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ORIGINAL PAPER

EFFECT OF SULPHUR FERTILIZATION ON THE CONTENT OF COMPONENTS INFLUENCING FODDER QUALITY IN COCKSFOOT (*DACTYLIS GLOMERATA* L.)*

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Abstract

The nutritional value of animal feed primarily depends on its chemical composition (content of crude protein, crude ash, crude fibre, crude fat and nitrogen-free extracts), which is to some extent determined by fertilization with sulphur. The aim of the study was to evaluate the effect of different sulphur application regimes on selected quality parameters of cocksfoot *Dactylis glomerata* L. A field experiment was conducted on brown eutrophic soil. Sulphur was applied in two doses: 10 kg S ha^{-1}, and 20 kg S ha^{-1}. In addition, each experimental treatment was fertilized with 120 kg N ha^{-1}, 30 kg P ha^{-1}, 100 kg K ha^{-1}, and 15 kg Mg ha^{-1}. During the growing period from March to September, air temperature and precipitation were monitored. Fertilization with sulphur was found to affect the content of crude fibre, crude ash, crude fat, cystine and methionine in cocksfoot, while no correlation was observed between the content of these chemical components and the weather conditions. The concentration of nitrogen-free extracts did not vary significantly in response to sulphur application, but the content of these compounds correlated with the average precipitation sum, air temperature, and Selyaninov's coefficient. Sulphur application did not affect the crude protein content, which was significantly correlated with the temperature and Selyaninov's coefficient during the growing season of cocksfoot. The results of the field experiment suggest that sulphur fertilization of cocksfoot increased the quality of the fodder.

Keywords: crude protein, crude fibre, crude ash, crude fat, nitrogen-free extracts, cystine and methionine.

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INTRODUCTION

Most soils in Poland are acidic or very acidic, which is a cause of increased leaching of sulphur (S) into the soil profile and its limited availability for plants (Malarz et al. 2011). The reduction in emissions of sulphur compounds into the atmosphere has led to a deficiency of this element in the soil and, consequently, in crop production (Patorczyk-Pytlik et al. 2008). Over the years, Polish researchers have been improving analytical methods and tests of plants and soils aimed at diagnosing sulphur application requirements and evaluating sulphur supply to crop plants (Skwierawska et al. 2016). Once present in excess in the environment, sulphur has now become an element which limits crop production, and symptoms of sulphur deficiency have been in plants with both high and low sulphur requirements (Barczak et al. 2013). Sulphur deficiency in the soil has an adverse effect not only on crop yield and quality, but also on the uptake and metabolism of other nutrients, such as nitrogen (Kaczor, Zuzańska 2009).

Sulphur is a macroelement that plays an important role in nutrition and fertilization of crop plants (Mugford et al. 2011). Crop requirements for sulphur depend on the expected yield, species, use of organic and mineral fertilizers, soil moisture content, type of a soil profile, climate and the hydrological conditions in a field. The main function of sulphur in the plant is as to be the core element of two essential amino acids cystin, cystein and methionine, and to participate in the composition and functioning of many coenzymes (Scherer 2001). Sulphur is needed for physiological functions in the plant; it has biological, protective and economic significance because plants that are well supplied with this macronutrient are less vulnerable to biotic stresses and more resistant to diseases, as a result of which produce higher yields. Sulphur improves the nutritional and consumption value of harvested crops. Sulphur plays the key role in nitrogen utilization by the plants (Na, Salt 2011). Sulphur deficiency in the growing environment of plants is conducive to their higher accumulation of nitrogen but a decreased concentration of albuminoid nitrogen, which indicates the important role of S in protein synthesis. Sulphur deficit contributes to the inhibition of photosynthesis. Negative consequences of a low sulphur concentration in plants include leaf chlorosis and inhibition of the growth of the youngest leaves, which reduces the green area index, thereby determining the course of photosynthesis (Podleśna 2005). Sulphur application increases the content of essential amino acids, particularly methionine, cysteine and cystine (Skwierawska et al. 2016).

