydrothermal conditions during the research period. The use of selenium fertilization in doses of ≤20 g Se · ha^{-1} cannot be considered as a factor increasing the yield of green fodder. There is no economic justification for the use of this element in the accepted doses as a beneficial ingredient in the nutrition of forage grasses, which – as suggested by Feng et al. [2013] – would increase plant resistance to environmental stresses. The grasses reaction to drought stress was the same, regardless of the dose of Se fertilization applied. In further studies, it could be checked whether there is an effect of Se fertilization in doses higher than 20 g Se · ha^{-1} on the yielding of grasses, however, it should be taken into account that it is a very expensive component, and the profitability of its use only for the possible yield-generating effect is highly questionable.

Table 3 shows the results of determination of selenium content in the dry matter of forage grasses treated with various doses of this element. The statistical analysis showed a significant difference between the average selenium content in the 1st, 2nd and 3rd harvests. It was found that the obtained results of selenium content differ significantly depending on the applied fertilizer dose. The interaction between the mean Se contents for the 1st, 2nd and 3rd swaths and the applied fertilizer dose is statistically significant.

Table 3. The effect of fertilization with different doses of selenium on its content in dry matter of fodder grasses in three harvests (average of three repetitions ±standard deviation, SD)

Tabela 3. Wpływ nawożenia zróżnicowanymi dawkami selenu na jego zawartość w suchej masie traw pastewnych w trzech pokosach (średnia z trzech powtórzeń ±odchylenie standardowe, SD)

Dose of selenium Dawka selenu [g · ha^{-1}]	Content of selenium in dry matter Zawartość selenu w suchej masie [mg · kg^{-1}]		
	Harvest No. 1 Pokos 1.	Harvest No. 2 Pokos 2.	Harvest No. 3 Pokos 3.
0	N/D	N/D	N/D
5	N/D	N/D	N/D
10	N/D	N/D	N/D
15	0.08 ±0.02	0.02 ±0.03	N/D
20	0.18 ±0.08	0.07 ±0.02	N/D

The value N/D should be understood as the lack of selenium in the sample or its content below the limit of determination of the method.

Wartość N/D należy rozumieć jako brak selenu w próbce bądź jego zawartość poniżej progu oznaczalności metody.

Plants not fertilized with selenium (control sample), growing on the soil on which the experiment was established, were not able to accumulate in the yield the amount of Se that could be determined by the method used. Similarly, plants fertilized with Se in doses of ≤ 10 g · ha^{-1}. Only selenium in the dose of ≥ 15 g · ha^{-1} caused a detectable increase in the content of this element in the dry matter of forage grasses. Therefore, in order to increase the Se content in the yield of fodder grasses, it is reasonable to use this element in doses of ≥ 15 g · ha^{-1}, while lower doses should be considered ineffective.

The average Se content in the dry matter of the first harvest fertilized with selenium in the dose of 20 g · ha^{-1} is two and a quarter times higher than the average content of this element in the first harvest of grasses fertilized with the dose of 15 g Se · ha^{-1}. In the second harvest, the content of selenium in the dry matter of forage grasses, compared to the first harvest, decreased four times in the sample fertilized with the dose of 15 g Se · ha^{-1} and over 2.5 times after fertilization with the dose of 20 g Se · ha^{-1}. This is in line with the studies of other authors, which showed that this element is mainly deposited in the aerial parts of plants [Wesołowski 2006]. In the experiment, a significant amount of the applied selenium was brought out with the yield of the first cut. The element detected in the second harvest was most likely from the reserves accumulated in the underground parts of the plants, although some of it could have been absorbed from the soil, the surface of which was also sprayed with liquid fertilizer. In the third harvest, selenium was no longer detected. The reason for this was most likely the depletion of selenium reserves in the root system. Also, the selenium in the root zone could have been washed away by rain. Thus, the effect of one-time foliar fertilization with selenium applied before harvest is noticeable only in the first and to a lesser extent in the second harvest.

It should be taken into account that selenium applied to the soil in the same doses as used in the experiment may be accumulated in the yield of fodder grasses in a significantly smaller amount. Rutkowska et al. [2004] state that foliar fertilization of winter triticale with selenium is the most effective method of providing this grass with this element. Selenium in soil application is more susceptible to environmental losses, related to, among others, leaching, switching to chemical forms that are more difficult for plants to absorb, or with ion antagonism.

