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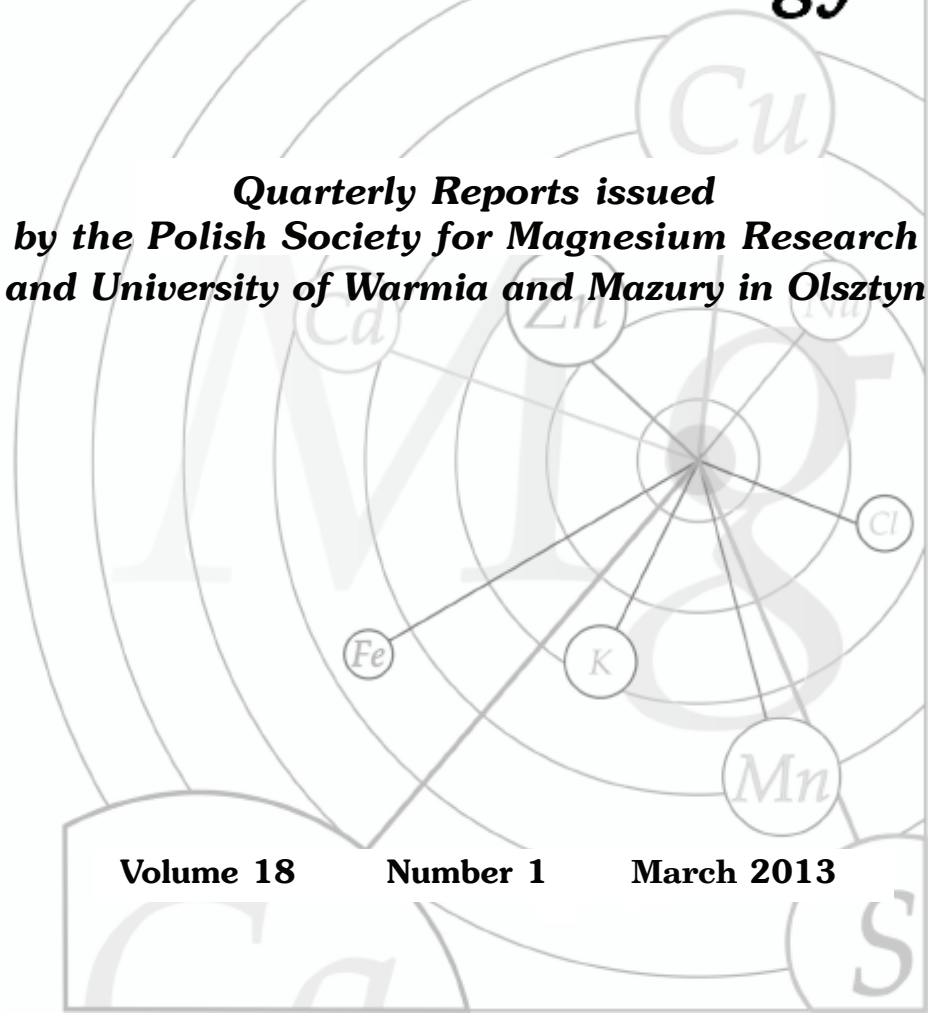

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ORIGINAL PAPERS

SODIUM AS AN ELEMENT INCREASING NITROGEN PRODUCTIVITY – A CASE STUDY ON SUGAR BEET

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Abstract

The objective of the study was to evaluate the effect of sodium-enriched nitrogen fertilizers against the background of pre-sowing sodium fertilization on sugar beet productivity, including technological quality of taproots. A field experiment, completed in 2001-2003, consisted of two main factors: (i) pre-sowing sodium application (0, 30 kg Na ha^{-1} in the form of NaCl), (ii) a set of nitrogen fertilizing variants, composed of two sub-levels: one consisting of four nitrogen rates (0, 90, 120, 150 kg N ha^{-1}) and the other one comprising three chemical N fertilizer forms [(i) ammonium nitrate, 34%, AN, (ii) mixture of ammonium and sodium nitrates, 26%N + 6% Na (ASN1), (iii) mixture of ammonium and sodium nitrates, 21%N + 13% Na (ASN2)]. Depending on a nitrogen rate, the fertilizers were applied on two or three dates. The first N rate was applied only as ammonium nitrate. The in-season application of nitrogen and sodium as the 2nd and the 3rd rate of nitrogen allowed for discrimination of sodium rates, ranging from 0 to 44.2 kg Na ha^{-1}. The effect of soil applied sodium was significant in the 2nd and 3rd year of study. The highest yields of taproots and sugar, despite changeable weather conditions, were harvested on the 120 kg N ha^{-1} treated plot. The response of sugar beet plants to in-season applied sodium was varied and depended on soil available sodium content and the course of weather during the growing season. The strongest response occurred in 2003, characterized by both the lowest amount of available soil sodium and shortage of water. The necessity of sodium application, as a nutritional factor increasing yields of taproots and sugar, was clearly demonstrable under low soil sodium content (< 5 mg kg^{-1} soil). Then, the optimum rate of in-season applied Na in the form of ASN1 ranged from 14.8 to 29.5 kg Na ha^{-1}. The available sodium content, from 10 to 12 mg kg^{-1} soil, defined the upper limit of sodium fertilizer application. At that sodium fertility level, 7.4 kg Na ha^{-1} should not be exceeded. The highest unit N productivity, as attributed to the 90 kg N ha^{-1} treatment, responded positi-

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vely to soil and in-season applied sodium. Therefore, it can be concluded that soil and/or in-season applied sodium can improve productivity of unit nitrogen, provided that a nitrogen rate will be reduced by up to 30 kg N ha^{-1} in comparison to its optimum rate.

Key words: nitrogen rates, sodium nitrate, NaCl, sugar yield, nitrogen productivity.

SÓD JAKO PIERWIASTEK ZWIĘKSZAJACY PRODUKCYJNOŚĆ AZOTU – NA PRZYKŁADZIE BURAKA CUKROWEGO

Abstrakt

Celem badań była ocena wpływu nawozów azotowych wzbogaconych w sód na tle przedsiewnego nawożenia sodem na produkcyjność buraków, włącznie z jakością technologiczną korzeni. Eksperyment polowy (w latach 2001-2003) zawierał dwa czynniki nawozowe: (i) przedsiewne stosowanie sodu (0 i 30 kg Na ha^{-1}, w formie NaCl), (ii) zestaw wariantów azotowych, ujętych w dwa podpoziomy. Pierwszy, wyznaczony przez dawki azotu (0, 90, 120 i 150 kg N ha^{-1}), oraz drugi, wynikający ze składu chemicznego testowanych nawozów [(i) (saletra amonowa, 34% N (AN), (ii) mieszanina saletry amonowej i sodowej, 26% N + 6%Na (ASN1), (iii) mieszanina saletry amonowej i sodowej, 21% N + 13% Na (ASN2)]. W zależności od dawki azotu, nawozy stosowano w dwóch lub trzech terminach. Pierwszą dawkę azotu, przedsiewnie, stosowano tylko w formie saletry amonowej. Nawozy azotowe stosowane w drugiej i trzeciej dawce zawierały również sód. W ten sposób zróżnicowano pogłówne dawki sodu w zakresie od 0 do 44,2 kg Na ha^{-1}. Wpływ doglebowo zastosowanego sodu był istotny w drugim i trzecim roku badań, prowadząc do wzrostu plonu korzeni i cukru. Największy plon korzeni i cukru, niezależnie od warunków pogodowych, otrzymano stosując 120 kg N ha^{-1}. Reakcja buraka cukrowego na pogłówne nawożenie sodem była zmienna, warunkowana dostępnością tego składnika w glebie oraz przebiegiem warunków pogodowych. Największy przyrost plonu korzeni zanotowano w 2003 roku. Stwierdzono wówczas najmniejszą zawartość przyswajalnego sodu w glebie oraz niedobór wody w okresie wegetacji. Konieczność stosowania sodu jako czynnika żywieniowego zwiększającego plony korzeni i cukru ujawniła się jednoznacznie w stanowiskach ubogich w przyswajalny sód (<5 mg Na kg $gleby^{-1}$). W tych warunkach optymalna dawka sodu w formie ASN1 wynosiła od 14,8 do 29,5 kg Na ha^{-1}. Zawartość przyswajalnego sodu w glebie od 10 do 12 mg Na kg^{-1} wyznaczała górny poziom stosowania nawozów sodowych. W tym zakresie wielkość pogłównej dawki sodu nie powinna przekraczać 7,4 kg Na ha^{-1}. W wariancie z 90 kg N ha^{-1}, charakteryzującym się największą produkcyjnością jednostkową azotu, odnotowano wzrost tego wskaźnika w reakcji na doglebowe i pogłówne stosowanie sodu. Można więc stwierdzić, że doglebowe i pogłówne nawożenie buraka cukrowego sodem prowadzi do zwiększenia produkcyjności jednostkowej azotu. Warunkiem koniecznym jest redukcja dawki tego składnika nawet o 30 kg N ha^{-1} w porównaniu z dawką optymalną uzyskaną w warunkach bez nawożenia sodem.

Słowa kluczowe: dawki azotu, saletra sodowa, NaCl, plon cukru, produktywność azotu.

INTRODUCTION

Despite many potential ways to use sugar beet, including production of bio-ethanol or bio-gas, in Europe this crop is predominantly grown to produce sugar (MÄRLANDER et al. 2003, VENTURI, VENTURI 2003). Harvested yields of sugar depend on many natural and agro-technical factors, including weath-

er conditions during the growing season. According to FRECLETON et al. (1999), the weather is responsible for 26% to 79% of yield variability. Drought in summer months (July and August) can be most detrimental to the final yield of beets. Nevertheless, the negative impact of water shortage on the growth of beet plants and sugar yield can be partly compensated for by a highly sophisticated fertilization system, in which required nutrients, their rate and application timing are managed properly (MÄRLANDER et al. 2003).

