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ORIGINAL PAPER

SULPHUR AS A FACTOR INFLUENCING THE CONTENT OF NITROGEN COMPOUNDS IN SEEDS OF NARROW-LEAFED LUPIN*

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Abstract

In view of the predicted increase in sulphur deficiency in crop production, a study was undertaken to assess the effect of fertilization with this nutrient on criteria determining the biological value of narrow-leafed lupin seeds (total and true protein, their ratio, nitrates and amino acid composition). The study was based on a three-year, three-factor field experiment, in which the Elf cultivar of narrow-leafed lupin (*Lupine Angustifolius* L.) was grown on light soil with low sulphur content. The experiment included foliar and soil application of sulphur and its elemental and ionic forms. The application doses were 0, 20, 40 and 60 kg S ha^{-1}. In all years of the study, the sulphur application dose was found to significantly determine the total and true protein content as well as their ratios in the lupin seeds. Application of just 20 kg S ha^{-1} caused a significant increase in true protein in the lupin seeds and in the proportion of true protein in the total protein, as compared to the control. The CS index for all the essential amino acids showed that the first limiting amino acid in the lupin seed protein was sulphur-containing methionine. As the dose of applied sulphur increased, there was an overall increase in $CS_{meth.}$ and EAAI. Given the positive effect of sulphur, particularly in the elemental form, on the protein content and amino acid composition of lupin seeds and in reducing the content of harmful nitrates, the use of this nutrient in fertilization of this species is recommended.

Keywords: Amino acids**,** *Lupinus angustifolius* L.*,* nitrates, protein, sulphur.

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INTRODUCTION

A new problem in agriculture is the growing sulphur deficiency, caused by the extremely strict environmental protection standards imposed at the end of the 20th century. Literature reports in recent years point to progressive sulphur deficiency in the soils of many regions the world (Scherer 2009, Jamal et al. 2010, Lucheta, Lambais 2012, Degryse et al. 2017, Shoman 2017), including Poland (Szulc 2008, *Monitoring*… 2019). This is primarily the result of ecological measures placing restrictions on emission of sulphur compounds into the atmosphere (Scherer 2009). A significant role has been played by international cooperation to reduce emissions of SO_2 and prevent its spread beyond national borders (*Directive 2001/80/EC of the European Parliament and of the Council…*). Sulphur deposition in Europe decreased from over 100 kg S year^{-1} in 1970 to 5-20 kg S year^{-1} in 2005 (Szulc, 2008). In 90% of samples of Polish soils, the available S does not exceed 16.5 mg SO_4^{2-} kg^{-1}, and in 2010 as many as 94% of profiles tested were classified as low in sulphur (*Monitoring*… 2019). According to many authors (Scherer 2009, Barczak 2010, Zaman et al. 2014), given the important functions of sulphur in plant metabolism and its positive effect on crop yield and quality, this element should not be overlooked when planning fertilization of crops. Species sensitive to sulphur deficiency, besides the families *Brassicaceae* and *Liliaceae*, include representatives of the *Fabaceae* family (Szulc et al. 2014). The role of sulphur in fertilization of plants of this family is important due to the specific character of their nitrogen metabolism (Khan, Mazid 2011) Insufficient sulphur in the soil can lead to nitrogen-fixing disturbances in *Fabaceae*, as the activity of both ferredoxin and nitrogenase, which are crucial to the process, requires the presence of sulphur (Khan, Mazid 2011). Similarly, protein biosynthesis is determined by sulphur supply to plants (Cazzato et al. 2012). Sulphur-containing methionine is a limiting amino acid reducing protein quality in *Fabaceae* (Barczak 2010). Aulakh (2003) showed that the seed yield of legumes increases by 18-33% as a result of sulphur application, and in a study with chickpea Shoman (2017) found that application of 0-200 kg S ha^{-1} increased the yield by 84-87%.