Several grass species are used to produce animal feeds on arable land, including *Dactylis glomerata* L. An adequate content of nutrients is particularly important in the cultivation of grasses for fodder. Grasses and mixtures of grasses and pulses are a source of fibre, energy and protein essential to ruminants. In order to attain high levels of animal production, it is important to ensure high nutritional quality of bulky feeds, and in particular to increase the content of sulphur-containing amino acids essential for maintaining proper metabolism in mammals.

Therefore, the aim of the study was to evaluate the effect of varied sulphur application on selected quality parameters of cocksfoot *Dactylis glomerata* L.

MATERIAL AND METHODS

In 2011-2014 a field experiment was carried out at the Experimental Station in Zamość, (50°42′ N, 23°12′ E), University of Life Sciences in Lublin. The plant tested was the Amba cultivar of cocksfoot (*Dactylis glomerata* L.). In each year of the experiment, the grass was harvested three times: in May, July and September, always in the last ten days of a month. The experiment was conducted on brown eutrophic soil with the textural composition of silt loam (Polish Soil Classification 2011). Characteristics of the soil are presented in Table 1.

Table 1

Basic characteristics of the soil prior to the field experiment

Parameter	Value
pH in 1 mol dm^{-3} KCl	6.7
Hydrolytic acidity (Hh)	7.5 mmol H^+ kg^{-1}
Available phosphorus	274.0 mg P kg^{-1} (very high level)
Available potassium	127.1 mg K kg^{-1} (medium level)
Available magnesium	106.0 mg Mg kg^{-1} (high level)
Total sulphur	170.2 mg S kg^{-1}
Sulphate sulphur (S-SO_4)	15.1 mg S kg^{-1} (low level)
Exchangeable calcium	106.40 mmol (+) kg^{-1}
Exchangeable magnesium	7.85 mmol (+) kg^{-1}
Exchangeable potassium	7.15 mmol (+) kg^{-1}
Exchangeable sodium	2.75 mmol (+) kg^{-1}
Cation exchange capacity (CEC)	137.6 mmol (+) kg^{-1}

Sulphur was applied in two doses: 10 kg S ha^{-1}, and 20 kg S ha^{-1}. In each experimental treatment, 100 kg K ha^{-1} was applied. Potassium was applied in the form of KCl (49.8% K) on the plots without sulphur fertilization, while on the ones fertilized with 10 kg S ha^{-1} or 20 kg S ha^{-1} KCl was combined with potassium sulphate. Potassium was divided into two equal parts, of which one was applied in early spring before the start of plant growth, and the other one was given after the first harvest. In the field experiment, all sulphur was applied prior to the start of plant growth, in the form of potassium sulphate (K_2SO_4 – 41.5% K, 18% S). In each year of the experiment, the applied doses of nitrogen, phosphorus and magnesium were the same. Nitrogen was applied at 120 kg N ha^{-1} in the form of ammonium nitrate, phosphorus at 30 kg P ha^{-1} in the form of triple *superphosphate*, and magnesium in the amount of 15 kg Mg ha^{-1} in the form of magnesium chloride hexahydrate. Phosphorus and magnesium were applied once in early spring, and nitrogen was supplied three times: 60 kg prior to the start of plant growth, 30 kg after the first harvest and the remainder during the second harvest.

Every year after each harvest, plant material collected from each fertilization variant was dried and ground to obtain representative samples. The following were determined in this material: crude protein (N determined by the Kjeldahl's method x 6.25) (Radkowski, Kuboń 2007), fat (the Soxhlet method, according to PN-A-79011-4:1998), crude ash (gravimetric method according to PN-A-79011-8:1998), and crude fibre (ISO 6541:1981, a modified Scharrer method). The content of nitrogen-free extracts was calculated as the difference between the content of dry matter and the sum of crude protein, crude fat, crude ash and crude fibre (Łysoń, Biel 2016). The content of methionine and cystine was determined according to Schram et al. 1954.

