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ORIGINAL PAPERS

CONCENTRATION AND POOLS OF TRACE ELEMENTS IN ORGANIC SOILS IN THE IZERA MOUNTAINS

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Abstract

Concentrations of trace elements in organic soils are a result of natural accumulation or due to anthropogenic factors. A field study was carried out in May 2008 on selected sites in the Izera Mountains. Soil samples were collected from 8 profiles of organic soils. Four soil profiles were located on the plateau meadow called Hala Izerska, at the altitude of 835-850 m a.s.l. The other 4 profiles were located in the mountain range known as the Grzbiet Wysoki, at 909-990 m a.s.l. The concentration and pools of heavy metals were determined according to the elevation, depth in soil profile, content of organic matter and soil pH. The content of trace elements (Pb, Cu, Zn) was determined after wet mineralization of samples in nitric acid, using a Flame Atomic Absorption Spectrometer. Pools of trace elements were recalculated into g m^{-2} in the 0-30 cm layer of soil. The aim was to determine the influence of altitude on concentrations of trace elements in organic soils profiles. Organic soils from the Grzbiet Wysoki and Hala Izerska showed significant contamination, mainly with lead and zinc. The elements were most abundant in surface horizons. With an increasing depth into the soil profile, the content of trace elements decreased. The arithmetic means showed strong dominance of lead on zinc and copper in the examined profiles. The Grzbiet Wysoki, due to its higher location above the sea level, is more exposed to atmospheric deposition of contaminants than Hala Izerska. However, the concentration of Cu is higher in organic soils from Hala Izerska. Statistical analysis showed significant positive correlation between the Pb concentration and altitude. With an increasing altitude, the content of Pb in soil also increased. Zinc and lead correlate highly

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negatively with the depth into the soil profile. With an increasing depth, the content of these elements decreases.

Key words: soil, peat, trace elements, mountains, mountain peatlands, the Izera Mountains.

KONCENTRACJA I ZASOBY PIERWIASTKÓW ŚLADOWYCH W GLEBACH ORGANICZNYCH Z OBSZARU GÓR IZERSKICH

Abstrakt

Koncentracja pierwiastków śladowych w glebach organicznych jest wynikiem naturalnej akumulacji, lub spowodowana czynnikami antropogenicznymi. Badania terenowe wykonano w maju 2008 r. na wybranych obszarach Gór Izerskich (Hala Izerska oraz Wysoki Grzbiet). Materiał glebowy pobrano z 8 profilów gleb organicznych. Próbki do badań z Hali Izerskiej pochodziły z 4 profilów zlokalizowanych na wysokości od 835 do 850 m.n.p.m, natomiast 4 profile wykonane na Wysokim Grzbiecie występowały na wysokości od 909 do 990 m.n.p.m. Koncentrację oraz zasoby pierwiastków śladowych określono na tle wysokości nad poziomem morza, głębokości w profilu, zawartości materii organicznej oraz odczynu gleby. Zawartość Pb, Cu, Zn oznaczono po mineralizacji próbek na mokro w stężonym kwasie azotowym z użyciem aparatu AAS. Zasoby pierwiastków śladowych w glebie przeliczano na g m^{-2} w 0-30 cm warstwie gleby. Celem pracy było określenie wpływu czynnika wysokościowego na akumulację badanych pierwiastków w profilu gleb organicznych. Zawartość badanych pierwiastków śladowych (Pb, Zn, Cu) w glebach organicznych Wysokiego Grzbietu oraz Hali Izerskiej wskazuje na znaczne zanieczyszczenie tych gleb, głównie Pb i Zn. Koncentracja wszystkich analizowanych metali jest największa w poziomach powierzchniowych i z reguły maleje wraz ze wzrostem głębokości. Średnie arytmetyczne zasobów wykazały zdecydowaną dominację Pb nad Zn i Cu w profilach analizowanych gleb. Obszar Wysokiego Grzbietu ze względu na wyższe położenie jest bardziej narażony na atmosferyczną depozycję zanieczyszczeń. Koncentracja miedzi jest wyższa w profilach glebowych Hali Izerskiej. W analizie statystycznej wykazano znaczącą dodatnią korelację między koncentracją Pb a czynnikiem wysokościowym. Wraz ze wzrostem wysokości nad poziomem morza wzrastała zawartość Pb w glebie. Cynk i ołów ujemnie silnie koreluje z głębokością w profilu glebowym, wraz ze wzrostem głębokości maleje zawartość tych pierwiastków.

Słowa kluczowe: gleba, torf, pierwiastki śladowe, góry, torfowiska górskie, Góry Izerskie.

INTRODUCTION

Increased concentrations of trace elements in soil are mainly caused by anthropogenic contamination. Major anthropogenic sources of trace elements include fossil fuel combustion, non-ferrous metal production and combustion of gasoline (Pacyna et al. 2001). These contaminants can be transported over long distances. Most trace elements in terrestrial ecosystems originate from atmospheric wet and dry deposition (Anicic et al. 2009) Organic soils are often used as references in studies of the impact of human activity on contamination with trace elements (Martínez-Cortizas et. al. 1999). These soils are formed under temporary or permanent waterlogged conditions from the partial decomposition of peat-forming plants (ZACCONE et al. 2007). Ombrotrophic bogs are particularly useful for assessment of the degree of trace element pollution from atmospheric deposition. This kind of peat soil is supplied with water mainly by atmospheric depositions (rain or snow) (KVAERNER et al. 2008). One of more important factors which can influence the trace element content in soils is altitude (GERDOL et al. 2006). It plays a significant role as it alters the atmospheric dry or wet deposition of trace elements.

The aim of this study was to determine influence of altitude on the trace elements concentrations in the profiles of examined organic soils.

STUDY AREA

The study was carried out in the Izera Mountains (the Western Sudetes), a mountainous region situated in southwestern Poland (Figure 1) at 50°49'- 50°51' N and 15°18'-15°29' E, with the maximum elevation of 1126 m a.s.l. The Izera Mountains lie in highland climatic regions with the longest winter duration of 110 days and snow cover duration of 116 days. The westerly and northerly Atlantic wet winds contribute to a high level of precipitation and wetness. The average air temperature is -5°C in January and 13°C in July, while the average annual precipitation is 1500 mm. The vegetation growth period lasts 175 days and starts in late April (MIGOŃ et al. 2003). The Izera Mountains are crisscrossed by valleys of several rivers and creeks.

Fig. 1. Localization of Izera Mountains

The Izera Valley is situated between the Grzbiet Wysoki and Grzbiet Średni ranges. Geomorphological processes, affected by the temperature and hydrological regime, continue to shape the mountains. Numerous flattened peaks, often with little runoff, favor the formation of alpine-like peatlands, which in total cover nearly 300 hectares. The peatlands in the Izera Mts. are considered to be a specific type of mountain subarctic peat bogs and share many features with northernmost peat bogs, particularly those in Scandinavia (MATUŁA et al. 1997). Peatlands in the Izerska Mts. developed on granites andgneisses. The analyzed sites in the Grzbiet Wysoki represent ombrotrophic peatlands with characteristic communities of *Eriophoro vaginati-Sphagnetum recurvi* class (profile 1), *Caricetum nigrae* class (profile 3), Calmagrostis villosae-Piceetum sphagnetosum class (profile 4). Site 2 is covered by replacement communities with *Molinia caerulea*. The plant communities growing on Hala Izerska (a meadow and pasture) belong to class *Scheucherio-Caricetae fuscae* (profiles 5 and 6) or constitute a forest site with class *Oxycocco-Sphagneta* with *Pinus sylvestris* – profile 7 and *Carex acutiformis* – profile 8 (POTOCKA 1996). The examined organic soils profiles were classified as Typical sapric peat soils or Typical hemic peat soils (PTG 2011) and Sapric or Hemic Histosols according to the WRB classification (WRB 2006). Profile 1 from the Grzbiet Wysoki was classified as Peaty-Gley soils (PTG 2011) and Histic Gleysols (WRB 2006). The Izera Mountains are located in an area called the Black Triangle (crossed by the Polish, Czech and German borders). For many years, it has been heavily polluted many power plants located in Northern Bohemia, Saxony and Lower Silesia. Additionally, the long-range pollution transport form North-Western Bohemia and Eastern Saxony has aggravated the local pollution (STRZYSZCZ et al. 2001).

METHODS

Soil samples were collected with an Instorf sampler from 8 sites located on two ombrotrophic peatlands: one in the Grzbiet Wysoki (4 profiles) and the other one on Hala Izerska (4 profiles). The sampling sites lay within an elevation range from 835-850 m.a.s.l (Hala Izerska) and 909-990 m.a.s.l (the Grzbiet Wysoki). The following properties were determined in the collected soil samples: soil colour according to the Munsell Color System, ash content by peat combustion in a muffle furnace at 550°C for 6 hours, bulk density measured in undisturbed samples, organic carbon determined by dry combustion using an automatic analyzer (CS – MAT 5500), total nitrogen assayed by standard Kjeldahl technique, pH in H_2O and 1 mol dm^{-3} KCl potentiometrically at the soil : solution ratio of 1:2.5, total content of trace elements (Zn_t, $Cu_{t,}$ Pb_t,) with flame atomic absorption spectroscopy (FAAS).

The samples were digested in nitric acid (HNO_3). Pools of trace elements at the depth of 0-30 cm were calculated using formulas: metal (g m^{-2}) = a (mg kg^{-1}) · b (g cm^{-3}) · c (mm)/1000 (a – metal content, b – bulk density, c – depth). Statistica 9.0 software (StatSoft Inc., Tulsa, OK) was used to test the statistical significance of trends in changes of soil properties. For each sites, Pearson correlation coefficients were determined between the concentration of trace elements, depth, altitude, pH and soil organic matter. Differences between pools of elements in soils from Hala Izerska and the Grzbiet Wysoki were calculated using the mean standard deviation (SD) and basic analysis of variance (CV).

RESULTS AND DISCUSSION

The examined organic soils are strongly acidic. The range of pH_{KCl} values was from 2.3 to 3.8 (Table 1). The pH_{KCl} values below 4 are characteristic of ombrotrophic bogs, which are supplied with water mainly by weakly acidic precipitation (Berset et al. 2001, De la Rosa et al. 2003). The determined soil organic carbon (SOC) values ranged from 265 to 535 g kg^{-1}. The lowest SOC was observed in horizons under the moorshing process. This phenomenon is broadly reported in the literature (Sokołowska et al. 2005, Kogel-Knabner 2002). It is caused by mineralization of organic matter and its release into the atmosphere. The highest SOC was found in peat horizons consisting hemic and sapric material. Arithmetic means of the total nitrogen content in soils did not exceed 20 g kg^{-1}. Values of this parameter in all samples are estimated between 5.1-35.1 g kg^{-1} (Table 1).

Table 1

Concentrations of trace elements and some other properties of organic soils horizons

Soil horizon	Depth (cm)	Soil colors	pH_{KCl}	C-total (g kg^{-1})	N og (g kg^{-1})	Pb	Zn	Cu
						(mg kg^{-1})		
Oi	5-20* 11**	10YR 8/3*** 7.5 YR 8/3	2.3-3.4 2.8	377-480 438	11.8-26.2 17.5	15.8-207 117	13.9-134 53.7	6.4-50.5 18.5
Oe	4-18 9	10YR 8/4 7.5YR 8/4	2.3-3.2 2.7	399-510 456	5.1-20.7 14.9	42.4-246 126	12.5-58.5 33.5	1.8-35.2 12.1
Oa	7-58 29	10YR 7/4 7.5YR 4/4	2.4-3.8 3.1	322-535 451	7.8-35.1 17.1	12.0-94.9 40.1	5.8-70.4 30.2	2.7-35.9 14.0
M	10-20 16	10YR 5/4 7.5YR 6/4	2.7-3.3 2.9	265-464 391	8.0-20.7 13.6	15.0-243 96.2	24.3-122 65.5	2.2-36.4 18.8

Key: M – moorsh, O – organic, a – sapric peat, e – hemic peat, i – fibric peat,
*range (minimum-maximum), **arithmetic mean, ***soil color range

According to the Polish ground quality standards (*Ordinance*…2002), the content of trace elements in the examined soils exceeds the permissible level set for group A areas (legally protected areas), except for the zinc concentration in organic soils in Hala Izerska, which was below the maximum threshold. Generally, the highest levels of trace elements appeared in the surface horizons. With an increasing depth into the soil profile, the content of trace elements decreases. The analyzed organic soils are mostly polluted with lead. The content of this element was within a wide range of 12.0--246 mg kg^{-1}. Among the analyzed trace elements, the content of copper was significantly lower. The concentration of this element in soils did not exceed 50 mg kg^{-1} (Table 1). ETTLER et al. (1999) in their study on ombrotrophic peatlands in the Czech Izera Mountains, presented very similar concentrations of trace elements in soil.

The calculated pools of trace elements in the 0-30 cm soil layer showed significant dominance of lead above the other determined elements. The pools of Pb in soils from the Wysoki Grzbiet ranged from 3.33 to 9.46 g m^{-2}. Profile 2, localized at 990 m a.s.l., had the highest content of Pb. Soils from Hala Izerska are characterized by smaller pools of lead (Table 2). The abundance of zinc pools was estimated at 1.14 to 5.73 g m^{-2} for soils from the Grzbiet Wysoki and 1.22 to 2.87 g m^{-2} for soils from Hala Izerska. Both lead and zinc created more abundant pools in organic soils from the Grzbiet Wysoki. A reverse situation was determined for copper. Richer pools of this element were characteristic of soils from Hala Izerska (Tables 2 and 3). Profile 5 had a particularly high concentration of copper, which may have been caused by its localization in an old riverbed of the Izera River. The river transported material eroded from surrounding areas, which then settled in the riverbed (WU et al. 2011). This may indicate that the copper resources are the result of natural accumulation, not shaped by the atmospheric deposition. The results showed that altitude strongly affected the Pb concentration. Organic soils in the higher Izera Mountains contained more of this element. No such correlation was proven for the copper and zinc concentrations (Figure 2). The Grzbiet Wysoki is an orographic barrier that stops air masses, which carry pollution. Thus, higher Pb concentrations are found at at higher altitudes. ERISMAN et al. (2003) mentioned that dry or wet deposition of gases and particles was affected by factors which influenced the turbulent transport, especially wind velocity, but also by the relief and height of the land.

Statistical analysis showed a significant positive correlation between Pb concentration and altitude factor (r=0.48). As the altitude increased, so did the content of Pb in soil. Also GERDOL et al. (2006) described strong correlation between Pb concentrations and altitude. Zinc (r=-0.45) and lead (r=-0.53) negatively strongly correlated with the depth of a soil profile. The content of these elements decreases with the profile's depth. Higher concentrations of organic carbon in topsoil was mentioned elsewhere (YIN et al.

Table 2

Pools of trace elements in upper horizons of examined soils

Profile No.	Layer (cm)	Trace elements calculated (g m^{-2})					
		Pb		Zn		Cu	
1/GW	0-10	0.66	3.33	0.83	2.12	0.38	0.57
	10-20	1.25		0.65		0.12	
	20-30	1.42		0.64		0.07	
2/GW	0-10	4.62	9.46	2.32	5.73	0.69	1.17
	10-20	3.23		2.09		0.40	
	20-30	1.62		1.31		0.08	
3/GW	0-10	0.88	5.92	0.57	1.85	0.11	0.63
	10-20	3.12		0.68		0.34	
	20-30	1.92		0.60		0.18	
4/WG	0-10	2.90	7.68	0.34	1.14	0.42	0.86
	10-20	2.70		0.42		0.33	
	20-30	2.08		0.39		0.10	
5/HI	0-10	3.24	6.84	0.98	2.87	0.95	2.26
	10-20	3.24		0.98		0.95	
	20-30	0.36		0.92		0.37	
6/HI	0-10	0.28	0.76	1.25	2.65	0.54	1.02
	10-20	0.27		1.07		0.44	
	20-30	0.21		0.33		0.05	
7/HI	0-10	1.41	3.91	0.44	1.22	0.26	0.65
	10-20	1.41		0.44		0.26	
	20-30	1.09		0.33		0.14	
8/HI	0-10	0.85	2.56	0.41	1.22	0.14	0.41
	10-20	0.85		0.41		0.14	
	20-30	0.85		0.41		0.14	

Key: GW – the Grzbiet Wysoki, HI – Hala Izerska

2002). The reaction of the analyzed soils depended on the altitude and soil profile's depth (Table 4). The acidification in soil from the Grzbiet Wysoki is caused by higher precipitation at a higher altitude. There is more precipitation at higher altitude (Boruvka et al. 2009).

Table 3

Statistical parameters of trace elements pools in examined soils

Trace element (n=4)	localization of profile	x	SD	dx	CV
Pb	GW	6.60	2.614	3.08	0.143
	HI	3.52	2.563		
Zn	GW	2.71	2.055	0.72	0.544
	HI	1.99	0.893		
Cu	GW	0.81	0.272	0.28	0.545
	HI	1.09	0.823		

Key: x – arithmetic mean, SD – standard deviation, dx – difference of arithmetic mean, CV – coefficient of variation, n – number of samples

Table 4

Coefficients of correlation between concentrations of trace elements and properties of examined organic soils

Value	Pb_t	Zn_t	Cu_t	pH	C-total
Height	0.48*	0.23	-0.21	-0.62*	0.09
Depth	-0.53*	-0.45*	-0.07	0.71*	0.18
Pb_t		0.33*	0.24	-0.29	-0.25
Zn_t			0.36*	-0.17	-0.10
Cu_t				0.13	-0.21
pH					-0.21

Key: n=37, correlation ratio significant at *p<0.05

Also, some correlation was observed between the trace elements, e.g. zinc concentration is determined by lead and copper. Significant positive correlation is observed between these elements. Strong correlation was often observed between Pb and Zn of the anthropogenic origin, mainly from coal-fired power plants (Strzyszcz et al. 2001). None of analyzed trace elements showed significant correlation with organic carbon. This situation is typical of organic soils and often described in available literature (Bogacz 2010, Bogacz et al. 2011).

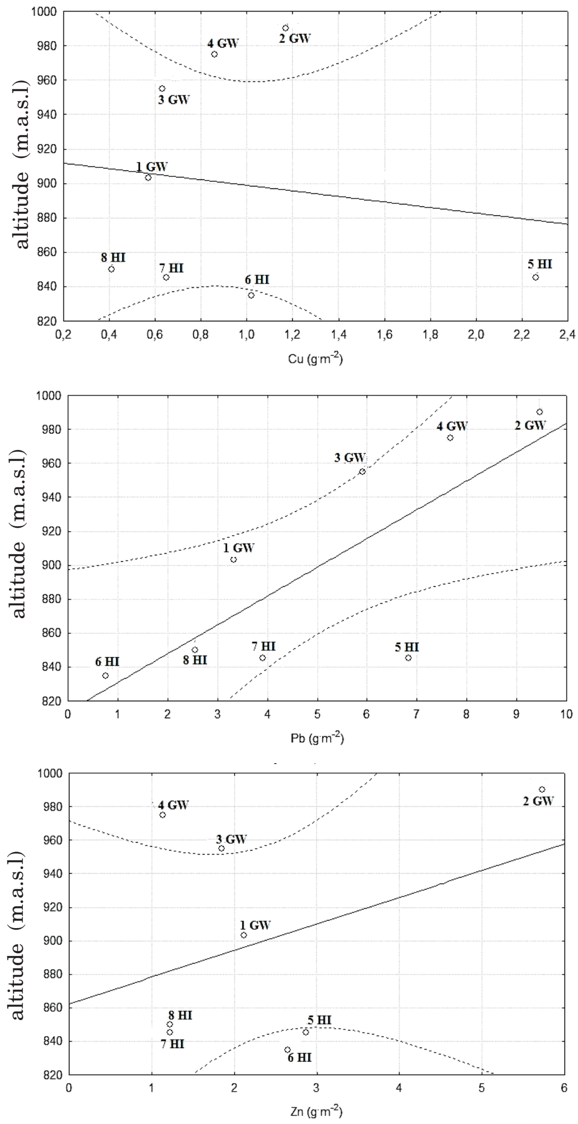

Fig. 2. Relationship between altitude and pools of trace elements

CONCLUSIONS

1. Pools of trace elements were strongly differentiated by the location of examined soils on different altitudes above sea level. The concentration of lead was particularly strongly dependent on on the altitude.

2. The highest concentrations of lead and zinc were observed in the epipedons of organic soils, which proves the anthropogenic origin of these elements and their atmospheric deposition.

3. At higher altitudes, lower values of pH were observed in soil, which suggests strong acidification of these soils.

4. The statistical analysis showed significant differences between pools of lead in soils in the Grzbiet Wysoki and Hala Izerska.

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INFLUENCE OF DIFFERENT METHODS OF CROPPING AND WEED CONTROL ON THE CONTENT OF Cu AND Zn IN FODDER MAIZE (*ZEA MAYS* L.) AND ON THEIR UPTAKE BY MAIZE

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Abstract

Strip intercropping is the practice of growing two or more species of plants in strips wide enough for independent mechanical cultivation, yet narrow enough to allow interaction between the species. This can affect not only crop yield but also competition in the uptake of nutrients and thus the chemical composition of the plants. The aim of this study was to assess the impact of strip intercropping and three methods of weed control on the content of zinc and copper in maize and on uptake of these components by maize. The study was conducted in 2004-2006 on a private farm located in the village of Frankamionka in the administrative district (*powiat*) of Zamość. It was based on a field experiment conducted on clayey silt soil with grain-size distribution of clay and a moderate Zn and Cu content. The experiment design was a split-plot randomized complete block in four replications. The factors taken into account were two methods of cultivation: sole cropping and strip cropping (common bean, dent maize, and spring wheat in adjacent strips) and three methods of weed control: mechanical – weeding of interrows twice; mechanical-chemical – the herbicide Gesaprim 90 WG 1.5 kg ha^{-1} + weeding of interrows once; and chemical – the herbicides Gesaprim 90 WG 1.5 kg ha^{-1} + Milagro 040 SC 1.5 L ha^{-1}. Fodder maize was grown for silage and harvested during milky-wax maturity. The content of copper and zinc in the dry matter of maize was determined by atomic absorption spectrophotometry (AAS) after digestion in HNO_3 (extra pure) in accordance with PN-EN ISO 6869:2002. On average for the experiment, strip cropping of maize with common bean and spring wheat reduced zinc content in maize, but in successive years of the study, the impact of the cultivation methods was varied. Strip cropping significantly increased the copper content in the plants in comparison with sole cropping. Zn and Cu content varied depending on the location of a row in strip cropping.

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Location adjacent to a strip of beans was more favourable to zinc accumulation in the biomass of maize, while copper content was the highest in maize plants from rows adjacent to wheat, and lowest when grown next to a bean strip.

Strip cropping significantly decreased zinc uptake by maize, but the influence of cropping methods on copper uptake was not significant. The uptake of zinc and copper was the highest when the chemical weed control method was used, and resulted mainly from the high biomass of maize. The results confirm the impact of strip cropping on competition in the uptake of nutrients and on their content and total uptake.

Key words: maize, strip cropping, copper, zinc, content, uptake.

ZAWARTOŚĆ Cu I Zn W KUKURYDZY PASTEWNEJ *(ZEA MAYS* L.) ORAZ ICH POBRANIE Z PLONEM W ZALEŻNOŚCI OD METOD UPRAWY I REGULACJI ZACHWASZCZENIA

Abstrakt

Uprawa współrzędna pasowa polega na uprawie dwóch lub więcej gatunków roślin w pasach wystarczająco szerokich, aby umożliwić niezależną mechaniczną uprawę, a jednocześnie dość wąskich, aby zachodziło ich wzajemne oddziaływanie. Może to wpływać nie tylko na plonowanie roślin, ale również konkurencję w pobieraniu składników pokarmowych, a co za tym idzie skład chemiczny roślin. Celem pracy była ocena wpływu współrzędnej uprawy pasowej i trzech metod regulacji zachwaszczenia na zawartość cynku i miedzi w kukurydzy oraz pobranie tych składników z plonem. Badania przeprowadzono w gospodarstwie indywidualnym położonym we wsi Frankamionka w powiecie zamojskim, w latach 2004-2006. Eksperyment polowy założono na glebie o składzie granulometrycznym pyłu ilastego, o średniej zasobności w Zn i Cu, metodą podbloków losowanych w układzie zależnym split-plot, w czterech powtórzeniach. Badanymi czynnikami były dwa sposoby uprawy: siew czysty i uprawa współrzędna pasowa (w sąsiadujących pasach fasola zwyczajna, kukurydza pastewna, pszenica jara) oraz trzy metody regulacji zachwaszczenia: mechaniczna – dwukrotne opielanie międzyrzędzi; mechaniczno-chemiczna – herbicyd Gesaprim 90 WG 1,5 kg ha^{-1} + jednokrotne opielanie międzyrzędzi; chemiczna – herbicydy Gesaprim 90 WG 1,5 kg ha^{-1}+ Milagro 040 SC 1,5 l ha^{-1}. Kukurydzę pastewną uprawiano na zielonkę i zbierano w fazie dojrzałości mleczno-woskowej. Zawartość miedzi i cynku w suchej masie kukurydzy oznaczono metodą absorpcyjnej spektrofotometrii atomowej (ASA) po mineralizacji w HNO_3 (ekstra czystym) zgodnie z normą PN-EN ISO 6869:2002. Uprawa współrzędna pasowa kukurydzy z fasolą zwyczajną i pszenicą jarą średnio w doświadczeniu wpływała na zmniejszenie zawartości cynku w kukurydzy, jednak w kolejnych latach badań wpływ metod uprawy był zmienny. Uprawa współrzędna wpływała zaś istotnie na zwiększenie zawartości miedzi w roślinach w porównaniu z ich uprawą w siewie czystym. Zawartość Zn i Cu zmieniała się w zależności od położenia rzędu w uprawie pasowej. Sąsiedztwo z pasem fasoli sprzyjało gromadzeniu większej ilości cynku w biomasie kukurydzy, natomiast zawartość miedzi była najwyższa w roślinach z rzędów sąsiadujących z pszenicą, zaś najniższa w sąsiedztwie pasa fasoli. Uprawa pasowa współrzędna wpływała istotnie na zmniejszenie pobrania cynku z plonem kukurydzy, zaś wpływ metod uprawy na pobranie miedzi nie był istotny. Pobranie cynku i miedzi z plonem roślin było największe w warunkach stosowania chemicznej metody regulacji zachwaszczenia i wynikało głównie z ilości biomasy kukurydzy. Uzyskane wyniki potwierdzają wpływ współrzędnej uprawy pasowej na konkurencję w pobieraniu składników pokarmowych oraz ich zawartość i pobranie z plonem.

Słowa kluczowe: kukurydza, współrzędna uprawa pasowa, miedź, cynk, zawartość, pobranie.

INTRODUCTION

In recent years, maize has begun to play an important role in the Polish agriculture as animal feed, human food and raw material for industry (SZYMAŃSKA et al. 2009). It is a popular crop because it can produce high yield of both green matter and highly nutritional grain (BILSKI et al. 1997). The content of nutrients in maize depends on the plant development phase, cultivar-specific properties, soil richness and cultivation conditions (SZYSZKOWSKA et al. 2007, SIMIC et al. 2009, SKOWROŃSKA, FILIPEK 2009). Strip cropping involves growing two or more species of plants in strips wide enough to allow independent mechanical cultivation, yet narrow enough so that the species interact. This system not only affects crop yield, but also competition in the uptake of nutrients, with the power and direction of this competition depending on the nutrient and on the neighboring plants (GHAFFARZADEH et al. 1998, LI et al. 2001b, GŁOWACKA 2010). Cultivated plants are a source of food for people and are used as animal feed in fresh or processed form. Therefore, their feeding value and proportions of their components are very important for the health of humans and animals (GRZYŚ 2004, GRAHAM et al. 2007). Copper and zinc are important nutrients in plants. Zinc is a component of many enzymes and is involved in the metabolism of carbohydrates, proteins and phosphorus compounds (ALLOWAY 2004, GONDEK 2010). It participates in the synthesis of auxins and increases plant tolerance to drought and disease (KABATA-PENDIAS, PENDIAS 1999). It is also one of the most important elements conditioning the proper functioning of a human body (SALGUEIRO et al. 2000). It is a micronutrient that regulates protein metabolism (SZLEGEL-ZAWADZKA 2001) and – together with copper, boron, and manganese – has a significant effect on the carbohydrate metabolism (ALAALAOUI-SOSSE et al. 2004). Copper is an activator of many enzymes involved in nitrogen metabolism and a conveyor of electrons in various biochemical reactions in plants. In the Polish literature, few studies have dealt with the impact of strip cropping on the yield of crop plants (BURCZYK 2003, GŁOWACKA 2008) and macronutrient uptake (GŁOWACKA 2010, GŁOWACKA et al. 2011). No information is available about the possible impact of strip cropping on the uptake of trace elements by crops.

The aim of this study was to assess the impact of strip cropping and sole cropping and of different methods of weed control on the content of copper and zinc in fodder maize and on their uptake by maize.

MATERIAL AND METHODS

The study was conducted in 2004-2006 as a field experiment on a private farm located in the village of Frankamionka in the administrative district (*powiat*) of Zamość. The field experiment was set up in a split-plot randomized block design in four replications. It was conducted on clayey silt soil with granulometric composition. The soil was slightly acidic (pH in 1 mol KCl 6.5) with 19 g kg^{-1} of organic matter and average content of available forms of zinc and copper (6.7-8.2 mg Zn kg^{-1}; 3.2-3.3 mg of Cu kg^{-1}).

The examined factors were as follows: I. method of cultivation, and II. weed control method. I. method of cultivation a) sole cropping, in which the size of plots was 23.75 m^2 for sowing and 17 m^2 for harvest, with 10 rows of maize spaced at 50 cm sown on each plot; b) strip cropping, in which three crops – common bean, fodder maize and spring wheat – were grown side by side in strips 2.5 m wide - five rows of maize were planted in each maize strip, spaced at 50 cm. The size of maize plots was 11.75 m^2 for sowing and 10.5 m^2 for harvesting. II. weed control method: a) mechanical – weeding of interrows twice; b) mechanical-chemical – the herbicide Gesaprim 90 WG 1.5 kg ha^{-1} (a.i. atrazine 135 g ha^{-1}) + weeding of interrows once; c) chemical – the herbicides Gesaprim 90 WG 1.5 kg ha^{-1} (a.i. atrazine 135 g ha^{-1}) + Milagro 040 SC 1.5 L ha^{-1} (a.i. nicosulfuron 60 g ha^{-1}).

The foder maize cultivar Veritis (FAO 230-240) was sown between 25th April and 5th May. Uniform mineral fertilization was applied consisting of 160 kg N ha^{-1}, 40 kg P ha^{-1} and 108 kg K ha^{-1}. Maize was harvested at the milky-wax stage (BBCH 79/83) in the second third of September. Spring wheat was harvested in the second third of August (BBCH 89), and beans - in the third week of August or first week of September (BBCH 88/89). Detailed description of the experiment methodology is given in an earlier study (GŁOWACKA 2008). Meteorological conditions during the experiment were variable and are shown in Table 1.

Table 1

Rainfall and air temperature in April - September versus long-term means (1971-1988), according to the Meteorological Station in Zamość

Rainfall (mm)							
Years	Apr	May	June	July	Aug	Sep	Σ Apr-Sep
2004	46.3	50.1	34.9	145.0	71.9	36.3	384.5
2005	45.4	98.2	69.5	33.6	52.7	15.8	315.3
2006	58.4	54.0	43.5	28.3	144.8	0.8	329.8
Means for 1971-2005	44.1	65.5	78.9	98.4	54.3	52.2	393.5
Temperature (°C)							
2004	9.6	13.5	18.1	19.4	19.7	14.3	2891
2005	9.7	15.4	17.5	21.8	18.7	13.3	2948
2006	10.5	14.8	18.4	23.3	19.0	16.8	3141
Means for 1971-2005	7.9	14.1	16.8	18.4	17.8	12.9	2690

Every year prior to harvest, two plants were randomly collected from the middle rows of each plot. In addition, from each plot with strip cropping two plants were collected from the border rows adjacent to spring wheat and common bean and from the middle row. After the plants were crushed, dried, ground and wet mineralized in analytically pure HNO_3, copper and zinc content was determined by atomic adsorption spectrophotometry (AAS) according to PN-EN ISO 6869:2002. The results were converted to dry weight. Analyses were performed at the Central Agroecological Laboratory of the University of Life Sciences in Lublin. Based on the content of the elements determined in maize and the yield volume (GŁOWACKA 2008), the uptake of zinc and copper was calculated. The results were analyzed statistically by analysis of variance (KALA 2009). Differences between means were assessed by Tukey's test. The significance of differences was determined with 95% probability.