There are numerous balance studies that analyze the effect of selenium concentration in feed rations on the metabolic changes of this element in cattle with various uses. It is assumed that the full coverage of the cattle's selenium requirements is provided by the content of approx. 0.3 mg Se $\cdot$ kg DM^{-1} of fodder [Haremza et al. 1988; Niwińska and Andrzejewski 2014]. None of the results obtained in the experiment meets this condition. Fertilization of fodder grasses with selenium doses ≤ 20 g $\cdot$ ha^{-1} cannot be regarded as an effective method of indirect selenium supply of cattle, which would allow for the elimination of feed additives from cattle feeding.

Further research on fertilization doses higher than 20 g Se $\cdot$ ha^{-1} should be undertaken and due to the short duration of the fertilization effect (it is maintained only in the swath following fertilizer application), three fertilization should be considered (before the first harvest, after the first harvest and after the second harvest). However, it is doubtful whether this method – as suggested in the introduction – would be a way to simplify the feeding of cattle. In agricultural practice, there is a problem of uneven selenium application on grasslands, which could result in exceeding the permissible Se content in one batch of feed and its deficiency in another batch. The livestock farmer does not have access to expensive laboratory methods to determine Se content prior to feeding. For now, a better solution seems to be to use ready-made, safe and easy-to-use feed additives characterized by a constant selenium content determined by the manufacturer.

CONCLUSIONS

1. Foliar feeding of fodder grasses with selenium in the form of sodium selenate in doses of $\leq$20 g Se $\cdot$ ha^{-1} has no statistically significant impact on the yield of green fodder. The results of the research do not justify the conclusion that selenium in doses $\leq$20 g $\cdot$ ha^{-1} has a toxic effect on plant growth and development. They also do not allow to consider its role as a beneficial element in the nutrition of fodder grasses.
2. Only foliar Se fertilization in the doses of 15 and 20 g Se $\cdot$ ha^{-1} increases its content in the dry matter of forage grasses. However, this content is insufficient in terms of cattle's demand for this element.
3. The effect of Se fertilization is maintained only in the two harvests following application of Se. No Se was detected in the plant material in the third harvest.

4. Further research should be undertaken on fertilization doses higher than 20 g Se · ha^{-1} and due to the short duration of the fertilization effect, three selenium applications should be considered (before the first mowing, after the first mowing and after the second mowing).

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WPŁYW NAWOŻENIA SELENEM TRAW PASTEWNYCH NA PLONOWANIE I ZAWARTOŚĆ SE W ROŚLINACH

Streszczenie. Celem badań było określenie wpływu nawożenia dolistnego mieszanki traw pastewnych selenem w dawkach 5, 10, 15, 20 g · ha^{-1} na zawartość pierwiastka w plonie trzech pokosów oraz ustalenie wpływu tego zabiegu na plonowanie traw. Trawy koszono trzykrotnie, każdorazowo mierzono plon zielonki i oznaczano zawartość Se w suchej masie metodą ICP-OES. Selen w dawkach ≤20 g · ha^{-1} nie zwiększył istotnie plonu zielonki. W suchej masie roślin z próby kontrolnej oraz roślin nawożonych Se w dawkach 5 i 10 g · ha^{-1} nie wykryto selenu. Dawka 15 g · ha^{-1} zwiększyła zawartość Se do 0,08 ±0,02 mg · kg s.m.$^{-1}$ w pierwszym pokosie oraz do 0,02 ±0,03 mg · kg s.m.$^{-1}$ w drugim pokosie. Selen w dawce 20 g · ha^{-1} podniósł zawartość tego pierwiastka do 0,18 ±0,08 mg · kg s.m.$^{-1}$ w pierwszym i do 0,07 ±0,02 mg · kg s.m.$^{-1}$ w drugim pokosie. W trzecim pokosie Se nie wykryto. Oznaczona zawartość Se w przypadku żadnej z dawek nie była wystarczająca dla zabezpieczenia potrzeb bydła.

Słowa kluczowe: biofortyfikacja w selen, jakość paszy, zawartość Se w paszy