During the growing period from March to September, air temperature and precipitation were monitored; the means for each year are presented in the paper (Table 2). The highest precipitation sum in the growing season was noted in the first year of the experiment, and the lowest one occurred in

Table 2

Weather conditions during the cocksfoot growing season from March to September

Growing season	March	April	May	June	July	August	September	Mean air temperatures (°C)
Air temperatures (°C)								
1	3.1	11.6	16.1	19.9	20.1	20.2	16.7	15.4
2	5.0	10.8	16.6	19.5	23.6	20.7	16.2	16.1
3	-1.3	9.9	17.5	20.2	21.6	22.0	13.4	14.8
Precipitation (mm)								precipitation sum (mm)
1	7.90	39.5	23.6	101.8	177.6	89.1	1.80	441.3
2	13.1	51.5	55.7	88.9	20.0	62.1	36.6	327.9
3	13.8	49.3	108.2	120.5	44.2	11.2	79.3	426.5
Selyaninov's coefficient								mean Selyaninov's coefficient
1	0.60	1.14	0.47	1.71	2.85	1.43	0.04	1.18
2	0.77	1.59	1.08	1.52	0.27	0.97	0.75	0.99
3	4.91	1.66	2.01	1.99	0.66	0.17	1.97	1.91

the second year. In the whole experiment, the mean temperature during the growing period was higher than the long-term average for 1976-2000 (12.3°C). The air temperature and amount of precipitation from March to September were used to calculate the Selyaninov's hydrothermal coefficient (Skowera, Puła 2004).

The results were analyzed statistically. The data were subjected to analysis of variance and the means were separated by the Tukey's test ($p \leq 0.05$). The Pearson's correlation coefficients were calculated.

RESULTS AND DISCUSSION

Generally, mineral fertilization has a beneficial effect on the chemical composition of animal feeds obtained from permanent grassland. Grasses, despite being classified among plants with low sulphur requirements, respond positively to fertilization with this element (Scherer 2001). Research indicates that sulphur applied in the form of sulphate fertilizers is a significant factor with regard to cocksfoot yield (Kaczor, Brodowska 2009). Cocksfoot has low sulphur requirements, but the lack of fertilization with this nutrient led to its content in unfertilized plants fall below the values considered optimal for grasses. The need for sulphur to achieve a proper growth and development of plants is partly explained by its beneficial effect on photosynthesis, protein biosynthesis, and synthesis of lignin and fatty acids (Gondek, Gondek 2010). The quality of fodder for ruminants is to a certain extent described by quality indicators such as the content of protein, crude fibre, crude ash, fat, and amino acids, including sulphure containing ones.

The crude protein content is a characteristic trait for cultivars and species of grasses. It is also linked to habitat fertility and especially to soil nitrogen content. In the present study sulphur application did not significantly affect the amount of crude protein in the dry weight of cocksfoot (Table 3). In the successive years of cocksfoot cultivation, the mean concentration of crude protein in the dry weight of cocksfoot ranged from 92.5 to 137.7 g kg^{-1}, which is consistent with literature data regarding its mean content (Grzelak 2010). The highest crude protein content was noted in the plants that were not fertilized with sulphur in the third year of the field experiment. The present study confirms earlier reports by Radkowski, Grygierzec (2004), who found similar levels of crude protein in green plants of permanent meadows (from 116.0 to 120.0 g kg^{-1}). According to Bruinenberg et al. (2001), the mean content of protein in grasses may change if mowing is too late, because in this phase the content of indigestible substances usually increases in grasses. An insufficient sulphur content in plants inhibits nitrogen metabolism, having a negative effect on the yield and the biological and technological value of the crop (Barczak et al. 2013). The crude protein content in grasses may vary between cuts. Research has confirmed it for *Phleum pratense*, in