RESULTS AND DISCUSSION

Maize yields varied significantly in different years of the study (Table 2). Such substantial differences in yields may have resulted from weather conditions in different growing seasons. In 2005 and 2006, rainfall was much lower than in 2004 and the long-term average. Moreover, precipitation was very unevenly distributed in 2006. In the first year, the yield of maize grown in sole cropping was significantly higher than in the strip cropping. However, on average for the whole period of the experiment, the cultivation method did not significantly affect maize yields. As might have been expected, of the three methods of weed control, the mechanical method had the least favourable effect on maize yield. The differences between the mechanical-chemical and chemical methods were within statistical error. In strip cropping, the yield from the border rows was only slightly higher than that obtained in sole cropping (GŁOWACKA 2008). Zinc content varied significantly as influenced by weather conditions during the study, methods of cultivation and weed control methods (Table 3). The zinc content in the biomass of maize ranged from 7.4 to 17.6 mg kg^{-1} d.m. This can be regarded as low, as GORLACH and MAZUR (2002) state that zinc content in plants is 20-1,500 mg kg^{-1} d.m. Studies by other authors have found that strip cropping increased total yield and allowed for more efficient use of nutrients (ZANGH, LI 2003). LI et al. (2001a) found that wheat was more competitive in the uptake of nutrients (N, P, K) than maize or soy accompanying it in strip cropping. The present study also found that strip cropping affected the uptake of trace elements, but the change was not unidirectional, particularly with respect to zinc. On average for the experiment, maize grown in strip cropping with common bean and spring wheat contained slightly but significantly less zinc than in sole cropping. However, analysis of the particular years shows that

Table 2

Yield of maize depending on the method of cropping and weed control (Mg d.m. ha^{-1})

Method of cultivation	Weed control*	Years			Average
		2004	2005	2006	
Sole cropping	A B C	20.6 22.9 23.2	14.6 15.9 18.7	12.3 16.7 16.1	15.8 18.5 19.3
Strip cropping – mean for plot	A B C	18.8 19.8 20.9	14.0 17.9 18.4	11.6 15.3 15.2	14.8 17.7 18.2
LSD (α = 0.05)		n.s.	n.s.	n.s.	n.s.
Average for factors					
Sole cropping	–	22.2^{b}	16.4	15.4	17.9
Strip cropping – mean for plot	–	19.9^{a}	16.8	14.0	16.9
LSD (α = 0.05)		0.94	n.s	n.s.	n.s.
–	A	19.7^{a}	14.3^{a}	11.9^{a}	15.3^{a}
–	B	21.3^{b}	16.2^{b}	16.0^{b}	18.1^{b}
–	C	22.0^{b}	18.5^{c}	15.7^{b}	18.8^{b}
LSD (α = 0.05)		1.45	1.81	3.05	1.59
Years	2004 2005 2006	21.0^{c} 16.6^{b} 14.5^{a}			
LSD (α = 0.05)		1.65			

*Weed control: A – mechanical, B – mechanical-chemical, C – chemical
a, b, c – mean in columns marked with the same letter do not differ significantly

strip cropping decreased the zinc content in maize only in the first year of the experiment. In the second year, higher zinc content was found in maize from strip cropping, while in the third year the cultivation methods did not produce a significant effect. This indicates strong influence of the weather on both yield-stimulating effect of strip cropping, as noted in other studies (Francis 1986), and on the chemical composition of plants in comparison with sole cropping.

In strip cropping, zinc content in maize varied depending on the row in a strip (Figure 1). On average, irrespective of the method of weed control, the highest zinc content was in the maize growing in the row adjacent to beans and the lowest – in the middle row. These results are consistent with the results of a study by Głowacka et al. (2011) on the impact of strip cropping on content and uptake of magnesium by maize. Analysis of the changes in zinc content in plants from different rows, including the method of weed control, shows that they were similar when the mechanical-chemical and

Table 3

Content of zinc in maize depending on the method of cropping and weed control (mg kg^{-1} d.w.)

Method of cultivation	Weed control*	Years			Average
		2004	2005	2006	
Sole cropping	A	15.1^{c}	7.4^{a}	15.5^{c}	12.7^{b}
	B	17.6^{e}	9.0^{b}	13.4^{a}	13.3^{c}
	C	12.5^{b}	12.6^{d}	16.3^{d}	13.8^{d}
Strip cropping – mean for plot	A	11.1^{a}	10.9^{c}	17.6^{e}	13.2^{c}
	B	12.1^{b}	11.5^{c}	13.2^{a}	12.2^{a}
	C	16.2^{d}	11.3^{c}	14.1^{b}	13.8^{d}
LSD (α = 0.05)		0.81	0.95	0.71	0.28
Average for factors					
Sole cropping	–	15.1^{b}	9.7^{a}	15.0	13.24^{b}
Strip cropping – mean for plot	–	13.1^{a}	11.2^{b}	14.9	13.07^{a}
LSD (α = 0.05)		0.29	0.34	n.s.	0.12
–	A	13.1^{a}	9.1^{a}	16.5^{c}	12.9^{a}
–	B	14.8^{c}	10.2^{b}	13.2^{a}	12.8^{a}
–	C	14.3^{b}	11.9^{c}	15.2^{b}	13.8^{b}
LSD (α = 0.05)		0.44	0.52	0.39	0.25
Years	2004	14.1^{b}			
	2005	10.4^{a}			
	2006	15.0^{c}			
LSD (α = 0.05)		0.25			

*Weed control: A – mechanical, B – mechanical-chemical, C – chemical
a, b, c – mean in columns marked with the same letter do not differ significantly

chemical methods were applied, but somewhat different in the case of the mechanical method (Figure 2).

The copper content in aerial plant organs is usually between 5 and 20 mg kg^{-1} d.m. (Kabata-Pendias, Pendias 1999). Hence, the copper content in maize determined in this study can be said to be low but within this range. Strip cropping significantly increased the copper content in the maize compared to sole cropping (Table 4). The variations in the copper content in maize, depending on the row within a strip, were different than in the case of zinc. The copper content was lower in maize plants from the row adjacent to common bean strips than in the middle row or the row adjacent to spring wheat, with no significant differences between the two latter variants (Figure 3). It may have ben so because wheat was harvested earlier than beans and therefore stopped competing with maize for minerals. Similarly, Głowacka et al. (2011) reported that strip cropping affects the calcium con-

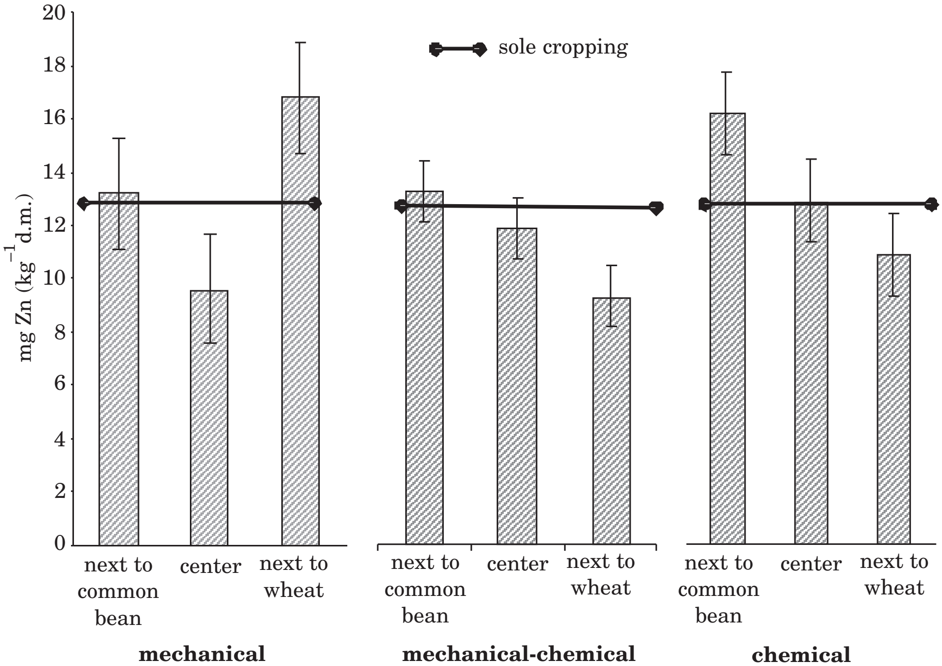

Fig. 1. The influence of adjacent row on zinc content in maize

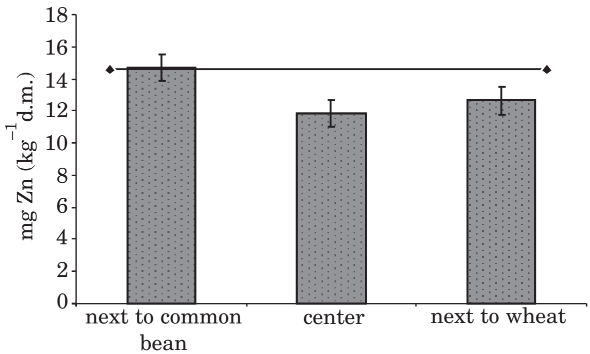

Fig. 2. The influence of adjacent row and weed control method on zinc content in maize

tent in plants. Maize growing in the row adjacent to the wheat strip contained more macronutrients than maize from the row adjacent to common bean. When Li et al. (2001b) studied the impact of strip cropping of maize and wheat on changes in the uptake of N, P and K, they found significantly lower uptake in the earlier stages of maize development and faster uptake in the later growth period (after the wheat harvest), as compared to sole cropping. As a result, at the stage of full maturity the content of these

Table 4

Content of copper in maize depending on the method of cropping and weed control
(mg kg^{-1}d.w.)

Method of cultivation	Weed control*	Years			Average
		2004	2005	2006	
Sole cropping	A B C	5.90^{d} 5.17^{b} 5.49^{c}	4.94^{c} 4.63^{b} 5.33^{e}	4.42^{a} 5.62^{c} 6.56^{e}	5.09 5.14 5.79
Strip cropping – mean for plot	A B C	4.62^{a} 5.55^{c} 6.71^{e}	4.50^{a} 5.17^{d} 5.32^{e}	6.25^{d} 4.96^{b} 7.51^{f}	5.12 5.22 6.51
LSD (α = 0.05)		0.153	0.07	0.074	n.s.
Average for factors					
Sole cropping	–	5.52^{a}	4.97	5.53^{a}	5.34^{a}
Strip cropping – mean for plot	–	5.63^{b}	4.99	6.24b	5.62^{b}
LSD (α = 0.05)		0.05	n.s.	0.026	0.04
–	A	5.26^{a}	4.72^{a}	5.33^{a}	5.12^{a}
–	B	5.36^{b}	4.90^{b}	5.29^{a}	5.18^{a}
–	C	6.10^{c}	5.32^{c}	7.03^{c}	6.15^{b}
LSD (α = 0.05)		0.08	0.04	0.040	0.27
Years	2004 2005 2006	5.57^{b} 4.98^{a} 5.88^{b}			
LSD (α = 0.05)		0.37			

*Weed control: A – mechanical, B – mechanical-chemical, C – chemical
a, b, c, d, e, f – mean in columns marked with the same letter do not differ significantly

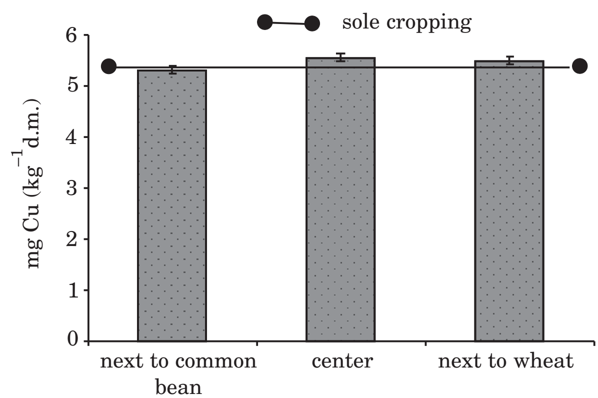

Fig. 3. The influence of adjacent row on copper content in maize

elements in the maize in strip cropping was similar or slightly higher than in sole cropping. Skowrońska and Filipek (2009) report that the most intensive uptake of micronutrients, especially copper, occurs between 109 and 132 days after the sowing of maize.

Changes in the copper content in plants from each row were similar in the variants of mechanical-chemical and chemical weed control methods, but different from the variant in which only mechanical weeding was applied (Figure 4).

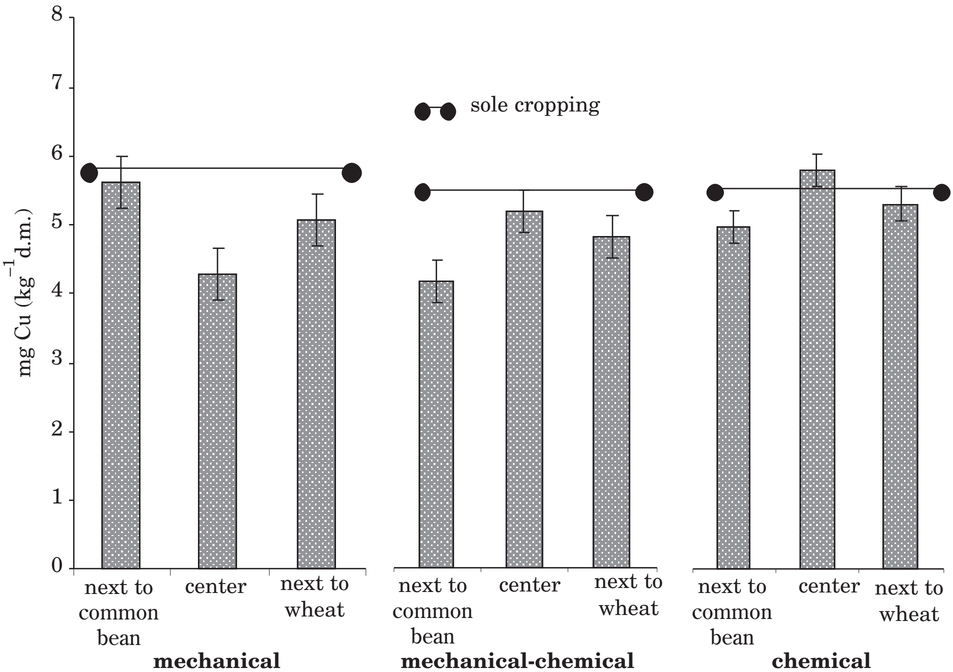

Fig. 4. The influence of adjacent row and weed control method on copper content in maize

The soil on which maize was grown on had an average content of bioavailable zinc and copper (extracted in 1 mol dm^{-3} HCl). The low content of zinc and copper in maize supports the claim that the uptake of these elements by plants depends on their total content in soil and on other factors that affect their phytoavailability (Garcia-Mina et al. 2004). Complex interactions take place in soil between macro- and micronutrients, and these processes are significantly influenced by the soil pH (Grzyś 2004). Moreover, research by Korzeniowska and Stanisławska-Glubiak (2004) indicate that the solutions used in the analysis, i.e. 1 mol dm^{-3} HCl, 0.1 mol dm^{-3} HCl, and DTPA, extracted significantly more zinc than a plant can take up.

Weeds are often more efficient in the uptake of nutrients than crops, hence widespread occurrence of weeds may limit availability of elments to crops. Plants that produce less yield contain more micronutrients per unit mass produced in relation to their availability in soil than high-yielding plants. At higher yields, plants are unable to take up from the soil sufficient quantities of nutrients, which are 'diluted' in the plant biomass (CAKMAK 2004). This, however, was not confirmed in my study. The highest copper content was noticed in maize from sites where the chemical weed control method alone was used, which also favoured the production of large amounts of biomass (Table 4). On average, significantly less copper was accumulated by plants cultivated under the mechanical and mechanical-chemical weed control methods, and the differences between these methods were not significant. Also, Pearson's correlation coefficient (r = 0.556, $p < 0.01$) confirms the positive relationship between the yield of maize and Cu content in the biomass. On average for the whole experiment, zinc content was also the highest in maize weeded chemically (Table 3). However, in the successive years, the impact of the weed control methods was changeable. On average for the experiment, no significant interaction was confirmed between the methods of cultivation and weed control in shaping the content of Cu in maize. It may be noted that under the mechanical weed control method, in contrast to the mechanical-chemical and chemical methods, the zinc content in maize was higher in strip cropping. This may have resulted from the effect of strip cropping identified and discussed elsewhere (GŁOWACKA 2007), in which weed infestation of maize was controlled mechanically and weed competition was therefore weaker. The weather conditions significantly influenced the content of zinc and copper in the maize. The lowest content of these microelements in the dry matter of maize was noted in 2005, when there was little rainfall in July and August. The highest content of zinc and copper was recorded in 2006. Copper is arrested in soil by organic matter and released during its decomposition. Soil moisture is an important factor affecting the decomposition of organic matter and, consequently, the release of copper to the soil solution and availability of this element to plants (STANISŁAWSKA-GLUBIAK, KORZENIOWSKA 2010). The effect of rainfall on the bioavailability of micronutrients and their higher content in plants is confirmed by other authors (RAJCAN, SWANTON 2001, KLIKOCKA 2011). However, there are also some contradictory reports, for example a three-year experiment carried out by KRASKA (2011), in which a higher content of trace elements (Cu, Zn, Fe and Mn) was obtained in wheat grain in a year when there was less rainfall, but it was evenly distributed throughtout the entire growing season.

The intake of zinc by maize determined in this study (an average of 240 g per 1 ha) is similar to the results reported by RABIKOWSKA and PISZCZ (2004). On average, significantly less zinc was accumulated by maize grown in strip cropping with spring wheat and common beans than in sole cropping (Table 5). According to CZUBA (2000), the uptake of copper is 118 g of maize for

Table 5

Uptake of zinc with maize yield depending on the method of cropping and weed control ($g\ ha^{-1}$)

Method of cultivation	Weed control*	Years 2004	Years 2005	Years 2006	Average
Sole cropping	A B C	310.9^{bc} 401.7^{d} 288.8^{b}	107.9^{a} 143.8^{a} 234.2^{c}	225.8 213.2 303.3	214.9 252.9 275.4
Strip cropping – mean for plot	A B C	208.2^{a} 239.4^{a} 337.8^{c}	152.4^{b} 204.5^{c} 207.0^{c}	245.0 235.6 260.5	201.9 226.5 268.4
LSD (α = 0.05)		44.44	38.20	n.s.	n.s.
Average for factors					
Sole cropping	–	333.8^{b}	162.0^{a}	247.4	247.7^{b}
Strip cropping – mean for plot	–	261.8^{a}	188.0^{b}	247.1	232.2^{a}
LSD (α = 0.05)		15.77	13.55	n.s.	12.79
–	A	259.5^{a}	130.2^{a}	235.4^{a}	208.4^{a}
–	B	320.6^{b}	174.2^{b}	224.4^{a}	239.7^{b}
–	C	313.3^{b}	220.6^{c}	281.9^{b}	271.9^{c}
LSD (α = 0.05)		24.22	20.82	44.03	19.65
Years	2004 2005 2006	297.8^{c} 175.0^{a} 247.2^{b}			
LSD (α = 0.05)		21.45			

*Weed control: A – mechanical, B – mechanical-chemical, C – chemical
a, b, c, d – mean in columns marked with the same letter do not differ significantly

1 ha. However, in a study conducted by RABIKOWSKA and PISZCZ (2004), maize uptake was 51.6 g of Cu per 1 ha. In our study, on average for the experiment, maize took up 95.5 g of copper from 1 ha (Table 6), while the effect of the cultivation method on the copper uptake was negligible. The total uptake of zinc and copper was the highest when chemical weed control was used and the lowest in the case of mechanical weeding (Tables 5 and 6). This was caused by the yield volumes. The close relationship between the uptake of these micronutrients and yield is confirmed by Pearson's correlation coefficient, $r = 0.945$ ($p < 0.001$) for copper and $r = 0.910$ ($p < 0.001$) for zinc. Positive correlation was verified between the uptake and content of copper – $r = 0.786$ ($p < 0.001$) and zinc – $r = 0.649$ ($p < 0.001$). Similar results were obtained by MAZUR and SIENKIEWICZ (2009), who report that the uptake of Cu and Zn were significantly correlated with the content of these trace elements in the biomass of maize ($r = 0.66$ for Cu and $r = 0.65$ for Zn).

Table 6

Uptake of copper with maize yield depending on the method of cropping and weed control (g ha^{-1})

Method of cultivation	Weed control*	Years			Average
		2004	2005	2006	
Sole cropping	A B C	121.4^{b} 118.1^{bb} 127.2^{b}	72.1^{ab} 73.8^{ab} 99.4^{c}	54.1 93.9 105.9	82.6 95.3 110.8
Strip cropping – mean for plot	A B C	87.0^{a} 109.9^{b} 140.6^{d}	62.9^{a} 92.4^{bc} 97.88^{bc}	72.2 75.7 114.3	74.0 92.7 117.6
LSD (α = 0.05)		18.32	24.50	n.s.	n.s.
Average for factors					
Sole cropping	–	122.2^{b}	81.8	84.7	96.2
Strip cropping – mean for plot	–	112.5^{a}	84.41	87.4	94.8
LSD (α = 0.05)		6.50	n.s.	n.s.	n.s.
–	A	104.2^{a}	67.5^{a}	63.2^{a}	78.3^{a}
–	B	114.0^{a}	83.1^{b}	84.8^{b}	94.0^{b}
–	C	133.9^{b}	98.63^{c}	110.1^{c}	114.2^{c}
LSD (α = 0.05)		9.99	13.39	18.23	7.84
Years	2004 2005 2006	117.4^{c} 95.5^{b} 86.0^{a}			
LSD (α = 0.05)		8.54			

*Weed control: A – mechanical, B – mechanical-chemical, C – chemical
a, b, c, d – mean in columns marked with the same letter do not differ significantly

CONCLUSIONS

1. Strip cropping of maize with common bean and spring wheat significantly increased copper content, without affecting its uptake by the maize, compared with sole cropping. Although strip cropping decreased the zinc content and uptake on average for the whole experiment, the influence of the cultivation method varied substantially from year to year.

2. The copper content in maize and the uptake of copper and zinc were the highest in the chemical weed control variant. The effect of the weed control method on the zinc content was irregular and variable between the years.

3. The research revealed that the zinc and copper uptake by maize depended on the species of the adjacent crops. Maize grown adjacent to bean strips accumulated more zinc. A higher copper content was noted in maize grown next to wheat strips. However, more detailed research needs to be conducted in order to improve our understanding of the mechanism involved in this process.

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RESPONSE OF ORAL MUCOSA TO CONTACT WITH CLASS 4 TITANIUM

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Abstract

Although titanium dental implants are characterized by great biocompatibility, both electrochemical and galvanic corrosion may take place in the oral environment, even in the cases of full osseointegration of implants. The aim of the study was to evaluate processes occurring in the gingival mucous membrane collected from dental implants after a period of healing. In the gingival tissues in contact with implants fully integrated with the bone, infiltrations composed of subpopulations of T lymphocytes (CD45R0 and CD25) and Langerhans cells (S-100 positive) were found. The presence of immunologically competent cells in the infiltrations indicated that the titanium implant was recognized by the host's immune system. The lack of clinical symptoms of hypersensitivity may suggest local tolerance to a correctly healed intraosseous dental implant.

Key words: titanium implants, local tolerance, hypersensitivity.

REAKCJA BŁONY ŚLUZOWEJ JAMY USTNEJ NA KONTAKT Z TYTANEM KLASY IV

Abstrakt

Pomimo że tytanowe implanty zębowe charakteryzują się wysoką biokompatybilnością, to w środowisku jamy ustnej, nawet w przypadkach pełnej osteointegracji implantów, może dochodzić zarówno do elektrochemicznej, jak i galwanicznej korozji. Celem pracy była ocena procesów zachodzących w błonie śluzowej dziąsła pobranej znad implantów po okresie ich wgajania. W tkankach dziąsła kontaktujących się z w pełni zintegrowanymi z kością implantami wykazano nacieki złożone z subpopulacji limfocytów T (CD45R0 i CD25) oraz komórek Langerhansa (S-100 pozytywnych). Obecność komórek immunologicznie kompe-

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tentnych w naciekach świadczy o rozpoznaniu tytanowego wszczepu przez układ immunologiczny gospodarza. Brak klinicznych objawów nadwrażliwości może sugerować wytworzenie miejscowej tolerancji na prawidłowo wgojony śródkostny wszczep stomatologiczny.

Słowa kluczowe: implanty tytanowe, tolerancja miejscowa, nadwrażliwość.

INTRODUCTION

Although titanium is thought to be highly biocompatible, an increasing number of reports suggest its various local effects and even a negative influence on patients' general health as well as disruptions during a healing processes. (THOMAS et al. 2006, NAWAZ et al. 2007). *In vitro* and *in vivo* studies show that despite the passive activity of the external layer of oxides, both electrochemical and galvanic corrosion may occur in the environment of the oral cavity. Thus, titanium ions causing discolouration of gingiva, oedema, *gingivitis stomatitis*, skin rash, erythema as well as delayed healing may be released (CHATURVEDI 2009, KOIKE et al. 2001). The low pH and the high concentration of fluorine ions damage the protective layer of oxides, leading to disorders in the osseointegration process and to changes in soft tissues (CHATURVEDI 2009). Numerous macrophages with the cytoplasm containing titanium particles were found in the gingiva surrounding implants that were lost as a result of fracture or a loss of osseointegration. It is believed that the process of phagocytosis may stimulate macrophages to release inflammatory mediators, which lead to activation of osteoclasts and, in consequence, to bone resorption (OLMENDO et al. 2003).

Sporadic cases of hypersensitivity in the oral cavity are explained by asmaller number of dendritic cells in the mucosa and lower permeability of mucous membrane in comparison with the skin (BASS et al. 1993, SCHRAMM et al. 2000). Moreover, it is estimated that in order to cause a hypersensitive response in soft tissues, 5-12-fold greater exposure to allergens is necessary. On the other hand, the glycoproteins included in saliva form a protective barrier on the surface of a titanium implant that prevents the direct contact of the metal with the mucosa (THOMAS 2000, BASS et al. 1993).

The processes observed in soft tissues have been diagnosed and documented on biopsy material almost exclusively in cases of failure of the implantological treatment (GISLASON et al. 2004, PIATELLI et al. 1998). There is no evidence demonstrating that in patients with full implant integration and without any pathological clinical symptoms, changes in the gingival mucosa could prove the release of titanium ions.

Aim of the study

The aim of the study has been to analyzed changes in soft tissues of the oral mucosa which may result from the contact with a titanium intraosseous implant during the period of osseointegration.

MATERIAL AND METHODS

The studies were conducted on clinical material collected from 15 patients, including 9 women and 6 men aged 40-69 (mean age 52). For this study, two-stage intraosseous screw implants: Osteoplant-Hex (PL), Neoss (USA), Ilerimplant (E), made of class 4 titanium were inserted (Table 1). In total, 43 implantations were carried out, including 25 procedures in the maxilla and 18 in the mandible. In the first stage of the implantation, control material was collected from a patient before inserting the intraosseous part, during the preparation of the mucous lobe on the alveolar ridge, from the area of the planned implant bed. After 4-6 months of osseointegration, during the procedure of implant exposure, proper material for the study was collected from the same patient, including fragments of mucosa in direct contact with the titanium intraosseous implant during its healing period. In all the cases, clinical and radiological examination confirmed complete implant osseointegration. After fixing in Bouin's solution (picric acid + formaldehyde + acetic acid, 6 h, room temperature), fragments of the gingival mucosa were routinely dehydrated and immersed in paraffin. 5 μm thick cross-sections of the mucous membrane were placed on microscope slides. 5160 paraffin fragments placed on 1290 slides were prepared for morphological studies.

Table 1

Description of research group

Number of patients	Sex F/M	Age	Place of implantation Maxilla/mandible	Number of implants	Implantation system
15	9/6	52	9/6	43	Osteoplant-Hex Neoss Illerimplant

First, an analysis of the structure of the gingival mucosa was conducted so that which features changing independently of the conditions (before and after contact with an implant) or changing on consecutive cross-sections of a given fragment were excluded. In further examinations, the occurrence of cell clusters over a surface above 0.01 mm^2 was evaluated with an aid of the software programme MicroImage, v. 4.0 (Olympus, MS Windows 98) coupled with the light microscope Olympus BX 50. Individual types of cells in the clusters were identified according to the expression of typical markers using the immunocytochemical technique ABC (Table 2).

Non-parametric Wilcoxon and Mann-Whitney tests were applied for statistical analysis. The correlation significance was checked with Student's t-test. The hypotheses were verified at $p < 0.05$. The statistical analysis of the results was performed with the use of Statistica v.6.1 (Statsoft, Inc.).

Table 2

Panel of antibodies (Ab) recognising surface or intracellular antigens (Ag) in gingival mucosa cells and commercial kits used in the immunocytochemical studies

Antibody (Ab)	Antigen (Ag)	Company	KIT	Labelled cell type
Rabbit N1573	S-100	Dako	Dako Cytomation LSAB2 System-HRP	Dendritic cells
Mice UCHL1	CD45RO	Novum Biologicals	Vectastain Elite ABC Kit	T lymphocytes
Mice 4C9	CD25	NovoCastra	Vectastain Elite ABC Kit	T lymphocytes
Mice HM47/A9	CD79a	NovoCastra	Vectastain Elite ABC Kit	B lymphocytes, plasmatic cells

RESULTS

In the mucosa from above the titanium implants, attention was drawn to the occurrence of cell infiltrations located in the subepithelial layer and in the neighbouring region of the epithelium (Figure 1). As shown in Figure 2, changes of this type were found in 11 patients, while in 6 patients they were observed only on single cross-sections. In 5 patients, the surface of the infiltrations was several times larger, and in 2 of these patients, cell infiltrations were found in the comparative material as well, and their surface increased statistically significantly after contact with the implant ($p < 0.05$).

The occurrence of the infiltrations did not depend on the type of implants or the number of implanted pillars in a given patient. In patients with more implants, infiltrations were observed next to all implants, although they differed in size. Detailed analysis of subsequent cross-sections of the mucosa collected above the implants allowed us to trace mononuclear cells which migrated from the subepithelial infiltrations to the epithelium, populating its successive layers. Within the epithelium, a significant number of mononuclear cells concentrated on a small area.

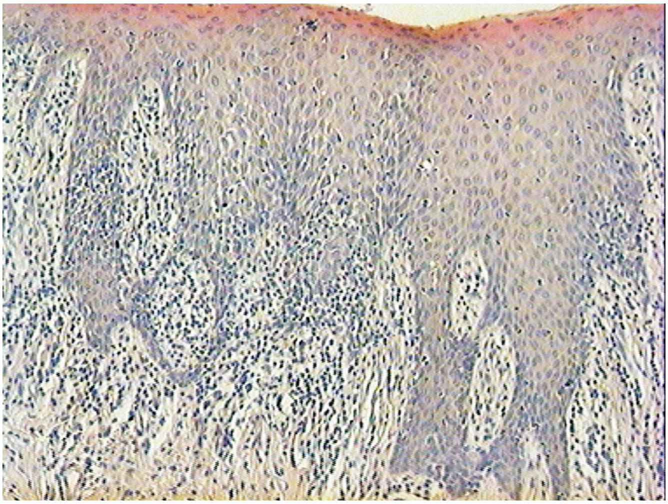

Fig. 1. Fragment of mucosa collected above an implant. Abundant infiltrations of mononuclear cells in the subepithelial layer infiltrating the epithelium visible. H+E. Magnification x 25

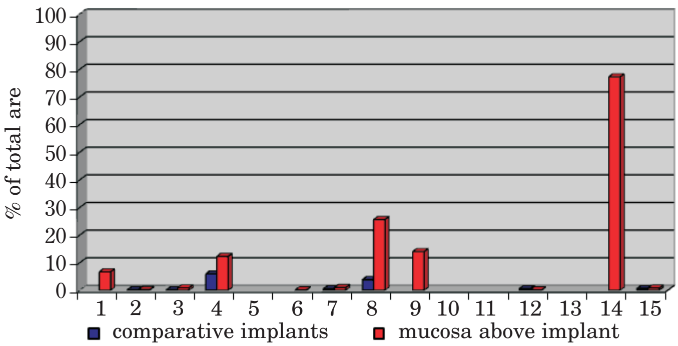

Fig. 2. Occurrence of cell infiltrations in individual patients (marked with consecutive numbers) and their area in the mucosa after contact with an implant and in comparative material

On all the cross-sections, a statistically significant (at $P < 0.05$) increase in the number of Langerhans cells was found in the epithelium of the mucosa covering the intraosseous implants, expressed by their great density 51 (± 30.9) per mm^2 in relation to the comparative material. The density of S-100 positive cells per unit of an epithelium area rose several times in most patients (Figure 3).

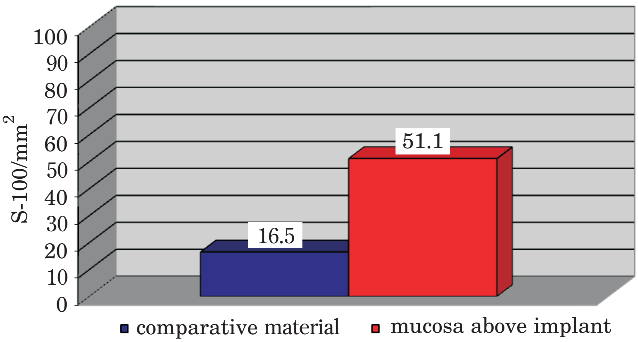

Fig. 3. Distribution of Langerhans cells density (S-100 positive) in mucosal epithelium after direct contact with an implant and in comparative material

In the material collected above implants, changes in the distribution of the investigated cells were also found within the epithelium, where many of the mononuclear cells creating characteristic clusters were S-100 positive. It should be emphasized that within these groupings, positive reaction cells made pairs with S-100 negative mononuclear cells of a lymphoid type. A statistically significant ($P < 0.05$) increase in S-100 positive cells both in the epithelium and in the subepithelial layer of the mucous membrane above the implant in relation to the comparative material collected from these patients is noteworthy. Furthermore, concentration of a considerable number of S-100 positive cells in the subepithelial zone in the infiltrations is a characteristic finding. However, no correlation between an increase in the number of S-100 cells and an area of the infiltrations was found.