Potassium and sodium play the most important role in controlling the response of sugar beet plants to water shortage (GRZEBISZ et al. 2002). The effect of sodium on plants' growth and yielding has long been a subject of scientific controversy (RÖMER et al. 2004). The key biological and physiological functions of this element in sugar beet plants are related to its ability to replace potassium in its ordinary functions. A classical example of its physiological impact is regulation of the cell's osmotic potential and turgor (SUBBARAO et al. 2003). Contribution of potassium ions to the cell's total osmotic potential, depending on the plant type, ranges from 53 to 96%. It is well recognized that importance of other nutrients such as calcium, magnesium and especially sodium increases when potassium deficit appears. Plants classified as halophytes compensate high external osmotic pressure by accumulating sodium ions in the vacuole (FLOWERS 1985). It is supposed that this mechanism also works in sugar beet plants. Using data from hydroponics experiments with fodder beets, SUBBARAO et al. (1999) showed that sodium may replace up to 96% of the impact produced by potassium on the cell's osmotic potential. Therefore, sugar beet plants supplied with sodium instead of potassium do not show symptoms of potassium deficiency (WAKEEL et al. 2009). Experiments conducted under water stress have revealed an increased content of sodium in older leaves of sugar beet plants, which elevates the rate of photosynthesis (NIAZI et al. 2000). It is supposed that beet plants under water stress but well supplied with sodium are able to transport potassium to the most physiologically sensitive organs. Therefore, these findings raise a question about the impact of sodium on sugar beet productivity under conditions of water shortage in summer months.

One of the biggest controversies is stirred by the impact of sodium on nitrate transport within the plant. Although potassium is a key nutrient responsible for transport of nitrates in plants, sodium ions can increase their accumulation in sugar beet plants. The same phenomenon has been observed in the case of sulphate and chloride ions (SUBBARAO et al. 2003, HOFFMANN 2005). These findings underline the importance of sodium in controlling nitrogen use efficiency.

Sugar beet fertilization with sodium raises some doubts among agronomists regarding its optimum rates and effect on the quality of taproots and on sugar yield. In many scientific reports and papers, positive effects of sodium are demonstrated (HANSEN 1994, FRECLETON et al. 1999, WAKEEL et al. 2010). Some other reports underline the lack of response or even negative influence on taproot quality (VON BRAUNSCHWEIG 1983, MILFORD et al. 2000, RÖMER et al. 2004).

The key objective of this study was to assess the effect of sodium applied to soil as pre-sowing fertilization and/or in the form of mixtures of ammonium and sodium nitrates on nitrogen use efficiency, considered as a prerequisite of good yields of taproots and sugar.

MATERIAL AND METHODS

Field investigations were conducted during three consecutive seasons: 2001, 2002, 2003, on a farm in Sadki (Poland: 52º08'N; 16º47'E). The experimental design comprised two factors, as follows:

1) soil applied sodium: 0, 30 kg Na ha^{-1} (as NaCl);
2) a set of nitrogen fertilizing variants, composed of two sub-levels: one consisting of four rates of nitrogen fertilization (0, 90, 120 and 150 kg N ha^{-1}) and the other one comprising three types of nitrogen fertilizers: (i) ammonium saltpeter (AN), (ii) mix of ammonium and sodium nitrate, 26% N + 6%Na (ASN1), (iii) mix of ammonium and sodium nitrate, 21% N + 13%Na (ASN2).

The basic rate of sodium was applied two weeks before sugar beet sowing. The first N rate of 60 kg N ha^{-1} in the form of pure ammonium nitrate was applied before sugar beet sowing. The remaining rates of nitrogen fertilizers, enriched with sodium, were applied in accordance to the experimental design, at BBCH 14/16 and BBCH 19/37 stages of sugar beet (according to MEIER et al. 2001). The composition of 10 nitrogen variants was as follows: control – without nitrogen (0), $60_{AN}+30_{AN}$ (AN_{90}); $60_{AN}+30_{ASN1}$ ($ASN1_{90}$); $60_{AN}+30_{ASN2}$ ($ASN2_{90}$); $60_{AN}+60_{AN}$ (AN_{120}); $60_{AN}+60_{ASN1}$ ($ASN1_{120}$); $60_{AN}+60_{ASN2}$ ($ASN2_{120}$); $60_{AN}+60_{AN}+30_{AN}$ (AN_{150}); $60_{AN}+60_{ASN1}+30_{ASN1}$ ($ASN1_{150}$); $60_{AN}+60_{ASN2}+30_{ASN2}$ ($ASN2_{150}$). The rates of sodium applied together with nitrogen were different, depending on the composition of the carrier, i.e., type of nitrogen fertilizer. For ASN1, sodium rates were it 7.4, 14.8 and 22.1 kg Na ha^{-1} and for ASN2 they were 14.8, 29.5 and 44.2 kg Na ha^{-1}.

The area of each treatment, replicated four times, was 54 m^2 (10 m × 12 rows). Field trials were established on fields cropped with spring barley in the preceding year. Post harvest residues of the cereal and white mustard, grown as green manure, were the only sources of the introduced organic matter. Phosphorus and potassium were applied, irrespective of the treatment, in rates of 21 kg P ha^{-1} (single super-phosphate, 19% P_2O_5 i 0,2% B), and 100 kg K ha^{-1} (muriate of potash, 60% K_2O).

The soil under the experiment was typical soil in Poland. It developed from glacial loamy sands, was poor in organic matter but rich in basic macronutrient. Its fertility declined in the order: 2001 > 2002 > 2003. The content of available sodium also decreased in that order. The amount of mineral nitrogen (N_{min} = N-NH_4 + N-NO_3), measured down to 60 cm, was high (Table 1).

Table 1

Physical and chemical properties of soil

Year	Soil layer (m)	Clay (g kg^{-1})	Corg (g kg^{-1})	pH_{KCl}	P*	K*	Mg**	Na**	Nmin*** (kg ha^{-1})
					(mg kg^{-1})				
2001	0.0-0.3	80	9.2	7.4	132	231	40	10.2	98
	0.3- 0.6	140	5.3	7.3	77	196	40	12.0	114
2002	0.0- 0.3	30	8.1	6.9	141	173	53	5.0	65
	0.3- 0.6	110	3.8	6.6	41	107	66	4.2	67
2003	0.0- 0.3	40	6.7	7.0	97	147	45	3.5	96
	0.3- 0.6	110	2.5	6.0	41	97	44	2.5	78

Extracting solution: *lactate buffer, pH 3.55; **0.0125 mol $CaCl_2$; ***0.01 mol $CaCl_2$.

The weather conditions during the study showed high year-to-year variability. The total of precipitations (in mm) from April to October was: 553 in 2001, 633 in 2002 and 412 in 2003. With respect to rainfall, the worst situation occurred in 2003. The total amount of rainfall in August and September 2003 was just 40 mm, while the required amount of water is estimated at 145 mm. In 2001 and 2002, the sum of precipitation in August and September was 150 and 169 mm, respectively, which satisfied the crop's water requirement.

Sugar beet plants were harvested at the technological maturity growth stage (BBCH 49) in the first decade of October from 16.20 m^2 (six rows per 6 m). The technological value of taproots, such as sugar content polarization (S), α-amino-N (α-N), potassium (K) and sodium (Na), were determined using a Venema auto-analyzer (Type IIG). Sugar concentration was determined in extracts (0.3% aluminium sulphate) by using the polarimetric method; K and Na were assayed photometrically and α-N was determined fluorimetrically with o-phthaldehyde (OPA). These basic characteristics of taproots were then used to calculate some technological indices such as (Buchholz 1995):

1) standard molasses loss, SML = 0.12 · (K+Na) + 0.24 · α-N + 1.08 (%);
2) recoverable sugar, RS = S – SML (%);
3) processing efficiency (recovery), PE = (100 · RS) · S^{-1} (%);
4) recoverable (white) sugar yield, $Y_S = (Y_B \cdot RS) \cdot 100^{-1}$ (t ha^{-1});

where: K – content of potassium in mmol 100^{-1} g of fresh taproots; Na – content of sodium in mmol 100^{-1} g of fresh taproots; α-N – content of α-amino-N in mmol 100^{-1} g of fresh taproots; Y_B – beet (taproots) yield in t ha^{-1}; S – polarimetric determined sugar content in beet in %.

The efficiency of N fertilizer was evaluated using two classical parameters:

1) partial factor productivity nitrogen, PFP_N:

$$PFP_N = \frac{YS}{DN} (\text{kg kg}^{-1});$$

Table 2

Effect of sodium chloride fertilization on sugar beet yield

Year	Treatment	Taproots yield (t ha^{-1})	Leaves yield (t ha^{-1})	Taproots/leaves ratio
2001	- Na	64.1^{a}	32.7 ^{a}	0.52 ^{a}
	+ Na	64.4 ^{a}	30.4 ^{a}	0.48 ^{a}
2002	- Na	53.7 ^{a}	23.2 ^{a}	0.43 ^{a}
	+ Na	56.4 ^{b}	25.1 ^{b}	0.45 ^{a}
2003	- Na	51.0 ^{a}	23.9 ^{a}	0.47 ^{a}
	+ Na	56.1 ^{b}	25.0 ^{a}	0.44 ^{a}
Mean	- Na	56.2 ^{a}	26.6 ^{a}	0.47 ^{a}
	+ Na	59.0 ^{b}	26.8 ^{a}	0.46 ^{a}

Means with the same letter are not significantly different at α=0.05 (Tukey's test).