Table 3

Concentration of selected nutrients in the dry weight of cocksfoot

Growing season		1				2				3				Mean 1-3	LSD $_{0.05}$
Swath		I	II	III	mean 1	I	II	III	mean 2	I	II	III	mean 3		
	Sulphur dose	(g kg^{-1}d.m.)													
Crude protein	0 kg S ha^{-1}	105.0	108.8	115.6	109.8	115.0	85.50	100.0	100.2	16.12	13.53	11.56	13.74	11.58	n.s.
	10 kg S ha^{-1}	85.06	106.9	104.4	99.00	116.2	80.60	80.60	92.50	158.1	112.5	101.3	124.0	105.1	
	20 kg S ha^{-1}	108.8	98.80	95.00	100.9	110.0	90.00	88.10	96.00	165.0	111.9	105.6	127.5	108.1	
Crude fibre	0 kg S ha^{-1}	252.0	260.0	256.0	256.0	259.0	263.0	267.0	263.0	270.0	271.0	267.0	269.3	262.8	0.48
	10 kg S ha^{-1}	265.0	271.0	264.0	266.7	255.0	261.0	260.0	258.7	260.0	267.0	269.0	265.3	263.6	
	20 kg S ha^{-1}	274.0	275.0	264.0	271.0	244.0	247.0	250.0	247.0	252.0	254.0	259.0	255.0	257.7	
Crude ash	0 kg S ha^{-1}	90.00	91.00	91.00	90.70	92.00	94.00	94.00	93.30	93.00	95.00	94.00	94.00	92.70	0.17
	10 kg S ha^{-1}	92.00	94.00	91.00	92.30	98.00	95.00	95.00	96.00	95.00	96.00	96.00	95.70	94.70	
	20 kg S ha^{-1}	93.00	97.00	92.00	94.00	100.0	98.00	100.0	99.30	100.0	100.0	101.0	100.3	97.90	
Crude fat	0 kg S ha^{-1}	19.00	21.00	23.00	21.00	17.00	17.00	18.00	17.30	22.00	22.00	21.00	21.70	20.00	0.15
	10 kg S ha^{-1}	25.00	22.00	22.00	23.00	20.00	20.00	20.00	20.00	25.00	24.00	23.00	24.00	22.30	
	20 kg S ha^{-1}	29.00	28.00	28.00	28.30	20.00	20.00	18.00	19.30	20.00	19.00	16.00	18.30	22.00	
Nitrogen-free extracts	0 kg S ha^{-1}	534.0	519.2	514.4	522.5	517.0	540.5	521.0	526.2	453.8	476.7	502.4	477.6	508.8	n.s.
	10 kg S ha^{-1}	532.4	506.1	518.6	519.0	510.8	543.4	544.4	532.9	461.9	500.5	510.7	491.0	514.3	
	20 kg S ha^{-1}	495.2	501.2	521.0	505.8	526.0	545.0	543.9	538.3	463.0	515.1	518.4	498.8	514.3	
		(g kg^{-1} d.m.)													
Cystine	0 kg S ha^{-1}	2.37	2.51	2.60	2.49	2.58	2.68	2.65	2.64	2.69	2.62	2.59	2.63	2.59	0.10
	10 kg S ha^{-1}	2.55	2.51	2.52	2.53	2.78	2.65	2.76	2.73	2.74	2.75	2.82	2.77	2.68	
	20 kg S ha^{-1}	2.61	2.43	2.68	2.57	2.74	2.81	2.75	2.77	3.00	3.21	2.95	3.05	2.80	
Methionine	0 kg S ha^{-1}	1.92	2.16	1.85	1.98	1.86	1.96	1.99	1.94	2.20	1.90	2.02	2.04	1.98	0.13
	10 kg S ha^{-1}	2.23	2.12	1.96	2.10	2.05	1.95	2.11	2.04	2.10	2.00	2.12	2.07	2.07	
	20 kg S ha^{-1}	2.26	2.20	2.08	2.18	2.24	2.15	2.03	2.14	2.28	2.18	2.16	2.21	2.18	