The morphometric analysis demonstrated the distribution of cell types found in the subepithelial infiltrations. In all the analysed cases, most of the cells within the infiltrations were CD45-positive. In five cases evaluated in detail, the percentage of CD45RO cells increased significantly from 45.7% in the comparative material to 81.3% after contact with an implant (Figure 4). In the same patients, the average percentage of CD25-positive cells also increased from 21% to 42.9% (Figure 5). It was noticed that CD45RO- and CD25-positive cells were the most numerous group of cells in the infiltrations within the epithelium, beside S-100 positive cells. In the material collected above an implant, B lymphocytes and plasmatic cells were usually found among cells creating small clusters around vessels, and only single ones were dispersed in the connective tissue.

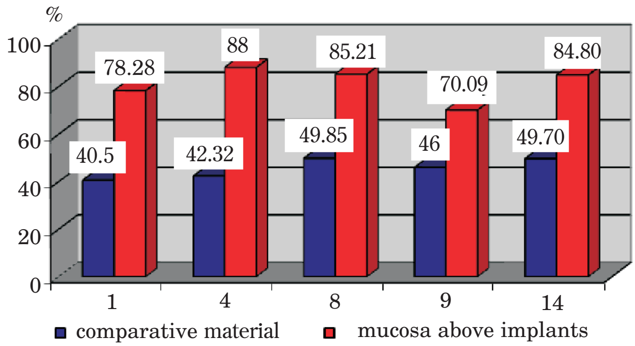

Fig. 4. Presence of CD45RO-positive T lymphocytes in cell clusters in the group of the patients with infiltrations in the mucosa above implants and in comparative material

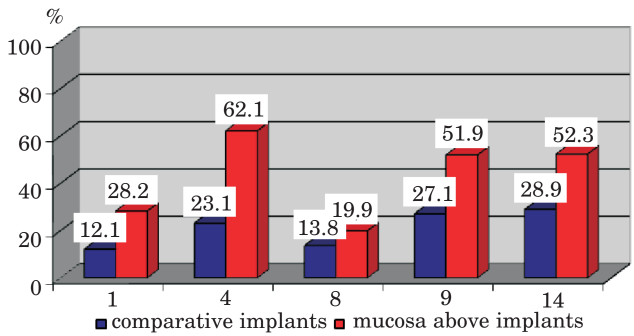

Fig. 5. Presence of CD25-positive T lymphocytes in cell clusters in the group of the patients with infiltrations in the material after contact with an implant and in comparative material

DISCUSSION

During the research, in most patients cell infiltrations located in the subepithelial region infiltrating the epithelium were found in the mucosa collected above implants. The infiltrations were tentatively defined as cell clusters covering an area above 0.01 mm of a cross-section. In most cases, such cell infiltrations occurred on single cross-sections, which limited the possibility of further research by immunocytochemical methods. For this reason, detailed phenotypic analysis of cells in infiltrations was only possible in 5 cases in which the infiltrations appeared on consecutive cross-sections. In the other cases, B or T lymphocytes and Langerhans cells were present in infiltrations or in their direct vicinity.

On the basis of a semi-quantitative analysis, it was determined that CD45R0-positive T lymphocytes were the dominant cell population in the infiltrations. Moreover, in the same infiltrations, a considerable percentage of cells included a subpopulation of T lymphocytes with the CD25 expression, accompanied by Langerhans cells. It is noteworthy that together as the number of T lymphocytes in infiltrations in the subepithelial zone increased, so did the count of Langerhans cells, which may suggest that this is a "cumulative area" for cells migrating to the lamina propria of the gingival mucosa, from which these cells relocate further to the epithelium.

The direct contact of Langerhans cells with T lymphocytes observed within the epithelium indicates their interactions. Studies on dogs conducted by Pongnarisorn et al. pointed to the possible occurrence of cell infiltrations in gingival mucosa in cases of implants properly integrated with the bone, without any symptoms of inflammation. Similarly to the results presented in this study, some authors have demonstrated that T lymphocytes constituted the most numerous group of cells in the infiltrations. The occurrence of Langerhans cells was not taken into consideration in those studies (PONGNARISON et al. 2007). In cases of inflammatory changes of *periimplantitis* type, B lymphocytes and plasmatic cells were considered to be the most abundant group (GUALINI et al. 2003, BERGLUNDH et al. 2004). As shown in the studies by Sanz et al., cell infiltrations constituted more than 65% of the total area of biopsy specimens in inflammatory processes occurring in the mucous membrane around titanium implants (SANZ et al.1991).

In recent years, attention has been drawn to the subpopulation of CD25+ immunoregulatory T lymphocytes which control the activity of T lymphocytes in response to organ-specific autoantigens (ITOH et al. 1999, SUN et al. 2006). As follows from the research on the phenomenon of tolerance by CAVANI et al. (1998, 2000), the role of regulatory T lymphocytes consists in inhibiting the immunological response through secretion of Interleukin-10, which leads to a decrease in the extent of cell damage. T lymphocytes also have a direct influence on dendritic cells[1] . An increase in the number of CD25-positive T lymphocytes in the mucosa after contact with a titanium implant points to the well-known mechanism of these cells acting in the case of contact hypersensitivity to nickel. Nickel ions, similarly to ions of other metals such as chromium, cobalt, gold as well as titanium, are haptens which are capable of binding to different extracellular and intracellular proteins (CZARNOBILSKA et al. 2007, ŚPIEWAK et al. 2006). Research into the mechanism of reactions taking place locally shows that the protein + hapten complex is recognized by Langerhans cells, which transport it to neighbouring lymph nodes and present to CD4 and CD8 lymphocytes. These cells, then, migrate to the site of the hypersensitive reaction. CD8 T lymphocytes may locally exert cytotoxic influence on keratinocytes, inducing their apoptosis. When

[1]Cell family including Langerhans cells.

this happens, CD4 cells perform the role of regulatory cells, either stimulating or inhibiting the hypersensitive reactions (BÜDINGER et al. 2000, SAINT-MEZARD et al. 2004, MARTIN 2004).

Studies of the induction of tolerance to nickel drew attention to the role of CD4+CD25+ regulatory lymphocytes. In allergic patients, as well as in people without any symptoms of hypersensitivity, the presence of cells allergic to nickel was demonstrated. It was proven that clinical manifestation of hypersensitivity symptoms depended on the count of CD4+CD25+ cells and their activity (CAVANI et al. 2003, CAVANI 2005).

In vitro and *in vivo* studies show that despite the passive activity of the external layer of oxides, titanium ions are released as a result of electrochemical and galvanic corrosion in the oral cavity environment. Clinical manifestation of this phenomenon includes gingiva discolouration, oedema of soft tissues, *gingivitis*, *stomatitis*, skin rash, erythema, itch as well as delayed healing (KOIKE et al. 2007, CHATURVEDI 2009). The presence of titanium ions was also revealed in soft tissues collected above implants correctly integrated with the bone, not accompanied by any clinical symptoms (MAKUCH et al. 2011).

Due to their characteristic image, the morphological changes occurring in the mucosa after contact with a titanium implant, observed in this study, point to an immunological process. The question arises whether these changes are a reflection of cell interactions inducing local tolerance in response to a titanium intraosseous implant. Such an assumption is undoubtedly supported by the increase in counts of Langerhans cells and T lymphocytes (CD45RO, CD25). Owing to a similar cell composition and the type of changes in the epithelial cells, the observed processes resemble a hypersensitive response to nickel. The lack of any clinical symptoms in patients subjected to implantation may be associated with a high increase in CD25-positive T lymphocytes in the mucosa collected above the implants. More detailed explanation of the processes occurring in soft tissues as a result of the contact with a titanium intraoseous implant requires further research on a larger group of patients.

CONCLUSIONS

1. In the healing process, cell infiltrations composed of a subpopulation of T lymphocytes and Langerhans cells appear in areas that are in contact with an implant.

2. The characteristic cell composition of the infiltrations within the gingival mucosa indicates that in areas of direct contact with an implant, processes of an immunological character take place.

3. The lack of clinical symptoms of hypersensitivity as well as the presence of immunologically competent cells may suggest that a titanium intraosseous implant is recognised by the immune system, with local tolerance being induced.

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EFFECT OF FOLIAR APPLICATION OF SALICYLIC ACID ON THE RESPONSE OF TOMATO PLANTS TO OXIDATIVE STRESS AND SALINITY

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Abstract

The aim of the study has been to evaluate the effect of an increased salt concentration in a nutrient solution and foliar application of salicylic acid and $KMnO_4$ (the latter causing oxidative stress) on the yield, fruit quality and nutritional status of tomato plants. Salinity stress was stimulated by elevating the electrical conductivity (EC) of a nutrient solution by a proportional increase in the content of all macro- and micronutrients. In 2009-2010, tomato plants were grown on rockwool, in a heated foil tunnel. The experiment included two sub-blocks with two EC levels (2.5 and 4.5 mS cm^{-1}). Within each sub-block, the following foliar application variants were distinguished: 1. control, without foliar application; 2. salicylic acid (SA); 3. SA/$KMnO_4$. In the SA/$KMnO_4$ combination, solutions of these compounds were applied alternately every 7 days. SA was applied in the concentration of 0.01%, while the concentration of $KMnO_4$ was 0.1%. Foliar treatments were conducted at 7-day intervals from the 3rd cluster flowering stage until ten days before the first harvesting of fruits. Irrespective of the EC of the nutrient solution, foliar application of SA as well as SA/$KMnO_4$ had no significant effect on the tomato yield, total acidity and dry matter or soluble sugar content in fruits. Neither did it affect significantly the mineral status of plants except for an increase in the Mn level induced by SA/$KMnO_4$. A significantly higher content of ascorbic acid together with a decreased content of phenolic compounds and free amino acids resulted from the foliar application of SA and SA/$KMnO_4$. Salicylic acid counteracted the oxidative stress caused by $KMnO_4$.

Key words: tomato, salicylic acid, $KMnO_4$, salinity, yield, fruit quality, mineral content.

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WPŁYW DOLISTNEJ APLIKACJI KWASU SALICYLOWEGO NA REAKCJĘ ROŚLIN POMIDORA NA STRES OKSYDACYJNY I ZASOLENIA

Abstrakt

Celem badań było określenie wpływu zwiększonej koncentracji soli w pożywce oraz dolistnej aplikacji kwasu salicylowego i $KMnO_4$ (stymulowanie stresu oksydacyjnego) na plonowanie, jakość owoców oraz stan odżywienia roślin pomidora w składniki mineralne. W badaniach stymulowanie stresu zasolenia osiągano przez zwiększenie przewodności elektrycznej (EC) pożywki, w wyniku proporcjonalnego zwiększenia zawartości wszystkich makro- i mikroskładników pokarmowych. Rośliny pomidora uprawiano na wełnie mineralnej w ogrzewanym tunelu foliowym, w latach 2009-2010. Badania obejmowały dwa podbloki ze zróżnicowanym poziomem EC pożywki (EC 2.5 i 4.5 mS cm^{-1}). W każdym podbloku wyróżniono kombinacje z dolistną aplikacją roślin: 1) kontrola – bez dolistnej aplikacji, 2) kwas salicylowy (SA), 3) SA/$KMnO_4$. SA i $KMnO_4$ były aplikowane przemiennie, co 7 dni. SA aplikowano w stężeniu 0,01%, a $KMnO_4$ – 0,1%. Zabiegi opryskiwania roślin roztworami wykonywano co 7 dni od momentu kwitnienia trzeciego grona, a zakończono na 10 dni przed pierwszym zbiorem owoców. Bez względu na EC pożywki dolistna aplikacja roślin SA oraz SA/$KMnO_4$ nie miała istotnego wpływu na plonowanie, kwasowość owoców oraz zawartość suchej masy i cukrów rozpuszczalnych w owocach oraz na stan odżywienia liści roślin w makro- i mikroskładniki, z wyjątkiem zwiększenia zawartości Mn w przypadku przemiennej aplikacji SA/$KMnO_4$. Wykazano zwiększenie zawartości kwasu askorbinowego w owocach pomidora oraz zmniejszenie zawartości związków fenolowych i sumy wolnych aminokwasów wskutek dolistnego zastosowania SA oraz SA/$KMnO_4$. Kwas salicylowy przeciwdziałał stresowi oksydacyjnemu stymulowanemu przez $KMnO_4$.

Słowa kluczowe: pomidor, kwas salicylowy, $KMnO_4$, zasolenie, plonowanie, jakość owoców, stan odżywienia roślin.

INTRODUCTION

The influence of osmotic stress on plants in both *in vitro* and *in vivo* conditions is relatively well documented (MARSCHNER 1995). Nevertheless, most scientific papers describe response of plants to salinity (salinity of growth media, soils and substrates) caused by application of high doses of a particular salt, i.e. NaCl (ARFAN et al. 2007, STEVENS et al. 2006, TARI et al. 2002). With just a few exceptions, sodium chloride is not a typical mineral fertilizer. Thus, in intensive crop production (including protected soilless cultivation), salinity of soil or substrate is not caused by excessive concentrations of Na^+ and Cl^- ions alone, but by a much wider spectrum of cations and anions introduced with mineral fertilizers. It is even recommended to use chloride-free and low-sodium fertilizers in protected cultures. In this type of crop production, medium salinity is most commonly caused by excessive concentration of K^+, NH_4^+, Na^+, NO_3^-, SO_4^{2-} and BO_3^{3-} as well as Na^+ and Cl^-, with the latter two being ballast elements in applied fertilizers (AUERSWALD et al. 1999, JAROSZ et al. 2011). Currently, soilless or hydroponic culture, a specific method of crop production, predominates in greenhouses and foil tunnels (GAJC-WOLSKA et al. 2008). In this type of plant production, the plant shoot weight to substrate volume ratio is unfavorable. In agricultural practice, an increase in the salt concentration in substrates used for hydroponic cultivation results from the presence of all cations and anions introduced with a nutrient solution into the root environment (AUERSWALD et al. 1999, GRATTAN, GRIEVE 1999).

Endogenous salicylic acid (SA) is said to act like a growth regulator. It functions as an indirect signal stimulating many physiological, biochemical and molecular processes and therefore it affects the plant growth and development (KLESSIG, MALAMY 1994, MALAMY et al. 1990). Numerous studies have documented the influence of endo- and exogenous SA on the content of photosynthetic pigments in leaves (YILDIRIM et al. 2008), on plant photosynthesis (FARIDUDDIN et al. 2003) and on nitrogen metabolism owing to SA producing a positive impact on the activity of nitrate reductase (FARIDUDDIN et al. 2003, MIGUEL et al. 2002, JAIN, SRIVASTAVA 1981), on the synthesis of secondary plant metabolites and on antioxidant activity (ERASLAN et al. 2007) or the improved plant tolerance to heavy metals (GUO et al. 2009, POPOVA et al. 2008, GUO et al. 2007, METWALLY et al. 2003). In the context of the present study, the following findings seem particularly interesting: the beneficial effect of SA on plant adaptation (resistance, increased tolerance) to stress factors including heat (LIU et al. 2006, SHI et al. 2006, LARKINDALE, HUANG 2005), low temperature (KANG et al. 2003), fungal or bacterial infection (LEE et al. 1995) and excessive salinity of soil or nutrient solutions, i.e. osmotic stress (ARFAN et al. 2007, SAWADA et al. 2006, STEVENS et al. 2006, TARI et al. 2002). Studies on tomato cultivation have revealed that exogenous application of SA into a nutrient solution (TARI et al. 2002), soil (STEVENS et al. 2006) or sprayed over leaves (HE, ZHU 2008) improved the plant's tolerance to osmotic stress caused by high concentration of NaCl. However, there are few reports on the influence of exogenous salicylic acid on plants exposed to salinity stress induced by an elevated concentration of other than ions Na^+ and Cl in the rhizosphere.

Potassium permanganate ($KMnO_4$) is a strong oxidant, which is commonly used in medicine owing to its antifungal and antibacterial properties. It is also applied in chemical reactions as an oxidizing agent. Relatively little is known about its influence on plants. The information provided by MOLLENHAUER (1959) and KNOTH (1981) indicates that application of $KMnO_4$ can be recommended when preparing microscope slides from plant samples. Noteworthy is the study conducted by CHEN and YEH (2005) on use of $KMnO_4$ for removal of *Chodatella* sp. algae from water. Results presented by these authors indicate that the response of *Chodatella* sp. to $KMnO_4$ included a release of extracellular organic matter and promotion of algal cell aggregation, which was additionally stimulated by increased water hardness. Foliar application of $KMnO_4$ could be of some importance for disinfection or control of fungal spores or bacteria on plant leaves and shoots during cultivation as a measure to prevent infection. On the other hand, such a treatment may increase the oxidative stress in epidermal cells, particularly in plants grown in a protected culture, whose leaves develop a thinner cuticular layer.

The available literature lacks information on the effect or possible use of exogenous salicylic acid to stimulate the plant's resistance or mitigate the negative symptoms of oxidative stress caused by $KMnO_4$ in plant cultivation. In practice, this type of plant stress can be caused by numerous agrochemicals (including chemical plant protection products), which may have oxidative properties. It is worth emphasizing that in large-scale protected cultivation, the co-occurrence of both stress factors – salinity and oxidative stress –is possible. No studies documenting the impact of SA application on plants exposed to both types of stresses have been presented so far.

The aim of this study has been to evaluate the effect of an elevated EC in a nutrient solution and of foliar application of salicylic acid (SA) and $KMnO_4$ (the latter stimulating oxidative stress) on yield, fruit quality and nutritional status of tomato plants. The idea has been to induce salinity stress by proportionally increasing the content of all macro- and micronutrients in the nutrient solution.

MATERIAL AND METHODS

The study, which was conducted in 2009-2010, consisted of trials on cv. Admiro F_1 tomato (*Lycopersicon esculentum* Mill.). Tomato plants were cultivated in a foil tunnel, on slabs filled with Grotop Master Dry rockwool (Grodan®). An open system plant fertigation was applied. The plants were divided into two sub-blocks, each treated with a nutrient solution characterized by a different EC: 2.5 and 4.5 mS cm^{-1}. Within each sub-block, the following foliar application variants were distinguished:

1) control – plants without foliar spraying,
2) plants sprayed with 0.01% solution of salicylic acid,
3) plants sprayed alternately with 0.01% salicylic acid or 0.1% $KMnO_4$.

The plants were sprayed at 7-day intervals from the 3rd cluster flowering stage until ten days before the first fruit harvest. In total, the plants were sprayed four times (four treatments every seven days). In combination 3, the first plant spraying included salicylic acid, while the treatment conducted seven days later consisted of a solution of potassium permanganate. Spraying was performed so as to cover all plants, including flowers, with the solution (approximately 2000 dm^3 of solution ha^{-1}). In order to improve the efficiency of the treatment, Superam 10 AL (Danmar) was added to the working solution as an adjuvant.

Plant fertigation with nutrient solutions of two different EC values (2.5 or 4.5 mS cm^{-1}) started at the 2^{nd} cluster flowering. Until then, plants from both sub-blocks had been fertigated with a nutrient solution of the same EC value. During the vegetative growth period, the pH of the nutrient solution was adjusted to 5.5 in all the variants.

The higher EC, i.e. 4.5 mS cm^{-1}, was obtained by a proportional increase of the concentration of all mineral nutrients in relation to their levels in the nutrient solution with EC equal 2.5 mS cm^{-1}. The composition of a medium with EC 2.5 and 4.5 mS cm^{-1} was as follows (in mg dm^{-3}): N 200 and 310, P 50 and 78, K 300 and 470, Mg 60 and 94, Ca 210 and 330, Fe 1.80 and 2.8, Mn 0.60 and 0.94, Zn 0.33 and 0.51, B 0.33 and 0.51, Cu 0.05 and 0.08, Mo 0.05 and 0.08, respectively. In the subsequent plant growth stages, N:K ratios were modified as recommended. Under intensive light exposure and high temperature, automatic compensation of the solution's EC was initiated in both series so as to obtain the maximum value of 0.5 mS cm^{-1}. Single and two-component fertilizers were used for making the nutrient solution. Microelements were introduced in the form of Superba Mikromix and Tenso (Yara) as well as ammonium molybdate.

Each sub-block consisted of 108 plants, 36 of which represented a study variant (12 plants × 3 replications). The crop density was 2.5 plants per m^2. The plants were pruned to a single stem and the topping was conducted above the 5^{th} cluster.

The nutritional status of tomato plants was assessed according to the content of N, P, K, Ca, Mg, S as well as Fe, B, Cu, Mn, Mo and Zn in leaves. The determinations were made on the fifth (from the top) fully developed leaf harvested when fruits from the 3^{rd} cluster were 3-4 cm in diameter. The nitrogen content was analyzed using Kjeldahl method, while the other macro- and micronutrients were determined using ICP-OES after mineralization in nitric acid. The dry matter content was assessed by drying leaf samples at 105°C.

Ripe fruits from all clusters were harvested and classified as marketable and non-marketable yield. Marketable yield included fully colored fruits, without visible deformations and above 35 mm in diameter.

The chemical composition was analyzed on ripe fully coloured fruits, similar in size, harvested from the 3^{rd} cluster. For each replication of every variant, eight fruits were chosen, washed in distilled water, dried and homogenized. The following assessments were made on the fruit pulp: the content of dry matter by drying at 70°C, ascorbic acid using Tillmans method and total acidity by titration method. Spectrophotometric methods were applied to determine the levels of soluble sugars (with antrone), phenolic compounds (with Folin-Ciocalteu reagent(and total free amino acids (with ninhydrine).

As the results of all determinations in both years of the study were comparable, statistical analysis was conducted on mean values. The results were verified using a two-way analysis of variance with the following factors: foliar application and nutrient solution EC. Statistical significance of differences between means was analyzed using Duncan's test at $P < 0.05$.

All the results are presented in Tables 1-4 as means from 2009-2010.

RESULTS

Tomato yield (both total and marketable) was significantly affected only by the EC of a nutrient solution. In contrast, no significant effect of foliar application or its interaction with the EC values on tomato yield was found (Table 1). The higher EC of a nutrient solution (4.5 mS cm^{-1}) reduced total and marketable yield by 1.04 and 1.05 kg m^{-2}, respectively, compared to tomato plants cultivated on a medium with the EC equal 2.5 mS cm^{-1}.

Table 1

Effect of foliar application and EC of nutrient solution on yield of tomato (mean for years 2009-2010)

EC (mS cm^{-1})	Foliar application	Yield of tomato fruits (kg m^{-2})	
		total	marketable
2.5	control SA SA/$KMnO_4$	9.07 8.68 8.97	9.05 8.68 8.95
4.5	control SA SA/$KMnO_4$	7.99 7.72 7.90	7.96 7.71 7.87
Mean for: EC	2.5 4.5	8.91^b 7.87^a	8.89^b 7.84^a
foliar application	control SA SA/$KMnO_4$	8.54 8.20 8.43	8.51 8.19 8.41
Test *F* for:	EC foliar application interaction	*x* n.s. n.s.	*x* n.s. n.s.

x – significant differences at $P<0.05$; n.s. – non-significant difference;
Means within columns, marked with different letters differ at $P<0.05$.

Among all the tested tomato fruit quality parameters, that is dry matter, ascorbic acid, total acidity, soluble sugars, phenolic compounds and free amino acids (Table 2 – means for EC), the higher EC resulted in the production of tomato fruits with an increased content of dry matter and phenolic

Table 2

Effect of foliar application and EC of nutrient solution on fruit quality of tomato fruits (mean for years 2009-2010)

EC (mS cm^{-1})	Foliar application	Dry matter (%)	Ascorbic acid (mg 100 g^{-1} f.w.)	Acidity (g 100 g^{-1} f.w.)	Sugars (g 100 g^{-1} f.w.)	Phenolic compounds (mg 100 g^{-1} f.w.)	Amino acids (mg 100 g^{-1} f.w.)
2.5	control	6.06	10.08^{a}	0.369	3.16	35.25^{b}	87.41
	SA	6.11	12.50^{c}	0.352	2.60	29.46^{a}	69.00
	SA/$KMnO_4$	6.09	11.81^{bc}	0.357	2.78	29.87^{a}	71.37
4.5	control	6.53	10.58^{a}	0.386	3.02	35.03^{b}	82.48
	SA	6.21	11.67^{b}	0.341	3.12	34.38^{b}	70.49
	SA/$KMnO_4$	6.30	12.43^{bc}	0.344	3.01	35.12^{b}	73.05
Mean for:							
EC	2.5	6.09^{a}	11.46	0.359	2.85	31.52^{a}	75.93
	4.5	6.35^{b}	11.56	0.357	3.05	34.84^{b}	75.34
foliar application	control	6.29	10.33^{a}	0.377	3.09	35.14^{b}	84.94^{b}
	SA	6.16	12.08^{b}	0.347	2.86	31.92^{a}	69.74^{a}
	SA/$KMnO_4$	6.20	12.12^{b}	0.351	2.89	32.49^{a}	72.21^{a}
Test F for:	EC	x	n.s.	n.s.	n.s.	x	n.s.
	foliar application	n.s.	x	n.s.	n.s.	x	x
	interaction	n.s.	x	n.s.	n.s.	x	n.s.

x – significant differences at P<0.05, n.s. – non-significant difference;
Means within columns, marked with different letters differ at P<0.05.

compounds. Foliar spraying with SA and alternate application of SA/ $KMnO_4$ (Table 2 – means for foliar application) were similar in that they caused an increased ascorbic acid accumulation and reduced amounts of phenolic compounds and free amino acids in tomato fruits versus the control (plants without foliar treatment). Noteworthy is also the fact that the interaction between foliar application and nutrient solution's EC generated a statistically significant effect only on two tested fruit quality parameters: the level of ascorbic acid and phenolic compounds. When comparing the impact of foliar spraying with SA and SA/$KMnO_4$ independently for both sub-blocks (with the lower and higher EC value), a decrease in the ascorbic acid level was found only in plants treated with SA and grown in the solution of EC 4.5 mS cm^{-1} versus the plants cultivated in a nutrient solution with the lower EC. In both sub-blocks, fruits of plants treated with SA contained significantly more ascorbic acid than the control ones. Moreover, a significant decrease in the content of phenolic compounds was found in fruits of plants sprayed with SA and SA/$KMnO_4$ in the sub-block with EC 2.5 mS cm^{-1} when compared to the control as well as all to the combinations with plants cultivated in a nutrient solution of the higher salinity (EC 4.5 mS cm^{-1}).

No significant influence of EC, foliar application of SA or SA/$KMnO_4$ or interaction between EC and foliar treatments on the dry matter content in tomato leaves was found (Table 3). Taking into consideration the analyzed macro- and micronutrients, a significant increase in N, K, Cu and Mo and a decrease in the Ca content were noted in leaves of plants cultivated in a nutrient solution with the higher EC (Tables 3 and 4 – means for EC value). Among all the mineral nutrients determined in tomato leaves, foliar treatment had a significant effect only on Mn (Table 4, means for foliar application). When compared to the control and SA application, the foliar SA/$KMnO_4$ treatment contributed to an increased accumulation of manganese in leaves. The correlation between the EC value of a nutrient solution and foliar application was statistically significant only for the S and Zn content in leaves (Tables 3 and 4). Foliar application of SA led to a significant but relatively small increase in the sulphur level in leaves of plants grown on a nutrient solution with EC 4.5 mS cm^{-1} compared to plants from the sub-block with the lower EC value. Regarding Zn, the foliar application of SA/$KMnO_4$ in the sub-block with the higher EC value significantly decreased the content of this element in tomato leaves in comparison to plants grown on a nutrient solution with EC 2.5 mS cm^{-1}. In the remaining combinations with foliar application, no significant differences in the content of S and Zn were found between plants from both sub-blocks.

Table 3

Effect of foliar application and EC of nutrient solution on the content of macronutrients in tomato leaves (mean for years 2009-2010)

EC (mS cm^{-1})	Foliar application	Dry matter (%)	N	P	K	Ca	Mg	S
			(% d.w.)					
2.5	control	12.47	3.53	0.799	4.16	2.35	0.559	1.041^{ac}
	SA	12.08	3.51	0.788	4.06	2.46	0.572	0.987^{ba}
	SA/$KMnO_4$	12.93	3.49	0.767	4.02	2.44	0.574	1.006^{abc}
4.5	control	12.65	3.76	0.837	4.37	1.96	0.529	0.948^{b}
	SA	12.69	3.76	0.886	4.43	2.27	0.574	1.070^{c}
	SA/$KMnO_4$	12.60	3.80	0.781	4.46	2.14	0.514	1.079^{c}
Mean for:								
EC	2.5	12.50	3.51^{a}	0.784	4.08^{a}	2.42^{b}	0.568	1.011
	4.5	12.65	3.77^{b}	0.834	4.42^{b}	2.13^{a}	0.539	1.032
foliar application	control	12.56	3.64	0.818	4.26	2.16	0.544	0.994
	SA	12.39	3.63	0.837	4.24	2.37	0.573	1.028
	SA/$KMnO_4$	12.77	3.64	0.774	4.24	2.29	0.544	1.042
Test *F* for:	EC	n.s.	*x*	n.s.	*x*	*x*	n.s.	n.s.
	foliar application	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*x*

x – significant differences at $P<0.05$, n.s. – non-significant difference;
Means within columns, marked with different letters differ at $P<0.05$.

Table 4

Effect of foliar application and EC of nutrient solution on the content of micronutrients in tomato leaves (mean for years 2009-2010)

EC (mS cm^{-1})	Foliar application	Fe	B	Cu	Mn	Mo	Zn
		(mg kg^{-1} d.w.)					
2.5	control	106.16	35.33	8.59	40.64	2.16	21.06^{ab}
	SA	101.06	33.69	7.68	40.29	1.92	19.75^{a}
	SA/$KMnO_4$	105.15	33.99	7.98	55.78	1.99	23.44^{b}
4.5	control	97.57	30.95	8.54	42.00	2.14	21.60^{ab}
	SA	103.16	34.04	9.15	47.64	2.32	21.80^{ab}
	SA/$KMnO_4$	94.72	31.40	8.50	58.19	2.23	20.72^{a}
Mean for:							
EC	2.5	104.12	34.34	8.08^{a}	45.58	2.02^{a}	21.42
	4.5	98.48	32.69	8.73^{b}	49.28	2.23^{b}	21.37
foliar application	control	101.87	33.14	8.57	41.34^{a}	2.15	21.33
	SA	102.11	33.87	8.41	43.97^{a}	2.12	20.77
	SA/$KMnO_4$	99.93	32.69	8.24	56.98*b*	2.11	22.08
Test *F* for:	EC	n.s.	n.s.	*x*	n.s.	*x*	n.s.
	foliar application	n.s.	n.s.	n.s.	*x*	n.s.	n.s.
	interaction	n.s.	n.s.	n.s.	n.s.	n.s.	*x*

x – significant differences at $P<0.05$, n.s. – non-significant difference;
Means within columns, marked with different letters differ at $P<0.05$.

DISCUSSION

Salinity stress – effect of the EC of a nutrient solution on plants

Tomatoes belong to plant species moderately tolerant to salinity stress. The yield reduction among plants cultivated on a nutrient solution of EC 4.5 mS cm^{-1} compared to plants treated with a nutrient solution of EC 2.5 mS cm^{-1} was most probably due to salt stress. The effect of salinity on plant growth and yield is relatively well documented in numerous reports. Well-known are the effects caused by increased values of osmotic pressure within the rhizosphere (soil solution, nutrient solution in hydroponics) on plants, manifested by growth inhibition, blue-green colour of leaves or a slender phenotype. These changes are caused by the increased biosynthesis of ABA (inhibitor of plant growth and development) under salt stress conditions. ABA pays an important role in enhancing plants' tolerance to salinity (MARSCHNER 1995). Improved tolerance to salt stress is obtained by the higher expression of genes responsible for the biosynthesis of osmoprotectants and stress proteins. This effect, however, is achieved at substantial energetic expense, which eventually contributes to the growth retardation and reduction of plant yield. Under stress conditions (irrespective of the etiology of stress), phenolic compounds are more intensively synthesized in plants (WU et al. 2005, ALI, ABBAS 2003, RIVERO et al. 2001). In the present study, increased levels of phenolic compounds were determined in fruits of plants cultivated on a nutrient solution with EC 4.5 mS cm^{-1}. Another cause of the higher accumulation of phenolic compounds in these plants could have been the improved nitrogen status of plants, which led to the increased activity of enzymes responsible for its biosynthesis (MATSUYAMA, DIMOND 1973).

One of the physiological responses of plants to osmotic stress is an increased demand for potassium, which is explained by the participation of K^+ ions in osmoregulation (MARSCHNER 1995). In the research conducted by WEST and TAYLOR (1980), a higher uptake of mineral nutrients (Na and Cl) was observed in tomato plants subjected to NaCl-induced salinity stress and the effect was positively correlated to an increase in the temperature and moisture of the substrate. In the present study, changes in the content of all mineral nutrients analyzed in leaves of plants cultivated under the higher EC value included increased concentrations of K, N, Cu and Mo and a reduced level of Ca. These results demonstrate clearly that when the salinity stress is induced by a proportional increase in the concentration of all macro- and micronutrients in a nutrient solution, plants can selectively regulate the uptake of particular mineral nutrients. The reduced accumulation of Ca in plants cultivated on a nutrient solution of EC 4.5 mS cm^{-1} (when compared to EC 2.5 mS cm^{-1}) could have resulted from the well-known antagonism between K^+ and Ca^{2+} (during its uptake by plant roots), but also from the fact that these two cations participate in the regulation of water relations albeit producing opposite effects. With an increased demand for K^+ by plants grown at 4.5 mS cm^{-1} EC, a reduction in the Ca^{2+} uptake was observed. On the other hand, the improved nitrogen status found in these plants could have been caused by the synergistic action of K^+ on the NO_3^- uptake. As a result, the demand for Mo was higher as well. This element is a cofactor for nitrate reductase, an enzyme responsible for the reduction of nitrate ions (CAMPBELL 1999). It is worth mentioning than effects induced by salinity stress could have included an impaired uptake of calcium, in extreme cases leading to its deficiency (MARSCHNER 1995). In our study, a reduction in the total yield was observed, with no relationship between a lower calcium status of plants and the incidence of blossom-end rot in tomato fruits. The difference between total yield and marketable yield was created by harvesting smaller tomato fruits. It should be underlined that the aforementioned interactions between the influence of a nutrient solution's salinity level on the mineral nutrition of plants had no significant effect on the dry matter content in leaves. Nonetheless, the dry matter content was found to have increased in fruits of plants subjected to salt stress.