2) agronomic efficiency of fertilizer, AE_N:

$$AE_N = \frac{Y_{S(N)} - Y_{S(0)}}{D_N} (\text{kg kg}^{-1});$$

where: Y_S = white (recoverable) sugar yield (kg ha^{-1}); $Y_{S(N)}$ and $Y_{S(0)}$ – white sugar yield with and without N-fertilization (kg ha^{-1}); D_N – rate of N (kg ha^{-1}).

All sets of collected data were subjected to analysis of variance (Fisher-Snedocor's method) for each year separately and for the interaction between year and experimental treatment, using computer software Statistica 9. In the calculation procedure (analysis of variance), the nitrogen fertilizer rates and chemical composition of tested fertilizers were considered as levels of the same factor. For F-test showing significant differences, Tukey's test (HSD) at the probability level of α = 0.05 was additionally performed to compare mean values. Stepwise variable selection was performed in order to find out relationships between white sugar yield and other parameters.

RESULTS AND DISCUSSION

The three-year average values implicitly indicate that pre-sowing application of sodium in the rate of 30 kg Na ha^{-1} is a measure which increases yields of sugar beets and recoverable sugar. The yield stimulating effect of this nutrient was in fact achieved though an increase in the taproot yield. A significant yield increase was found in two of the three years. In 2002, the

relative increase was about 5% ($p \leq 0.018$), but in 2003 it went up to 10% ($p \leq 0.001$). In the third year, the yield was reduced due to shortage of water in August and September, as mentioned above. Yields of tops and taproot to top ratios did not show any response to soil applied sodium despite the year-to-year weather variability (Table 2). The same trend in the response of sugar beet to sodium application has been observed by HANEKLAUS et al. (1998).

Table 3

Taproot and leaf yields depending on the rate and chemical composition of in-season applied N-fertilizer; means for treatments with and without NaCl fertilization (t ha^{-1})

Treatment	Taproots yield				Leaves yield			
	2001	2002	2003	mean	2001	2002	2003	mean
0	59.5 ^{a}	45.4 ^{a}	51.3 ^{a}	52.1 ^{a}	27.8 ^{a}	18.1 ^{a}	19.5 ^{a}	21.8 ^{a}
AN_{90}	62.5 ^{ab}	54.4 ^{b}	51.9 ^{ab}	56.2 ^{ab}	28.4 ^{a}	24.0 ^{ab}	25.3 ^{abc}	25.9 ^{ab}
$ASN1_{90}$	67.7 ^{ab}	57.3 ^{b}	51.6 ^{ab}	58.9 ^{b}	34.7 ^{a}	27.9 ^{b}	23.0 ^{ab}	28.5 ^{b}
$ASN2_{90}$	65.5 ^{ab}	57.0 ^{b}	54.9 ^{ab}	59.2 ^{b}	31.7 ^{a}	22.0 ^{ab}	25.1 ^{abc}	26.3 ^{ab}
AN_{120}	67.9 ^{b}	52.8 ^{ab}	55.5 ^{ab}	58.8 ^{ab}	32.8 ^{a}	22.9 ^{ab}	23.5 ^{ab}	26.4 ^{ab}
$ASN1_{120}$	64.6 ^{ab}	58.9 ^{b}	57.7 ^{b}	60.4 ^{b}	30.3 ^{a}	27.8 ^{b}	29.8 ^{c}	29.3 ^{b}
$ASN2_{120}$	62.3 ^{ab}	59.6 ^{b}	57.2 ^{ab}	59.7 ^{b}	32.0 ^{a}	25.5 ^{b}	26.7 ^{bc}	28.1 ^{b}
AN_{150}	65.9 ^{ab}	52.3 ^{ab}	51.9 ^{ab}	56.7 ^{ab}	33.6 ^{a}	24.1 ^{ab}	23.5 ^{ab}	27.1 ^{ab}
$ASN1_{150}$	64.4 ^{ab}	56.7 ^{b}	51.0 ^{ab}	57.4 ^{ab}	33.3 ^{a}	25.9 ^{b}	22.3 ^{ab}	27.2 ^{ab}
$ASN2_{150}$	62.4 ^{ab}	55.6 ^{b}	52.5 ^{ab}	56.8 ^{ab}	31.1 ^{a}	23.2 ^{ab}	25.4 ^{abc}	26.6 ^{ab}

Means with the same letter are not significantly different at a=0.05 (Tukey's test).

Positive effect of sodium application on sugar beet yield can be partly explained by differences in the soil available content of both potassium and sodium. In 2001, the content of both nutrients was high, providing good conditions for supply of both nutrients. The content of soil available potassium above 20 mg kg^{-1} soils is a prerequisite for a high rate of sugar beet growth and yielding (WOJCIECHOWSKI et al. 2002). Absence of any response of sugar beet to an available sodium content above 10 mg kg^{-1} soil could be used as an indicator of soil sodium self-sufficiency. Moderate response, as found in 2002, and strong response in 2003 are related to an insufficient content of available soil sodium and to the shortage of water that occurred in 2003. These results are in agreement with those reported by DRYCOTT, DURRANT (1976). There are some reports underlying increasing adaptability of sugar beet plants to prolonged drought, provided that they are well supplied with sodium (BLOCH et al. 2006). Some authors stress that sodium is much more effective in preventing the negative response of sugar beet to water shortage than potassium (DRYCOTT, DURANT 1976, HAMPE, MARSCHNER 1982).

The effect of nitrogen treatments on sugar beet yields was significant in all the three years at $p \leq 0.0447$, $p \leq 0.00014$ and $p \leq 0.022$ in 2001, 2002 and 2003, respectively (Table 3). In the present study, no significant interaction has been found for the NaCl x N-treatments. The assessment of the response of sugar beet to the applied nitrogen fertilizers involves first of all an evaluation of the yields harvested on the control plot, i.e. without any addition of external nitrogen. As presented in Table 1, the general level of soil fertility was high, allowing high efficiency of soil mineral nitrogen (N_{min}), as presented below for the harvested quota of taproots (Y_B):

$$Y_B = 0.176N_{min} + 21.74 \quad \text{for } R^2 = 0.99 \text{ and } n = 3,\ p = 0.001,$$

where: Y_B – taproot yield, t ha^{-1}; N_{min} – mineral nitrogen, kg N ha^{-1} in the soil layer 0-0.6 m.

Therefore, any differences between the control plot and experimental treatments can be explained by the composition of nitrogen fertilizers and weather in each of the growing seasons. In 2001, the highest yield of taproots was harvested from the plot fertilized with 120 kg N ha^{-1} in the form of AN (Table 3). However, in comparison to the control plot, this increase was only 14%. A positive effect but not a significant one was attributed to sodium only when applied in the 90 kg N ha^{-1} treatment. A higher rate of nitrogen caused a slight yield decrease, but there are different explanations. Some authors relate yield decrease to an antagonism between sodium and other cations – calcium, magnesium (WAKEEL et al. 2009). In 2002, the highest yield of taproots was recorded in the plot fertilized with 120 kg N ha^{-1}, but applied as ASN2. The relative yield increase compared to the control plot was high, slightly above 30%. However, the same level of yield was attributed both to all 90 kg N ha^{-1} treatments and to 150 kg N ha^{-1} treatments with sodium. These data clearly indicate that sodium application to soil poor in this nutrient can rise significantly yields of both taproots and tops. In the third year (2003), characterized by excessive water shortage in the late season, the highest yield increase versus the control was attributed to the treatment consisting of 120 kg N ha^{-1} and ASN1 fertilizer. The yield averaged over years verifies the significant impact of sodium applied during the season, irrespective of the year-to-year variability. The highest yield of taproots was recorded in the treatment fertilized with 120 kg N ha^{-1} in the form of ASN1 (Table 3). However, the same level of yield was noted for the 90 kg N ha^{-1} in the form of ASN1 or ASN2 and for the 120 kg N ha^{-1} in the form of ASN2 ($p \leq 0.0011$). It can be concluded that the optimum rate of sodium applied in the season ranges from 14.8 to 29.5 kg Na ha^{-1}. This amount of sodium should be recommended on soil generally poor in sodium. The results suggest that it is necessary to substitute, even partly, some ammonium saltpeter by sodium nitrate (HENKENS 1971). In contrast, on soil reach in sodium, the effect of applied sodium nitrate can be controversial, as pointed out by ALLISON et al. (1994).

This study showed distinctly that the technological quality of taproots depends to a great extent on the course of weather during the growing season and on applied N rates (Table 4). Both factors had the strongest effect on the concentration of sugar and nitrogen compounds (α-N). At the same time, these two taproot quality indicators showed a contrary response to the annually changing weather conditions. Variation in the potassium and sodium concentrations was much lower. Sodium applied to soil did not affect taproot quality, thus indirectly revealing its positive impact on sugar beet growth. A complex qualitative parameter called sugar loss achieved a much higher value in 2002 than in the other two years. Its variation was in accordance with changes in the concentration of α-N compounds (Table 4).