In view of the predicted growth in plant-production sulphur deficiency (Scherer 2009, Shoman 2017, *Monitoring*… 2019) and the need to maintain high crop quality, a study was undertaken to determine the effects of application of sulphur fertilizers on quality criteria of crops. Fertilization with sulphur, due to its important physiological role in nitrogen metabolism in legume plants, is assumed to significantly affect the biological value of narrow-leafed lupin seeds. The aim of the research was to evaluate the effect of different methods, forms and doses of sulphur application on the content of total and true protein in narrow-leafed lupin seeds, their proportions, and the content of nitrates as a mineral form of nitrogen.

MATERIALS AND METHODS

Plant material, growth conditions, and mineral fertilizer treatments

The research was based on a field experiment carried out in 2009-2011 at the Experimental Station (53°26′ N, 17°79′ E) of the University of Science and Technology in Bydgoszcz (northern Poland). The experiment was set up in three replications, within a randomized split plot design. The soil was a Haplic Luvisol (IUSS Working Group WRB, 2015) with the textural composition of loamy sand (light soil according to agronomic soil categories). The soil represented a good rye complex, and belonged to soil valuation class IIIb. It had acidic pH and moderate content of available forms of phosphorus, potassium and magnesium. Interestingly, the level of sulphate S-SO_4^{2-} in the soil where the experiment was performed qualifies it as soil of low sulphur content.

The following factors were considered in the field experiment:

– factor A – means of application of substances containing sulphur: soil application before sowing and foliar application;

– factor B – form of sulphur: elemental sulphur in the form of Siarkol Extra and ionic sulphur in the form of sodium sulphate (VI);

– factor C – dose of applied sulphur in kg S ha^{-1}: 0, 20, 40 (20+20) and 60 (20+20+20).

The plants were sprayed with the solutions of Siarkol Extra and sodium sulphate (VI) at a concentration of 3.3% at the following growth stages (designated according to the European Union BBCH scale):

– 20 kg S ha^{-1} was applied once prior to bud-setting (BBCH: 45-50);

– 40 kg S ha^{-1} was applied at two stages: 20 kg S ha^{-1} prior to bud setting (BBCH: 45-50) and 20 kg S ha^{-1} at the beginning of flowering (BBCH: 53-59);

– 60 kg S ha^{-1} was applied at three stages: 20 kg S ha^{-1} prior to bud setting (BBCH: 45-50), 20 kg S ha^{-1} at the beginning of flowering (BBCH: 53-59), and 20 kg S ha^{-1} at full flowering (BBCH: 64-67).

Sulphur application was accompanied by uniform pre-sowing application of phosphorus and potassium: phosphorus at a dose of 30 kg P ha^{-1} in the form of 40% triple superphosphate, and potassium at 60 kg K ha^{-1} as 60% potassium chloride. The area of the plots for harvest was 15 m^2. The preceding crop in each year of the study was spring barley.

Laboratory analysis procedure

Content of the following was determined in lupin seeds of all the experimental treatments (Łoginow et al. 1990):

– total nitrogen (N_{total}) by the Kjeldahl method (distillation was carried out in a Kjeltec 2200 Foss);

– protein nitrogen ($N_{protein}$) by the Kjeldahl method following precipitation of protein with trichloroacetic acid (Kjeltec 2200 Foss);
– nitrate nitrogen ($N\text{-}NO_3$) by the colorimetric method with phenoldisulfonic acid (DR-2000).

The products of the content of total, protein and nitrate nitrogen, together with appropriate conversion factors, were used to calculate the content of total protein ($6.25 \times N_{total}$), true protein ($6.25 \times N_{protein}$) and nitrates ($4.43 \times N\text{-}NO_3$).

Statistical analysis

The results were statistically analysed by ANOVA, using Statistica software (SAS Institute, Cary, North Carolina, USA). Three-factor analysis of variance was performed in a randomised block design in a mixed model. The significance of differences between the means for respective fertilization treatments (different letters in the tables) was determined by the Tukey range test ($p \leq 0.05$).

Meteorological conditions in 2009-2011

The field experiment was performed in an area with an average annual air temperature of 7.8°C and precipitation generally not exceeding 450 mm, and about 300 mm for the growing period (means from 1949-2011). Field work begins at the beginning of April and the growing period usually lasts 205-230 days.