Influence of SA and $KMnO_4$ foliar application and its interaction with the EC of a nutrient solution

There is a wealth of research results indicating that exogenous salicylic acid increases plant tolerance/resistance to salinity (He, Zhu 2008, Arfan et al. 2007, Sawada et al. 2006, Stevens et al. 2006, Tari et al. 2002). It is therefore puzzling that foliar application of salicylic acid in a relatively high dose of 0.01% had no effect on yield and mineral nutrition (with the exception of sulphur) of plants exposed to salt stress compared to plants grown on a nutrient solution with the lower value of EC. One possible explanation is that our experiment was conducted in a foil tunnel. In protected culture, droplets of working solution dry on the leaf surface very rapidly. The absorption of compounds applied with the working solution depends strongly on how long the leaves remain moist. This process may also be resumed after rehydration of the dry deposit (formed after water evaporation) on the leaf surface by its humidification with water vapour. The level of rehydration depends on air humidity as well as the POD (Point of Deliquescence) of particular compounds applied through foliar spraying (Schönherr 2002). When a mixture of salts (organic compounds) is sprayed, a change in the ionic strength of the solution occurs depending on its concentration. As a consequence, the value of the POD, which is difficult to estimate experimentally, also changes. It should be mentioned that the POD is not temperature-dependent (Kolthoff et al. 1969). Generally, organic compounds used commonly for foliar applications are characterized by high values of the POD (Schönherr 2002), a characteristic responsible for the low level of rehydration of the dry deposit formed on the plant's surface.

It can be assumed that due to the rapid drying of working solution droplets, absorption of compounds applied foliarly (salicylic acid and $KMnO_4$) was hampered. Additionally, in our study, the rehydration of the dry deposit formed on leaves was hindered by a relatively low air humidity in the foil tunnel (as well as the limited dew deposition on plants). Visual observations revealed small violet spots (from evaporated $KMnO_4$ solution) on leaves of plants treated alternately with SA/$KMnO_4$. Moreover, it can be suspected that most of the applied SA (in both tested combinations) could remain on the leaf surface (dry deposit) as the absorption of organic compounds is generally slower than that of dissociated cations and anions when applied foliarly (Schönherr 2002). This can also explain why SA, a compound which theoretically protects plants from potential yield loss caused by a higher EC of a nutrient solution, did not produce any effect. It is worth mentioning that Salehi et al. (2011), who examined tomatoes grown in a subtropical climate in pots irrigated with saline water (0, 4, 8 and 12 dS m^{-1}), reported that foliar application of SA (in concentrations of 0, 10^{-6}, 10^{-4} and 10^{-2} mol) did not significantly affect fresh and dry weight of shoots or the number of flowers per plant. However, these authors suggest further studies including lower SA doses. In this context, the study by Mady (2009) seems particularly relevant. It was conducted on a field plantation of tomatoes, where foliar application of 50 and 100 mg SA dm^{-3} contributed to an increase in the number of branches and leaves per plant, leaf area per plant, dry weight of leaves as well as the concentration of photosynthetic pigments, N, P, K, Fe, Zn, Mn, total carbohydrates and crude protein in leaves.

The results of our study indirectly indicate that both compounds applied through foliar spraying were to some extent absorbed by plants, but the rate of their absorption was insufficient to significantly affect the fruit yield or mineral nutrition of plant leaves. The exceptions included an increase in the Mn content in leaves of plants treated alternately with SA/$KMnO_4$ and an improved sulphur status in leaves owing to a foliar application of SA in the sub-block with the higher EC level. Salicylic acid plays a role of an indirect signal triggering numerous biochemical processes in plants (MALAMY et al. 1990, KLESSIG, MALAMY 1994). The induction of plants' resistance to salinity is related to the stimulated synthesis of protective proteins, and because sulphur amino acids are required in this process, the plants' demand for sulphur increases.

The applied compounds were found to have produced a relatively small influence on the quality of tomato fruits. However, the results on ascorbic acid and phenolic compounds in fruits are interesting. Plant spraying with solutions of SA and SA/$KMnO_4$ increased the content of ascorbic acid in fruits from both sub-blocks when compared to the control. It seems that SA may have stimulated the synthesis of ascorbic acid while reducing the phenolic content, particularly in the sub-block with the lower EC value. The results obtained in the sub-block with the lower EC indicate that SA effectively counteracted the oxidative stress caused by foliar application of $KMnO_4$. What was intriguing was the comparable levels of ascorbic acid noted in fruits of plants from both sub-blocks and treated with SA/$KMnO_4$. It seems that salt stress (induced by application of a nutrient solution with the higher EC value) did not weaken the cultivated plants, hence the temporary oxidative stress (caused by foliar application of $KMnO_4$) had no significant effect on the their growth and development. It is possible that plants exposed to a constant stress of the high EC value managed to adapt to unfavorable conditions. Frequently, the plant's adaptation or "immunization" to one stress factor contributes to its increased tolerance/higher sensitivity threshold to other stressors. This is indirectly confirmed by our study, where an increase in the content of phenolic compounds occurred in plants treated with the foliar application of SA and SA/$KMnO_4$ in the sub-block with EC 4.5 mS cm^{-1} compared to plants from analogous combinations in the other sub-block. The effect of a higher synthesis of phenolic compounds in response to salinity stress was observed previously (WU et al. 2005, ALI, ABBAS 2003). Nevertheless, it is surprising why the spraying of tomato plants with SA and SA/$KMnO_4$ reduced the level of phenolic compounds in the plants treated with the lower EC value. SA is one of the phenolic compounds present in plants and an application of its exogenous forms, at least theoretically, could increase the content of phenolics and other secondary metabolites as its derivatives. The influence of exogenous salicylic acid on the accumulation of phenolic compounds and ascorbic acid in plants is most likely dependent on the SA concentration and method of application (also with respect to simultaneous introduction of other compounds), cultivation conditions and species-specific variation. In the experiment conducted by Smoleń and Sady (2012), a double application of SA in the concentration of 10 mg dm^{-3} as well as a simultaneous application of urea+Mo+BA+sucrose+SA contributed to a comparable decrease in the accumulation of ascorbic acid, with no effect on the phenolic content in radish roots in comparison to the control. In carrot cultivation, an application of 0.5 mmol SA kg^{-1} of soil resulted in a higher accumulation of carotenoids and anthocyanins in storage roots (Eraslan et al. 2007).

CONCLUSIONS

1. Plant cultivation on a nutrient solution of a higher electrical conductivity (EC) value caused a significant reduction of tomato fruit yield accompanied by an increased content of N, K, Cu and Mo and a decrease in the Ca level in leaves. The higher EC value of a nutrient solution increased the accumulation of dry matter and phenolic compounds in tomato fruits.

2. Foliar application of salicylic acid and salicylic acid/$KMnO_4$ had no significant influence on the fruit yield, total acidity and content of soluble sugars in fruits or the nutritional status of leaves with respect to macro- and micronutrients, with the exception of an increased Mn content in plants treated alternately with salicylic acid and $KMnO_4$.

3. An increased content of ascorbic acid and reduced accumulation of phenolic compounds and free amino acids were noted in tomato fruits following the foliar application of salicylic acid and salicylic acid/$KMnO_4$.

4. Results of the determination of ascorbic acid and phenolic compounds in tomato fruits from the sub-block with the lower EC value indicate that salicylic acid effectively counteracted the oxidative stress stimulated by a temporary foliar application of $KMnO_4$. No such relationship was found in the sub-block with EC 4.5 mS cm^{-1}, most probably due to the plant's adaptation (resistance) to the main stress factor, i.e. salinity.

5. No influence of exogenous salicylic acid applied foliarly was found such as the prevention of fruit yield reduction caused by the high EC level (salt stress).

6. A significant interaction between foliar application of the compounds and the EC level was observed for the content of ascorbic acid and phenolic compounds in fruits as well as the S and Zn accumulation in leaves.

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EFFECT OF VARIED NPK FERTILISATION ON THE YIELD SIZE, CONTENT OF ESSENTIAL OIL AND MINERAL COMPOSITION OF CARAWAY FRUIT (*CARUM CARVI* L.)

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Abstract

Fertilisation is the main factor stimulating yields of herbaceous plants, including yields of active substances per area unit.

The aim of the research was to evaluate the yielding and chemical composition of fruits of cv. Kończewicki caraway grown in a pure stand and supplied different rates of mineral fertilizer. The results are derived from a controlled, one-factor, micro-plot experiment set up with four replications in a split-plot design. The experimental factor consisted of NPK mineral fertilisation introduced into soil at the following rates per hectare: A_0 – without mineral fertilisation, A_1 – 103.9 kg NPK (37.5 kg N+17.6 kg P+4 8.8 kg K), A_2 – 209.8 kg NPK (75 kg N+35.2 kg P+ 99.6 kg K), A_3 – 314.7 kg NPK (112.5 kg N+52.8 kg P+ 149.4 kg K). Mineral fertilisation in the control increased the caraway fruit yield, the yield of essential oil as well as the content of total nitrogen. The rates of 209.8 kg NPK ha^{-1} and 314.7 kg NPK ha^{-1} caused a distinct decrease in the content of essential oil in caraway fruits compared with the unfertilized treatment and with the fertilisation rate of 103.9 kg NPK ha^{-1}. Mineral fertilisation, in general, significantly decreased the content of total phosphorus, calcium, magnesium and sodium, although it did not differentiate substantially the content of potassium in caraway fruits.

Key words: caraway, NPK fertilisation, macronutrients, essential oil.

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WPŁYW ZRÓŻNICOWANEGO NAWOŻENIA NPK NA WIELKOŚĆ PLONU, ZAWARTOŚĆ OLEJKU ETERYCZNEGO I SKŁAD MINERALNY OWOCÓW KMINKU ZWYCZAJNEGO (*CARUM CARVI* L.)

Abstrakt

Nawożenie jest głównym czynnikiem podnoszenia plonów roślin zielarskich oraz wydajności substancji czynnych z jednostki powierzchni. Celem badań była ocena plonowania oraz składu chemicznego owoców kminku zwyczajnego odmiany Kończewicki uprawianego w siewie czystym, pod wpływem zróżnicowanego nawożenia mineralnego. Wyniki pochodzą ze ścisłego, jednoczynnikowego doświadczenia mikropoletkowego, założonego w czterech powtórzeniach metodą losowanych bloków. Czynnikiem doświadczenia było nawożenie mineralne NPK zastosowane doglebowo w następujących dawkach na 1 ha: A_0 – bez nawożenia mineralnego, A_1 – 103,9 kg NPK (37,5 kg N+17,6 kg P+48,8 kg K), A_2 – 209,8 kg NPK (75 kg N+35,2 kg P+99,6 kg K), A_3 – 314,7 kg NPK (112,5 kg N+52,8 kg P+149,4 kg K). Nawożenie mineralne w odniesieniu do obiektu kontrolnego powodowało zwiększenie plonu owoców kminku zwyczajnego, plonu olejku eterycznego, a także zawartości azotu ogólnego. Pod wpływem dawek 209,8 kg NPK ha^{-1} oraz 314,7 kg NPK ha^{-1} stwierdzono wyraźne zmniejszenie zawartości olejku eterycznego w owocach w porównaniu z obiektem bez nawożenia i nawożonego dawką 103,9 kg NPK ha^{-1}. Nawożenie mineralne powodowało na ogół istotne zmniejszenie zawartości fosforu ogólnego, wapnia, magnezu oraz sodu, natomiast nie różnicowało wyraźnie koncentracji potasu w owocach kminku.

Słowa kluczowe: kminek zwyczajny, nawożenie NPK, makroelementy, olejek eteryczny.

INTRODUCTION

Caraway (*Carum carvi* L.) is one of the oldest spices and medicinal plants in the world. It is a biennial plant, which grows in the wild and on plantations. Caraway is one of the most popular spices, hence its production in Poland reaches 2 thousand ha (SEIDLER-ŁOŻYKOWSKA 2009). Poland is the world's leader in the production of this species. Its high content of essential oil, often above 4% (KLUSZCZYŃSKA 2002), makes the Polish caraway very highly valued on the global markets.

Fruits of *Fructus Carvi* (*Fructus Cari carvi*) collected in the second year of growth are used to make medications and as a spice (PANK, KRŰGER 1998), whilst both fruits and essential oil (*Fructus et Oleum Carvi)* serve as pharmacopeial material. Caraway fruits typically contain up to 82 cm^3 kg^{-1} of essential oil, which mainly consists of carvone (52%) and limonene (45%) (OLLE, BENDER 2010). Fruits also include fatty oil (up to 22%), protein compounds (up to 25%) as well as sugars, flavonoids, organic acids, coumarin derivatives, mineral salts and other compounds (KLUSZCZYŃSKA 2002). The extracts from caraway fruits have mostly relaxant, carminative, bile-forming, cholagogic, diuretic, diaphoretic, lactating, anti-inflammatory, tranquilising, expectoranting and antibacterial effects (DYDUCH et al. 2006). They stimulate the secretion of saliva, gastric acid and pancreatic juice, but also inhibit fermentation processes in intestines (KLUSZCZYŃSKA 2002).

A high demand from the herbaceous plant processing industry for high quality material calls for launching research on raising the yielding of plants without decreasing the content of active compounds. For caraway fruits, the content of essential oil, particularly carvone, is extremely important (SEIDLER-ŁOŻYKOWSKA 2009). Better yields and quality of harvested produce can be achieved when suitable agronomic technologies are implemented, (KWIATKOWSKI, KOŁODZIEJ 2005), especially proper mineral fertilisation (KOZERA, NOWAK 2010, KOZERA, MAJCHERCZAK 2011). The biochemical processes which stimulate the formation and accumulation of specific compounds are connected with assimilation, respiration as well as the uptake and absorption of minerals. Deficiency as much as excess of particular minerals can disturb the biosynthesis and, consequently, decrease the content of active compounds. Production of herbal plants is distinguished by an effort to achieve a high content of active substances, the key criterion in yield quality assessment, possibly by application of low rates of fertilisation.

The aim of the research was to evaluate the yielding and content of minerals and essential oil in fruits of caraway grown at different rates of mineral fertilisation.

MATERIAL AND METHODS

The research was based on the results of a controlled, multiple, one-factor, micro-plot experiment carried out in 2006-2009 at the Experimental Station of the Faculty of Agriculture and Biotechnology, University of Technology and Life Sciences in Bydgoszcz, located at Wierzchucinek (53°26' N, 17°79' E). Caraway was grown in a pure stand. The experiment was set up in a split-plot design with four replications. The experimental factor was NPK fertilisation applied at the following rates per hectare: A_0 – without mineral fertilisation, A_1 – 103.9 kg NPK (37.5 kg N +17.6 kg P+48.8 kg K), A_2 – 209.8 kg NPK (75.0 kg N+35.2 kg P+99.6 kg K), A_3 – 314.7 kg NPK (112.5 kg N+52.8 kg P+149.4 kg K). In the first year, Polifoska 6 (6-20-30) was applied pre-sowing at rates of 200 kg ha^{-1}, 400 kg ha^{-1} as well as 600 kg ha^{-1}. Nitrogen fertilisation was supplemented with ammonium nitrate, applied after plant emergence at rates 75, 150 and 225 kg ha^{-1}, respectively. In the second year, Polifoska 6 was used at the onset of plant growth while the ammonium nitrate treatment was performed 2 to 3 weeks later.

In both years, spring barley was the preceding crop. The experiment was performed in Haplic Luvisol, good rye complex soil of neutral reaction (pH_{KCl} – 6.7). The content of available forms of phosphorus was 63 mg P kg^{-1}, potassium – 114 mg K kg^{-1}, magnesium – 51 mg Mg kg^{-1} of soil.

Caraway's growth and development largely depended on the weather during in the plant growth season. The mean air temperature over the vegetative period, from March to October in the sowing year and from March through July in the harvest year was similar to the multi-year mean for the above months (Table 1). However, the two years were completely different in total rainfall. In the whole growing season of 2006/2007, there was a deficit of precipitations, for example in June and July 2006 the mean total rainfall corresponded to just 40.2% and 34.3% of the mean multi-year total for these months, respectively. The shortage of rainfall inhibited vegetation, especially the root system, which – when well developed – enhances the

Table 1

Air temperature (°C) and precipitation (mm)

Month	Growing cycles						Mean - year	
	2006/2007		2007/2008		2008/2009			
	temp.*	precip.**	temp.	precip.	temp.	precip.	temp.	precip.
March	-1.5	27.4	5.0	47.9	3.0	61.2	1.8	24.3
Apr	7.1	77.0	8.5	17.6	7.6	38.7	7.3	27.8
May	12.5	59.9	13.8	73.1	13.2	11.5	13.0	42.4
June	16.8	21.8	18.2	105.5	17.6	15.5	16.2	54.2
July	22.4	24.2	18.0	104.7	19.2	58.7	18.0	70.6
Aug	16.6	129.0	17.8	42.1	17.8	95.5	17.5	54.7
Sep	15.2	40.6	12.4	37.6	12.4	20.2	13.2	40.4
Oct	9.6	12.1	6.9	19.9	8.4	80.0	8.3	32.2
March--Oct	12.3	392.0	12.6	448.4	12.4	381.3	11.9	346.6
March	5.0	47.9	3.0	61.2	2.4	43.7	1.8	24.3
Apr	8.5	17.6	7.6	38.7	9.8	0.4	7.3	27.8
May	13.8	73.1	13.2	11.5	12.3	85.3	13.0	42.4
June	18.2	105.5	17.6	15.5	14.3	57.4	16.2	54.2
July	18.0	104.7	19.2	58.7	18.6	118.0	18.0	70.6
March--July	12.7	348.8	12.1	185.6	11.5	304.8	11.3	219.3

*mean monthly air temperature, **sum of monthly precipitation

success of overwintering of plants and fruit-bearing in the successive year. In April 2007, the total rainfall was 10.2 mm lower than the long-term mean. Heavy rains in June (105 mm) and July (104.7 mm) delayed the caraway harvest. In the successive vegetative period (2007/2008), the rainfall deficit occurred in May and June 2008, when the mean total rainfall fell down to 27.1% and 28.6% of the multi-year total, respectively. In the 2008/2009 season, the rainfall deficit in April 2009 accounted for 98.6% and slowed down the caraway vegetation, while the heavy rain in July resulted caused uneven fruit ripening.

The harvest took place in June and July. It was performed manually, by cutting plants from an area of 3 m^2 area and then drying them up. Ripe caraway was threshed in a small plot harvester. For the purpose of chemical analyses, representative samples of 0.50 kg of fruits each were taken from each plot. Once they were ground and wet mineralized in sulphuric acid (VI), the following were determined: total nitrogen with Kjeldahl method; total phosphorus by colorimetry with ammonium; potassium, calcium and sodium molybdate with the flame photometry method; magnesium by atomic absorption spectroscopy. The concentration of essential oil extracted from ground caraway fruits was determined with the direct method (Farmakopea Polska 2008) by distillation with water vapour in a Deryng apparatus with a closed water cycle. Based on the yield size and the content of essential oil in the fruits, oil efficiency per hectare was calculated.

The analyses were performed at the Laboratory of Safe Food Production Technology Support, in an apparatus purchased under the project titled *Second Stage of the Establishment of a Regional Innovation Centre*.

The results were statistically verified using the analysis of variance for a one-factor design, and the differences between means were evaluated with Tukey's test at the level of significance of $\alpha = 0.05$.

RESULTS AND DISCUSSION

In the present research, the mean yield of caraway fruits was 0.97 t ha^{-1} (Table 2). The yields from the first two research years were significantly lower than in the third year: 0.97, 0.70 versus 1.24 t ha^{-1}, and fell in the lower limits of the yielding potential of that species, which ranges from 0.6 to 2.5 t ha^{-1} (Bouwmeester et al. 1995). Caraway yield depends on the local weather conditions, which can drastically depress the profitability of growing this herb whenever they turn unfavourable for the species (Bouwmeester, Smid 1995). Another factor which affects caraway yields is the type of soil on which it is grown (Bouwmeester et al. 1995).

The tested mineral fertilisation had a significant effect on caraway yielding. Caraway fruit yield was distinctly higher in all the fertilization treatments than in the control. Significantly the highest fruit yield was obtained at the rate of 314.7 kg NPK ha^{-1}. It was 0.42 t ha^{-1} higher than the yield collected from the control. A significant yield increase was also recorded after the application of mineral fertilisation at the rate of 209.8 kg NPK ha^{-1} (A_2): 0.37 t ha^{-1} higher than the control. The fruit yield increase for treatments A_1 was also significant: 0.22 t ha^{-1}. According to Kołodziej (2006), fertilisation is the key factor increasing yields of herbal plants and the efficiency of active substances per area unit. Soil deficit of any of the three basic nutrients, especially nitrogen, can significantly decrease yields and the content of active substances (Kordana et al. 1998).

Table 2

Yielding and essential oil content in fruits of caraway

Functional quality	Year	Fertilisation treatments				Mean
		A_0	A_1	A_2	A_3	
Fruits yield (t ha^{-1})	2007	0.72	0.94	1.09	1.14	0.97
	2008	0.62	0.67	0.75	0.77	0.70
	2009	0.81	1.21	1.42	1.50	1.24
	2007-2009	0.72	0.94	1.09	1.14	0.97
	$LSD_{0.05}$ for: years – 0.053, fertilisation – 0.068 fertilisation in years: 2007 – 0.095, 2008 – 0.080, 2009 – 0.219					
Essential oil content ($cm^3 kg^{-1}$)	2007	48.8	49.4	46.9	45.6	47.7
	2008	53.8	54.9	52.4	51.3	53.1
	2009	51.3	51.9	50.0	48.1	50.3
	2007-2009	51.3	52.0	49.7	48.3	50.3
	$LSD_{0.05}$ for: years – 0.97, fertilisation – 0.75 fertilisation in years: 2007 – 2.64, 2008 – n.s., 2009 – 2.43					
Essential oil yield (kg ha^{-1})	2007	35.06	46.52	50.94	51.98	46.13
	2008	33.34	36.55	39.01	39.34	37.06
	2009	41.65	62.90	71.25	72.42	62.05
	2007-2009	36.68	48.66	53.73	54.58	48.41
	$LSD_{0.05}$ for: years 2 1.497, fertilisation – 1.349 fertilisation in years: 2007 – 2.553, 2008 3.022, 2009 – 4.353					

A_0 – with no mineral fertilisation, A_1 – 103.9 kg NPK ha^{-1}, A_2 – 209.8 kg NPK ha^{-1},
A_3 – 314.7 kg NPK ha^{-1},
n.s. – non-significant differences

Interestingly, although rate A_3 contained 50% more nutrients than A_2, the caraway fruit yield collected from that treatment was just 4.6% higher, hence the difference was not significant. Some authors have considered the economic aspect of higher fertiliser rates in caraway cultivation. The report by Floot (1990) showed that 75 kg N ha^{-1} is the highest and economically viable nitrogen rate for this species.

Essential oils are among the most common active substances found in plants and their therapeutic properties are broadly used in treatment of illnesses (Król, Kapka-Skrzypczak 2011). In caraway, the essential oil is found in an entire plant, although fruits are the richest source – 1-6% (Sedláková et al. 2003). Fruits harvested in the present experiment had a relatively high mean content of essential oil: 50.3 cm^3 kg^{-1} (Table 2). The highest content of oil was found in fruits collected from the fertilisation treatment with the lowest rate of NPK (A_1): 52.0 cm^3 kg^{-1}. However, it was not significantly higher than in the control. Any successively higher NPK fertilisation rate significantly decreased the content of oil to 3.12% (A_2) and 5.85% (A_3) of the control. Similar tendencies were observed in each year. Significantly the highest oil content was determined in caraway fruits collected in 2008 (an average of 53.1 cm^3 kg^{-1}), but the inter-treatment differences were not significant. The lowest content of oil, 47.7 cm^3 kg^{-1}, was found in schizocarps harvested in 2007. Results of studies on caraway reported in recent years are varied, one reason being highly changeable and different weather conditions during the plants' growth. Thus, it is difficult to evaluate the effect of fertilisation on the content of essential oil in herbal materials. In the present study, the lower total rainfall in the second year of vegetation (2008) enhanced the accumulation of oil in fruits, whereas more rainfall in 2007 decreased its content in schizocarps. According to RŐHRICHT et al. (1996), the oil content in material is more strongly affected by habitat conditions than nitrogen fertilisation. EZZ EL-DIN et al. (2010) reported an increase in the oil content in caraway fruits under the effect of increasing nitrogen rates. As reported by DZIDA (2007), more intensive nitrogen fertilisation increased the oil content in thyme herbage.

It is possible to improve oil efficiency by increasing its content in plant material or by raising plant yield per area unit. High yields are unattainable without providing plants with adequate growth and development conditions, including optimal mineral fertilisation. With that in mind, it is essential to look for such fertilisation methods which will increase yield of herbal material but not decrease its content of active substances. Another consideration is to ensure high quality of the final product and production profitability (BIELSKI et al. 2011). The mean essential oil yield in the tested caraway fruit yield was 48.41 kg ha^{-1}.There was a significantly higher oil yield from the fertilised variants than from the control (Table 2). The highest essential oil yields in caraway fruits were found after the application of 314.7 kg NPK ha^{-1} (54.58 kg ha^{-1}) and 209.8 kg NPK ha^{-1} (53.73 kg ha^{-1}). The yields were 48.8% and 46.5% higher, respectively, than from the control. Significantly the lowest mean oil yield (37.06 kg ha^{-1}) was in 2008 while the highest oil efficiency per hectare was found in 2009 (62.05 kg ha^{-1}).

Caraway fruits are also a rich source of potassium, magnesium, phosphorus as well as micronutrients (KLUSZCZYŃSKA 2002). In the present research, a significant effect of NPK fertilisation on the chemical composition of caraway fruit was found (Table 3). All the different rates of NPK fertiliser clearly increased the mean content of total nitrogen in fruits. The highest total nitrogen content was reported in the fertilisation treatment with the rate of 314.7 kg NPK ha^{-1}, and the difference was 11.6% compared with the control.

Considerable variation was observed in the mean content of total nitrogen in fruits between the years: from 29.69 g kg^{-1} (2008) to 36.00 g kg^{-1} (2007). Significantly most nitrogen was recorded in fruits collected in 2007.

Interestingly, rates A_2 and A_3 were the only ones which induced a clear increase in total nitrogen in fruits, as compared with the control. At the lowest rate (103.9 kg NPK ha^{-1}), there was an increase in the mean content of N in fruits, although the differences were not significant. DZIDA (2007), reporting on the effect of increasing nitrogen fertilisation on the chemical composition of thyme herbage, also observed increased accumulation of minerals, including total nitrogen. Nitrogen is an essential element in agrotechnical practise employed for caraway cultivation (EVENHUIS et al. 1999). In plants, this is a very mobile element, which is indispensable in biosynthesis of protein, a key building material of the plant, whose content in caraway fruits reaches 25% (KLUSZCZYŃSKA 2002). Nitrogen is involved in enzymatic and metabolic processes; it participates in synthesis and transfer of energy; it is also a component of chlorophyll (EZZ EL-DIN et al. 2010).

This research has demonstrated a significant effect of fertilisation on the total phosphorus accumulation in fruits (Table 3). In fruits from the fertilisation treatments with phosphorus, the content of this element was significantly lower than the control. The biggest decrease in the content of phosphorus was in fruits from the fertilisation treatment with the rate of 209.8 kg NPK ha^{-1} (the difference reached 6.3%). The highest mean content of total phosphorus in fruits (7.01 g kg^{-1}) was noted in 2009. In the other years, its content was less than 20.3% (2007) and 16.5% (2008). Interestingly, the NPK fertiliser rates in the first year (2007) did not result in any significant changes in the content of total phosphorus in fruits, as compared with the control, which was analogous to the results reported by DZIDA and JAROSZ (2006a), who did not show any distinct effect of varied nitrogen and potassium fertilisation on the content of phosphorus in marjoram herbage.

No significant effect of NPK fertilisation on the potassium content in fruits of *Carum carvi* L. was demonstrated. Its content ranged from 16.22 g K kg^{-1} in fruits from treatment A_1 to 16.79 g K kg^{-1} from treatment A_2 (Table 3). DZIDA and JAROSZ (2006b), investigating savory herbage, recorded similar effects produced by nitrogen-potassium fertilisation. The fertiliser rates did not differentiate the content of potassium in 2009, although it was higher than in fruits from the control. The effect of the applied fertilisation rates on the potassium content in 2007 and 2008 was not obvious. In 2008, a significant increase in the potassium content in fruits was reported up to rate A_2. On the other hand, in 2007, rate A_1 significantly decreased the content of potassium versus the control, whilst the other rates did not differentiate it, which implies an evident effect of the weather conditions on potassium in fruits. Other reports point to a considerable variation in the content of that element in herbaceous plants depending on the region where they grow (AJASA et al. 2004) and the soil's abundance in available forms of nutrients (SHEDED et al. 2006).

The average content of calcium in caraway fruits was 5.96 g Ca kg^{-1}. Similarly to phosphorus, the different mineral fertilisation levels significant-

Table 3

Macronutrients content in caraway fruits (g kg^{-1})

Nutrient	Year	Fertilisation treatments				Mean
		A_0	A_1	A_2	A_3	
Total nitrogen	2007	34.13	35.25	36.96	37.66	36.00
	2008	28.00	28.95	30.03	31.78	29.69
	2009	32.92	34.31	35.77	36.58	34.89
	2007-2009	31.68	32.84	34.25	35.34	33.53
	$LSD_{0.05}$ for: years – 0.137, fertilisation – 0.099 fertilisation in years: 2007 – n. s., 2008 – 0.338, 2009 – 0.317					
Total phosphorus	2007	5.73	5.53	5.53	5.59	5.59
	2008	6.20	5.71	5.63	5.87	5.85
	2009	7.16	7.12	6.71	7.06	7.01
	2007-2009	6.36	6.12	5.96	6.17	6.15
	$LSD_{0.05}$ for: years – 0.137, fertilisation – 0.099 fertilisation in years: 2007 – n. s., 2008 – 0.338, 2009 – 0.317					
Potassium	2007	16.85	15.53	16.48	16.48	16.34
	2008	15.34	15.72	16.06	15.52	15.66
	2009	16.96	17.42	17.82	17.56	17.44
	2007-2009	16.38	16.22	16.79	16.52	16.48
	$LSD_{0.05}$ for: years – 0.393, fertilisation – n.s. fertilisation in years: 2007 – 0.693, 2008 – 0.268, 2009 – n.s.					
Calcium	2007	7.19	6.63	5.96	5.73	6.38
	2008	7.10	6.80	6.70	6.25	6.71
	2009	5.36	4.99	4.46	4.36	4.79
	2007-2009	6.55	6.14	5.71	5.45	5.96
	$LSD_{0.05}$ for: years – 0.139, fertilisation – 0.135 fertilisation in years: 2007 – 0.308, 2008 – 0.378, 2009 – 0.425					
Magnesium	2007	3.70	3.30	3.16	3.62	3.44
	2008	4.29	3.95	3.99	3.93	4.04
	2009	3.93	3.62	3.58	3.63	3.69
	2007-2009	3.97	3.62	3.58	3.72	3.72
	$LSD_{0.05}$ for: years – 0.103, fertilisation – 0.065 fertilisation in years: 2007 – 0.319, 2008 – 0.239, 2009 – 0.206					
Sodium	2007	0.31	0.27	0.25	0.27	0.27
	2008	0.34	0.29	0.29	0.34	0.32
	2009	0.31	0.27	0.27	0.29	0.29
	2007-2009	0.32	0.28	0.27	0.30	0.29
	$LSD_{0.05}$ for: years – 0.017, fertilisation – 0.022 fertilisation in years: 2007 – 0.027, 2008 – 0.006, 2009 – n.s.					

A_0 – with no mineral fertilisation, A_1 – 103.9 kg NPK ha^{-1}, A_2 – 209.8 kg NPK ha^{-1},
A_3 – 314.7 kg NPK ha^{-1},
n.s. – non-significant differences

ly decreased the content of that element in fruits compared with the unfertilised treatment. This tendency was observed in all the years (Table 3). Kołodziej (2011), who investigated the effect of NPK fertilisation on the chemical composition of European goldenrod, found a decrease in the content of calcium in the herbage of that plant.