Sophisticated management of nitrogen on a sugar beet plantation should take into account three aspects: (i) N fertilizer rate, (ii) N application tim-

Table 4

Response of sugar beet quality to experimental factors

Year and levels of treatments	Quality parameters					
	polarization S (%)	a-N (mmol kg^{-1})	K (mmol kg^{-1})	Na (mmol kg^{-1})	loss SML (%)	recovery PE (%)
Year						
2001	17.65 ^{a}	23.4 ^{b}	40.2 a	3.5 ^{a}	2.16 ^{a}	87.8 ^{ab}
2002	17.78 ^{a}	24.1 ^{b}	51.9 ^{ab}	5.4 ^{b}	2.33 ^{b}	86.9 ^{a}
2003	20.53 ^{b}	19.0 ^{a}	57.8 ^{b}	4.4 ^{ab}	2.27 ab	88.9 ^{b}
NaCl- fertilization						
- Na	18.56 ^{a}	22.5 ^{a}	49.1 ^{a}	4.4 ^{a}	2.25 ^{a}	87.8 ^{a}
+ Na	18.75 ^{a}	21.9 ^{a}	50.9 ^{a}	4.5 ^{a}	2.26 ^{a}	87.9 ^{a}
Treatments of N-fertilization						
0 (without N)	19.34 ^{c}	15.3 ^{a}	46.6 ^{a}	3.7 ^{a}	2.04 ^{a}	89.4 ^{b}
AN_{90}	18.89 ^{abc}	21.2 ^{ab}	48.4 ^{a}	4.3 ^{a}	2.21 ^{ab}	88.2 ^{ab}
$ASN1_{90}$	18.60 ^{ab}	23.3 ^{ab}	50.8 ^{a}	4.1 ^{a}	2.28 ^{ab}	87.6 ^{a}
$ASN2_{90}$	18.68 ^{abc}	20.7 ^{ab}	50.4 ^{a}	4.7 ^{a}	2.22 ^{ab}	88.0 ^{ab}
AN_{120}	18.82 ^{abc}	19.8 ^{ab}	50.2 ^{a}	3.7 ^{a}	2.19 ^{ab}	88.3 ^{ab}
$ASN1_{120}$	18.60 ^{ab}	21.5 ^{ab}	49.8 ^{a}	4.4 ^{a}	2.23 ^{ab}	88.0 ^{ab}
$ASN2_{120}$	18.58 ^{ab}	22.6 ^{ab}	50.1 ^{a}	5.2 ^{a}	2.27 ^{ab}	87.7 ^{a}
AN_{150}	18.47 ^{ab}	26.8 ^{b}	50.0 ^{a}	4.5 ^{a}	2.36 ^{b}	87.1 ^{a}
$ASN1_{150}$	18.40 ^{ab}	25.7 ^{b}	53.8 ^{a}	5.3 ^{a}	2.39 ^{b}	87.0 ^{a}
$ASN2_{150}$	18.16 ^{a}	24.7 ^{b}	49.8 ^{a}	4.8 ^{a}	2.31 ^{b}	87.2 ^{a}

Means with the same letter are not significantly different at a=0.05 (Tukey's test).

ing during the growing season, (iii) N fertilizer chemical composition. As a rule, all of these factors are important for recoverable sugar yield (MÄRLANDER et al. 2003). This general observation is fully supported by the present study. Nevertheless, the effect of nitrogen on taproot quality was to some extent modified by applied sodium. Some key indicators of beet quality such as sugar and α-N concentration showed a strong but contrary response to increasing N rates. The effect of applied sodium on sugar concentration was generally negative, decreasing in accordance with the increasing rates of both N and Na. Plants fertilized with the highest N rate showed a very high concentration of α-N compounds, which showed a tendency to decrease in response to external supply of sodium (Table 4). Concentration of potassium and sodium, in spite of raising rates of both nitrogen and sodium, did not show any significant changes. However, it has been observed a serious impact of both nitrogen and sodium fertilizers on sodium concentration in taproots. Some qualitative parameters such as loss of sugar and sugar recovery showed a higher response to N than to sodium rates. At the same time, both parameters were inversely related to changes in α-N concentration. The obtained results are not in agreement with a thesis presented by HANEKLUAS et al. (1998), who underline positive aspects of sodium application on sugar beets yield but at the same negative on taproots quality.

Yield of recoverable sugar is considered as a product of yield of taproots and their qualitative characteristics. This study showed that yield of taproots explained 60%, 86% and 92% of sugar yield variability in 2001, 2002, 2003, respectively (Table 5). This simple comparison fully supports a hypothesis proposed by Hanekluas et al. (1998) about the dominating effect of sodium on taproot yield. However, analysis of the influence of these two yield forming components on sugar yield variability showed a much more complicated picture, as presented in Table 5. The two last equations (nos 11 and 12) indirectly indicate importance of sugar beet quality as a factor significantly responsible for both N and Na rates (Table 5).

On average, the highest yield of recoverable sugar, up to 9.95 t ha^{-1}, was harvested in 2001. Statistically, the same yield was recorded in 2003 (9.80 t ha^{-1}). Application of NaCl significantly influenced white sugar yield in 2002 and 2003 (Figure 1). In 2001, the effects of tested N-fertlizers were negligible, but addition of sodium, excluding the 90 kg N ha^{-1} treatment, showed negative impact on the harvested volume of sugar (Figure 2). Nevertheless, the plot fertilized with nitrogen in the form of ASN1 should be considered as interesting for future studies on nitrogen use efficiency in sugar beet. In 2003, characterized by a low content of soil available sodium and unfavorable growth conditions, sugar beet plants fertilized with 120 kg N ha^{-1} and sodium in the rate of 22.1 kg Na ha^{-1} produced the highest yield of sugar. In 2002, yields of sugar were much lower but the highest ones occurred in the treatment of 120 kg N and 14.8 kg Na ha^{-1}. In 2002, the relative sugar yield increase due to sodium application was 7.3%

Table 5

Recoverable sugar yield as a function of taproot yield, YB (t ha^{-1}) and sugar content, S (%) depending on the year and treatment of NaCl fertilization

No	Year	Equation	R^2 value	n
1.	2001	$Y_S = 1.841 + 0.1263\ Y_B$	$R^2 = 0.60^{**}$	$n = 20$
2.		$Y_S = -13.109 + 0.1556\ Y_B + 0.7400\ S$	$R^2 = 0.99^{**}$	$n = 20$
3.	2002	$Y_S = 0.4587 + 0.1460\ Y_B$	$R^2 = 0.86^{**}$	$n = 20$
4.		$Y_S = -12.171 + 0.1566\ Y_B + 0.6778\ S$	$R^2 = 0.99^{**}$	$n = 20$
5.	2003	$Y_S = -0.8850 + 0.1933\ Y_B$	$R^2 = 0.46^{**}$	$n = 20$
6.		$Y_S = -13.264 + 0.1719\ Y_B + 0.6744\ S$	$R^2 = 0.99^{**}$	$n = 20$
7.	mean	$Y_S = 3.2046 + 0.1077\ Y_B$	$R^2 = 0.46^{**}$	$n = 60$
8.		$Y_S = -10.886 + 0.1662\ Y_B + 0.5748\ S$	$R^2 = 0.99^{**}$	$n = 60$
9.	-Na treatments	$Y_S = 2.9766 + 0.1094\ Y_B$	$R^2 = 0.58^{**}$	$n = 30$
10.		$Y_S = -10.7837 + 0.1666\ Y_B + 0.5677\ S$	$R^2 = 0.99^{**}$	$n = 30$
11.	+Na treatments	$Y_S = 3.437 + 0.3339\ S$		$n = 30$
12.		$Y_S = -10.7779 + 0.1634\ Y_B + 0.5780\ S$	$R^2 = 0.99^{**}$	$n = 30$

*, ** – significant level for $p \leq 0.01$ and 0.001, respectively

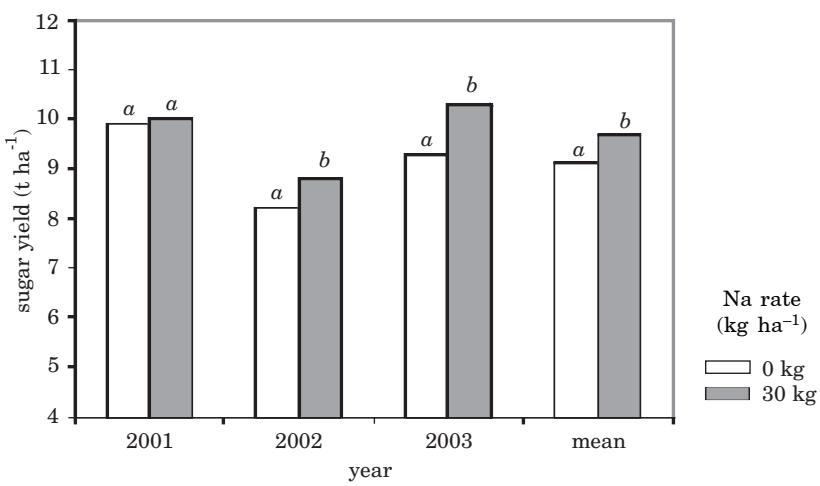

Fig. 1. Effect of NaCl fertilization on recoverable sugar yield (means for N-treatments). Means with the same letter are not significantly different at α=0.05 (Tukey's test)