For a more complete characterization of the weather conditions during the research period, Selyaninov's hydrothermal coefficient was calculated for the months of the narrow-leafed lupin's growing season:

$$K = P / 0.1 \Sigma t,$$

where: P – total monthly precipitation in mm,
t – total monthly air temperature >0°C

The values for Selyaninov's coefficient (Table 1) show high variation in the weather conditions in different years of the study. The highest temperature and precipitation fluctuations occurred in the 2010 growing season, when the most severe water deficits of the entire research period were recorded (Selyaninov's coefficient averaged 0.43 in June and 0.35 in July, while in April it attained its highest mean value (K=3.62), indicating

Table 1

Selyaninov coefficient values throughout the research period

Years	Months				
	April	May	June	July	August
2009	1.57	2.18	0.68	0.56	0.85
2010	3.62	1.55	0.43	0.35	2.51
2011	0.69	1.71	1.93	1.88	0.76

extremely moist conditions). More stable conditions in terms of temperature and precipitation were recorded in 2009, which was distinguished from the other research years by low precipitation from June to August.

RESULTS AND DISCUSSION

The mean content of total and true protein in the narrow-leafed lupin depending on the research year fell within a relatively wide range and was 285.6-332.6 g kg^{-1} and 232.7-271.1g kg^{-1} (Table 2). In 2009 and 2010,

Table 2

Content of total and true protein in the seeds of narrow-leafed lupin

Factor of study		Factor level	Years of study		
			2009	2010	2011
Total protein – 6.25 N_{total} (g kg^{-1})					
A	application method	soil fertilizer	329.4	299.4	286.3
		foliar fertilizer	331.9	299.4	285.6
B	form	elemental	332.5^{a}	300.6^{a}	286.3^{a}
		ionic	328.8^{b}	297.5^{b}	285.6^{a}
C	dose (kg S ha^{-1})	0	329.6^{b}	298.1^{b}	283.8^{b}
		20	330.0^{b}	299.4^{b}	285.6^{ab}
		40	335.0^{a}	300.6^{b}	286.9^{ab}
		60	335.6^{a}	304.4^{a}	288.8^{a}
mean			331.6	299,9	286.1
True protein – 6.25 $N_{prot.}$ (g kg^{-1})					
A	application method	soil fertilizer	270.0	234.4	240.6
		foliar fertilizer	271.9	231.3	233.8
B	form	elemental	271.3^{a}	234.4^{a}	236.9^{a}
		ionic	270.6^{a}	231.3^{b}	236.9^{a}
C	dose (kg S ha^{-1})	0	262.5^{c}	225.6^{b}	231.3^{c}
		20	271.3^{b}	233.8^{a}	236.3^{b}
		40	274.4^{ab}	235.0^{a}	239.4^{ab}
		60	276.3^{a}	236.3^{a}	241.3^{a}
mean			271.1	232.7	237.1
Ratio of true protein to total protein (%)					
A	application method	soil fertilizer	81.9^{a}	78.4^{a}	84.0^{a}
		foliar fertilizer	81.9^{a}	77.3^{b}	81.8^{b}
B	form	elemental	81.5	78.0	82.8
		ionic	82.4	77.7	82.9
C	dose (kg S ha^{-1})	0	79.6^{b}	77.4^{b}	80.4^{b}
		20	82.3^{a}	77.7^{ab}	82.8^{a}
		40	81.8^{a}	78.5^{a}	83.5^{a}
		60	82.3^{a}	77.8^{a}	83.6^{a}
Mean			81.7	77.9	882.7

the hydrothermal conditions were more favourable to total protein accumulation than in 2011. In the first two years of the study, the values of Selyaninov's coefficient in June and July were very low, indicating dry and very dry conditions (Table 1). According to Song et al. (2016), precipitation deficits and high sun exposure at the stage of generative organ formation are conducive to higher protein content in seeds of soybean. The conditions least favourable to total protein accumulation in lupin seeds occurred in 2011, when relatively low temperature was accompanied by high soil moisture in the period prior to pod filling. High variation in the protein content of harvested crops, depending on weather conditions, has been emphasized in research on the role of sulphur in agrotechnical practices for spring barley (Staugaitis et al. 2014), while Klikocka (2004) stresses that the effect of the weather factor on the protein content in potato is considerably stronger than that of the form or application dose of sulphur.