As a result of mineral fertilisation, the magnesium content in fruits was lower than in the control by 8.8% (rate A_1), 9.8% (rate A_2), and by 6.3% (rate A_3), respectively. Such a tendency occurred in all the years. Most magnesium was accumulated in caraway fruits collected in 2008 (4.04 g Mg kg^{-1}). In the other two years (2007 and 2009), the content was 14.9% and 8.7% lower, respectively (Table 3). The literature stresses that magnesium is essential for various physiological processes, in which it plays numerous roles, for example it participates in the formation of chlorophyll, controls the water balance of plants and facilitates the synthesis of many chemical compounds (Panak 1997). The plants take up magnesium throughout the vegetative period, but mostly during the most intensive growth. The content of this nutrient in plants depends on its availability in soil, which can be changed quite considerably by soil moisture (Mikiciuk, Seidler 2004). Golcz et al. (2003) point to a higher demand of plants for magnesium when their yielding is stimulated by nitrogen fertilisation. A high demand of the tested caraway plants for Mg could have decreased its content in the fruits.

The NPK mineral fertilisation, as compared with the control, clearly decreased the content of sodium in caraway fruits (Table 3). The significantly lowest content of this element (0.27 g Na kg^{-1}) was determined in fruits collected from the fertilisation treatment with the medium NPK rate (A_2). The highest sodium content, like that of magnesium, was recorded in fruits collected in 2008 (0.32 g Na kg^{-1}). The year 2009 was exceptional in that that the mineral fertilisation did not differentiate significantly the sodium content in caraway fruits. Amounts of sodium available to plants depend mostly on the content of available forms of Na in soil, total rainfall and plant species (Adaszyńska, Swarcewicz 2011). The sodium content in herbs is not high relative to amounts of other macronutrients (Arceusz et al. 2008).

Based on results of this experiment, it was concluded that the yield volume and quality were highly varied by the weather conditions during the growing seasons in each year. Interestingly, as caraway has a two-year growth cycle, it is especially sensitive to rainfall deficit during the vegetative period, which can inhibit flowering and fruit-bearing until the third year of growth (Kołodziej 2010). It is very important to ensure all the best agrotechnical conditions to caraway plants, especially in the first year of cultivation, in order to avoid the delay of flowering to the third year of cultivation. Under the influence of the highest rate (314.7 kg NPK ha^{-1}), the highest mean fruit yield and the essential oil yield were produced, which suggests that the essential oil yield is more strongly correlated to the fruit yield increase than to the changes in its content in schizocarps. The yield increase is one of the ways of increasing the efficiency of essential oil per area unit in oilseed crops (BIELSKI et al. 2011). Under the fertilisation dose mentioned above, the fruits also accumulated most total nitrogen. The content of total nitrogen, total phosphorus as well as magnesium and sodium in fruits reported after the application of 314.7 kg NPK ha^{-1} was significantly higher than after the rate of 209.8 kg NPK ha^{-1}, which is recommended for caraway growing in soils moderately rich in available forms of nutrients (KOŁODZIEJ 2010). The NPK rate of 209.8 kg NPK ha^{-1} resulted in fruits accumulating significantly more essential oil and calcium than fruits from plants treated with the highest NPK rate. The use of the lowest NPK rate (103.9 kg NPK ha^{-1}) enhanced the accumulation of essential oil in fruits, whose content of which was distinctly higher compared with the other fertilisation treatments. The present research results show that optimisation of caraway fertilisation, mostly to produce a high yield of high-quality material, is a promising option, which is worth further investigations.

CONCLUSIONS

1. Increasing mineral fertilisation rates, as compared with the control, resulted in caraway fruits producing a significantly higher fruit yield, higher yield of essential oil and improved total nitrogen content.

2. The rates of 209.8 and 314.7 kg NPK ha^{-1} distinctly decreased the content of essential oil in caraway fruits compared with the control or the rate of 103.9 kg NPK ha^{-1}.

3. In general, the application of mineral fertilisation resulted in caraway fruits presenting a significant decrease in the content of total phosphorus, calcium, magnesium and sodium. However, the potassium content was not so evidently differentiated.

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EFFECT OF THE BIOSTIMULATOR ASAHI SL ON THE MINERAL CONTENT OF EGGPLANTS (*SOLANUM MELONGENUM* L.) GROWN IN AN UNHEATED PLASTIC TUNNEL*

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Abstract

Eggplants, known for their exceptional taste, are low in calories and constitute a rich source of potassium, phosphorus, magnesium, calcium, iron, manganese, copper and aluminum. They require a long and warm growing season, since adverse environmental conditions lead to growth inhibition, blossom fall and fruit drop. The application of eco-friendly biostimulators, such as Asahi SL, contributes to improving the growth and development of eggplants through increasing their stress tolerance and environmental adaptability, thus resulting in a higher yield.

A two-factorial experiment in a split-plot design with three replications was conducted in 2010-2011. The leaves of two eggplant cultivars, Black Beauty and Violetta Lunga, were sprayed with a 0.1% solution of Asahi SL. Seedlings were grown in a heated greenhouse, in line with the generally observed standards for eggplant cultivation. Eight-week-old seedlings were planted in the ground, at 50x60 cm spacing, in an unheated plastic tunnel. Starting from 30 May, at 10-day-intervals, eggplants were sprayed three times with the biostimulator Asahi SL at a concentration of 0.1%. Plants sprayed with water served as control. The fruits were harvested at the stage of commercial maturity, when normal sized and colored but still unripe.

The objective of this study was to determine the concentrations of macronutrients and micronutrients in fruits of two eggplant cultivars, grown in an unheated plastic tunnel and sprayed with the biostimulator Asahi SL.

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The mineral content of eggplants was determined in mid-August, on an average sample of marketable fruit yield collected in each treatment. The application of Asahi SL led to a significant decrease in total nitrogen levels in the fruits of cv. Black Beauty, and a significant increase in potassium concentrations in the fruits of both eggplant cultivars. The biostimulator increased Cu accumulation in fruits of both cultivars, and it significantly decreased Fe accumulation in cv. Violetta Lunga in comparison with the control treatment.

The fruits of cv. Violetta Lunga harvested from control plants were characterized by normal Ca:Mg and Ca:P ratios. The broadest K:Mg ratio was noted in cv. Violetta Lunga in both treatments. The K:(Mg+Ca) ratio was higher in the fruits from control plants of cv. Black Beauty.

Key words: eggplants, Asahi SL, foliar application, macronutrients, micronutrients.

WPŁYW STYMULATORA ASAHI SL NA ZAWARTOŚĆ SKŁADNIKÓW MINERALNYCH W OWOCACH OBERŻYNY (*SOLANUM MELONGENUM* L.) Z UPRAWY W NIEOGRZEWANYM TUNELU FOLIOWYM

Abstrakt

Owoce oberżyny oprócz niezwykłego smaku są cennym źródłem składników odżywczych. Są niskokaloryczne, bogate w potas, fosfor, magnez, wapń, żelazo oraz mangan, miedź i glin. Rośliny wymagają długiego i ciepłego okresu wegetacyjnego, a w mniej sprzyjających warunkach środowiskowych następuje zahamowanie wzrostu oraz opadanie kwiatów i zawiązków owoców. Stosowanie biostymulatorów, np. Asahi SL, które są bezpieczne dla środowiska, może być jednym ze sposobów poprawy warunków wzrostu i rozwoju roślin przez zwiększenie odporności oberżyny na czynniki stresowe oraz szybsze przystosowanie się do mniej sprzyjających warunków, a w konsekwencji zwiększone plonowanie.

Dwuczynnikowe doświadczenie, w układzie losowanych podbloków w trzech powtórzeniach, przeprowadzono w latach 2010-2011. Do badań wytypowano dwie odmiany oberżyny: Black Beauty oraz Violetta Lunga, w których uprawie stosowano dolistnie 0,1% preparat Asahi SL. Oberżynę uprawiano z rozsady, którą przygotowano w ogrzewanej szklarni na parapetach, wg ogólnie przyjętych zasad dla tego gatunku. Ośmiotygodniową rozsadę wysadzono w gruncie nieogrzewanego tunelu foliowego w rozstawie 50x60 cm. Od 30 maja, w odstępach 10-dniowych, trzykrotnie wykonywano oprysk preparatem Asahi SL w stężeniu 0,1%. W obiekcie kontrolnym rośliny opryskiwano wodą. Zbiór owoców przeprowadzano sukcesywnie, w fazie ich dojrzałości użytkowej, gdy owoce były wyrośnięte i wybarwione, ale niedojrzałe.

Celem badań była ocena zawartości podstawowych makro- i mikroelementów w owocach dwóch odmian oberżyny z uprawy roślin opryskiwanych biostymulatorem Asahi SL w nieogrzewanym tunelu foliowym.

Słowa kluczowe: oberżyna, Asahi SL, aplikacja dolistna, makro- i mikroelementy.

INTRODUCTION

The eggplant (*Solanum melongena* L.), also known as the aubergine, is highly valued for its taste, nutritional and health benefits (GOLCZ et al. 2005, ADAMCZEWSKA-SOWIŃSKA, KOŁOTA 2010). In additional to their extraordinary taste, eggplants are low in calories and constitute a rich source of essential nutrients that should be included in a balanced diet, as well as potassium, phosphorus, magnesium, calcium, iron (MICHAŁOJĆ, BUCZKOWSKA 2008, MARKIEWICZ et al. 2011), manganese, copper and aluminum (RAIGÓN ET AL. 2008). Until quite recently, eggplants were not grown in backyard gardens in Poland (SĘKARA et al. 2007, SĘKARA 2010), mostly due to their higher temperature requirements compared with tomatoes and peppers (GAJEWSKI et al. 2006). Under unfavorable environmental conditions, warm-climate plants are exposed to stress which adversely affects their growth and development, reducing the yield and quality of fruit. In Poland, eggplants can be grown in unheated greenhouses (SĘKARA 2010), but ground frost in the fall can pose a threat to developing fruit (KOWALSKA, BUCZKOWSKA 2004).

Eco-friendly biostimulators, such as Asahi SL whose active ingredients (nitrophenols) occur naturally in plants, may be applied to improve the growth and development of crops. Biostimulators increase plant resistance to stress factors (heat, drought, disease, mechanical injury) and their environmental adaptability, thus contributing to a higher fruit yield (CZECZKO, MIKOS-BIELAK 2004, SŁOWIŃSKI 2004).

The aim of this study was to determine the concentrations of selected macronutrients and micronutrients in the fruits of two eggplant cultivars, grown in an unheated plastic tunnel and sprayed with the biostimulator Asahi SL.

MATERIAL AND METHODS

A two-factorial experiment was conducted in 2010-2011 in the garden of the Research and Experimental Station of the University of Warmia and Mazury in Olsztyn. Eggplants were grown in an unheated plastic tunnel in a split-plot design with three replications. The leaves of two eggplant cultivars, Black Beauty and Violetta Lunga, were sprayed with a 0.1% solution of Asahi SL.

Seedlings were grown in a heated greenhouse, in line with the generally observed standards for eggplant cultivation and Polish Standard BN-88/9125-08 – Vegetable Seedlings. Each year, on 22 March, seeds were sown in boxes for seedlings, filled with substrate. On 6 April, seedlings were planted out in pots 10 cm in diameter, filled with peat substrate of the following chemical composition: $N–NO_3$ – 100, P – 80, K – 215, Ca – 1240, Mg – 121 mg dm^{-3}, pH in H_2O – 5.9, salt concentration – 1.5 g dm^{-3}. Eight-week-old, hardened off seedlings were planted in the ground, at 50x60 cm spacing, 24 plants per pot. Starting from 30 May, at 10-day-intervals, eggplants were sprayed three times with the biostimulator Asahi SL at a concentration of 0.1%. Plants sprayed with water served as control. The fruits were harvested at the stage of commercial maturity, when normal sized and colored but still unripe (30- to 40-day-old buds). They were hard and firm, with the texture of a firm sponge but without developed seed cases. Harvest began towards the end of July and ended in the first week of October. The mineral content of eggplants was determined in mid-August, on a sample of four fruits collected in each treatment, which were comminuted, dried at 65°C to constant weight and ground in an electric grinder. At the laboratory of the Chemical and Agricultural Station in Olsztyn, the plant material was mineralized with concentrated sulfuric acid and assayed for the content of total nitrogen by the potentiometric method, phosphorus by the vanadium-molybdenum method, potassium by flame photometry, magnesium by atomic absorption spectrometry (AAS), calcium by flame photometry, copper and iron by atomic absorption spectrometry (AAS). The study was carried out under Accreditation Certificate no. AB 277 issued by the Polish Center for Accreditation in Warsaw.

The results were processed statistically by analysis of variance. The significance of differences between means was estimated by Tukey's test at α=0.05. All calculations were performed using Statistica software.

RESULTS AND DISCUSSION

Due to their specific taste and flavor, eggplants are becoming increasingly popular among vegetable consumers. Eggplant fruits are rich in organic and mineral compounds, whose concentrations vary depending on the cultivar, growing conditions and the degree of ripeness. Minerals help maintain normal acid-base balance and are stored in the skeletal system (MICHAŁOJĆ, BUCZKOWSKA 2008, MARKIEWICZ et al. 2011, WADAS, MIODUSZEWSKA 2011).

As demonstrated by CZECZKO and MIKOS-BIELAK (2004), the concentrations of chemical components (dry matter, sugars, vitamin C, protein) in vegetable plants change in response to biostimulators. Crops of the family Solanaceae (tomatoes, potatoes) treated with Asahi SL were characterized by increased saccharose concentrations in fruits and tubers, but the noted changes were statistically non-significant. The macronutrient and micronutrient content of eggplant fruits determined in the present experiment was similar to that reported by MICHAŁOJĆ and BUCZKOWSKA (2008). A statistical analysis revealed that neither the cultivar nor the biostimulator had a significant effect on macronutrient concentrations in eggplants (Table 1). The levels of P, Mg and Ca in the fruits of both cultivars were not affected by Asahi SL. The application of Asahi SL led to a significant decrease in total nitrogen levels in the fruits of both eggplant cultivars, which was particularly high in cv. Black Beauty. Potassium is a very important mineral for the proper functioning of cells, tissues and organs in the human body. The potassium content of eggplant fruits ranged from 2.49 to 2.56 g kg^{-1} d.m., and it was higher in plants of both cultivars treated with Asahi SL.

Table 1

Concentrations of selected macronutrients and micronutrients in fruits of eggplants sprayed with Asahi SL (means of 2010-2011)

Cultivar	Biostimulator	Macronutrients (g kg^{-1} d.m.)					Micronutrients (mg kg^{-1} d.m.)	
		total N	P	K	Mg	Ca	Cu	Fe
Black Beauty	control	2.07	0.34	2.49	0.13	0.34	0.16	7.01
	Asahi SL	1.88	0.31	2.56	0.15	0.35	0.22	6.49
	Mean	1.98	0.33	2.53	0.14	0.34	0.20	6.75
Violetta Lunga	control	1.96	0.36	2.49	0.12	0.38	0.29	9.95
	Asahi SL	1.90	0.32	2.51	0.12	0.38	0.39	5.24
	Mean	1.93	0.34	2.50	0.12	0.38	0.34	7.60
Mean	control	2.02	0.35	2.49	0.13	0.36	0.23	8.48
	Asahi SL	1.89	0.32	2.54	0.14	0.37	0.31	5.87
$LSD_{0.05}$								
Cultivar		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.13
Biostimulator		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.97
Interaction		0.10	n.s.	0.05	n.s.	n.s.	0.22	1.88

The copper content of eggplant fruits was significantly affected by the cultivar x biostimulator interaction. Higher copper concentrations were noted in the fruits of cv. Violetta Lunga and cv. Black Beauty sprayed with Asahi SL, and the differences relative to control plants reached 0.10 mg kg^{-1} d.m. and 0.06 mg kg^{-1} d.m., respectively. The iron content of eggplant fruits varied widely, from 5.24 to 9.95 mg kg^{-1} d.m.. Significantly higher iron accumulation was observed in the fruits of cv. Violetta Lunga. Asahi SL decreased iron concentrations by 2.61 mg kg^{-1} d.m., in comparison with the control treatment. The interaction between the experimental factors was significant. Higher iron levels were determined in control plants of both cultivars. Dobrzański et al. (2008) and Łyszkowska et al. (2008) found that the application of various biostimulators contributed to a significant yield increment in vegetable crops and a minor increase in the content of dry matter and nitrates, but further research is needed to confirm the above findings. Very good results were obtained when Asahi SL was applied in combination with herbicides to sugar beets whose roots contained increased concentrations of sugars, nitrogen, potassium and sodium (Kositorna, Smoliński 2008). In contrast to the findings of Kositorna and Smoliński (2008) and analogously to our study, Matysiak et al. (2011) demonstrated that edible parts of plants treated with Asahi SL contained significantly less nitrogen. Asahi SL had varied effects on the potassium content of sugar beet roots, but a significant increase in the concentrations of this macronutrient was observed in only one year of the study.

According to Kotowska and Wybieralski (1999), the quality of edible plant parts is determined by both the concentrations and ratios of macronutrients and micronutrients, in particular the K:Mg, Ca:Mg and K:(Mg+Ca) ratios. In the current study, the ratios between the analyzed elements in eggplant fruits varied between the cultivars and between plants treated and untreated with Asahi SL (Table 2).

Table 2

Ratios of selected elements in edible parts of eggplants sprayed with Asahi SL (means of 2010-2011)

Cultivar	Biostimulator	Ratios			
		Ca:Mg	Ca:P	K:Mg	K:(Ca+Mg)
Black Beauty	control	2.62	1.00	19.15	5.30
	Asahi SL	2.33	1.13	17.07	5.12
	Mean	2.43	1.03	18.07	5.27
Violetta unga	control	3.17	1.06	20.75	4.98
	Asahi SL	3.17	1.19	20.75	4.98
	Mean	3.17	1.12	20.75	4.98
Mean	control	2.88	1.03	19.92	5.13
	Asahi SL	2.70	1.16	18.70	5.05

As demonstrated by Majkowska-Gadomska and Wierzbicka (2008), and Majkowska-Gadomska (2006, 2009, 2010), the optimal Ca:Mg ratio in edible parts of vegetables should approximate 3, and the Ca:P ratio should be within the 1.2-2.2 range. The Ca:P ratio is very important in children's nutrition. The fruits of cv. Black Beauty were characterized by lower values of the Ca:Mg and Ca:P ratios (Table 2). The fruits of cv. Violetta Lunga harvested from control plants had normal Ca:Mg and Ca:P ratios. According to Radkowski et al. (1999), the K:Mg and K:(Mg+Ca) ratios should be 6 and 1.6 – 2.2, respectively. The broadest K:Mg ratio was noted in cv. Violetta Lunga in both treatments. The K:(Mg+Ca) ratio was higher in fruits from control plants of cv. Black Beauty.

CONCLUSIONS

1. The application of Asahi SL led to a significant decrease in total nitrogen levels in fruits of cv. Black Beauty, and a significant increase in potassium concentrations in fruits of both studied eggplant cultivars.

2. The biostimulator increased Cu accumulation in fruits of both cultivars, and it significantly decreased Fe accumulation in cv. Violetta Lunga in comparison with the control treatment.

3. The application of Asahi SL had varied effects on the quality of eggplant fruits, which were characterized by similar ratios of the analyzed elements in both treatments, including normal Ca:Mg and Ca:P ratios. The broadest K:Mg ratio was noted in cv. Violetta Lunga in both treatments. The K:(Mg+Ca) ratio was higher in the fruits from control plants of cv. Black Beauty.

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EFFECT OF SULFUR FORMS ON CONCENTRATIONS OF CADMIUM AND NICKEL SOLUBLE IN 1 MOL HCl dm^{-3} IN ARABLE SOILS

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Abstract

Heavy metal contamination of arable soils remains one of the world's most serious environmental problems. The mobility of heavy metals in soil is determined by soil conditions, environmental factors and human activity. The immediate and residual effects of sulfur fertilization on the concentrations of heavy metals in soil are an important consideration when monitoring changes in environmental conditions in agricultural areas.

The objective of this study was to determine the effect of increasing doses of sulfate and elemental sulfur on changes in the concentrations of cadmium and nickel soluble in 1 mol HCl dm^{-3} in soil samples collected at a depth of 0-40 and 40-80 cm. A three-year field experiment was conducted on Dystric Cambisols (FAO) of the texture of heavy loamy sand. Soil samples were collected from each plot, prior to the establishment of the trials, after each harvest and before sowing the consecutive crop. The soil samples were used to determine the concentrations of Cd and Ni in soil (extractions with 1 mol HCl dm^{-3}, the ratio between soil and extraction - 1:10) by the AAS method using a Schimadzu AA apparatus.

Irregular changes in soluble cadmium concentrations were observed in soil fertilized with sulfate and elemental sulfur. No distinct trends were noticed in response to sulfate and elemental sulfur fertilization. The effect of sulfur was noticeable only in the third year of the experiment. Sulfur application contributed to a decrease in the soluble nickel content of soil. Sulfate and elemental sulfur fertilization did not increase the levels of cadmium and nickel soluble in 1 mol HCl dm^{-3}. The soil can be used for growing high-quality horticultural and agricultural crops safe for human consumption.

Key words: fertilizer, sulfate sulfur, elemental sulfur, cadmium, nickel, soil.

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OCENA WPŁYWU FORM SIARKI NA ZMIANY ZAWARTOŚCI KADMU I NIKLU ROZPUSZCZALNYCH W 1 MOL HCl dm^{-3} W GLEBIE UŻYTKOWANEJ ROLNICZO

Abstrakt

Zanieczyszczenie metalami ciężkimi gleb użytkowanych rolniczo to jeden z problemów środowiskowych w wielu częściach świata. Określenie możliwości uruchamiania metali ciężkich jest procesem złożonym zależnym od czynników glebowych, środowiskowych i działalności człowieka. Zbadanie bezpośrednich i następczych efektów nawożenia siarką na zmiany zawartości metali ciężkich w glebie ma znaczenie dla monitorowania zmian przyrodniczych warunków na obszarach rolniczych.

Celem badań była ocena wpływu nawożenia wzrastającymi dawkami siarki siarczanowej i elementarnej na zmiany zawartości form kadmu i niklu rozpuszczalnych w 1 mol HCl dm^{-3} w dwóch poziomach gleby: 0-40 i 40-80 cm. Trzyletnie doświadczenie polowe założono na glebie brunatnej, kwaśnej o składzie granulometrycznym piasku gliniastego mocnego. Glebę do analiz chemicznych pobierano wiosną i jesienią. Formy rozpuszczalne kadmu i niklu ekstrahowano z gleby roztworem 1 mol HCl dm^{-3}, a ich zawartość oznaczono metodą absorpcyjnej spektrometrii atomowej. Wyniki analiz chemicznych gleby opracowano statystycznie metodą analizy wariancji.

Po nawożeniu siarką siarczanową i elementarną zmiany zawartości form rozpuszczalnych kadmu w glebie przebiegały nieregularnie. Nie uwidoczniła się żadna tendencja w zmianie zawartości omawianej formy pierwiastka. Działanie siarki uwidoczniło się dopiero w trzecim roku trwania eksperymentu. Zastosowanie siarki wpłynęło na zmniejszenie zawartości form rozpuszczalnych niklu w glebie. Nawożenie siarką siarczanową i elementarną nie spowodowało zwiększenia zawartości form kadmu i niklu rozpuszczalnych w 1 mol HCl dm^{-3}. Gleba nadaje się do uprawy żywności o wysokim stopniu bezpieczeństwa. Może być zagospodarowana pod uprawę roślin ogrodniczych i rolniczych.

Słowa kluczowe: nawożenie, siarka siarczanowa, siarka elementarna, Cd, Ni, gleba.

INTRODUCTION

The mobility of heavy metals in soil is determined by soil conditions, environmental factors and human activity. The bioavailability and desorption of heavy metals are affected by soil properties, including organic matter content, pH, iron oxide content, and redox conditions. Soil pH usually exerts the strongest effect due to the solubility and speciation of heavy metals in the soil solution (MÜHLBACHOVÁ et al. 2005, JIAN-LING et al. 2010).

Soil is a non-renewable resource and it should be adequately protected and used rationally to produce high quality crops (KAYA et al. 2009), which is often ignored when industrialization and agricultural development become the top priorities. According to NEDERLOF and RIEMSOLIJK (1995), TEMMINGHOFF et al. (1997), FÄSSLER et al. (2010a,b) and VIOLANTE et al. (2010), the toxicity of heavy metals and their availability to plants increase due to soil acidification caused by sulfur deposition. KAYSER et al. (2000) demonstrated that the application of elemental sulfur and a decrease in soil pH increased heavy metal solubility in soil. In the Province of Warmia and Mazury, average heavy metal concentrations in arable soils are considerably lower than in other regions of Poland. The above soils have a natural (0°) heavy metal content (TERELAK et al. 2001).

Previous research findings into the residual effects of sulfur on agricultural ecosystems have been inconsistent and contradictory, which prompted us to carry out the present experiment. The objective of this study was to determine the effect of increasing doses of sulfate and elemental sulfur on changes in the concentrations of cadmium and nickel soluble in 1 mol HCl dm^{-3} in soil samples collected at a depth of 0-40 and 40-80 cm.

MATERIAL AND METHODS

A three-year field experiment was conducted in North-East Poland from 2000 to 2002. The exact location was far from larger industrial plants emitting sulfur compounds or any big cities. The soil content of sulfur in the soil was not caused by human activity.

The trial was set up on Dystric Cambisols (FAO) of the texture of heavy loamy sand. Initially, the soil had the following properties: $pH_{(KCl)}$ = 5.30, mineral nitrogen 24.0, sulfate sulfur 4.10, available phosphorus 34.5 and potassium 110.0 mg kg^{-1} of soil. The annual rates of sulfate sulfur ($SO^{2-}{}_4$-S) and elemental sulfur (S^{-0}-S) were: S_1 – 40, S_2 – 80 and S_3 – 120 kg ha^{-1}. Air-dry soil was passed through a 1 mm mesh sieve.

A permanent experiment was established in a random block design and consisted of eight fertilization treatments with four replications: 1) unfertilized control, 2) NPK, 3) NPK + S_1-SO_4, 4) NPK + S_2-SO_4, 5) NPK +S_3-SO_4, 6) NPK +S_1-S^0, 7) NPK +S_2-S^0, 8) NPK +S_3-S^0. The following compounds were applied to introduce given elements: ammonium nitrate (34% N) or ammonium sulphate (20.5% N), triple superphosphate (20.1% P), potassium salt (49.8% K) or potassium sulphate (43.7% K), potassium sulphate (17% S), ammonium sulphate (24% S) or elemental sulfur. The NPK rates (Table 1) depended on the crop species and soil fertility. No heavy metals were added to the soil during the experiment.

Table 1

Applied doses of NPK in the experiment

Year	kg (ha^{-1})		
	N	P	P
2000	200.0	52.5	180.0
2001	160.0	60.0	183.0
2002	90.0	80.0	111.0

Soil samples were collected from each plot at 0-40 and 40-80 cm depths prior to the onset of the trials, after each harvest and before sowing the consecutive crop. Air-dry soil was passed through a 1 mm mesh sieve. The soil samples were used to determine the concentrations of Cd and Ni in soil (extractions with 1 mol HCl dm^{-3}, the ratio between soil and extraction – 1:10) by the AAS method using a Schimadzu AA apparatus.

The results of the yields and chemical analysis of soil were processed statistically with the analysis of variance for a two-factorial experiment in a random block design, using the form of sulfur as factor a and rate of sulfur as factor *b*.

RESULTS AND DISCUSSION

A three-year field experiment was carried out to determine the effect of fertilization with sulfate or elemental sulfur at doses of 40, 80 and 120 kg ha^{-1} on concentrations of cadmium and nickel soluble in 1 mol HCl dm^{-3} in soil samples collected from 0-40 cm and 40-80 cm soil horizons.

Changes in the soil pH resulting from sulfate and elemental sulfur fertilization have been described in detail by SKWIERAWSKA et al. (2008). Over the three-year experimental period, the application of sulfate and elemental sulfur significantly affected soil pH in the 0-40 cm horizon. When applied in a dose of 120 kg, S-SO^{2-}_4 and S-S^0 caused a significant decrease in the soil pH in comparison with the control treatment. In the deeper soil layer, pH values remained relatively stable and lower than in the topsoil in the corresponding treatments. The results of Duncan's test at a significance level of 0.05 show that during the three years the average soil pH was the lowest in the treatment with a triple sulfate dose, and the differences relative to the other doses were statistically significant.

Cadmium is one of the most toxic and mobile metallic elements (BASTA et al. 2005, ZHAO, MASAIHIKO 2007). Before the experiment, the content of cadmium soluble in 1 mol HCl dm^{-3} in both sampled soil horizons (0-40 cm and 40-80 cm) was comparable, but slightly lower in the deeper layer than in the topsoil (Tables 2 and 3). Similar trends were observed in the first and second year of the study, when neither the sulfur form nor its dose exerted a significant effect on changes in cadmium concentrations in the soil. The impact of sulfur was not observed until the third year. At the end of the experiment, the soluble cadmium content of the 0-40 cm soil layer increased in the treatments fertilized with sulfate and elemental sulfur at a dose of 40 kg ha^{-1}. In the 40-80 cm horizon, sulfate and elemental sulfur had no significant effect on soluble cadmium levels in soil (Table 3). Before sowing spring barley, the cadmium content of soil increased insignificantly, particularly in the treatment fertilized with 120 kg S-SO^{2-}_4 ha^{-1}. After three years

Table 2

Effect of different rates and forms of sulfur on the content of cadmium soluble in 1 mol HCl dm^{-3} in soil at 0-40 cm depth (mg Cd kg^{-1} soil)

Treatments	Before experiment	After cabbage harvest	Before onion sowing	After onion harvest	Before barley sowing	After barley harvest
0	0.080	0.090	0.054	0.072	0.055	0.096
NPK	0.085	0.095	0.055	0.072	0.094	0.119
NPK+ S_1-SO^{2-}_4	0.080	0.087	0.072	0.078	0.113	0.132
NPK+ S_2-SO^{2-}_4	0.089	0.098	0.048	0.063	0.115	0.105
NPK+ S_3-SO^{2-}_4	0.077	0.087	0.058	0.061	0.053	0.116
NPK+S_1-S^{-0}	0.069	0.072	0.047	0.076	0.095	0.120
NPK+S_2-S^{-0}	0.089	0.091	0.062	0.045	0.064	0.093
NPK+S_3-S^{-0}	0.096	0.111	0.039	0.077	0.070	0.119
$LSD_{-0.05}$						
a	n.s.	n.s.	n.s.	n.s.	0.0163	n.s
b	n.s.	n.s.	n.s.	n.s.	0.0230	n.s.
a x *b*	n.s.	n.s.	n.s.	n.s.	0.0326	0.0274

SO^{2-}_4 – sulfate sulfur; S^0– elementary sulfur; S_1 – 40 kg ha^{-1}, S_2– 80 kg ha^{-1}, S_3– 120 kg ha^{-1}, *a* – form of sulfur, *b* – dose of sulfur,
a x *b* interaction,
*n.s. – non-significant difference

Table 3

Effect of different rates and forms of sulfur on the content of cadmium soluble in 1 mol HCl dm^{-3} in soil at 40-80 cm depth (mg Cd kg^{-1} soil)

Treatments	Before experiment	After cabbage harvest	Before onion sowing	After onion harvest	Before barley sowing	After barley harvest
0	0.037	0.047	-	0.070	0.045	0.013
NPK	0.036	0.046	-	0.072	0.127	0.029
NPK+ S_1-SO^{2-}_4	0.038	0.038	-	0.078	0.082	0.033
NPK+ S_2-SO^{2-}_4	0.039	0.042	-	0.063	0.046	0.021
NPK+ S_3-SO^{2-}_4	0.032	0.032	-	0.061	0.113	0.030
NPK+S_1-S^{-0}	0.036	0.028	-	0.076	0.067	0.039
NPK+S_2-S^{-0}	0.035	0.035	-	0.045	0.092	0.017
NPK+S_3-S^{-0}	0.031	0.033	-	0.077	0.079	0.021
$LSD_{-0.05}$						
a	n.s	n.s.		n.s.	n.s.	n.s
b	n.s.	n.s.	-	n.s.	n.s.	n.s.
a x *b*	n.s.	n.s.		n.s.	n.s.	n.s.

Key: cf. Table 2

of sulfate and elemental sulfur fertilization, cadmium concentrations decreased, which could have been due to the cadmium uptake by plants. FÄSSLER et al. (2010A) amended contaminated soil with elemental sulfur and ammonium sulfate to increase heavy metal bioavailability. As expected, elemental sulfur decreased the soil pH and increased its cadmium content. In the long term, sulfur fertilization contributed to an increased cadmium uptake by tobacco plants. Due to its high mobility, cadmium may be easily absorbed by plants (GONDEK 2010). As demonstrated by MCLAUGHLIN et al. (1998), an increase in $Na_2SO^{2-}{}_4$ doses is followed by an increase in the concentrations of active cadmium forms in the soil solution. Such an increase was also noted by KAYSER et al. (2001) after soil amendment with elemental sulfur.

Before the experiment, the content of nickel soluble in 1 mol HCl dm^{-3} in the 0-40 cm soil layer varied from 0.68 to 1.45 mg kg soil (Table 4).