Significance level: $p \leq 0$ 05; n.s. – no significant differences, d.m. – dry mass which the protein content was 94.5 g kg^{-1} in the first cut but increased in the second and third cuts (Łyszczarz 2001). The literature contains numerous examples of sulphur application causing an increase in the protein content, particularly in rapeseed, which is a sulphur-loving plant (Wielebski 2011). The link between sulphur application and increased protein concentration has also been confirmed by others (Podleśna 2005). Gondek and Gondek (2010) analyzed the effect of mineral fertilization on spring wheat yield and found that an additional application of sulphur caused an increase in crude protein in grain and straw of the plant. In contrast, Wieser et al. (2004) and Podleśna and Cacak-Pietrzak (2006) found no significant influence of sulphur application on the protein content. They did not find a distinct effect of sulphur application on the concentration of crude protein in the dry weight of plants in an experiment conducted on winter and spring wheat.

Crude fibre contains components of plant cell walls, mainly cellulose and hemicellulose, and is essential for proper digestion in the forestomach and digestive tract of cattle. The content of crude fibre in green forage should not exceed 300 g kg^{-1} DW (Nowak, Wołoszczyk 1995), and the optimal level is about 250-280 g kg^{-1} DW. According to dietary norms the minimum quantity of crude fibre in fodder should be 130 g kg^{-1} for cattle and 180-220 g kg^{-1} for dairy cows (Nutrition Standards IZ-INRA 2001). In the present study, the mean content of crude fibre in the dry matter of cocksfoot in the years of the experiment ranged from 247.0 to 271.0 g kg^{-1} (Table 3). Analysis of the means for the entire cocksfoot cultivation period reveals that application of sulphur at a rate of 20 kg S ha^{-1} significantly decreased the concentration of crude fibre in comparison with the rate of 10 kg S ha^{-1} (Table 3). A decrease in the content of crude fibre is beneficial because it is poorly digested and has a negative effect on the digestion of other feed components. Bruinenberg et al. (2001) emphasize that grasses should be mowed before heading, as later mowing causes a rapid decline in nutritional value.

Sulphur application in the experiment increased the content of crude ash (the non-organic portion of fodder, in which macronutrients predominate; Table 3). In the plants fertilized with 20 kg S ha^{-1}, the content of crude ash increased in the successive years by 36 g kg^{-1}, 65 g kg^{-1} and 7.4 g kg^{-1}. In an experiment on the chemical composition and nutritional value of winter rapeseed cultivars, Malarz et al. (2011) found that sulphur application had no significant impact on the crude ash content in the seeds or meal of this plant.

Crude fat significantly affects the energy value of feeds and digestive processes in cattle. The concentration of crude fat determined as the ether extract in bulky feeds ranges from 20 to 50 g kg^{-1} DW (Brzóska, Śliwiński 2011). In the present study, sulphur application in the three years caused a significant increase in the concentration of this component in the dry weight of cocksfoot (Table 3). In the available literature, most studies on the effect of sulphur application on the crude fat content are conducted on rapeseed. According to Wielebski (2011), sulphur application significantly reduces the crude fat content in seeds. Its concentration is also significantly influenced by weather conditions. Years with a high precipitation sum are particularly favourable to fat accumulation (Malarz 2011), which was confirmed in the present study. This correlation was particularly evident in the first year of cocksfoot cultivation, with the highest precipitation sum (Table 2).

Nitrogen-free extracts containing mainly monosaccharides, disaccharides, oligosaccharides, starch and products of starch hydrolysis (Brzóska, Śliwiński 2011) are an important energy component of feeds. In the present study, sulphur application did not affect their concentration in cocksfoot straw. The highest mean content of nitrogen-free extracts was noted in cocksfoot harvested in the second year of cultivation. According to Grzelak (2010), the concentration of carbohydrates depends on many factors, like the species of plant, the stage of growth, the time of mowing, and on sunlight, which is linked to photosynthesis and the water content in green forage. Sulphur, as a component of vitamin B_1, is indirectly responsible for carbohydrate metabolism in the plant. Thiamine, which contains sulphur, is a key component of enzymes engaged in glycolysis, the pentose phosphate pathway, and in the Krebs cycle (Goyer 2010).