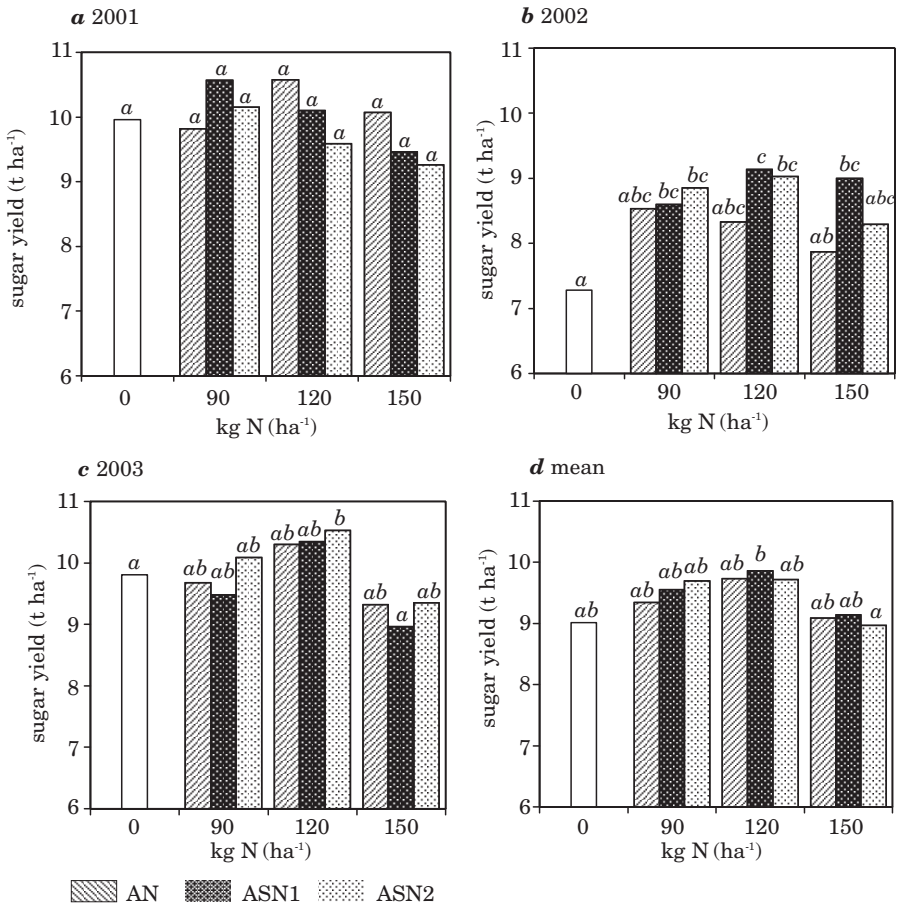

Fig. 2. Effect of nitrogen rate and type of fertilizers on recoverable sugar yield. Means for treatments with and without NaCl fertilization. Means with the same letter are not significantly different at α=0.05 (Tukey's test)

($p \leq 0.0013$), but in 2003 it rose to 10.7% ($p \leq 0.0001$). Yield of sugar, averaged over years and soil applied sodium, raised in accordance to the N rate up to 120 kg N ha^{-1} and Na rate up to 22.1 kg N ha^{-1} (Figure 2). These results suggest indirectly presence of N and Na interaction, which became evident only under relatively low N rates. The essential meaning of the above figures is higher productivity of applied N in the presence of sodium.

The interaction between nitrogen and sodium has been verified by using two indices, partial factor of nitrogen productivity (PFP_N) and net agronomic efficiency (AE_N). The first index describes unit nitrogen fertilizer productivity, which decreased significantly in response to increasing N rates but at the same time increased in response to soil applied sodium (Table 6).

Table 6

Response of indices of nitrogen fertilizer efficiency to in-season applied nitrogen and sodium (means for the years 2001-2003)

Treatment of fertilization	Partial factor productivity, PFPN (kg kg^{-1})			Agronomic N efficiency, AEN (kg kg^{-1})		
	-Na	+Na	mean	-Na	+Na	mean
AN_{90}	98.1	109.5	103.8 ^{a}	2.4	4.9	3.6 ^{a}
$ASN1_{90}$	102.4	109.7	106.1 ^{a}	6.7	5.1	5.9 ^{a}
$ASN2_{90}$	105.7	109.8	107.8 ^{a}	10.0	5.2	7.6 ^{a}
AN_{120}	80.0	82.3	81.2 ^{b}	6.4	0.2	3.3 ^{a}
$ASN1_{120}$	83.1	81.3	82.2 ^{b}	11.3	2.8	7.1 ^{a}
$ASN2_{120}$	78.9	83.0	81.0 ^{b}	7.1	4.6	5.9 ^{a}
AN_{150}	60.4	60.8	60.6 ^{c}	2.9	-2.0	0.5 ^{a}
$ASN1_{150}$	57.2	64.7	60.9 ^{c}	-0.2	1.9	0.8 ^{a}
$ASN2_{150}$	56.1	63.4	59.8 ^{c}	-1.3	0.6	-0.3 ^{a}
Mean	80.2 ^{m}	85.0 ^{n}	82.6	5.0 ^{m}	2.6 ^{m}	3.8

Means with the same letter are not significantly different at a=0.05 (Tukey's test).
Type of statistical differences: $m - n$ ® soil applied sodium; $a - c$ ® N × Na treatments

Explicit interaction between soil applied sodium and nitrogen treatments, also including sodium, has not been found. However, plants fertilized with sodium and 90 kg N ha^{-1} showed an increasing trend of unit N productivity, irrespective on broadcast applied sodium, The same positive trends are attributed to the 150 kg N treatment, but the PFP_N values were much lower. It could be therefore suggested that some of the applied nitrogen fertilizer can be successfully replaced by sodium. This hypothesis in illustrated in Figure 3, which shows that soil applied sodium allows one to decrease the optimum N rate by up to 30 kg ha^{-1}. The results are in agreement with the data reported by HANSEN (1994), who found that sodium applied in the rate of 60 kg Na ha^{-1} allowed a decrease in the optimum rate of fertilizer nitrogen from 120 to 80 kg N ha^{-1}. The other indicator of fertilizer N productivity, that is AE_N, did not show any response to the tested factors. However, the highest and positive trends should be attributed only to the 90 kg N ha^{-1} treatment (Table 6).

The study clearly revealed that the available sodium content (10-12 mg Na kg^{-1} soil) is sufficient to cover sugar beet requirements with respect to this element. However, a small rate of sodium up to 7.4 kg ha^{-1} (as sodium nitrate) can increase efficiency of applied nitrogen fertilizer, but the N rate should be reduced. Sugar beet plants cultivated under conditions of a low amount of soil available sodium should be fertilized, applying sodium fertilizers before sowing and during plant vegetation. The pre-sowing sodium

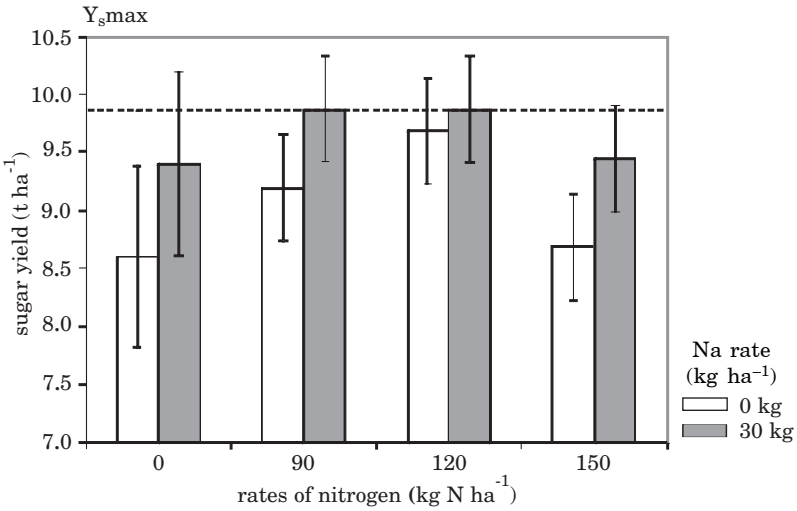

Fig. 3. Recoverable sugar yield response to N rates against the background of soil applied sodium (means for N-fertilizers). Vertical bars represent 0.95 confidence intervals at the α=0.05 level

application is a key factor affecting the N unit productivity, provided that N rates are significantly reduced. Under conditions dominating in Poland, a sodium rate should not be higher than 30 kg ha^{-1}.

CONCLUSIONS

1. The expected response of sugar beet and recoverable sugar yields to sodium fertilizers can be revealed only under conditions of low soil available sodium and water shortage during the growing season.

2. Response of qualitative characteristics of storage roots to sodium application is weak, showing a slight negative effect of sodium nitrate on sugar, but positive one on α-N concentration.

3. In-season application of fertilizer sodium can increase productivity of unit fertilizer nitrogen, but only under low amount of externally applied nitrogenous fertilizer.

4. The positive effect of soil applied sodium on nitrogen unit productivity is a basis for reduction in the nitrogen fertilizer rate in sugar beet production.

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CONTENT OF MACROELEMENTS IN FRUITS OF UKRAINIAN CULTIVARS OF HARDY KIWIFRUIT AND ACTINIDIA CHARTA DEPENDING ON THE WEATHER CONDITIONS DURING THE PHENOLOGICAL PHASES

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Abstract

Actinidia arguta and *Actinidia purpurea* are fruit-bearing vines that have been gaining increased recognition among consumers who expect tasty, natural food produced in an unpolluted environment. The berries of these plants, known as Chinese gooseberries, are smaller than the well-known kiwi fruit, have smooth skin and contain many valuable bioactive substances. The quality of food quality can be characterized, among others, by its content of mineral components, of which many are present in the fruits of these species.

The aim of the study was to determine the response of Ukrainian cultivars of *Actinidia arguta* and *Actinidia purpurea* grown in north-eastern Poland to weather conditions in phenological phases, expressed as a change in the content of macroelements in fruit. A correlation was found between the sum of temperatures and of precipitation in phenological phases in 2006-2011 and the content of macroelements in fruits of the following cultivars: Figurnaja, Kijewskaja Gibrydnaja, Kijewskaja Krupnopłodnaja, Purpurowa Sadowaja and Sientiabrskaja. The research demonstrated that the concentrations of Ca, N, P, Mg and Na in fruit of the examined actinidia cultivars were significantly affected, positively or negatively, by the interaction between the cultivars and meteorological factors in individual phenophases. It was found that the content of macroelements in fruits of cv. Kijewskaja Krupnopłodnaja, Purpurowa Sadowaja and Sientiabrskaja was not significantly dependent on the daily temperatures in any of the examined phenophases. However, these cultivars significantly responded to the content of phosphorus, nitrogen, magnesium and sodium, depending on the sum of precipitation in the two first phenophases. For the culti-

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var Figurnaja, sums of temperatures in the phase from fruit setting to harvest were a factor significantly affecting the magnesium content. On the other hand, the sodium content in fruits of this cultivar was significantly negatively correlated with the sum of temperatures in the phase between the beginning of flowering and fruit setting.