Table 4

Effect of different rates and forms of sulfur on the content of nickel soluble in 1 mol HCl dm^{-3} in soil at 0-40 cm depth (mg Ni kg^{-1} soil)

Treatments	Before experiment	After cabbage harvest	Before onion sowing	After onion harvest	Before barley sowing	After barley harvest
0	1.34	1.44	0.78	0.95	0.80	0.367
NPK	0.89	0.93	1.01	1.15	0.56	0.592
NPK+ S_1-$SO^{2-}{}_4$	0.68	0.68	1.29	1.28	0.55	0.505
NPK+ S_2-$SO^{2-}{}_4$	1.35	1.35	1.13	1.11	0.82	0.556
NPK+ S_3-$SO^{2-}{}_4$	1.45	1.65	0.80	0.89	0.92	0.501
NPK+S_1-S^{-0}	0.83	0.85	1.36	0.93	0.65	0.539
NPK+S_2-S^{-0}	1.09	1.11	0.73	0.99	0.57	0.470
NPK+S_3-S^{-0}	1.00	1.09	1.51	0.97	0.76	0.516
$LSD_{-0.05}$						
a	n.s	n.s.	n.s.	0.1305	n.s.	n.s
b	0.2191	0.2191	0.2460	n.s.	0.0962	0.0974
a x *b*	0.3098	0.3098	0.3479	n.s.	n.s.	n.s

Key: cf. Table 2

In the first year of the study, the nickel content of the 0-40 cm horizon increased with increasing sulfate doses. The experimental factors had no significant influence on changes in nickel concentrations in the 40-80 cm soil layer (Table 5).

In the spring, in the second year of the study, sulfur fertilization at all applied doses contributed to an increase in the soluble nickel content of the 0-40 cm soil layer. Sulfur form had no significant effect on nickel concentra-

Table 5

Effect of different rates and forms of sulfur on the content of nickel soluble in 1 mol HCl dm^{-3} in soil at 40-80 cm depth (mg Ni kg^{-1} soil)

Treatments	Before experiment	After cabbage harvest	Before onion sowing	After onion harvest	Before barley sowing	After barley harvest
0	0.80	0.90	-	0.31	0.39	0.178
NPK	1.13	1.14	-	0.34	0.30	0.413
NPK+ S_1-SO^{2-}_4	1.35	1.35	-	0.64	0.35	0.306
NPK+ S_2-SO^{2-}_4	1.09	1.11	-	0.63	0.17	0.290
NPK+ S_3-SO^{2-}_4	1.01	1.18	-	0.64	0.32	0.265
NPK+S_1-S^{-0}	1.30	1.37	-	0.48	0.29	0.433
NPK+S_2-S^{-0}	0.98	0.99	-	0.71	0.30	0.162
NPK+S_3-S^{-0}	1.01	1.01	-	0.66	0.19	0.178
$LSD_{-0.05}$						
a	n.s	n.s.	-	n.s.	n.s.	n.s
b	n.s.	n.s.		n.s.	n.s.	n.s.
a x *b*	n.s.	n.s.		n.s.	n.s.	n.s.

Key: cf. Table 2

tions in soil. After onion harvest, the application of sulfate sulfur resulted in an increase in nickel concentrations in the 0-40 cm horizon, compared with elemental sulfur. Increasing doses of sulfate sulfur led to a minor decrease in the concentrations of nickel soluble in 1 mol HCl dm^{-3} in soil. Changes in the nickel content of the 40-80 cm soil layer were not affected by sulfur dose or form.

In the third year, before sowing spring barley, the concentrations of nickel soluble in 1 mol HCl dm^{-3} in the 0-40 cm horizon were in most cases lower than in the corresponding treatments in the previous two years. The application of 120 kg ha^{-1} of both sulfur forms led to an increase in the nickel content of soil in comparison with the other sulfur doses. This could have resulted from the drop in the soil pH, which increased the nickel content and bioavailability. Sulfur decreases the pH of soil and increases the solubility, availability and mobility of heavy metals (MARTINEZ et al. 2000, CUI et al. 2004).

Sulfur fertilization generally increased soluble nickel concentrations in the soil, relative to the NPK treatment. In the deeper soil layer, neither the sulfur form nor its dose had a significant effect on changes in nickel concentrations.

At the end of the experiment, the soluble nickel content of the 0-40 cm soil layer ranged from 0.367 to 0.592 mg kg soil. A decrease in soluble nickel levels was observed in comparison with the previous two years. The application of elemental sulfur at a dose of 80 kg led to a decrease in the concentration of nickel soluble in 1 mol HCl dm^{-3} compared with the NPK treatment, probably due to an increased nickel uptake by the tested plants. Our results corroborate the findings of SUMMERS et al. (2003) and HOLAH et al. (2010) in this respect. Sulfur fertilization had no significant influence on changes in soluble nickel concentrations in the 40-80 cm horizon (Table 5). However, a steady decrease in the concentrations of nickel soluble in 1 mol HCl dm^{-3} in soil was noted, compared with the corresponding treatments in the past two years.

CONCLUSIONS

1. Irregular changes in the concentrations of cadmium soluble in 1 mol HCl dm^{-3} were observed in soil fertilized with sulfate and elemental sulfur. The effect of sulfur was noticeable only in the third year of the experiment.

2. Sulfur application contributed to a decrease in the soluble nickel content of soil.

3. The applied sulfur doses had no significant effect on the concentrations of cadmium and nickel soluble in 1 mol HCl dm^{-3} in soil. The analyzed soil can be used for growing high-quality horticultural and agricultural crops safe for human consumption, particularly for infants and children.

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INFLUENCE OF HORSE BREED AND HOUSING SYSTEM ON THE LEVEL OF SELECTED ELEMENTS IN HORSE'S HAIR

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Abstract

The aim of the study was to compare the levels of selected toxic elements in hair of horses of two breeds, held in areas free of industrial pollution. The research was conducted on free range Hucul mares (n=40) and Purebred Arabian mares (n=40) kept in a mixed stable-pasture system. The content of 9 elements (arsenic, barium, cadmium, mercury, germanium, lithium, lead, tin and strontium) was determined in each trial. The hair of Purebred Arabian mares was characterized by significantly higher levels of arsenic, barium, mercury, lead, tin and strontium. The differences were statistically significant. Cadmium and germanium, on the other hand, were significantly higher in the hair of Hucul mares. Lithium was found to be on a similar level in both breeds i.e., 0.150 mg kg^{-1} of dry mass in Hucul horses and 0.142 mg kg^{-1} of dry mass in Purebred Arabian horses. There was a significant positive correlation between the levels of mercury and arsenic observed in the hair of horses of both breeds. Arabian mares displayed a very high correlation between the levels of barium and lead, barium and tin, and between tin and lead.

Keywords: horses, breed, hair, toxic elements.

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POZIOM WYBRANYCH PIERWIASTKÓW W SIERŚCI KONI Z UWZGLĘDNIENIEM RASY I SYSTEMU UTRZYMANIA

Abstrakt

Celem badań było porównanie poziomu wybranych metali toksycznych w sierści koni dwóch ras utrzymywanych na terenach wolnych od zanieczyszczeń przemysłowych. Badaniom poddano klacze rasy huculskiej (n=40) utrzymywane systemem bezstajennym i klacze czystej krwi arabskiej (n=40) utrzymywane systemem stajenno-pastwiskowym. Określono poziom 9 pierwiastków (arsenu, baru, kadmu, germanu, rtęci, litu, ołowiu, cyny, strontu) w każdej z prób. W sierści klaczy czystej krwi arabskiej wykazano znacznie wyższą zawartość arsenu, baru, rtęci, ołowiu, cyny i strontu. Różnice te były statystycznie istotne. Natomiast zawartość kadmu i germanu była wyraźnie wyższa w sierści klaczy huculskich. Na zbliżonym poziomie u obu ras oznaczono lit (0,150 mg kg^{-1} s.m. u koni huculskich i 0,142 mg kg^{-1} s.m. u czystej krwi arabskiej). W sierści koni obu ras stwierdzono istotną dodatnią zależność między poziomem arsenu i rtęci. U klaczy czystej krwi arabskiej wykazano bardzo wysokie korelacje między poziomem baru i ołowiu, baru i cyny, cyny i ołowiu.

Słowa kluczowe: konie, rasa, sierść, pierwiastki toksyczne.

INTRODUCTION

Trace elements are crucial for the proper functioning of all living organisms. Some of them (especially heavy metals), however, can induce metabolic disorders. Both humans and animals derive trace elements from plants, whose mineral composition is associated with the natural abundance of elements in soils or with industrial pollution. The growing public awareness on the hazards of environmental contamination compels monitoring of elements and other toxic substances in air, soil, food and living organisms. Tissues of herbivores can be efficient bio-indicators of current levels of environmental pollution (Maia et al. 2006, Smith et al. 2010).

Several sources imply that hair and fur are as good biological material as blood, plasma, enamel and soft tissues (Asano et al. 2005a, Farmer and Farmer 2000, Vermeulen et al. 2009, Gabryszuk et al. 2010, Kośla et al. 2011). They are characterized by non-invasive sampling as well as ease of transportation and storage. Moreover, the concentration of most trace elements in hair is much higher than in blood (Cizdziel, Gerstenberger 2004). In addition, the natural process of hair growth is a reflection of the ongoing metabolic changes which occur in the body over considerably long periods of time (Combs et al. 1982, Combs 1987).

Assays of the elemental composition of human hair for assessment of the level of minerals in a whole organism have been quite meticulously studied (Miekeley et al. 1998, Chojnacka et al. 2006, Szynkowska et al. 2009, Deshmukh et al. 2010, Gow et al. 2010). It is also claimed that determinations of the mineral content of farm animals' hair could be helpful to assess their nutritional status versus the requirements for certain substances or to determine their toxicity (HINTZ 2000, PALACIOS et al. 2002). Hair may also serve as a reliable source of data which facilitates monitoring of the body's mineral status owing to a relatively long period of deposition of bio-elements as well as its stability in comparison to blood.

Hair and fur are used to control the abuse of animals for therapeutic purposes as well as the application of steroids (DUNNETT, LEES 2003, ANIELSKI et al. 2005, GRATECOS-CUBRASI et al. 2006).

Providing that concentrations of certain elements in hair of horses may be taken as an indication of the need for their supplementation, the impact of such factors as breed, sex, hair colour, exposure to pollution and nutrition cannot be neglected (ASANO et al. 2002, MAIA et al. 2006).

The objective of the research was to compare the levels of selected trace elements in the hair of horses from two breeds kept in different housing systems, in a region free of industrial pollution.

MATERIAL AND METHODS

Animals

The research included 40 mares from each of two herds of horses. All the animals were kept in areas free of industrial pollution and grazed on pastures far from motorways. The Purebred Arabian horses between 3 and 25 years of age came from state-owned stables. All the horses were fed the same rations (3.5 kg oats, 4.0 kg hay, vitamin and mineral supplements), had an unlimited access to pasture and were kept in similar conditions: on pasture during the day and in stables overnight. The Hucul horses were kept on a private farm located in a mountainous area. The mares were aged 4-15 years. They had an unlimited access to grass on pasture and additionally received salt licks. They were kept in a free-range system all year round and were all healthy.

Sampling and mineral analysis

The hair designated for analysis was collected from all horses in early autumn. Hair samples of 500 mg each were obtained from the nape area nearest to the skin. The content level of 9 elements i.e., arsenic, barium, cadmium, germanium, mercury, lithium, lead, tin, and strontium was determined in each sample. Elemental analysis was conducted using an atomic emission spectrometer with inductively coupled plasma (ICP-OES) Optima 5300 DV by Perkin Elmer. The method was based on the readings achieved by atomic emission spectrometry using the optical technique.

Statistical analysis

The results were analyzed statistically, given the mean, standard deviation, and extreme values (minimum – min. and maximum – max.) Univariate analysis of variance ANOVA was performed. The significance of differences between means in herds was calculated using the student's t-distribution test. In order to estimate relationships between the levels of elements, their coefficients of correlation were calculated. The calculations were made using a Statistica 9.0 statistical package.

RESULTS

The average concentrations of the analyzed trace elements in horses' hair are summarized in Table 1. The hair of Purebred Arabian mares was characterized by significantly higher more arsenic, barium, mercury, lead, tin and strontium, and the differences were statistically significant. In contrast, the levels of cadmium and germanium were distinctly higher in hair of Hucul mares. Lithium was found to be on a similar level in both breeds (0.150 mg kg^{-1} of dry mass in Hucul horses and 0.142 mg kg^{-1} of dry mass in Purebred Arabian horses).

Table 1

Content of selected elements (mg kg^{-1} of dry mass) in horses' hair

Elements	Breed								Significance of difference	
	Hucul				Purebred Arabian					
	$\bar{x}$	SD	min	max	$\bar{x}$	SD	min	max	*P* value	
As	0.011	0.002	0.008	0.014	0.070	0.045	0.010	0.200	***	0.0000
Ba	0.144	0.085	0.057	0.431	0.204	0.135	0.060	0.800	*	0.0217
Cd	0.024	0.028	0.004	0.151	0.012	0.022	0.000	0.110	*	0.0354
Ge	0.023	0.007	0.011	0.037	0.015	0.002	0.011	0.020	***	0.0000
Hg	0.050	0.039	0.0004	0.121	0.096	0.009	0.070	0.120	***	0.0000
Li	0.150	0.080	0.054	0.493	0.142	0.159	0.013	0.760	ns	0.7798
Pb	0.081	0.064	0.000	0.229	0.124	0.074	0.048	0.479	**	0.0064
Sn	0.026	0.004	0.002	0.036	0.057	0.018	0.026	0.118	***	0.0000
Sr	2.393	0.958	0.890	5.530	2.775	0.712	1.670	4.690	*	0.0475

Correlation coefficients between concentrations of the elements analyzed in hair of Hucul horses are given in Table 2. Significant positive correlations were found between the levels of arsenic and mercury, arsenic and lithium as well as between arsenic and tin. Similar tendencies were noted

Table 2

Coefficients of correlations between levels of selected elements in Hucul horses

Elements	As	Ba	Cd	Ge	Hg	Li	Pb	Sn	Sr
As	*x*								
Ba	ns	*x*							
Cd	ns	ns	x						
Ge	ns	ns	ns	*x*					
Hg	0.3996* p=0.011	-0.3368* p=0.034	ns	0.3741* p=0.017	*x*				
Li	0.3709* p=0.018	ns	ns	0.4395* ** p=0.005	0.3855* p=0.014	*x*			
Pb	ns	ns	ns	ns	-0.4275* ** p=0.006	ns	*x*		
Sn	0.5922* ** p=0.000	ns	ns	0.3716* p=0.018	0.4900* ** p=0.001	ns	ns	*x*	
Sr	ns	ns	ns	ns	ns	0.4324* ** p=0.005	ns	ns	*x*

*significant at $P \le 0.05$; ** significant at $P \le 0.01$

Table 3

Coefficients of correlations between levels of selected elements Purebred Arabian horses

Elements	As	Ba	Cd	Ge	Hg	Li	Pb	Sn	Sr
As	*x*								
Ba	ns	*x*							
Cd	ns	ns	*x*						
Ge	ns	ns	ns	*x*					
Hg	0.4827* ** p=0.002	ns	ns	ns	*x*				
Li	ns	ns	ns	-0.3783* p=0.016	ns	*x*			
Pb	ns	0.7852* ** p=0.000	ns	ns	ns	ns	*x*		
Sn	ns	0.7809* ** p=0.000	ns	ns	ns	ns	0.7626* ** p=0.000	*x*	
Sr	ns	ns	ns	ns	0.3320* p=0.036	ns	ns	ns	*x*

*significant at $P \le 0.05$; ** significant at $P \le 0.01$

in respect of correlations between concentrations of germanium and mercury, germanium and lithium as well as between germanium and tin. Statistically significant positive correlation was also observed between concentrations of lithium and strontium. Negative correlation was, however, found between concentrations of mercury and barium. A similar tendency was observed between levels of mercury and lead.

The coefficients of correlation between concentrations of trace elements analyzed in the hair of Purebred Arabian horses are summarized in Table 3. Significant positive correlation, similarly to Hucul mares, was confirmed between the levels of arsenic and mercury. Significant positive correlation was also traced between the concentrations of barium and lead, barium and tin, tin and lead as well as between tin and lead, and mercury and strontium. Negative correlation was determined between the concentrations of lithium and germanium in the hair of Purebred Arabian mares.

DISCUSSION

In general, hair of herbivores is considered a better indicator of possible environmental pollution than human hair because these animals feed directly on plants (Nageeb Rashed, Soltan 2005). Animal hair is also considered good biological material for analysis of both essential and toxic elements (Combs 1987, Liu 2003, Asano et al. 2005a, Dobrzański et al. 200, Kaproń et al. 2010). Some authors also claim that elemental analysis of hair can serve as a good indicator of the nutritional status of animals (Hintz 2000), especially herbivorous ones, for which green forage is the basic source of nutrients.

Włostowski et al. (2006) suggest that a high concentration of cadmium in animals may be due to the low pH of soil, which accelerates migration of cadmium ions into plant tissues and therefore affects the quantity of cadmium absorbed by animals. This seems to confirm the results obtained in the tests reported herein. The soil under the pasture grazed by the Hucul horses were kept was characterized by a rather low pH. Madejon et al. (2009) demonstrated higher concentrations of certain elements in green biomass during autumn, which is attributable to a slower growth rate of plants than in spring. Thus, it seems that grazing horses during summer and autumn periods can result in higher levels of certain bio-elements in their hair.

Janiszewska and Cieśla (2002) reported higher concentrations of cadmium and lead, i.e., 0.196 mg kg^{-1} of dry mass, in the hair of half-blood horses during summer compared to 0.024 mg kg^{-1} d.m. for Hucul breed and 0.012 mg kg^{-1} d.m. for Purebred Arabian horses. The reason could be the fact that horses are more exposed to various sources of pollution during these periods. The above authors recomend supplementation of horses' diets with magnesium, zinc or selenium in order to limit the accumulation of potentially toxic cadmium and lead in animals.

Stachurska et al. (2011) obtained small differences in concentrations of heavy metals in hair and hoof horns of the Polish Konik depending on the housing system. The author also revealed that increased levels of minerals in hair, especially cadmium and lead, occurred during the winter feeding periods. In contrast, Kaproń et al. (2010) obtained lead concentrations at the level of 0.22 mg kg^{-1} d.m. for Polish Konik horses kept in the wild during summer. Less lead was found in the hair of horses analyzed during the present study (Table 1).

Gabryszuk et al. (2010), who investigated the levels of minerals in milk and hair of cows kept on conventional and organic farms, showed their relationship with the system of production. Anke et al. (1989) confirmed differences in the levels of cadmium in hair of mares and geldings held in Central Europe. They also noted that the intensity of cadmium accumulation was influenced by the race type, a finding also fact confirmed in our studies.

Asano et al. (2005b), when analyzing concentrations of several elements in hair of horses (using the PIXE method), observed a significant influence of the hair colour. Grey hair contained the lowest concentrations of Br, Ca, Se and Sr. With respect to the animals' gender, more mercury was found in hair of mares, lead was more abundant in hair of geldings while strontium was on a higher level in hair of stallions (Asano et al. 2005a).

Farmer and Farmer (2000) analyzed the content of some elements in tissues of animals held in regions exposed to industrial pollution and reported that the level of lead depended on its concentration in plants. In addition, the concentration of lead in hair was markedly higher than in soft tissues. However, no such dependency was observed for cadmium, which may suggest that its accumulation in the body depended on other factors as well. This seems to be corroborated by the study of Dobrzański et al. (2005) on the content of selected elements in hair of horses living in areas exposed to different levels of industrial pollution. The level of cadmium in the hair of horses from industrial areas was slightly higher, but the difference was not statistically significant. The authors cited above also observed positive correlation between levels of cadmium and lead, whilst in our study, correlation coefficients between these two elements were not significant. Because of the toxicity of lead, cadmium and mercury, concentrations of these metals in tissues of animals are often treated as realistic indicators of environmental pollution. The levels of arsenic, barium, germanium, lithium, tin and strontium in the hair of horses covered by the study show that both the housing system and nutrition may be modifying factors. This conclusion is supported the research conducted by Kabata-Pendias and Pendias (1993). However, the results obtained so far are difficult to interpret because not much research has been conducted on this question.

CONCLUSION

The content of minerals in the hair of domestic animals may vary depending on the breed and dietary factors. Too little information is available to elucidate the effect of these factors on horses. Further research will also be needed to evaluate the effect of a diet, regional differences and the reproductive status of animals.

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CONTENT OF MINERALS, TOTAL PROTEIN AND WET GLUTEN IN GRAIN OF SPRING WHEAT DEPENDING ON CROPPING SYSTEMS

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Abstract

A strict field experiment was established in 1988 at the Uhrusk Experimental Station, belonging to the University of Life Sciences in Lublin, south-eastern Poland. Results presented in this manuscript originate from the years 2006-2009, namely from 5 crop rotations and from a 16-20-year cereal monoculture. In a two-factor experiment, spring wheat of the cultivar Opatka was sown in different cropping systems. The first order factor in the experiment was the cropping system (CS): crop rotation (CR), and monoculture (M), whereas the second order factor were nitrogen doses: 90 kg N ha^{-1}, and 150 kg N ha^{-1}. The study demonstrated that wheat grain collected from CR was characterized by higher content of P, Ca, Fe and Zn compared to the grain harvested from M. Fertilization with 90 kg N ha^{-1} increased significantly the content of P, K, Ca, Fe and Zn in the grain compared to the fertilization with 150 kg N ha^{-1}. The cropping systems (CS) and nitrogen doses were observed to differentiate the content of protein and gluten in the grain. A higher content of protein was determined in the grain originating from M than in the grain harvested from CR. The content of protein and gluten was increased by the fertilization dose of 150 kg N ha^{-1} compared to the dose of 90 kg N ha^{-1}.

Key words: chemical composition, spring wheat, crop rotation, monoculture.

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ZAWARTOŚĆ SKŁADNIKÓW MINERALNYCH, BIAŁKA OGÓŁEM I GLUTENU MOKREGO W ZIARNIE PSZENICY JAREJ W ZALEŻNOŚCI OD SYSTEMU UPRAWY

Abstrakt

Ścisłe doświadczenie polowe założono w 1988 r. w Gospodarstwie Doświadczalnym Uhrusk należącym do Uniwersytetu Pyrodniczego w Lublinie. Prezentowane w pracy wyniki pochodzą z lat 2006-2009, tzn. 5 rotacji płodozmianu i 16-20-letniej monokultury zbożowej. W doświadczeniu 2-czynnikowym wysiewano pszenicę jarą odmiany Opatka w różnych systemach uprawy roślin. Czynnikiem pierwszego rzędu były systemy uprawy roślin: płodozmian i monokultura; czynnikiem drugiego rzędu – dawki azotu: 90 kg N ha^{-1}, 150 kg N ha^{-1}.

Wykazano, że ziarno pszenicy zebrane z płodozmianu zawierało więcej P, Ca, Fe i Zn niż pochodzące z monokultury. Nawożenie 90 kg N ha^{-1} istotnie wpływało na zwiększenie zawartości P, K, Ca, Fe i Zn w ziarnie, w stosunku do 150 kg N ha^{-1}. Systemy uprawy roślin oraz dawki N różnicowały zawartość białka i glutenu w ziarnie. Więcej białka zawierało ziarno pochodzące z monokultury niż płodozmianu. Zawartość białka i glutenu wzrastała pod wpływem dawki 150 kg N ha^{-1}, w stosunku do dawki 90 kg N ha^{-1}.

Słowa kluczowe: skład chemiczny, pszenica jara, płodozmian, monokultura.

INTRODUCTION

The quality and mineral composition of wheat grain depends on genotype, agro-climatic conditions and agrotechnology (Ranhort et al. 1995, Ruibal-Mendieta et al. 2005, Zeb et al. 2006). Khan and Zeb (2007) as well as Chowdhry et al. (1995) demonstrated significant differences in the chemical composition of various cultivars of wheat grain, which were due to different agro-climatic conditions and agrotechnology applied. As reported by Morris et al. (2009), the content of ash in grain was affected to a greater extent by the course of weather during wheat maturation than by its genotype. In the opinion of Araus et al. (1998), the highest concentrations of minerals are typical of grain exposed to draught at the early stage of maturation. In turn, Paris and Gavazzi (1972) as well as Eser et al. (1997) report that the chemical composition of grain is determined by the type of soil and its fertilization. Furthermore, Woźniak and Makarski (2012), and Kraska (2011) noted a higher content of ash in the grain of wheat sown in the ploughless than in the ploughing system. Habitat conditions and cultivar preferences also affect the content of protein and gluten in wheat grain (Morris et al. 2009). In a study by Peltonen and Virtanen (1994), high doses of nitrogen increased the protein content of grain. In turn, Woźniak (2007) demonstrated a higher content of protein and gluten in grain harvested from a crop rotation than from a monoculture.

Considering the above, the aim of this study was to evaluate the effect of cropping system and different doses of nitrogen fertilization on the chemical composition and quality of spring wheat grain.

MATERIAL AND METHODS

The experimental material was grain of spring wheat cv. Opatka from a crop rotation experiment established in 1988 at the Uhrusk Experimental Station belonging to the University of Life Sciences in Lublin, south-eastern Poland. The results presented in this manuscript originate from the years 2006-2009, namely from 5 crop rotations and from a 16-20-year cereal monoculture. The design of a longitudinal experiment is presented in Table 1. The cultivar Opatka belongs to high quality wheats characterized by good milling quality, very good flour strength and high resistance to sprouting.

Table 1

Design of a longitudinal field experiment

Rotation	Period	Crop rotation (CR)	Monoculture (M)
I	1988-1992	P - WTr - Pe - WTr	WTr
II	1992-1996	P - WTr - Pe - WTr	WTr
III	1997-2000	P - STr - Pe - STr	STr
IV	2001-2005	P - WW - Pe - WW	WW
V	2006-2009	Pe - SW - Pe - SB	SW

P – potato, WTr – winter triticale, Pe – peas, STr – spring triticale, WW – winter wheat, SW – spring wheat, SB – spring barley.

The experiment was established on Rendzina soil formed from limestone of light clay, rich in available forms of phosphorus (2.14 mg P kg^{-1}) and potassium (2.37 mg K kg^{-1}), and with pH_{KCl} =7.2. In this soil, the content of inorganic nitrogen (N) was 1.03 g kg^{-1}, whereas that of organic carbon (C) reached 7.60 g kg^{-1}. A two-factor experiment was established with the method of randomized sub-blocks with plots measuring 15 m^2. The first order factor was a cropping system (CS): crop rotation (CR), and monoculture (M), whereas the second order factor were nitrogen doses: 90 kg N ha^{-1}, and 150 kg N ha^{-1}. The dose of 90 kg N ha^{-1} was applied as follows: 50 kg N ha^{-1} before sowing, 20 kg N ha^{-1} at the 22/23 stage (Zadoks et al. 1974), followed by 10 kg N ha^{-1} (32/33 stage) and 10 kg N ha^{-1} (52/53 stage). The fertilization with 150 kg N ha^{-1} was applied in the same terms, with respective broadcasting doses of 70, 30, 30 and 20 kg N ha^{-1}.

The soil tillage for spring wheat was typical of the ploughing system and included pre-winter ploughing in the autumn and cultivation treatments in the spring. Plant protection was the same on all the plots and involved eradication of fungal diseases with Alert 375 SC fungicide (flusilazole + carbendazim) (32/33 stage) and Tilt Plus 400 EC (propiconazol + fenpropidin) (53/54 stage). Weed eradication in wheat crop was performed with the use of Chwastox Trio 540 SL herbicide (MCPA + mecoprop + dicamba) (23/24 stage).

Determinations of the content of mineral components in wheat grain were conducted after dry mineralization of the samples at a temperature of 600°C. The resultant ash was dissolved in 5 ml of 6M HCl, then each sample was filled up to the volume of 50 ml with redistilled water. Measurements were carried out with the method of Atomic Absorption Spectrometry with excitation in an acetylene-air flame in a UNICAM 939 apparatus. Protein and gluten were determined by the near-infrared (NIR) method using an Inframatic 9200 grain analyzer.

The data were analyzed statistically using the analysis of variance (ANOVA), and the means were compared by F-test protected LSD values calculated for $P < 0.05$ and $P < 0.01$. The information on agro-climatic conditions is presented in Table 2.

Table 2

Agro-climatic conditions at the Experimental Station in Uhrusk

Years	Months					Total or mean
	March	April	May	June	July	
Precipitation (mm)						
2006	36.2	32.0	98.8	35.2	47.6	249.8
2007	44.4	24.4	98.8	96.0	156.8	420.4
2008	50.0	49.0	69.0	38.0	117.0	323.0
2009	106.9	27.0	81.5	169.3	42.7	427.4
1963-2010	29.3	40.8	64.2	72.6	79.8	286.7
Air temperature (°C)						
2006	-2.2	8.8	13.5	17.0	21.5	11.7
2007	5.7	8.2	14.9	18.5	19.2	13.3
2008	3.8	8.3	13.7	17.9	18.2	12.4
2009	1.0	10.0	13.1	16.4	20.0	12.1
1963-2010	1.2	7.8	13.6	16.7	18.4	11.5

RESULTS

Contents of minerals in wheat grain were differentiated by nitrogen fertilization (Table 3). The dose of 150 kg N ha^{-1} increased significantly ash content of the grain (by 0.146 g kg^{-1} d.m.), compared to the dose of 90 kg N ha^{-1}. The content of phosphorus (P) in grain was found to depend on the cropping system (CS) and nitrogen (N) doses. The grain harvested from CR was characterized by a higher content of P (by 0.102 g kg^{-1} d.m.) than the

Table 3

Mineral composition of spring wheat grain (mean of 2006-2009)

Variable	Total ash (g kg^{-1} d.m.)	P (g kg^{-1} d.m.)	K (g kg^{-1} d.m.)	Mg (g kg^{-1} d.m.)	Ca (g kg^{-1} d.m.)	Mn (g kg^{-1} d.m.)	Fe (g kg^{-1} d.m.)	Zn (g kg^{-1} d.m.)
Crop rotation	18.18	2.59	2.82	1.05	0.59	23.69	26.20	19.22
Monoculture	18.25	2.48	3.56	1.06	0.54	24.23	24.68	15.99
90 kg N ha^{-1}	17.54	2.61	3.25	1.07	0.57	23.76	26.11	18.36
150 kg N ha^{-1}	18.88	2.46	3.13	1.04	0.56	24.16	24.77	16.85
LSD values								
Cropping systems	n.s	0.08*	0.23**	n.s.	0.03*	0.38**	0.76**	2.05**
Nitrogen doses	0.40**	0.12**	n.s.	n.s.	n.s.	0.27*	0.51*	1.31*
Cropping systems x nitrogen doses	n.s.	n.s.	0.39*	n.s.	n.s.	n.s.	n.s.	n.s.

n.s.: not significant; $^{*}P < 0.05$ and $^{**}P < 0.01$

grain originating from M. Also the fertilization dose of 90 kg N ha^{-1} increased (by 0.146 g kg^{-1} d.m.) P content of the grain, compared to the dose of 150 kg N ha^{-1}. The CS significantly differentiated also the content of potassium (K). In the grain originating from M, the content of K was higher (by 0.743 g kg^{-1} d.m.) than in that from CR. Also the CS x N interaction was observed to significantly affect K content of the grain. On CR plots the dose of 90 kg N ha^{-1} was increasing the content of K, compared to the dose of 150 kg N ha^{-1}. The content of magnesium (Mg) was at a similar level in CR and M plots and at fertilization doses of 90 and 150 kg N ha^{-1}. Also the CS x N interaction did not affect this parameter. In turn, calcium (Ca) content of the grain was differentiated by the cropping systems (CS). In the grain originating from CR the content of Ca was significantly higher than in the grain from the monoculture. The content of manganese (Mn) in wheat grain was differentiated by both CS and N doses. In the grain of wheat harvested from M, the content of Mn was higher (by 0.537 mg kg^{-1} d.m.) than in the grain from CR. Also the fertilization dose of 150 kg N ha^{-1} was observed to significantly increase Mn content of the grain (by 0.403 mg kg^{-1} d.m.), compared to the dose of 90 kg N ha^{-1}. The content of iron (Fe) in wheat grain depended on both CS and N doses. The grain of wheat harvested from CR was significantly richer in Fe than the grain originating from M. Also the fertilization dose of 90 kg N ha^{-1} had a significant effect on Fe content increase in the grain, when compared to the dose of 150 kg N ha^{-1}. Likewise zinc (Zn) content, which was significantly higher in the grain originating from plots with CR than with M. In turn, the nitrogen dose of 90 kg N ha^{-1} was increasing the content of this element in the grain, unlike the dose of 150 kg N ha^{-1}.

Out of the mineral components of the grain, the greatest variability was noted for K, Fe and Zn (Table 4). The content of K in the grain was characterized by higher variability (VC%) in CR than in M as well as on plots fertilized with the nitrogen dose of 150 kg N ha^{-1} than with 90 kg N ha^{-1}. In the case of Fe, higher VC values were determined in the grain harvested from M than from CR, and from plots fertilized with 90 kg N ha^{-1} than with 150 kg N ha^{-1}. Finally, Zn was characterized by over 2-fold higher value of VC in the grain from M than from CR.

The content of total protein in wheat grain depended on both CS and N doses (Table 5). The grain harvested from the monoculture (M) was characterized by a higher (by 0.9%) content of protein than the grain from crop rotation (CR). Also the fertilization dose of 150 kg N ha^{-1} was increasing protein content of the grain (by 0.5%) compared to the dose of 90 kg N ha^{-1}. In contrast, the content of gluten in the grain was determined only by nitrogen doses. A significantly higher content of gluten was noted in the grain of wheat fertilized with the nitrogen dose of 150 kg N ha^{-1} than with 90 kg N ha^{-1}.