The means of sulphur application (factor A), unlike factors B and C, had no significant effect on the content of total and true protein (Table 2). It is likely that the effectiveness of factor A depends mainly on air temperature and the amount and distribution of rainfall during the growing season. Foliar application of fertilizer has many advantages over soil application (Scherer 2009) . The nutrients applied in the solution act very rapidly, biological and chemical absorption can be avoided, and the treatment can be combined with pesticide application. However, this means of fertilization has serious disadvantages as well, i.e. dependence on weather conditions and the risk of leaf scorching. On the other hand, soil application entails losses of the nutrient due to absorption and leaching into the soil profile. In the present study, the effect of weather factors determining the efficiency of the means of application of sulphur fertilizers in each year was probably multi-faceted. In consequence, no statistically confirmed differences were noted in the content of true or total protein in the lupin seeds depending on factor A.

Especially in the dry years (2009 and 2010), content of both protein forms was generally higher in the treatments fertilized with elemental sulphur than in the Na_2SO_4 treatments. In 2011, with high precipitation levels, no differences were noted in the effect of the various sulphur forms.

The form of sulphur generally has a minor effect on the chemical composition of the crop (Scherer 2009, Degryse et al. 2017). In elemental form, sulphur is not soluble in water, which may be an advantage because it reduces losses of the nutrient through leaching. On the other hand, bacteria of the genus *Thiobacillus* must mediate its biological oxidation to sulphate (VI) form, which is available for plants. Due to the need for this transformation, sulphur in elemental form acts more slowly than the ionic form, especially in conditions of insufficient rainfall.

Of all factors, the sulphur dose most affected the level of the protein forms assayed in the lupin seeds. Their highest concentrations were recorded following application of 60 kg S ha^{-1}, with no significant differences noted between the doses of 40 and 60 kg S ha^{-1}. The year in which sulphur application, irrespective of its application and form, resulted in the greatest increases in the total and true protein content as compared with the control, was 2009.

The correlation between a good supply of sulphur to legumes (*Fabaceae*) and increased protein content, both in their vegetative parts and in the seeds, has been confirmed in reports by researchers such as Zhao et al. (1999) and Cazzato et al. (2012). Ganeshamurthy and Reddy (2000) showed that appropriate application of sulphur in agrotechnical practices for soya bean and pea resulted in an increased number and weight of nodules on the plant roots, intensifying the process of biological reduction of molecular nitrogen and thus protein synthesis. This can be explained by the presence of sulphur in nitrogenase and ferredoxin – enzymes involved in nitrogen assimilation (Scherer 2009). Their activity is conditioned by an appropriate spatial pattern, maintained by these enzymes owing to sulphide bonds formed by sulphide groups (–SH) of sulphur amino acids.

The measure of the efficiency of plant protein synthesis is the share of true protein in the total protein ($6{,}25\times N_{protein}/6{,}25\times N_{total}$), expressed as percentage. Despite considerable variation in the content of these protein forms in the lupin seeds between research years, the ratio was relatively stable, ranging on average from 77.8% to 82.9% (Table 2). Interestingly, in 2011, when the hydrothermal conditions were least favourable to nitrogen accumulation in the seeds, the ratio of true protein to total protein was highest. In two years (2010 and 2011) sulphur application to the soil was found to be more conducive to an increase in this value, and thus to the transformation of nitrogen compounds into protein, as compared to foliar application. In contrast to the reports by Brodowska (2004), who investigated spring cultivars of wheat and oilseed rape, and by Klikocka (2004) in a study on potato, who found that elemental sulphur had a more favourable effect than the ionic form in terms of the protein content in the harvested crops, although the present research on narrow-leafed lupin demonstrated no significant effect of the sulphur form on the content of the $N_{protein}/N_{total}$ ratio in any year of the study.