Sulphur-containing amino acids determine the biological value of protein (Gigolashvili, Kopriva 2014). Cysteine and the cysteine dipeptide cystine, as well as compounds containing an -SH group, take part in redox processes. A thiol group from cysteine is often part of the active centre of enzymes involved in redox reactions (Carfagna et al. 2011). Moreover, amino acids play a key role in maintaining the spatial conformation of protein by creating disulphide bridges, which are important for maintaining the tertiary structure of proteins. Key compounds for plant metabolism arise from cysteine: methionine, S-adenosynomethionine, S-methylmethionine, glutathione and, in consequence, phytochelatins (Gigolashvili, Kopriva 2014). Methionine is an essential amino acid of great importance in animal diets, because it is needed to produce S-adenosylmethionine, a donor of methyl groups necessary for synthesis of numerous metabolites, such as ethylene or polyamine (Davidian, Kopriva 2010). In the present study, the cystine content in the dry weight of cocksfoot was determined as cysteine and cystine combined (Table 3). The highest mean cystine content (3.05 g kg^{-1}) was noted in straw of cocksfoot fertilized with sulphur in the dose of 20 kg S ha^{-1} in the third year of the experiment. The highest methionine content (2.21 g kg^{-1}) was also noted in these plants. In the conditions of the present experiment, sulphur application caused a significant increase in the cystine and methionine content in the dry weight of cocksfoot in comparison with plants that were not fertilized with this nutrient.

In addition to agrotechnical factors, weather conditions significantly influence the yield of grasses (Dudek et al. 2004). During the experiment, the temperature and rainfall were varied (only means for the growing period in a given year are provided in the study). During the entire field experiment, an optimal value of the Selyaninov's coefficient was noted only in April and

June of 2012. This suggests that the cocksfoot growing period did not create optimal hydrothermal conditions for the grass. The correlation coefficients between the content of individual chemical components and the temperature, precipitation and Selyaninov's coefficient indicate that the hydrothermal conditions during the growing period did not significantly influence the content of crude fibre, crude ash, crude fat, cystine or methionine (Table 4). However,

Table 4

Correlation coefficients between the content of selected chemical components and atmospheric parameters in years 1-3

Selected chemical components	Mean air temperatures (°C)	Precipitation sum (mm)	Selyaninov's coefficient
Crude fibre	n.s.	n.s.	n.s.
Crude ash	n.s.	n.s.	n.s.
Crude fat	n.s.	n.s.	n.s.
Nitrogen-free extracts	0.91***	-0.66*	-0.91***
Crude protein	-0.89**	n.s.	0.95***
Cystine	n.s.	n.s.	n.s.
Methionine	n.s.	n.s.	n.s.

Significance level: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s. – no significant differences

precipitation was found to be significantly negatively correlated with the content of nitrogen-free extracts, while temperature was significantly positively correlated with their concentration and negatively correlated with the content of crude protein.

CONCLUSIONS

1. Differentiated sulphur application significantly affected parameters determining the quality of cocksfoot feed, such as crude fibre, crude ash, and crude fat, but did not affect the content of crude protein or nitrogen-free extracts.

2. Air temperature during the years of the study was significantly positively correlated with the concentration of nitrogen-free extracts, and negatively correlated with crude protein. The precipitation sum was negatively correlated with nitrogen-free extracts, which in turn were significantly negatively correlated with the Selyaninov's coefficient.

3. The results of the field experiment suggest that sulphur fertilization of cocksfoot is advisable because it significantly increases the methionine and cystine content in the plants and therefore improve the quality of the fodder.

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