Table 4

Variation coefficients (VC%) of the mineral composition of spring wheat grain

Variable	Total ash	P	K	Mg	Ca	Mn	Fe	Zn
Crop rotation	5.10	3.53	17.73	7.06	4.88	4.74	14.01	11.42
Monoculture	5.08	4.46	14.21	5.54	5.44	6.49	17.98	24.00
90 kg N ha^{-1}	3.90	2.83	18.57	6.72	7.46	6.26	19.74	19.83
150 kg N ha^{-1}	2.71	3.93	21.04	5.54	6.27	5.21	10.57	19.47

Table 5

Content of total protein and wet gluten in spring wheat grain (mean of 2006-2009)

Variable	Total protein		Wet gluten	
	mean (%)	VC (%)	mean (%)	VC (%)
Crop rotation	13.4	3.3	28.3	9.0
Monoculture	14.3	4.7	29.2	7.9
90 kg N ha^{-1}	13.6	5.9	28.5	10.0
150 kg Nha^{-1}	14.1	3.6	30.2	7.2
LSD values				
Cropping systems	0.4**	-	n.s.	-
Nitrogen doses	0.4**	-	1.5*	-
Cropping systems x nitrogen doses	n.s.	-	n.s.	-

*designations as in Table 3; VC – variation coefficient

DISCUSSION

The content of mineral components in wheat grain depended on both the cropping systems (CS) and nitrogen doses. In research by Kraska (2011), the content of ash in wheat grain was determined by soil tillage and skimmed intercrops. Ash content was also affected by cropping systems. Woźniak (2007) showed that grain of winter wheat originating from a monoculture was characterized by a higher ash content than grain harvested from a crop rotation. It may be speculated that this was due to a high contribution of fine grain with the poorly developed endosperm and a low 1000 kernel weight (Woźniak 2007). In the reported study, the content of ash in grain harvested from CR and M was at a similar level. The content of minerals in the grain was also affected by fertilization. The grain from plots fertilized with the nitrogen dose of 150 kg N ha^{-1} was characterized by a higher ash content than the grain from plots fertilized with 90 kg N ha^{-1}. Similar dependencies were demonstrated in studies by JACKOWSKA and BORKOWSKA (2002), PARIS and GAVAZZI (1972) as well as WOŹNIAK and MAKARSKI (2012). The cropping systems (CS) were found to exert a significant effect on the content of minerals in the grain. Higher content of P, Ca, Fe and Zn was assayed in the grain harvested from the crop rotation (CR) system than from the monoculture (M). It may be presumed that this was due to weaker absorption of minerals by plants from soil in the monoculture than in the crop rotation. As reported by COOK (1981) as well as STRUIK and BONCIARELLI (1997), under monoculture conditions the root system of cereals is more poorly developed than under crop rotation, which is linked with infestation of roots and the stem base with fungal diseases. WOŹNIAK and MAKARSKI (2012) demonstrated that the content of minerals in grain was also affected by preceding crops. Wheat harvested from plots after soybean was characterized by a higher content of ash, K and Mn than wheat originating from plots after pea. In contrast, wheat sown after pea had more Ca, Fe and Zn. Also KRASKA (2011) observed changes in the mineral composition of wheat grain after different preceding crops. The grain harvested after red clover was richer in Fe than the grain sown after other plants. In turn, JACKOWSKA and BORKOWSKA (2002) reported that a high content of nitrogen in soil was decreasing the bioavailability of some microelements to plants, and thus was reducing their content in grain. This was confirmed in the present study, where the fertilization dose of 90 kg N ha^{-1} significantly increased the contents of P, K, Ca, Fe and Zn in the grain compared to the dose of 150 kg N ha^{-1}.

The cropping system (CS) and N doses were also found to influence the content of total protein and gluten in the grain. A higher content of protein was determined in the grain harvested from the monoculture than in the grain from the crop rotation, which may be due to poorer grain filling with starch (WOŹNIAK 2007). The fertilization dose of 150 kg N ha^{-1} tended to increase the content of protein and gluten in the grain compared to the dose of 90 kg N ha^{-1}. These results are consistent with findings reported by ESER et al. (1997), JACKOWSKA and BORKOWSKA (2002), PELTONEN and VIRTANEN (1994), as well as WOŹNIAK and MAKARSKI (2012).

CONCLUSION

1. The cropping systems (CS) differentiated significantly the ash content and mineral composition of the grain. Wheat grain collected from the crop rotation (CR) was characterized by a higher content of P, Ca, Fe and Zn compared to grain harvested from the monoculture (M).

2. The applied fertilization with 90 kg N ha^{-1} increased significantly the content of P, K, Ca, Fe and Zn in the grain compared to the fertilization with 150 kg N ha^{-1}.

3. The cropping systems (CS) and nitrogen doses were also observed to differentiate the content of proteins and gluten in the grain. A higher content of protein was determined in the grain originating from M than that from CR. The content of protein and gluten increased under the influence of the fertilization dose of 150 kg N ha^{-1} compared to the dose of 90 kg N ha^{-1}.

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DISTURBANCES IN THE ACID-BASE AND ELECTROLYTE BALANCE AND CHANGES IN SERUM MINERAL CONCENTRATIONS IN CALVES DIAGNOSED WITH NUTRITIONAL MUSCULAR DYSTROPHY

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Abstract

Selenium is an essential nutrient, which is crucial for proper body function. Its role is complemented by vitamin E. Nutritional muscular dystrophy (NMD) is one of the main disorders caused by a selenium deficiency. NMD most often affects calves at the age of 4 to 6 weeks. The study was performed on 40 Holstein-Friesian (HF) calves divided into two groups of 20 animals each. Control group calves were administered an IM injection of selenium and vitamin E on the second day of life. The experimental group comprised calves with symptoms of NMD. Samples of the biceps femoris muscle were collected from six animals in each group for histopathological analyses to confirm changes in muscle parameters. Blood samples were obtained from all animals on three different dates. The following blood parameters were determined in laboratory analyses: pH, pCO_2, pO_2, HCO_3^-, BE, O_2SAT, the concentrations of Na^+, K^+, Cl^-, and Ca and P levels. A drop in pH, an increase in the partial pressure of carbon dioxide, a decrease in the partial pressure of oxygen, a significant decrease in bicarbonate ion concentrations and hemoglobin oxygen saturation as well as a base deficit were reported in the group of calves demonstrating symptoms of NMD. The above changes point to the development of uncompensated metabolic acidosis due to increased levels of pyruvic acid and lactic acid produced as a result of anaerobic processes that accompany muscle fiber degeneration. Minor fluctuations in sodium and chloride levels were observed throughout the experiment, but their concentrations remained within the norm in animal groups. Potassium levels were significantly hi-

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gher in the experimental group than in the control group. The serum concentrations of inorganic phosphorus and calcium were within the reference range in both groups. In calves, NMD leads to disruptions in the acid-base equilibrium and the electrolyte balance, which are manifested by uncompensated metabolic acidosis and hyperkalemia. Significant changes in calcium and phosphorus levels are not observed in the blood serum of calves affected by NMD.

Key words: NMD, calves, acid-base balance, electrolytes, serum mineral concentrations.

ZABURZENIA RÓWNOWAGI KWASOWO-ZASADOWEJ I ELEKTROLITOWEJ ORAZ ZMIANY STĘŻENIA WSKAŹNIKÓW MINERALNYCH SUROWICY W PRZEBIEGU POKARMOWEJ DYSTROFII MIĘŚNI CIELĄT

Abstrakt

Selen jest biopierwiastkiem odgrywającym istotną rolę w prawidłowym funkcjonowaniu ustroju. W organizmie jego działanie jest ściśle powiązane z witaminą E. Jednym z podstawowych zaburzeń powodowanych przez niedobór selenu jest pokarmowa dystrofia mięśni (PDM). Najczęściej na PDM zapadają cielęta w wieku 4-6 tygodni. Badania przeprowadzono na 40 cielętach rasy h-f podzielonych na dwie grupy po 20 zwierząt każda. Cielętom z grupy kontrolnej w 2. dniu życia podano domięśniowo preparat zawierający witaminę E i selen. Grupę doświadczalną, stanowiły cielęta wykazujące objawy pokarmowej dystrofii mięśni. Od 6 cieląt z każdej grupy pobrano bioptaty z mięśnia dwugłowego uda w celu wykonania badania histopatologicznego potwierdzającego zmiany w mięśniach. Od wszystkich cieląt pobrano trzykrotnie krew do badań laboratoryjnych. Oznaczono następujące parametry: pH, pCO_2, pO_2, HCO_3^-, BE, O_2SAT, stężenie Na^+, K^+, Cl^- oraz stężenie Ca i P. W grupie cieląt z objawami pokarmowej dystrofii mięśni wystąpił spadek pH, wzrost ciśnienia parcjalnego dwutlenku węgla, spadek ciśnienia parcjalnego tlenu, statystycznie istotny spadek stężenia jonów wodorowęglanowych i wysycenia hemoglobiny tlenem oraz pojawił się niedobór zasad. Zaobserwowane zmiany mogą sugerować rozwój kwasicy metabolicznej niewyrównanej związanej z wyższym poziomem kwasu pirogronowego i mlekowego powstającego w wyniku procesów beztlenowych towarzyszących rozpadowi włókien mięśniowych. Stężenia sodu i chlorków nieznacznie wahały się w trakcie trwania doświadczenia i pozostawały w granicach wartości referencyjnych, zarówno u cieląt grupy badanej, jak i kontrolnej. Poziom potasu w grupie doświadczalnej był istotnie wyższy w porównaniu z cielętami z grupy kontrolnej. Stężenie wapnia i fosforu nieorganicznego w surowicy cieląt z obu grup utrzymywało się w granicach norm referencyjnych. Pokarmowa dystrofia mięśni cieląt przebiega z zaburzeniami równowagi kwasowo-zasadowej i gospodarki elektrolitowej objawiającymi się kwasicą metaboliczną niewyrównaną i hiperkaliemią. W przebiegu pokarmowej dystrofii mięśni cieląt nie obserwuje się istotnych zmian w stężeniu wapnia i fosforu w surowicy.

Słowa kluczowe: PDM, cielęta, równowaga kwasowo-zasadowa, elektrolity, wskaźniki mineralne.

INTRODUCTION

Selenium is an essential trace element, which is necessary for proper body function. The element participates in various metabolic pathways in the body, and its role is complemented by vitamin E and sulfur-containing amino acids. The first references to the biological role of selenium, a component of glutathione peroxidase, were made in the 1970s. Since then, more than 30 selenium-containing proteins, mostly enzymes, have been identified (GHANY-HEFNAWY, TORTORA-PEREZ 2009). To date, scientists have been successful in describing the detailed biological function of only 10 selenoproteins from the above group. Low levels of selenium and/or vitamin E are the cause of nutritional muscular dystrophy (NMD, *dystrophia musculorum enzootia*). This condition, also known as white muscle disease, induces hyaline degeneration of skeletal muscle cells in various parts of the body, including the diaphragm and the heart muscle. NMD most often affects calves at the age of 4 to 6 weeks.

The acid-base balance is a condition where the optimal concentrations of hydrogen ions are maintained in intercellular and extracellular spaces as a result of various homeostatic control mechanisms in the body. Disturbances in that balance can be a primary source of disease, but they generally accompany other bodily dysfunctions, adding to their complexity and hindering the correct diagnosis and therapy. The diagnosis and treatment of acid-base imbalances requires the determination of pH, pCO_2, HCO_3 concentrations and the partial pressure of oxygen (pO_2). The above parameters are measured in arterial blood or arterialized capillary blood to fully identify the degree of blood oxidation and alveolar gas exchange (STOPYRA et al. 2012). In medical and veterinary practice, the acid-base balance is usually determined in samples of venous blood. This procedure is burdened by error due to hemostasis, which occurs when pressure is applied to the vein, and the activity of skeletal muscles during immobilization, which can produce false acidosis results. The above method also prevents precise determination of the partial pressure of carbon dioxide. Venous blood samples are generally used in evaluations of disorders that are not accompanied by complications (POMIANOWSKI et al. 2004).

Electrolytes are substances which, when dissolved in water, conduct electrical impulses essential for regulating vital bodily functions. They are charged ions, positive cations (sodium, potassium, calcium and magnesium) and negative anions (bicarbonates, chloride and phosphorus ions, sulfates, organic acids and protein components). Electrolytes control water distribution in the body, absorption, diffusion, the acid-base equilibrium, nerve and muscle function. An electrolyte balance is required for the body to perform its complex functions during significant changes in the diet, metabolism and kidney function (ROBERTS 2005).

MATERIAL AND METHODS

The study was performed on 40 HF calves. The control group comprised 20 healthy animals which were administered a single IM injection of selenium and vitamin E comprising tocopherol acetate – 50 mg and sodium selenate – 0.5 mg at a dose of 8 ml/calf on the second day of life. The experimental group consisted of animals demonstrating symptoms of NMD. Blood samples were collected from all animals on three different dates, at seven-day intervals, starting on day 5. Blood was sampled from the external jugular vein in the morning. On day 19, samples of the biceps femoris muscle were collected from six animals in each group to confirm changes in muscle parameters. The place of incision was shaved, disinfected and anesthetized by infiltration with a lignocaine solution. Muscle samples were obtained by scalpel incision of 0.8 x 0.8 cm with a depth of 0.7 cm. Muscle sections were immersed in a saline solution for 10 minutes, neutralized with 10% formalin and embedded in paraffin. Microtome sections were stained with hematoxylin and eosin (HE).

The acid-base balance and electrolyte concentrations in venous blood were determined with ion-selective electrodes using a RapidLab 348 analyzer (Siemens). The following parameters were identified: pH, the partial pressure of carbon dioxide (pCO_2), the partial pressure of oxygen (pO_2), bicarbonate ion concentrations (HCO_3^-), base excess (BE), hemoglobin oxygen saturation (O_2SAT) and the concentrations of Na^+, K^+ and Cl^-.

The content of minerals, calcium and phosphorus was determined using a Cormay Accent 200 automatic biochemical analyzer. Serum calcium levels were determined by the Arsenazo III colorimetric method (Cormay diagnostic kit), and phosphorus concentrations were measured by the colorimetric molybdenum ion test (Cormay diagnostic kit).

The results of laboratory tests were presented in SI units and processed in the Statistica 9.0 application. The significance of differences between test results and groups was determined by ANOVA at $p \leq 0.01$.

RESULTS AND DISCUSSION

Symptoms of NMD were not observed in experimental group calves (not administered the selenium and vitamin E supplement) on the first days of life. Signs of nutritional muscular dystrophy were reported in all experimental animals between day 10 and 17. The initial symptoms were non-specific, and they were manifested by apathy, lethargic behavior, decreased appetite and reluctance to move. They were followed by tremor in hind limbs, incorrect posture with widely spread limbs, a hunched-up spine, stilted gait and, in some cases, an elevated respiratory rate and cough. Several animals remained recumbent and their body temperature exceeded 40°C. Clinical symptoms of NMD were not observed in control group calves.

Numerous muscle fibers with hyaline degeneration of the sarcoplasm, granular degeneration of the sarcoplasm and striatal atrophy were observed in all biceps femoris samples stained by HE (Figure 1). Similar morphological changes in the muscles of ruminants affected by NMD were reported by HAFNER et al. (1996), BEYTUT et al. (2002), SOBIECH (2009) and TUNCA et al. (2009). HE-stained muscle samples from healthy calves showed correctly formed muscle fibers with clear striation.

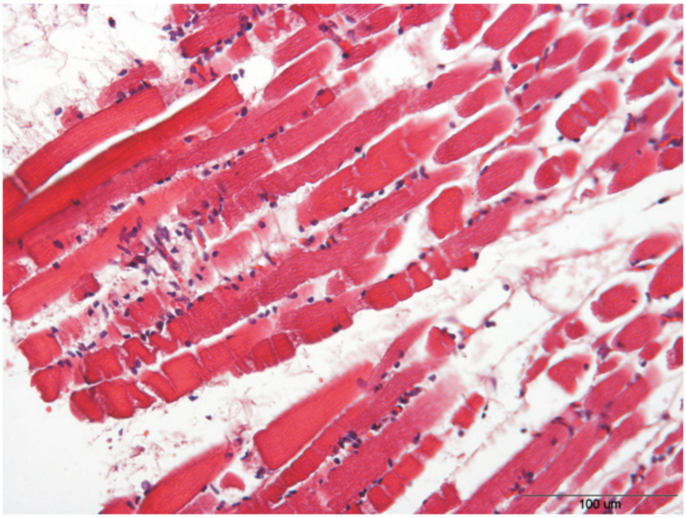

Fig. 1. Muscle of calf with symptoms of NMD. Muscle fibers with sarcoplasma degeneration (long arrow) and disappearance of cross-striation (short arrow) HE staining, magnification x 240

An analysis of the acid-base equilibrium in control and experimental animals did not reveal significant differences in test I (Table 1). The examined parameters were within the reference range. Numerous authors (LISBOA et al. 2002, TAVERNE 2008) have observed symptoms of mixed metabolic and respiratory acidosis in calves in the first hours of life. The described condition is intensified by the type of birth (natural, assisted or C-section) and post-natal vitality. Acidosis is compensated already on the second day postpartum.

A comparison of acid-base balance parameters in experimental and control animals revealed significant differences between groups in tests II and

Table 1

Parameters of blood acid-base balance

Examination	pH		pCO_2 (mmHg)		pO_2 (mmHg)		HCO_3^- (mmol l^{-1})		BE (mmol l^{-1})		O_2SAT %	
	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.
I	7.34 ±0.04	7.35 ±0.06	52.27 ±4.50	52.05 ±5.93	29.74 ±2.14	29.04 ±2.83	30.56 ±2.16	29.37 ±3.60	2.49 ±3.33	3.01 ±2.72	47.12 ±9.42	47.57 ±11.30
II	7.26^{B} ±0.04	7.34 ±0.05	54.17 ±3.11	51.31 ±5.37	28.28^{B} ±2.82	32.29 ±3.42	28.73 ±2.74	29.72 ±4.67	-1.67^{B} ±2.48	3.61 ±1.28	40.76 ±6.90	44.35 ±11.86
III	7.24^{B} ±0.04	7.37 ±0.06	56.67^{B} ±3.52	50.25 ±6.14	26.94^{B} ±2.29	32.70 ±3.81	25.84^{BA} ±3.13	30.05 ±4.52	-2.96^{B} ±1.04	3.80 ±1.69	34.28^{BA} ±5.45	44.01 ±9.21

A – statistically significant difference at $p \leq 0.01$ between examinations
B – statistically significant difference at $p \leq 0.01$ between groups

Table 2

Concentration of electrolytes and minerals in serum

Examination	Na^+ (mmol l^{-1})		K^+ (mmol l^{-1})		Cl^- (mmol l^{-1})		Ca (mmol l^{-1})		P (mmol l^{-1})	
	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.
I	138.20 ±4.35	137.10 ±4.67	4.50 ±0.64	4.79 ±0.48	98.30 ±3.85	100.65 ±4.88	2.75^{A} ±0.20	2.87^{A} ±0.17	2.13 ±0.35	2.18 ±0.49
II	137.35 ±3.87	138.65 ±5.53	5.57^{BA} ±0.63	4.51 ±0.35	99.20 ±3.14	99.35 ±4.53	2.57 ±0.27	2.55 ±0.28	2.12 ±0.41	2.17 ±0.44
III	137.10 ±3.14	136.80 ±4.72	5.87^{BA} ±0.55	4.70 ±0.44	98.45 ±4.36	99.80 ±4.27	2.54 ±0.24	2.54 ±0.35	2.17 ±0.43	2.14 ±0.40

A – statistically significant difference at $p \leq 0.01$ between examinations
B – statistically significant difference at $p \leq 0.01$ between groups

III (Table 1). A drop in pH, an increase in the partial pressure of carbon dioxide, a decrease in the partial pressure of oxygen, a significant decrease in bicarbonate ion concentrations and hemoglobin oxygen saturation as well as a base deficit were reported in calves demonstrating symptoms of NMD. The observed changes point to the development of uncompensated metabolic acidosis due to increased levels of pyruvic acid and lactic acid produced as a result of anaerobic processes that accompany muscle fiber degeneration. Similar results were reported by KATZ et al. (2009) in a case study of a foal with NMD and by SOBIECH, KULETA (2002), who analyzed the acid-base equilibrium in goats affected by NMD.

Minor fluctuations in sodium and chloride levels were observed throughout the experiment, but their concentrations remained within the norm in both groups of animals (Table 2). Our results corroborate the findings of GRZEBUŁA (1989) who did not report changes in the concentrations of the above electrolytes in a study of foals with clinical symptoms of NMD. KOZAT et al. (2009) observed hyponatremia and hypochloremia in a foal with NMD. In the above study, changes in sodium and chloride levels were noted at advanced stages of NMD, which were accompanied by secondary pathological conditions resulting from impaired membrane permeability and the release of electrolytes from damaged muscle fibers. In our study, the absence of significant variations in sodium and chloride concentrations between control and experimental groups indicates that the examined animals were not affected by equally advanced pathological changes.

Tests II and III revealed a significant increase in potassium ion concentrations in the blood serum of experimental calves (Table 2). In this group of animals, potassium levels were significantly higher than in control. The presence of hyperkalemia can be attributed to a close correlation between the concentrations of K^+ ions and pH. An increase in the pH of blood lowers potassium ion levels, whereas a drop in pH leads to higher concentrations of K^+ ions. The above processes rely on the exchange of hydrogen and potassium ions between extracellular and intracellular spaces. This exchange can be induced by a primary increase in hydrogen ion concentrations (lower pH) in extracellular fluid which leads to the transfer of ions into the cell. In line with the law of electrical neutrality of bodily fluids, potassium ions move in the opposite direction, which leads to an increase in their plasma concentrations (HOLTENIUS 1990, DUBOSE 2000). The above phenomenon was observed in diseased cattle, in which progressing metabolic acidosis was accompanied by an increase in potassium ion levels.

The serum concentrations of inorganic phosphorus and calcium remained within the reference range in both groups of animals – Table 2 (EGLI, BLUM 1998, JEZEK et al. 2006). Minor fluctuations in calcium and phosphorus levels were observed, and significantly higher calcium concentrations in the serum of both groups noted in the first test can be attributed to the absorption of calcium from colostrum (STEINHARDT et al. 1993, SOBIECH et al. 2008). PAVLATA

(2001) and KATZ et al. (2009) did not report significant variations in serum levels of calcium and phosphorus in animals affected by NMD, and their findings are consistent with our results. Elevated calcium concentrations in the blood serum of lambs with NMD were noted by KOZAT (2009), who attributed this increase to sarcolemmal damage and the opening of calcium channels. No significant correlation between selenium supplementation and serum mineral concentrations was observed in studies of goats (ORDEN et al. 2000, HAYASHIDA et al. 2003).

CONCLUSIONS

1. Nutritional muscular dystrophy in calves involves disorders of the acid-base equilibrium and the electrolyte balance, which are manifested by uncompensated metabolic acidosis and hyperkalemia.

2. Significant changes in the serum concentrations of calcium and phosphorus are not observed in calves affected by nutritional muscular dystrophy.

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REVIEW PAPERS

THE ROLE OF MAGNESIUM IN CARDIAC ARRHYTHMIAS

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Abstract

Magnesium plays an essential role in many fundamental biological reactions, as it is involved in more than 300 metabolic reactions. Deficiency of magnesium may result in many disorders, including cardiac arrhythmias. As intravenous magnesium has a high therapeutic to toxic ratio and a minimal negative inotropic effect, it has long been used in treatment and prevention of cardiac arrhythmias. Several studies have shown the beneficial effect of intravenous infusion of Mg during an attack of supraventricular tachycardia, indicating that the effectiveness of magnesium depends on the type of tachycardia. Magnesium has also an advantageous effect in the case of atrial fibrillation, considered a criterion of the effectiveness of atrial fibrillation acute treatment at the ventricular rate $\leq$ 100 min^{-1}.

As intravenous magnesium seems to be useful in prevention and treatment of various cardiac arrhythmias, it is a common component of a complex antiarrhythmic therapy.

Key words: magnesium, arrhythmia, supraventricular tachycardia, atrial fibrillation.

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ROLA MAGNEZU W ARYTMIACH SERCA

Abstrakt

Celem pracy była ocena magnezu w arytmiach serca. Magnez odgrywa istotną rolę w wielu podstawowych procesach biologicznych, ponieważ bierze udział w ponad 300 reakcjach metabolicznych. Niedobór magnezu może spowodować wiele zaburzeń, w tym zaburzenia rytmu serca. Ze względu na wysoki stosunek efektywności terapeutycznej do toksyczności, magnez od lat jest stosowany w leczeniu i zapobieganiu arytmiom serca. Liczne badania wykazały korzystny wpływ dożylnej infuzji magnezu podczas ataku częstoskurczu nadkomorowego, wskazując jednocześnie, że skuteczność magnezu zależy od rodzaju częstoskurczu. Magnez ma również korzystny wpływ w przypadku migotania przedsionków, jeżeli za kryterium skuteczności przyjąć uzyskanie rytmu komorowego $\leq$ 100 min^{-1}.

Ze względu na korzystny wpływ magnezu w zapobieganiu i leczeniu różnych zaburzeń rytmu serca, stanowi on częsty element złożonej terapii przeciwarytmicznej.

Słowa kluczowe: magnez, arytmia, częstoskurcz nadkomorowy, migotanie przedsionków.

INTRODUCTION

Magnesium plays an essential role in many fundamental biological processes, as it is involved in more than 300 metabolic reactions. It participates in many enzymatic reactions, binding to a substrate or directly to an enzyme, thus altering its structure. Moreover, magnesium ions play a crucial role in the functioning of many ion channels, including cardiac Mg^{2+}-sensitive K^+ channels, which normally allow K^+ to pass more readily inward than outward. As magnesium regulates the outward K^+ movement, potassium is transported equally well in both directions when Mg^{2+} is absent. Therefore, magnesium deficiency may lead to a reduced amount of intracellular K^+, which disturbs the resting membrane potential of the heart muscle cells and results in cardiac arrhythmias (Rude, Shils 2006).

Cardiac conduction system

Cardiac arrhythmia is caused by a wrong rate or rhythm of the heartbeat (tachycardia: too fast heartbeat; bradycardia: too slow heartbeat), which are controlled by the cardiac conduction system (Figure 1). Each heartbeat begins with an electrical signal generated by the sinoatrial node (SAN), which is the dominant pacemaker site in the heart, located in the lateral right atrium (RA). Electrical impulses are conducted from the SAN to the rest of the RA and left atrium (LA) via specialized interatrial connections including the Bachmann's bundle. The electrical signal then moves downwards to a group of cells called the atrioventricular node (AVN), normally the only electrical connection between the atrium and ventricle. Electrical impulses transmitted through the AVN are conducted rapidly through the His bundle, which branches into a right and a left bundle. The terminal Purkinje fibers connect with the ends of the bundle branches, forming an

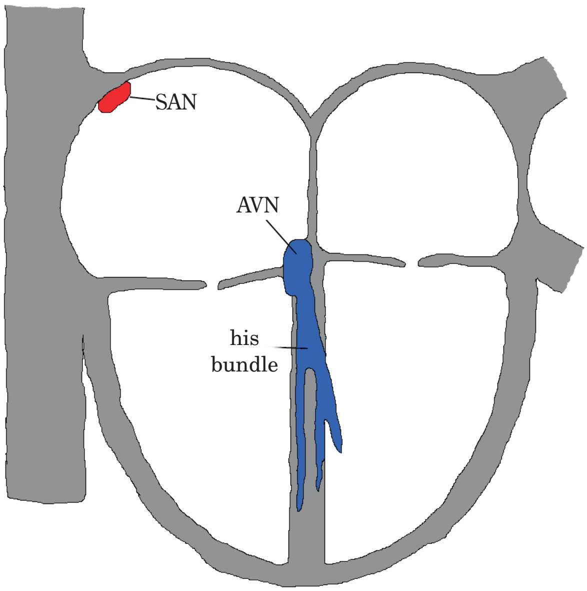

Fig. 1. Key elements of heart conduction system: SAN – sinoatrial node, AVN – atrioventricular node (based on: BENDER et al. 2011, modified)

interweaving network on the endocardial surface so that a cardiac impulse is transmitted almost simultaneously to cardiac muscle cells of both ventricles. Any malfunction during any part of this process can cause arrhythmia http://www.nhlbi.nih.gov/health/health-topics/topics/arr/ (BENDER et al. 2011).

Antiarrhythmic function of magnesium – the mechanism

Mechanisms of the antiarrhythmic function of magnesium are only partially known. As magnesium is a cofactor of the membrane Na-K pump, its deficiency can reduce the pump's activity, leading to partial depolarization and changes in the activity of many potential-dependent membrane channels (ANGUS, ANGUS 2001). Extracellular Mg can also penetrate into another ion channel, reducing the ion movement rate through the channel. Such an action on the L-type calcium channel makes magnesium a physiological calcium antagonist (HESS et al. 1986, WU, LIPSIUS 1990). Magnesium infusion has also been reported to increase significantly the sinus node recovery time (with correction of sinus node recovery time), atrioventricular node (AVN) conduction time during a sinus rhythm, atrial paced cycle length at which an AV node Wenckebach block occurred, AV node relative refractory period, functional refractory period and effective refractory period (DICARLO et al. 1986). As mentioned earlier, magnesium can also regulate the functioning of cardiac K^+ ion channels (RUDE, SHILS 2006).

Magnesium in various arrhythmias

Intravenous magnesium has a high therapeutic to toxic ratio and a minimal negative inotropic effect and has been used for years in treatment and prevention of cardiac arrhythmias. It is commonly believed that magnesium deficiency is an important pathogenetic factor responsible for supraventricular and ventricular arrhythmias. This dependence, however, is a complex one because magnesium is an intracellular ion and a systemic deficiency may coincide with normal magnesium blood levels.

Magnesium produces various electrophysiological effects, hence it has been reported to be efficient in prevention and treatment of various cardiac arrhythmias. Several studies have shown that intravenous infusion of Mg during an attack of supraventricular tachycardia (Figure 2) effectively pro-

Fig. 2. Electrocardiogram of a patient with supraventricular tachycardia

longs the RR interval, slows down the atrioventricular (AV) conduction and restores the sinus rhythm (WESLEY et al. 1981, STILES et al. 2007, SAGER et al. 1990, VISKEN et al. 1992). Invasive electrophysiological studies carried out on patients with paroxysmal supraventricular tachycardia indicate that the effectiveness of magnesium depends on the type of tachycardia. The study by STILES et al. (2007) revealed that intravenous (iv) magnesium extended the tachycardiac cycle to a greater extent in patients with the dual AVN conduction than those without it. This effect was also associated with a longer AH interval in patients with the dual AVN physiology than in ones without it. Besides, the presence of dual AVN conduction was more frequently associated with a reversion to the sinus rhythm. Research has also shown that an attack of recurrent supraventricular tachycardia in AVN could be interrupted by iv magnesium, while recurrent atrioventricular tachycardia was resistant to such therapy (STILES et al. 2007). Considering the above results, it is unsurprising that magnesium is commonly used in cases of supraventricular tachycardia. However, comparison of magnesium to adenosine or verapamil reveals that antiarrhythmic drugs, especially adenosine, are more effective than magnesium in relieving supraventricular tachycardia.

Atrial fibrillation (Figure 3) is a serious clinical problem, especially in elderly patients, because it is often associated with a high risk of thromboembolic complications and increased mortality. Successful electrical cardioversion restoring the sinus rhythm only partially solves the problem due to the high probability of the recurrence of arrhythmia (VOS 2004). As hypomagnesemia is diagnosed in 20-50% of patients with paroxysmal atrial

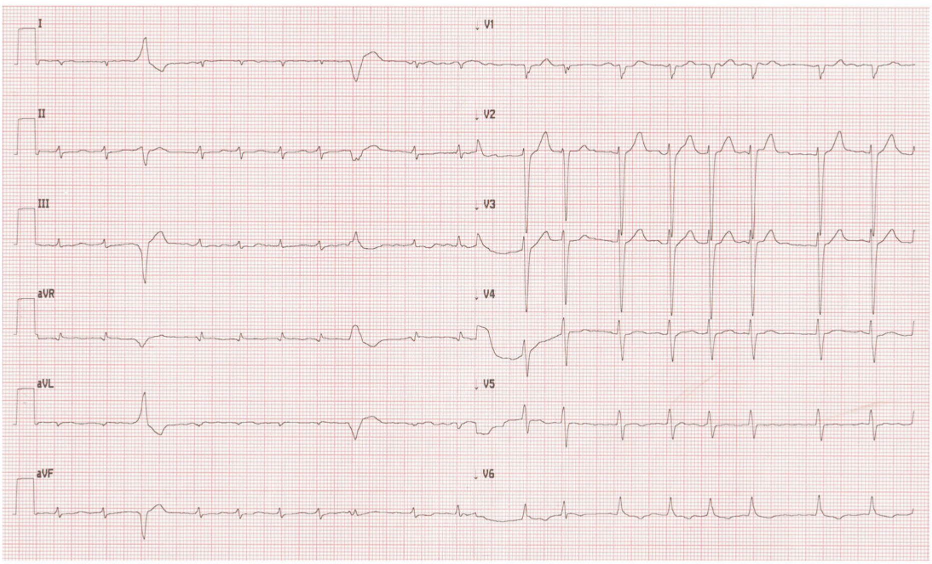

Fig. 3. Electrocardiogram of a patient with atrial fibrillation

fibrillation, supplementation with this cation is often used in the treatment and prevention of the recurrence of arrhythmia (DeCarli et al. 1985, Reinhart et al. 1991). Onalan et al. (2007) carried out a meta-analysis of 12 randomized controlled trials, comparing intravenous magnesium versus placebo or antiarrhythmic agents for the acute management of rapid atrial fibrillation. The analysis revealed that magnesium was effective in achieving the rate or rhythm control. Moreover, time to response was significantly shorter in the magnesium group while the risk of having a major adverse effect in the magnesium group was similar to that in the placebo group. Results of the meta-analysis data suggest that intravenous magnesium administration is an effective and safe strategy for the acute management of rapid atrial fibrillation (Onalan et al. 2007). Ho et al. (2007) carried out another meta-analysis of 10 randomized controlled trials, including a total of 515 patients with paroxysmal atrial fibrillation. The study revealed that intravenous magnesium was ineffective in converting atrial fibrillation to the sinus rhythm when compared to placebo or an alternative antiarrhythmic drug. However, compared to placebo, adding intravenous magnesium to digoxin increased the number of patients with a ventricular response <100 beats min.$^{-1}$ Comparing to calcium antagonists or amiodarone, intravenous magnesium was less effective in reducing the ventricular response but also less likely to induce significant bradycardia or atrioventricular block (Ho et al. 2007).