As in the study by Brodowska (2004), the sulphur application dose (factor C) was shown to have a much stronger effect than the means of application (factor A) and its form (factor B) on the ratio of true protein to total protein in the lupin seeds. Its value generally increased with respect to the treatments without sulphur after the application of just 20 kg S ha^{-1}. The highest application dose (60 kg S ha^{-1}) did not significantly affect the ratio as compared with the dose of 40 kg S ha^{-1}.

The increase in the share of true protein in its total content after sulphur application indicates intensification of protein synthesis when the supply of sulphur to plants is increased, confirming its essential role in nitrogen metabolism. This suggests that nitrogen uptake and biochemical changes in this nutrient were closely dependent on the presence of sulphur in the environment. Increasing the level of sulphur application improves plants' supply of this essential nutrient for protein synthesis and determines the content of both true and total protein, as well as their proportions, to a greater extent than the means of application or the form of sulphur fertilizer. The effect of these factors on these parameters depends to a greater extent on temperature and rainfall during the growing season than in the case of varied application doses.

Nitrates are important non-protein nitrogen compounds. Excessive nitrates in the diet are harmful to human health due to their oxidative properties. This form of nitrogen can be reduced to nitrites in the gastrointestinal tract of humans and animals and can induce methemoglobinemia, a disorder causing hypoxia in the central nervous system (Lundberg et al. 2008, Pobereżny et al. 2015). The presence of the reduced form of nitrogen ($N\text{-}NO_2^-$) is also conducive to the formation of mutagenic and carcinogenic nitrosamines. Another consequence of an excessive level of nitrates in the diet is oxidation of vitamin A, deficiencies of which interfere with protein synthesis (Kozłowska-Strawska, Badora 2013). A study on animals (Vough et al. 2006) found that the presence of nitrates in feed had a negative effect on body weight gain, reproduction, thyroid function, and the content of vitamins in the body.

In each year of the research, a significantly higher content of this form of nitrogen form was observed after the foliar application of sulphur than in the case of soil application; the difference was 5.3% on average for the three years (Table 3).

Table 3

Content of nitrates in the seeds of narrow-leafed lupin

Factor of study		Factor level	Years of study		
			2009	2010	2011
A	application method	soil fertilizer	218.8[b]	190.9[b]	191.4[b]
		foliar fertilizer	231.3[a]	200.6[a]	201.4[a]
B	form	elemental	214.3[b]	191.4[b]	190.6[b]
		ionic	235.8[a]	200.1[a]	202.2[a]
C	dose (kg S ha^{-1})	0	231.9[a]	206.1[a]	202.0[a]
		20	221.2[b]	196.6[bd]	197.8[a]
		40	222.9[b]	187.4[c]	189.2[bc]
		60	224.2[ab]	192.9[d]	196.5[ac]
Mean			225.1	195.7	196.4

Application of Na_2SO_4 led to greater accumulation of nitrates than the use of elemental sulphur (an average difference of 7.0%). Of all the factors studied, the sulphur application dose had the strongest effect on the content of nitrates in the seeds. Increasing application doses, as reported by Elwan and Abd El-Hamed (2011) for vegetables and by Filipek-Mazur and Gryzełko (2009) for white mustard, generally decreased the content of nitrates. On average for both application methods and both forms of sulphur, the use of 40 kg S ha^{-1} decreased nitrate content in lupin seeds by 13.5 mg kg^{-1} (6.3%), as compared with the control. The direction of changes in the content of nitrates shows that sulphur fertilization enhanced the nutritive value of the lupin seeds.

CONCLUSIONS

In the seeds of narrow-leafed lupin, the content of total and true protein, their ratio, and the content of nitrates in each year of the study were significantly dependent on sulphur application as well as on the weather conditions during the growing season. As compared to the means of application and the form of sulphur, the application dose was the factor that most strongly influenced the content of true and total protein, as well as the ratio of these two forms. In all years of the study, the application of 20 kg S ha^{-1} significantly increased the content of true protein in the lupin seeds and the ratio of true to total protein, as compared to the control. The nitrate content was generally reduced by sulphur application. Given the positive effect of sulphur, particularly its elemental form, on the nutritional value of lupin seeds, application of 20-40 kg S ha^{-1}, depending on the sulphur content in the soil, is recommended for cultivation of this species.

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