Key words: *Actinidia arguta, Actinidia purpurea*, climate factors, phenology, macroelements, correlation, cultivars, fruits.

ZAWARTOŚĆ MAKROELEMENTÓW W OWOCACH UKRAIŃSKICH ODMIAN AKTINIDII OSTROLISTNEJ I PURPUROWEJ W ZALEŻNOŚCI OD WARUNKÓW POGODOWYCH W FAZACH FENOLOGICZNYCH

Abstrakt

Aktinidia ostrolistna i purpurowa należą do owocodajnych pnączy, które w ostatnich latach zyskują coraz większe uznanie konsumentów oczekujących smacznej, naturalnej żywności wyprodukowanej w nieskażonym środowisku. Jagody tych roślin, zwane chińskim agrestem, są mniejsze od dobrze znanego owocu kiwi, mają gładką skórkę, zawierają wiele cennych substancji bioaktywnych. Jakość żywności można scharakteryzować m.in. uwzględniając zawartość składników mineralnych, które są także obecne w owocach tych gatunków.

Celem pracy było określenie zmian zawartości makroelementów w owocach ukraińskich odmian aktinidii ostrolistnej i purpurowej uprawianych w północno-wschodniej Polsce w zależności od warunków pogodowych w fazach fenologicznych. Wykazano korelacje między sumą temperatur i opadów w fazach fenologicznych w latach 2006-2011 a zawartością makroelementów w owocach odmian Figurnaja, Kijewskaja Gibrydnaja, Kijewskaja Krupnopłodnaja, Purpurowa Sadowaja i Sientiabrskaja. Stwierdzono, że koncentracja Ca, N, P, Mg i Na w owocach badanych odmian aktinidii zależała istotnie dodatnio lub ujemnie od współdziałania odmian z czynnikami meteorologicznymi w poszczególnych fenofazach. Zawartość makroelementów w owocach odmian Kijewskaja Krupnopłodnaja, Purpurowa Sadowaja i Sientiabrskaja nie zależała istotnie od sum temperatur dobowych w żadnej z omawianych fenofaz. Odmiany te reagowały natomiast istotnie na zawartość fosforu, azotu, magnezu i sodu w zależności od sum opadów w dwóch pierwszych fenofazach. W przypadku odmiany Figurnaja czynnikiem istotnie dodatnio oddziałującym na zawartość magnezu były sumy temperatur w fazie od zawiązywania owoców do ich zbioru. Zawartość sodu w owocach tej odmiany była istotnie ujemnie skorelowana z sumą temperatur w fazie od początku kwitnienia do zawiązywania owoców.

Słowa kluczowe: *Actinidia arguta, Actinidia purpurea*, czynniki klimatyczne, fenologia, makroelementy, korelacja, odmiany, owoce.

INTRODUCTION

Hardy kiwifruit [*Actinidia arguta* (Siebold et. Zucc.) Planch. Ex Miq.)] and actinidia charta [*Actinidia purpurea* Rehd. (*A. arguta* var. *purpurea* (Rehd.) C. F. Liang] are relatively young orchard plants. The first selections and varieties of those species emerged as late as in the mid 20th century. Most cultivars of *Actinidia arguta* and *Actinidia purpurea* have been selected or cultivated in China, New Zealand, North America, but also in Europe

(Williams et. al. 2003). In 1981, several varieties of those species were selected in the Botanical Garden in Kiev (Ukraine). Five of those varieties have been cultivated since 1996 in the garden of the University of Warmia and Mazury in Olsztyn (Bieniek 2012a). Starting from this year, a cultivation programme will be carried out in the Warsaw University of Life Sciences (SGGW) to obtain new cultivars with features adapted to the climatic conditions of Poland (Latocha, Krupa 2007). Adaptation of Ukrainian cultivars to the Polish climatic conditions also requires extensive experimental research before they can be recommended for production. As shown by the research carried out to date (Bieniek 2012a, Kawecki et al. 2001, 2004), cultivars selected in Ukraine and Russia are capable of producing fruits of the quality no worse than Chinese actinidia (kiwifruit). Latocha (2010), who examined other varieties of actinidia at the SGGW, found the highest content of most biologically active compounds in fruits of *A. arguta* and *A.purpurea* hybrids, although the content of mineral components was lower in the fruits of those genotypes than in *A. arguta.* As demonstrated by Latocha (2010) and Latocha and Jankowski (2011), actinidia fruits satisfy the requirements of consumers, who expect tasty, natural food produced in an unpolluted environment. According to Danilcenko et al. (2011), quality of food can be also described by its content of macroelements. They are necessary for proper growth and good health of bones and teeth. They ensure electrical transfer and act as cofactors in oxygen transport. Macroelements play an important role in enzymatic reactions, as well as in the protection of cells and lipids in biological membranes (Kang et al. 2007, Ekholma et al. 2007).

The research concerning determination of the mineral composition content of fruits of *Actinidia arguta* and *Actinidia purpurea* and their hybrid forms has been conducted by Bieniek (2012b), Ferguson and Ferguson (2003), Latocha and Krupa (2008), Latocha (2010), Skripczienko and Moroz (2002). These authors recommend fruit of the above species as a rich source of K, Fe, Cu and Mg. Many authors report that the concentration of mineral components in fruit depends on the cultivar, climatic and soil conditions, harvest dates (Milošević, Milošević 2012) and the level of irrigation and fertilization (Jaroszewska 2011). Fluctuations in the content of individual mineral components in fruits in consecutive seasons, found in the research by Latocha (2010) and Bieniek (2012b), can provide evidence for the significant effect of environmental conditions on their accumulation level. The climate in Poland is quite varied both year-to-year and within one year (Olesen et. al. 2007), the fact which can cause differentiation of the mineral composition of fruit. Phenophases are the reflection of plant adjustment to a moderate climate, in which only some seasons favour their growth and development. In some research institutions, phenological observations are treated as a sporadic element of research, and only a few experimental stations have data on the dates of beginning and duration of phenological phases of fruit trees (Licznar-Małańczuk 2004, after Kronenberg 1985). However, observations carried out under specific local geographical conditions indicate very high dif-

ferences in subsequent years of research as regards the commencement and duration of phenological phases of the flowering period due to the weather conditions (Licznar-Małańczuk 2004).

The aim of the study was to determine the response of Ukrainian cultivars of *Actinidia arguta* and *Actinidia purpurea* to the weather conditions during the phenophases manifested by a change of the content of macroelements in fruits.

MATERIAL AND METHODS

The study examined the effect of sums of mean daily temperatures and of precipitation in phenological phases in 2006, 2008, 2009, 2010 and 2011 on the content of macroelements (K, Ca, N, P, Mg, Na) in fruits of Ukrainian cultivars of *Actinidia arguta* and *Actinidia purpurea* and their hybrid forms grown in the garden of the Teaching and Experimental Station of the University of Warmia and Mazury in Olsztyn. The research was carried out on vines of the following cultivars: Sientiabrskaja (*Actinidia arguta* variety), Purpurowaja Sadowaja (*Actinidia purpurea*) and hybrid forms of *Actinidia arguta* and *Actinidia purpurea*: Figurnaja, Kijewskaja Gibrydnaja and Kijewskaja Krupnopłodnaja. Each cultivar was planted in five replications, and each shrub made a replication. Plants were grown in two rows with A-shape supports, in a 1.5 x 2 m spacing. The support was 4 m high. Five vines of the variety Weiki of *Actinidia arguta* were used as pollinators for those cultivars. In 2006-2011, plants were in their tenth to fifteenth years of vegetation. The research results of 2007 were not included because flowers of the examined cultivars were damaged by winter frosts and, consequently, chemical analyses of fruits were impossible. From the establishment of the experiment to the end of 2011, the plants did not require fertilization or chemical protection against diseases and pesticides or additional irrigation during the vegetation period. In the years of the study, 10-cm-thick bedding of bark of coniferous trees was spread around the shrubs. Plants grew on class IV soil of cereal-fodder strong complex. This is a highly loamy sand with the pH 6.85-7.52. The mineral composition of the soil was as follows: N – 9.66, P – 148.84, K – 57.83, Mg – 84.18 mg kg^{-1} d.m.

On the basis of observations in 2006-2011, the following developmental periods were determined for each cultivar: 1) bud swelling, 2) beginning of flowering, 3) fruit setting, 4) fruit harvest.

The period between the bud swelling and the beginning of flowering was the first phenological phase (1-2), the second phase was the period between the beginning of flowering and fruit setting (2-3) and the third was the time between fruit setting and harvest (3-4). In each of those phases, the values of temperatures and precipitation were added up. Tables 1 and 2

Table 1

Mean, minimum and maximum sums of temperatures of 2006-2011 in Olsztyn during phenological phases of Ukrainian cultivars of *Actinidia arguta and Actinidia purpurea*

Cultivar	ST 1-2				ST 2-3				ST 3-4			
	mean	min.	max.	SD	mean	min.	max.	SD	mean	min.	max.	SD
Figurnaja	457.0	350.0	553.5	85.0	488.3	381.7	538.0	63.2	1361.1	1261.3	1465.2	87.4
Kijewskaja Gibrydnaja	454.5	350.0	553.5	84.5	502.9	381.7	594.8	79.3	1365.0	1208.2	1465.2	101.8
Kijewskaja Krupnopłodnaja	485.4	389.4	586.3	75.0	491.1	321.4	566.4	99.4	1407.7	1223.7	1643.1	162.3
Purpurowaja Sadowaja	459.2	350.0	553.5	85.7	469.4	381.7	519.5	54.3	1346.0	1208.2	1465.2	92.1
Sientiabrskaja	463.4	350.0	553.5	87.8	457.3	381.7	519.5	63.1	1353.9	1208.2	1465.2	95.7

Explanations:
SD – standard deviation;
ST 1-2 – sums of temperatures in the first phenological phase (the period between the bud swelling and the beginning of flowering);
ST 2-3 – sums of temperatures in the second phenological phase (the period between the beginning of flowering and fruit setting);
ST 3-4 – sums of temperatures in the third phenological phase (the period between fruit setting and harvest.