According to Nair and Morillo, both meta-analyzes show that magnesium does not have advantage over conventional atrioventricular node blockers (calcium antagonists, beta-blockers) and antiarrhythmic agents (amiodaron, class I drugs). However, magnesium is better than placebo, particularly in combination with digoxin, if the ventricular rate $\leq$ 100 min^{-1} is taken as a criterion of the effectiveness of atrial fibrillation acute treatment (Nair, Morillo 2007). It seems that the role of magnesium in atrial fibrillation is secondary and limited to cases in which antiarrhythmic drugs are contraindicated or ineffective.

Magnesium might also be useful in postoperative atrial fibrillation, the most common complication after coronary artery bypass grafting. The ESC guidelines for the management of atrial fibrillation emphasize hypomagnesaemia as an independent risk factor for postoperative atrial fibrillation (Camm et al. 2010). Meta-analysis of seven double-blind, placebo-controlled, randomized clinical trials including 1,028 participants revealed that intravenous magnesium reduced the incidence of postoperative atrial fibrillation by 36%, encouraging the use of intravenous magnesium as an alternative to prevent postoperative atrial fibrillation after coronary artery bypass grafting (Gu et al. 2012). Moreover, it was found that administration of potassium/magnesium solution had a beneficial effect on the success rate of external biphasic electrical cardioversion in patients with persistent atrial fibrillation (Sultan et al. 2012). However, a study by Frick et al., carried out on 170 patients with atrial fibrillation, revealed no influence of oral magnesium on the recurrence rate of atrial fibrillation after elective cardioversion (Frick et al. 2000, Brugada 2000).

The significance of magnesium deficiency in ventricular arrhythmias has not been as thoroughly investigated as in supraventricular arrhythmias, although it was shown that magnesium increases the threshold for both ventricular tachycardia (Figure 4) and ventricular fibrillation (Ghani, Rabah 1977). Based on few and haphazard clinical observations, it is difficult to draw firm conclusions and recommend magnesium therapy. The effects of magnesium and potassium supplementation in stable patients with big (≥ 720/24 h) ventricular arrhythmia were assessed in a randomized, double-blind controlled MAGICA trial. It was found that a 3-week oral treatment with 6 mmol of magnesium and 12 mmol of potassium significantly reduced the incidence of arrhythmia compared to placebo (Zehender et. al. 1997). Despite such promising results, the authors limit the use of Mg/K as a therapeutic option only to non-life-threatening ventricular arrhythmias. The European and both American Cardiac Societies (ACC / AHA / ESC, 2006) recommend the use of Mg and K intravenously in severe cases or orally for chronic supplementation of patients with ventricular arrhythmias and to prevent sudden cardiac death, as such therapy has a beneficial effect on the electrophysiological substrate of ventricular arrhythmia (Zipes et al. 2006). This treatment is especially suitable for counteracting magnesium and/or potassium deficiency and should be considered as a complementary therapy when normal concentrations of electrolytes are diagnosed.

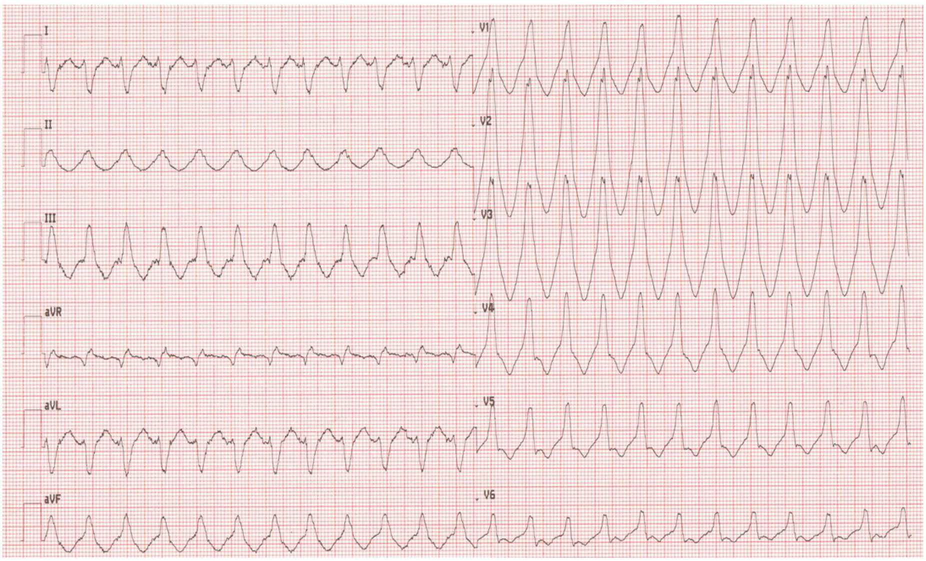

Fig. 4. Electrocardiogram of a patient with ventricular tachycardia

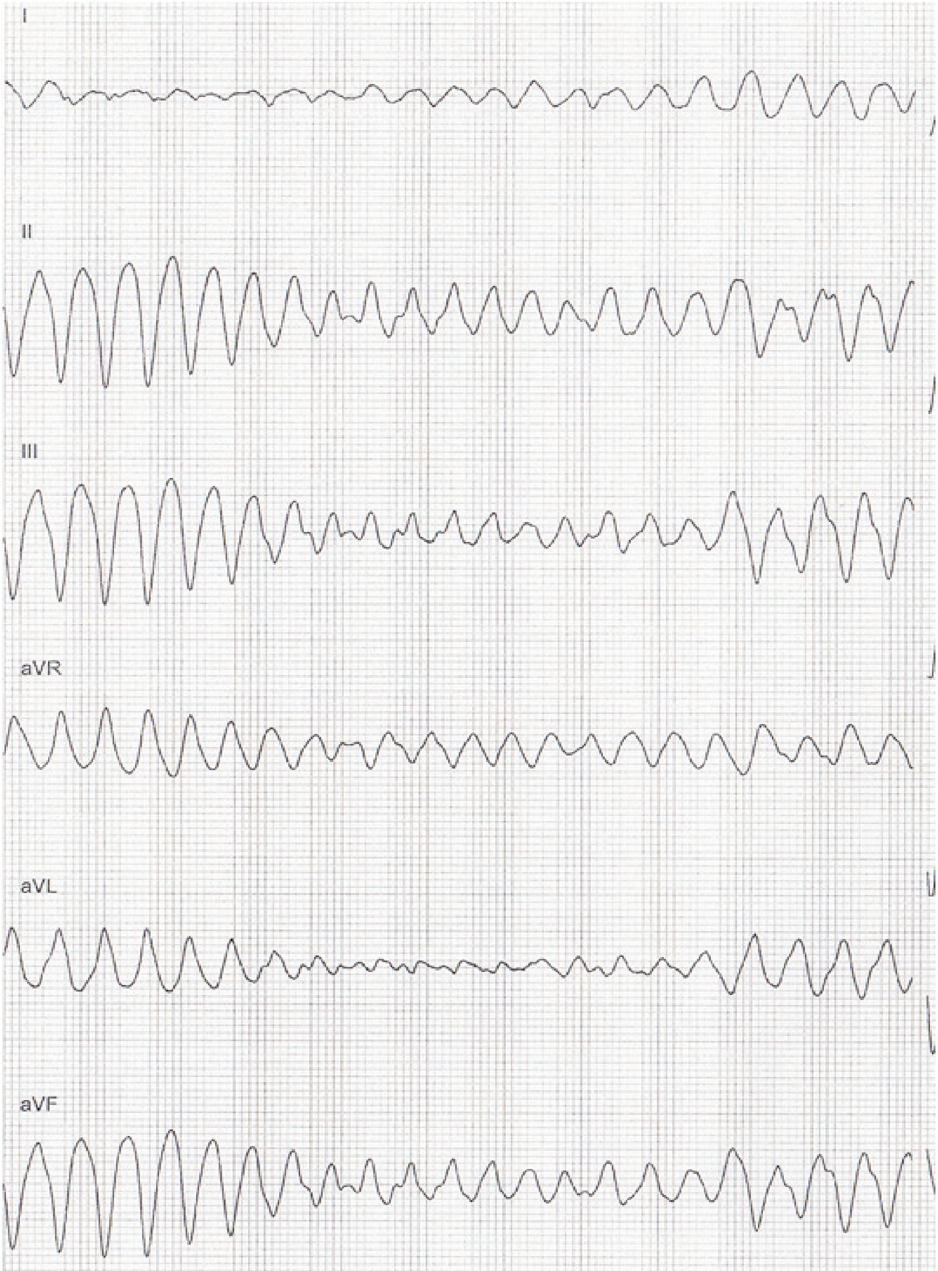

Fig. 5. Electrocardiogram of a patient with polymorphic ventricular tachycardia (torsade de pointes)

Polymorphic ventricular tachycardia (torsade de pointes; Figure 5) is an alternative form of ventricular tachycardia and can occur with or without QT prolongation. In cases with a normal QT interval, mostly induced by heart ischemia, antiarrhythmics (β-blockers, amiodarone) are recommended for therapy. In cases with a prolonged QT interval, the prolongation may be caused by antiarrhythmic drugs used in paroxysmal atrial fibrillation or by the deficiency of electrolytes. Then, administration of an intravenous bolus of magnesium effectively stops an attack of arrhythmia. Therefore, magnesium is recommended for the acute treatment of this form of arrhythmia (class IIa recommendation; ZIPES et al. 2006, HILTON et al. 1992).

CONCLUSIONS

Intravenous magnesium seems to be useful in prevention and treatment of various cardiac arrhythmias, being especially effective in cases of polymorphic ventricular tachycardia (torsade de pointes). Moreover, magnesium therapy is well-tolerated, with sporadic, mild and quickly subsiding adverse effects (heat, flushing, hypotension). Therefore, magnesium is a common component of a complex antiarrhythmic therapy. However, it should be noted that magnesium is not registered as an antiarrhythmic drug and should be treated rather as an adjuvant to antiarrhythmic therapy.

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EFFECTS OF SELENIUM ON ANIMAL HEALTH

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Abstract

Selenium is an essential trace element in the diet of humans and domesticated animals. It is a component of more than 30 selenoproteins, which play a significant role in the body. Selenoproteins protect cells from damage inflicted by free radicals, the cause of many chronic diseases. They also participate in the metabolism of thyroid hormones, control reproductive functions and exert neuroprotective effects. In addition to its anti-proliferative and anti-inflammatory properties, selenium stimulates the immune system. The role of selenium is aided by vitamin E and sulfur-containing amino acids. Selenium deficiency contributes to pathological changes in farm animals, which incur large financial losses each year. Low selenium levels can lead to the development of nutritional muscular dystrophy, also known as white muscle disease, in lambs, kids, foals, calves and poultry from birth to 3 months of age. Selenium deficiency may also cause exudative diathesis in poultry as well as dietary necrotic liver degeneration and mulberry heart disease in pigs. Parturition problems resulting from reduced tension of the muscular layer of the uterus, postparturient paraplegia, placental retention and purulent inflammations of the uterine lining are also attributed to low selenium levels. Selenium deficiency contributes to the formation of ovarian cysts and increased embryonic mortality in the first 3-4 weeks after insemination. Selenium and vitamin E facilitate neutrophil migration to the mammary gland, and they enhance the bactericidal effects of neutrophils, thus shortening and alleviating the symptoms of clinical mastitis. Selenium poisoning is rarely encountered, and it most often results from an overdose of selenium supplements. The most common forms of selenosis are chronic selenosis, referred to as alkali disease, and acute selenosis, popularly known as blind staggers.

Key words: selenium deficiency, selenoproteins, animals.

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SELEN I JEGO WPŁYW NA ZDROWIE ZWIERZĄT

Abstrakt

Selen jest niezbędnym składnikiem diety nie tylko ludzi, ale również zwierząt domowych. Wchodzi w skład ponad 30 białek zwanych selenoproteinami, które pełnią różnorodne funkcje, m.in.: pomagają zapobiegać uszkodzeniu komórek przez wolne rodniki, które uważa się za czynnik etiologiczny rozwoju przewlekłych chorób, uczestniczą w metabolizmie hormonów tarczycy, warunkują prawidłowy przebieg funkcji rozrodczych oraz działają neuroprotekcyjnie. Selen wykazuje działanie antyproliferacyjne i przeciwzapalne oraz stymuluje układ odpornościowy. Działanie tego pierwiastka jest ściśle powiązane z witaminą E i aminokwasami siarkowymi. Niedobór selenu odpowiedzialny jest za powstawanie wielu patologicznych stanów w organizmie zwierząt gospodarskich, co każdego roku generuje znaczne straty ekonomiczne. Schorzeniem powodowanym przez zbyt niski poziom selenu jest pokarmowa dystrofia mięśni (PDM), zwana też chorobą białych mięśni, występująca u jagniąt, koźląt, źrebiąt, cieląt oraz drobiu do 3. miesiąca życia. Do innych chorób uwarunkowanych niedoborem tego biopierwiastka należą: skaza wysiękowa drobiu, pokarmowe zwyrodnienie wątroby i choroba morwowego serca u świń. Niedoborom selenu przypisuje się również wpływ na trudne porody związane ze słabym napięciem mięśniówki macicy, zaleganie poporodowe, zatrzymanie łożyska oraz powstawanie ropnych zapaleń błony śluzowej macicy. Zbyt niski poziom tego pierwiastka może być przyczyną tworzenia się cyst na jajnikach i zwiększonej zamieralności zarodków w czasie 3-4 tygodni od inseminacji. Selen wraz z witaminą E usprawnia migrację neutrofili do gruczołu mlekowego oraz zwiększa ich zdolność do zabijania bakterii, co skraca czas trwania klinicznego zapalenia gruczołu mlekowego oraz łagodzi jego przebieg. Obecnie zatrucie selenem występuje rzadko, głównie jest związane z przedawkowaniem preparatów selenowych. Najczęściej opisywane są dwie postacie selenozy: przewlekła, zwana chorobą alkaliczną, i ostra, zwana ślepą kołowacizną.

Słowa kluczowe: selen, niedobór, selenoproteiny, zwierzęta.

INTRODUCTION

Selenium was discovered in 1818 by the Swedish chemist Berzelius, while he was producing sulfuric acid. The element was named after the Greek moon goddess, Selene (LENZ, LENS 2009). Selenium, sulfur and tellurium belong to group 6 (chalcogens) in the periodic table of the elements. Selenium rarely occurs in its elemental state in nature. It is a trace element with an estimated 0.00008% content of the earth's crust (BEDNAREK, BIK 1994a). Selenium concentrations in soil and the entire food chain vary considerably. In some countries, mainly in China and Brazil, very low (<0.1 mg kg^{-1}) as well as very high selenium soil levels (>0.5 mg Se kg^{-1}) are noted in areas distant only 20 km from one another (DHILLON, DHILLON 2003). In the United States, Ireland and India, the selenium content of soils may be as high as 100 mg Se kg^{-1} (LENZ, LENS 2009). In Poland, the most selenium-deficient areas are the southern parts of the country as well as the regions of Pomerania and Masuria (BEDNAREK, BIK 1994b). Our knowledge about the effects of selenium on living organisms has evolved significantly in the past decades. Perceived mostly as a toxic element in the past, selenium has been recognized as a vital contributor to human and animal health.

SELENOPROTEINS

Selenium is a vital component of various metabolic pathways in animals, and its role is complemented by vitamin E and sulfur-containing amino acids. Selenium is not accumulated in bodily organs or tissues. When ingested, it is incorporated into the functionally important group of selenoproteins, where, in combination with cysteine, a sulfur-containing amino acid, it is present mostly in the form of selenocysteine. The biological role of selenium as a component of glutathione peroxidase (GSH-Px) was first implied in 1973. Since then, more than 30 selenium-containing proteins, mostly enzymes, have been identified (HEFNAWY, TORTORA-PEREZ 2010). To date, scientists have been successful in describing detailed biological functions of only 10 selenoproteins from the above group. GSH-Px is one of the key selenoenzymes that has been characterized in detail. GSH-Px protects hemoglobin and fatty acids from oxidation, and it scavenges free radicals. The substrates for GSH-Px include reduced glutathione and, subject to enzyme specificity, hydrogen peroxide or phospholipid hydroperoxide. Four isoforms of the enzyme have been identified in mammals. They are: classic GSH-Px, also known as cytoplasmic peroxidase, which is found in all bodily tissues, plasma GSH-Px, an extracellular enzyme determined mostly in the kidneys and liver, gastrointestinal GSH-Px, and phospholipid-hydroperoxide GSH-Px which protects cell membrane phospholipids against oxidation and participates in the synthesis of prostaglandins and catecholamines (ARTHUR 2000). The first three GSH-Px isoforms are tetramers, whereas the latter is a monomer.

Iodothyronine deiodinases are also an important group of selenoenzymes which regulate the conversion of thyroxine (T_4) into 3,5,3'-triiodothyronine (T_3), a metabolically active thyroid hormone, or into reverse 3,5',3'-triiodothyronine (rT_3), an inactive hormone. Type 1 5'-iodotyronine deiodinase is present in the liver and kidneys, which, like the thyroid gland, produce hormone T_3. Its presence was also noted in the brain and in pituitary glands in ruminants. Type 2 5'-iodotyronine deiodinase is present in the brain and pituitary glands of all animal species. It catalyzes the transformation of T4 into T3 in tissues that are unable to capture the circulating T3. Type 3 5'-iodotyronine deiodinase converts T4 into rT3, and T3 – into diiodothyronine. It has been determined in the brain, skin and placenta, and it is responsible for inactivating thyroid hormones (ARTHUR 1997, CHADIO et al. 2006). Selenium deficiency can obstruct the above conversion process, which suggests that low levels of the investigated element can affect thyroid functions (HESS, ZIMMERMANN 2004).

Thioredoxin reductase is an enzyme and a selenoprotein that is found in all mammals. In the presence of electrons taken from NADPH, it catalyzes the reduction of oxidized thioredoxin and transfers the redox capacity of thioredoxin to cell proteins (LU et al. 2009). Our knowledge of the physiological functions of thioredoxin reductase continues to grow. Thioredoxins can donate electrons to redox enzymes, including ribonucleotide reductase and thioredoxin peroxidase. The discussed enzyme probably participates in DNA transcription and binding. Thioredoxins also act as growth factors, apoptosis inhibitors and hydroperoxidase reducers (ARNER, HOLMGREN 2000).

Selenoprotein P also plays an important role in the body. Selenoprotein P and plasma GSH-Px are the only identified plasma selenoproteins. The presence of selenoprotein P has been determined in the blood, liver, heart, kidneys and testes (BROWN, ARTHUR 2001). Until recently, researchers were of the opinion that selenoprotein P is an antioxidant responsible for selenium transport. The above observations were formulated based on high selenium concentrations in the discussed protein and its extracellular location. The proposed role of selenoprotein P is often questioned (DANIELS 1996). Selenoprotein P binds heparin proteoglycans in cells and the intercellular matrix, and it is also capable of binding metal ions. For this reason, selenoprotein P could also protect endothelial cells against oxidants (PERSSON-MOSCHOS 2000). Selenoprotein P concentrations decrease less rapidly in a selenium deficiency compared with GSH-Px (HILL et al. 1996).

Selenoprotein W, first isolated from rat muscle tissue, is one of the most recently identified selenoproteins (WHANGER 2000). It was determined mostly in muscles, spleen, testes, heart and brain. Its functions have not yet been fully investigated, but it has been suggested that selenoprotein W participates in muscle differentiation and development by protecting myoblasts against oxidative stress (LOFLIN et al. 2006).

The protein found in sperm mitochondria is a specific selenoprotein that determines the integrity of sperm tails. In selenium deficiency, its concentrations decrease significantly, sperm motility is weakened and spermatogenesis is impaired (URSINI et al. 1999, PFEIFER et al. 2001).

The group of selenium-containing proteins is also inclusive of selenoprotein K, an antioxidant in cardiomyocytes (LU et al. 2006), selenoproteins M and H which demonstrate neuroprotective activity (ZHANG et al. 2010), selenoprotein N which promotes muscle function (LESCURE et al. 2009), selenoprotein S whose deficiency could contribute to colorectal cancer in humans (SUTHERLAND et al. 2010), as well as proteins with less known functions, including selenoproteins T, O and I (LOPEZ-HERAS et al. 2011).

EFFECT OF SELENIUM ON THE IMMUNE SYSTEM

Selenium affects the immune system, and selenium compounds influence humoral immunity mechanisms and increase the levels of type M immunoglobulins (MAGGINI et al. 2007). Selenium supplementation of animal feed increases antibody levels, enhances the phagocytic activity of neutrophil granulocytes and macrophages and, when stimulated with myogens, increases T lymphocyte counts (HOFFMAN 2007, KAMADA et al. 2007). Selenium is indispensable in the production of the lymphocyte migration inhibition factor and interleukin 2 (WINTERGERST et al. 2007), which accelerates the proliferation, maturation and activity of T lymphocytes (SHRIMALI et al. 2008). T cells are particularly sensitive to Se deficiency because their cell membrane contains lipids that are more readily oxidized than the membrane lipids of B lymphocytes (ARTHUR et al. 2003). Selenium deficiency lowers the count and cytotoxic activity of T lymphocytes, an effect which is accompanied by decreased lymphotoxin production (HAWKES et al. 2001). A study on cows (CAO et al. 1992) has demonstrated significantly lower levels of lymphocyte proliferation (stimulated with concavalin A) in animals suffering from selenium deficiency than in the control group. In cows whose immunity was impaired due to a decrease in T lymphocyte counts, the administration of selenium supplements had an immunostimulating effect. Selenium supplementation intensified blastic transformation of splenic lymphocytes and prevented a decrease in lymphocyte proliferation (GHANY-HEFNAWY, TORTORA-PEREZ 2010).

The molecular mechanisms involved in the effect of selenium on the immune system have not been fully elucidated. Selenium could exert its effect aided by selenoenzymes – glutathione peroxidase and thioredoxin reductase. Those selenoenzymes are responsible for maintaining thiol groups on the surface of lymphocyte membranes in a reduced state, which significantly enhances the lymphocyte proliferative response to myogens, increases immunoglobulin production and boosts the killing activity of lymphocytes (AKYOL et al. 2007). Selenates increase the levels of reduced glutathione inside cells and maintain thiol groups in a reduced state (QIN et al. 2007). Selenium deficiency can aggravate an inflammatory process in the body, depressing the activity of selenoenzymes, which inhibit excessive synthesis of arachidic acid from linoleic acid (REINHARD et al. 2007). At normal concentrations of selenium, GSH-Px inhibits phospholipase A_2 and lowers the levels of arachidonic acid and its metabolites, the products for eicosanoid synthesis, in particular leukotriene B4. The synthesis of prostacyclin from arachidonate is intensified, and it inhibits the lipoxygenase metabolic pathway (JOHNSON et al. 2000). The effect of selenium on the immune system could also be produced via a different pathway. The use of sodium selenate as an immunostimulator influences the expression of α and β subunits of interleukin 2 on the surface of activated T and B lymphocytes, natural killer

(NK) cells and lymphokine-activated killer (LAK) cells, but it does not affect the endogenous concentrations of interleukin 1 (IL-1), interleukin 2 (IL-2) or interferon γ (IFN-γ). By binding to IL-2 receptors, IL-1 is internalized, and it induces the signal for the transition of activated cells from phase G1 (postmitotic) to phase S (DNA synthesis) of the cell cycle (JOZSEF, FILEP 2003).

Selenium deficiency inhibits neutrophil migration and disrupts the distribution of receptors on the neutrophil surface. The above can most probably be attributed to the oxidation of tubulin by excess H_2O_2 and the resulting damage to neutrophil microtubules (HADDAD et al. 2002). Neutrophils sampled from selenium-deficient animals were also characterized by impaired ability to produce and release free radicals for the extermination of foreign cells (YANG et al. 2004). Interestingly, high selenium doses also attenuate the immune response. In an *in vivo* study, excessive selenium concentrations inhibited the growth of cells in S and G2 phases of the cell cycle and decreased the synthesis of antibody proteins and prostaglandins (ZAGRODZKI 2004).

SELENIUM AND CANCER

Selenium is a key trace element with anti-neoplastic properties. Decreased Se blood concentration are often observed in cancer patients. The incidence of neoplastic diseases is significantly elevated in areas with low selenium concentrations in the soil (SANZ ALAEJOS et al. 2000). Several mechanisms of the anti-neoplastic action of selenium have been described. One of them is related to its antioxidant effect, namely the redox-dependent modulation of transcription factor functions which inhibits cell growth (SPYROU et al. 1995). Subject to the applied dose, selenium has a stimulatory or an inhibitory effect on the growth of animal tumors which are sensitive to the cytotoxic action of NK cells (KOLLER et al. 1986). Selenium also stimulates the production of anti-neoplastic metabolites; it inhibits angiogenesis and induces the apoptosis of cancer cells (COMBS, GRAY 1998).

SELENIUM TOXICITY

Selenium is a bioelement that plays key physiological functions, but the difference between what is considered an adequate dose of selenium and a toxic one is relatively small. Toxic effect of selenium on animals was first reported in the 1930s in South Dakota, where animals grazed on pastures rich in selenium and developed alkali disease and blind staggers due to acute or chronic selenium poisoning. The first mention about selenium toxicity was made by Marco Polo during his journey to China in 1295. He described symptoms of hoof rot disease in horses, which is contemporarily known as selenosis (TINGGI 2003). Selenosis or alkali disease affects horses, cattle, pigs and poultry. It is caused by high selenium concentrations in the soil, and by the consumption of plants growing in areas with elevated levels of alkaline compounds. In such environments, plants easily accumulate readily soluble and available selenium compounds. Plants that easily absorb selenium from the soil, thus posing a potential risk to grazing animals, include members of the families Leguminosae, Cruciferae and Compositae. They are referred to as selenophilic, selenium-bearing or selenium indicator plants. Plants which can absorb moderate or relatively high amounts of selenium without adverse consequences to consumers include wheat, barley, oats and maize (BEDNAREK, BIK 1994a). In animals, selenosis is observed in two clinical forms: chronic, known as alkali disease, and acute, referred to as blind staggers. Chronic selenosis leads to the loss of vitality, weight loss, hair loss, rough hair coat, hoof deformations, hoof necrosis, joint stiffness, myocardial atrophy, cirrhosis and anemia. In cattle, chronic selenosis lowers fertility by supporting the growth of ovarian cysts and prolonging anoestrus. Acute forms of selenosis are rarely diagnosed, and they result mainly from selenium overdose. In most cases, the disease affects the central nervous system, and the most common symptoms are dementia, unsteady gait, grinding of the teeth, salivation, colic and loss of vision (KOLLER, EXON 1986). The last stage of the disease is manifested by dyspnea and limb paralysis, and death usually results from respiratory failure (TINGGI 2003). Selenium is readily transferable through the placenta and it is secreted to milk, which is why symptoms of selenosis may be observed already in suckling animals (GUYOT et al. 2007). A single selenium dose of 1-6 mg kg^{-1} BW is lethal for most animal species (HOGUE 1970, WHANGER et al. 1996). Feed with a selenium content higher than 20-30 ppm leads to acute selenosis, and doses below 3-5 ppm cause chronic and subacute selenium poisoning (PANTER, JAMES 1990, NUTTAL 2006).

SELENIUM DEFICIENCY

In 1957, Schwarz and Foltz demonstrated that selenium plays a positive role in animal health. They noted that selenium supplementation of diets prevented liver necrosis in rats (SCHWARTZ, FOLTZ 1957). The selenium content of animal feed reflects concentrations of this trace element in soils on which crops are grown for green fodder, as well as the level of selenium availability to plants. Selenium availability is determined by the amount and type of selenium compounds present in the soil, plant species, climatic conditions, soil pH and the content of selenium antagonists, such as arsenic, sulfur and lead. Diseases caused by deficiency of selenium occur mostly in areas where soils are acidic and characterized by selenium deficiency or excess levels of selenium in the form of poorly available compounds, such as selenium sulfide. Higher disease rates are reported after cold and wet summers, and in areas intensively fertilized with superphosphate and sulfur (BEDNAREK, BIK 1994b). In metabolic patterns, marked differences in selenium distribution are reported, subject to dietary levels of this trace element. In selenium-deficient diets, this element is first incorporated into specific proteins with vital bodily functions (selenoprotein P, thyronine 5'-deiodinase), and successive amounts of ingested selenium are combined with non-specific proteins. Variations are also noted in tissue distribution of selenium. The brain, endocrine glands and reproductive organs are priority pathways of selenium absorption, before the liver, heart, skeletal muscles and erythrocytes, which explains why the latter organs are more susceptible to deficiency of selenium (FLORIAŃCZYK 1999). Selenium deficiency contributes to pathological changes in farm animals, which incur vast financial losses each year. Although selenium deficiency affects all animal species, ruminants, mostly lambs and goats, seem to be particularly susceptible. The adverse effects of selenium deficiency on animals were first documented in 1957, when low levels of selenium and vitamin E were recognized as a potential cause of nutritional muscular dystrophy (NMD) (MUTH et al. 1958). This condition, also known as white muscle disease, affects lambs, kids, foals, calves and poultry from birth to 3 months of age. NMD induces hyaline degeneration of skeletal muscle cells in various parts of the body, including the diaphragm, cardiac muscle and tongue (BEYTUT et al. 2002). The disease exists in two forms: acute, which affects the cardiac muscle, and subacute, which impairs mostly skeletal muscles. The clinical symptoms of acute NMD include tachycardia, arrhythmia, dyspnea at rest and cyanosis. In 60% of cases, acute NMD leads to sudden death. Chronic NMD is the most common form of the disease, and the affected animals have difficulty in standing up and maintaining a standing position. Changes in tongue muscles prevent suckling and swallowing, leading to milk discharge through the nostrils. Young animals affected by hyposelenosis are more susceptible to respiratory and gastric infections. Lower body gains are frequently reported (ALEMAN 2008). In adult individuals, selenium deficiency impairs fertility, contributes to the formation of ovarian cysts and increased embryonic mortality in the first 3-4 weeks after insemination (ISHII et al. 2002, HEMINGWAY 2003, PALMIERI, SZAREK 2011). Placental retention is one of the most frequently encountered fertility disorders that accompany selenium deficiency. Selenium-dependent GSH-Px protects the placenta which undergoes rapid degeneration after parturition. The enzyme metabolizes peroxides into less biologically active forms to protect cell membranes against the adverse consequences of oxidation that can lead to physical and chemical changes. Neutrophil damage caused by reactive oxygen species is yet another selenium-related cause of placental retention (RUTIGLIANO et al. 2008). Parturition problems resulting from reduced tension of the muscular layer of the uterus, postparturient paraplegia and purulent inflammations of the uterine lining are also attributed to low selenium levels. Selenium and vitamin E facilitate neutrophil migration to the mammary gland and enhance the bactericidal effects of neutrophils, thus shortening and alleviating the symptoms of clinical mastitis (MOEINI et al. 2009). The mastitis-metritis-agalactia syndrome (MMA) in pigs has been found to be closely correlated with selenium deficiency (HOSTETLER, KINCAID 2004). Low selenium levels also contribute to dietary necrotic liver degeneration and mulberry heart disease. Dietary necrotic liver degeneration, also known as toxic liver necrosis, affects mostly young, fast growing and apparently healthy pigs. In young animals, mulberry heart disease may lead to sudden death due to acute heart failure. Numerous spots and smudges (foci of cardiomyocyte degeneration), separated by extravased regions, are observed in the affected heart (SHARP et al. 1970). Low levels of selenium and vitamin E may be a cause of exudative diathesis in poultry. The disease affects mostly chicks aged 3-6 weeks and, less frequently, young turkeys, ducks and quails. It is manifested by subcutaneous edema, mainly in the area of the abdomen, chest and neck. In swollen areas, the skin takes on a purple-red color, and it ultimately turns greenish-blue. The disease leads to loss of appetite, weight loss and massive deaths. In poultry, selenium deficiency decreases egg laying and hatchability, and it inhibits feather growth (KOLLER, EXON 1986, SURAI 2002).

CONCLUSIONS

Selenium has a variety of functions. Owing to the antioxidant properties of glutathione peroxidase, selenium effectively neutralizes free radicals. This trace element is also essential to good health. The biological role of selenium-containing proteins needs to be explored in greater depth to validate positive health effects of selenium. Selenium is a promising element in prevention and treatment of various diseases, including cancer, but further work is needed to confirm the benefits of selenium supplementation in animals and humans.

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