Table 2

Mean, minimum and maximum sums of precipitation (SP) of 2006-2011 in Olsztyn during phenological phases of Ukrainian cultivars of *Actinidia arguta and Actinidia purpurea*

Cultivar	SP 1-2				SP 2-3				SP 3-4			
	mean	min.	max.	SD	mean	min.	max.	SD	mean	min.	max.	SD
Figurnaja	87.1	6.7	168.7	57.4	83.4	27.6	166.6	52.8	214.4	157.0	264.1	38.7
Kijewskaja Gibrydnaja	87.1	6.7	168.7	57.4	83.4	27.6	166.6	52.8	214.4	157.0	264.1	38.7
Kijewskaja Krupnopłodnaja	89.5	6.7	168.2	57.7	85.7	32.4	175.9	55.9	205.6	152.3	247.5	34.5
Purpurowaja Sadowaja	87.8	6.7	168.7	57.5	81.8	27.6	166.6	52.4	211.3	141.8	264.1	44.5
Sientiabrskaja	87.8	6.7	168.7	57.5	81.8	27.6	166.6	52.4	211.3	141.8	264.1	44.5

Key cf. Table 1

present the mean, minimum and maximum values, as well as standard deviation for those meteorological factors. The method of calculating the sum of temperatures expresses the empirical relations between plant development and the amount of heat they received in a given phenophase. It assumes a constant value of sums of temperatures for a specific phase of a given species (MIKKELSEN 1981).

Data originating from the Station of the Institute of Meteorology and Water Management in Olsztyn were used in calculations. Tables 3-8 show the values of the correlation coefficient between thermal conditions and precipitation in established phonological phases and the macroelement content in fruits for each cultivar.

Table 3

Correlation between the potassium content in fruits and the sum of air temperatures and precipitation in 2006-2011 in Olsztyn during phenological phases of Ukrainian cultivars of *Actinidia arguta and actinidia purpurea*

Factor	Cultivar				
	Figurnaja	Kijewskaja Gibrydnaja	Kijewskaja Krupnopłodnaja	Purpurowaja Sadowaja	Sientiabrskaja
ST 1-2	-0.252	0.211	-0.134	0.079	-0.365
SP 1-2	-0.391	-0.531	-0.457	-0.366	0.374
ST 2-3	-0.035	-0.159	-0.094	0.001	0.973
SP 2-3	0.546	0.608	0.633	0.757	0.845
ST 3-4	0.283	-0.472	-0.152	-0.530	-0.743
SP 3-4	-0.348	0.255	-0.464	-0.054	-0.873

Key cf. Table 1

Table 4

Correlation between the calcium content in fruits and the sum of air temperatures and precipitation in 2006-2011 in Olsztyn during phenological phases of Ukrainian cultivars of *Actinidia arguta and Actinidia purpurea*

Factor	Cultivar				
	Figurnaja	Kijewskaja Gibrydnaja	Kijewskaja Krupnopłodnaja	Purpurowaja Sadowaja	Sientiabrskaja
ST 1-2	-0.340	-0.377	0.041	0.156	0.914
SP 1-2	-0.791	-0.781	-0.580	-0.298	0.384
ST 2-3	-0.557	-0.682	-0.214	-0.599	-0.532
SP 2-3	-0.350	-0.453	-0.275	-0.320	-0.227
ST 3-4	0.090	0.232	0.566	0.185	0.060
SP 3-4	0.612	0.929*	0.764	0.760	0.280

Asterisks indicate correlation coefficient significant at α=0.05;
Key cf. Table 1

Table 5

Correlation between the nitrogen content in fruits and the sum of air temperatures and precipitation in 2006-2011 in Olsztyn during phenological phases of Ukrainian cultivars of *Actinidia arguta and Actinidia purpurea*

Factor	Cultivar				
	Figurnaja	Kijewskaja Gibrydnaja	Kijewskaja Krupnopłodnaja	Purpurowaja Sadowaja	Sientiabrskaja
ST 1-2	-0.710	0.920*	-0.723	0.457	0.730
SP 1-2	-0.767	0.408	0.152	0.238	1.000*
ST 2-3	-0.272	0.440	0.551	0.583	0.573
SP 2-3	-0.389	0.671	0.143	0.935*	0.809
ST 3-4	0.504	-0.911*	0.431	-0.828	-0.896
SP 3-4	0.507	0.042	-0.622	-0.433	-0.776

Asterisks indicate correlation coefficient significiant at α=0.05;
Key cf. Table 1

Table 6

Correlation between the phosphorus content in fruits and the sum of air temperatures and precipitation in 2006-2011 in Olsztyn during phenological phases of Ukrainian cultivars of *Actinidia arguta and Actinidia purpurea*

Factor	Cultivar				
	Figurnaja	Kijewskaja Gibrydnaja	Kijewskaja Krupnopłodnaja	Purpurowaja Sadowaja	Sientiabrskaja
ST 1-2	-0.566	-0.591	-0.370	-0.626	0.692
SP 1-2	-0.518	-0.402	-0.904*	-0.396	0.007
ST 2-3	-0.741	-0.294	-0.648	-0.699	-0.812
SP 2-3	-0.860	-0.941*	-0.521	-0.957*	-0.578
ST 3-4	0.484	0.667	0.527	0.919	0.433
SP 3-4	0.727	0.579	0.217	0.481	0.622

Asterisks indicate correlation coefficient significiant at α=0.05;
Key cf. Table 1

The content of macroelements was determined after fruit harvest at the harvest maturity stage. Chemical analyses were carried out in three replications. Tests were prepared directly after fruit harvest using 0.5 kg of randomly-selected fruits of each cultivar dried at 105ºC. The dried material was ground in a laboratory mill. Macroelements in fruits of actinidia were determined after digestion (1 g ground plant material) by wet mineralization (H_2SO_4 using a oxidant H_2O_2). The mineralized material was transferred to 200 cm^3 flasks. The following values were determined in the prepared samples:

– N – by the distillation method;
– P- colorimetrically, by the vanadium-molybdenum method;
– K, Ca, Na – by atomic emission spectroscopy ESA;
– Mg – by atomic absorption spectroscopy ASA.

The analyses were carried out on the basis of certified material CTA--VTL-2. The content of macroelements was determined according to Polish Norms PN-91/R-04014.

The following determination errors were included: N – 3%, P – 4.5%, K – 2%, Ca – 2.8%, Na – 7%, Mg – 1.5%.

Table 7

Correlation between the magnesium content in fruits and the sum of air temperatures and precipitation in 2006-2011 in Olsztyn in phenological phases of Ukrainian cultivars of *Actinidia arguta and Actinidia purpurea*

Factor	Cultivar				
	Figurnaja	Kijewskaja Gibrydnaja	Kijewskaja Krupnopłodnaja	Purpurowaja Sadowaja	Sientiabrskaja
ST 1-2	-0.782	-0.031	-0.608	-0.422	0.746
SP 1-2	-0.087	0.355	0.434	0.272	1.000*
ST 2-3	0.021	0.193	0.218	-0.013	0.553
SP 2-3	-0.465	-0.732	-0.732	-0.840	0.794
ST 3-4	0.888*	0.291	0.400	0.726	-0.885
SP 3-4	-0.451	0.240	-0.308	-0.074	-0.760

Asterisks indicate correlation coefficient significiant at α=0.05;
Key cf. Table 1

Table 8

Correlation between the sodium content in fruits and the sum of air temperatures and precipitation in 2006-2011 in Olsztyn during phenological phases of Ukrainian cultivars of *Actinidia arguta andAactinidia purpurea*

Factor	Cultivar				
	Figurnaja	Kijewskaja Gibrydnaja	Kijewskaja Krupnopłodnaja	Purpurowaja Sadowaja	Sientiabrskaja
ST 1-2	-0.787	-0.826	-0.417	-0.637	-0.983
SP 1-2	-0.802	-0.520	-0.952*	-0.247	-0.841
ST 2-3	-0.883*	-0.670	-0.698	-0.360	-0.044
SP 2-3	-0.470	-0.788	-0.435	-0.531	-0.367
ST 3-4	0.784	0.800	0.439	0.642	-0.518
SP 3-4	0.538	0.402	0.041	0.241	0.316

Asterisks indicate correlation coefficient significiant at α=0.05;
Key cf. Table 1

A detailed list of the macroelements and their content in fruits of the examined actinidia cultivars in individual years of the experiment has been published in BIENIEK (2012b). This study presents the mean content of the discussed elements in fruits of the examined actinidia cultivars in 2006-2011 (Table 9). The significance of differences was calculated using Tukey's HSD test at a level of significance α=0.01. In order to determine the relation between the content of macronutrients in fruits and weather conditions in phonological phases, linear correlation were analysed. The significance of the correlation coefficients was set at α=0.05. Calculations were performed with Statistica 9.1 software.

Table 9

The mean contents (g kg^{-1} d.m.) of macroelements in fruits of *Actinidia cultivars* in 2006-2011

Cultivar	K	Ca	N	P	Mg	Na
Figurnaja	12.31 ^{a}	3.13 ^{a}	9.43 ^{b}	2.99 ^{a}	0.67 ^{b}	0.72 ^{b}
Kijewskaja Gibrydnaja	16.57 ^{b}	3.03 ^{a}	8.96 ^{a}	3.03 ^{a}	0.68 ^{b}	0.60 ^{a}
Kijewskaja Krupnopłodnaja	18.83 ^{c}	3.51 ^{b}	9.53 ^{b}	3.28 ^{b}	0.61 ^{a}	0.66 ^{ab}
Purpurowaja Sadowaja	19.62 ^{d}	3.88 ^{c}	9.90 ^{c}	3.13 ^{ab}	0.73 ^{c}	0.59 ^{a}
Sientiabrskaja	16.42 ^{b}	4.86 ^{d}	12.21 ^{d}	4.14 ^{c}	0.81 ^{d}	0.66 ^{ab}

Means followed by the same letters do not differ at α=0.01.

RESULTS AND DISCUSSION

As shown in Table 1, the mean sum of daily temperatures and sum of minimum and maximum temperatures of 2006-2011 in the phase between bud swelling to the beginning of flowering were the highest for Kijewskaja Krupnopłodnaja – the cultivar started vegetation at the latest date. For the other cultivars, in the corresponding phenophase, the value of sums of minimum and maximum temperatures was the same. The lowest mean sum of daily temperatures was observed for cv. Kijewskaja Gibrydnaja, but in the next phenological phase, from the beginning of flowering to fruit setting, this meteorological factor showed a higher value. In addition, the sum of maximum temperatures was the highest for this cultivar, while the sum of minimum temperatures was the same as for other cultivars, except Kijewskaja Krupnopłodnaja. The lowest mean sum of temperatures in the period between fruit setting to harvest was recorded for Purpurowaja Sadowaja. This cultivar was distinguished by the earliest date of fruit ripening (BIENIEK 2012b). The sums of the minimum and maximum temperatures for the cultivars Kijewskaja Gibrydnaja, Purpurowaja Sadowaja and Sientiabrskaja in the examined phenophase showed the same value.

As shown by the analysis of sums of precipitation in individual phases of vegetation of the selected actinidia cultivars in 2006-2011, Kijewskaja Krupnopłodnaja stood out with its varied values of sums of mean precipitation in each of the examined period of development (Table 2). In the first two phenological phases, this value was the highest but in the last one, it was the lowest. The biggest differences, both in the mean sum of precipitation and in sums of minimum and maximum precipitation, were recorded in the phase between fruit setting and harvest. In that phenological phase, Purpurowaja Sadowaja and Sientiabrskaja had the same values of mean sums of precipitations, as well as the minimum and maximum sums of precipitation. Another pair of cultivars with the same properties was composed of Figurnaja and Kijewskaja Gibrydnaja. The cultivar Kijewskaja Krupnopłodna was distinguished by its variable values of the discussed factor.

Based on the data provided in Table 9, it can be claimed that the examined macroelements occur in actinidia fruits in the following, decreasing content: K>N>Ca>P>Mg>Na. Tables 3-8 show values of the correlation coefficient between thermal conditions and precipitation in the determined phenological phases and the content of macrooelements in fruits for each cultivar.

Potassium was the only macroelement for which no significant correlations were found as regards its content in fruits and the other examined factors (Table 3). Positive correlation coefficients for all the examined varieties in the phase between the beginning of flowering and fruit setting may also confirm a favourable relationship between the amount of precipitation in the phenological phase and the content of this element in fruit (Table 3). The highest mean potassium content in 2006-2011 was accumulated by fruit of Purpurowaja Sadowaja (Table 9), for which sums of precipitation in the period between the beginning of flowering and fruit setting had a positive effect on the accumulation of potassium (Table 3). Potassium is the second most important (after nitrogen) macroelement ensuring good growth and yielding of plants. As shown by the yield analysis for this cultivar in 2001-2009, it was also one of the most prolific varieties (Bieniek 2012a). Potassium is also required for obtaining good fruit colour, accumulation of acids and good taste.

Table 4 shows significant positive correlation between the calcium content in fruit from cv. Kijewskaja Gibrydnaja and the sum of precipitation in the phase between fruit setting to harvest. In the examined phenophase, both sums of precipitation and sums of temperatures showed a favourable effect on the growth of calcium content in fruits of all the analysed cultivars. On the other hand, in the phase between the beginning of flowering and fruit setting, sums of temperatures and precipitation had a negative effect on the content of calcium in fruits of all the examined cultivars. In the phase between bud swelling and the beginning of flowering, positive correlation coefficients for both sums of temperature and sums of precipitation were recorded only for Sientiabrskaja. This cultivar was characterized

by the highest total content of calcium in fruit. The lowest Ca content was found in fruits of Kijewskaja Gibrydnaja and Figurnaja cultivars (Table 9). Calcium is transferred from fruit to wood. In England, it was found that in the last three weeks before harvest, 20% of calcium was transferred from apples to other plant organs due to dry weather. Stress conditions during plant growth under very dry weather make water with Ca drain away from fruit to leaves. At the same time, low soil moisture reduces Ca availability for plants.

In the current experiment, a significant positive correlation coefficient was found for the interaction between sums of temperatures in the phase between bud swelling and the beginning of flowering and the content of nitrogen in fruits of cultivar Kijewskaja Gibrydnaja (Table 5). As reported by TROMP and OVAA (1971), in spring, just before bud bursting, nitrogen reserves are activated in shoots, which is manifested *inter alia* by intensive protein decomposition. On the other hand, sums of temperature in the phase between fruit setting and harvest had a significant negative effect on the content of this element in fruits of this cultivar. Significant positive values of the correlation coefficient for the content of nitrogen in fruit also confirm the relationship between the sum of precipitation in the phase between bud swelling and the beginning of flowering for cv. Sientiabrskaja, and in the phase between the beginning of flowering and fruit setting for fruits of cv. Purpurowaja Sadowaja. On the other hand, a negative value of the correlation coefficient in the subsequent phenophase, between fruit setting and harvest confirms the negative effect of sums of precipitation on the content of nitrogen in fruits of Kijewskaja Krupnopłodnaja, Purpurowaja Sadowaja and Sientiabrskaja. In addition, during the same phenological phase, the content of nitrogen in fruit was negatively affected by the sums of temperatures, particularly in case of Kijewskaja Gibrydnaja, where the correlation was significant, in fruit of Purpurowaja Sadowaja and Sientiabrskaja, where the effect was weaker. Fruits of the latter cultivar had the highest content of nitrogen (Table 9). Favourable sums of temperatures and precipitation in the first two phenological phases could have a significant effect on the content of this element in fruit of this cultivar, which is confirmed by the positive correlation coefficients (Table 5). Values of all correlation coefficients in these phases were negative only for cv. Figurnaja.

As regards the phosphorus content, significant negative correlations were observed for Kijewskaja Gibrydnaja and Purpurowaja Sadowaja and the sums of precipitation in the phase from the beginning of flowering to fruit setting, and for Kijewska Krupnopłodnaja and the sum of precipitation in the phase between bud swelling and the beginning of flowering (Table 6). Additional correlation coefficients, for all the cultivars, between sums of precipitation and temperatures in the phase between fruit setting to their harvest indicate the favourable effect of the examined meteorological factors on the growth of phosphorus content in fruits. The lowest content of phosphorus

was found in fruits of Figurnaja and Kijewskaja Gibrydnaja (Table 9). In this study, correlation coefficients in the first two phenological phases, both for sums of temperatures and precipitation, obtained for these cultivars were all negative, which could have affected the final content of this element in fruit. The highest content of phosphorus was found in fruits of Sientiabrskaja (Table 9). The increase of phosphorus in fruits of this cultivar may have resulted from the favourable course of climatic conditions in the first and in the last phenological phase. Phosphorus affects the formation of flower buds. In the differentiation period, tree buds accumulate significant amounts of organic phosphorus, and a shortage of this element highly reduces the number of flower buds (Taylor, Goubran 1975). It is important that the level of phosphorus should be at an optimum level in plant tissues both in the year of fructification and in the preceding year (Bould, Parfitt 1973). For the above cultivar, the role of phosphorus has not been elucidated. Bieniek (2012a) demonstrated that Sientiabrskaja is unsuitable for cultivation in climatic conditions of the 6 USDA zone, since it binds the fewest flower buds and produces the lowest yields.

Table 7 demonstrates that the effect of sums of temperatures in the phase between fruit setting and harvest had a highly significant positive influence on the magnesium content in fruit of Figurnaja cultivar, and the sum of precipitation in the phase between bud swelling to the beginning of flowering produced such influence on Sientiabrskaja.

Sientiabrskaja was also the cultivar with the highest content of magnesium in fruits (Table 9). As follows from Table 7, positive values of all correlation coefficients in first two phenological phases were found only for this cultivar. Lower content of magnesium was found in fruits of the hybrid forms of actinidia than in fruits of *Actinidia arguta* or *Actinidia purpurea* (Table 9). However, no significantly negative effect of the sums of temperatures or precipitation on concentration of this element in fruit of the examined cultivars was found in any of the phenological phases (Table 7). As regards the sodium content (Table 8), an opposite, significantly negative relation was found between sums of temperatures in the phase from the beginning of flowering and fruit setting for cv. Figurnaja, and between sums of precipitation in the phase between bud swelling to the beginning of flowering for cv. Kijewskaja Krupnopłodnaja.

Hołubowicz (1970) demonstrated the role of N, P, Ca and K elements in the regulation of flowering and fruiting. The experiment discussed in this study demonstrated that sums of temperatures and precipitation in the phase between fruit setting and harvest had a positive effect on the development of Ca and P content in fruit of all the examined cultivars.

The influence of temperature and its effect on the growth and development of various species of plants has been analysed in both Polish and foreign literature (Chmielewski et al. 2004, Dragańska et al. 2008, Kalbarczyk 2009, Kalbarczyk, Kalbarczyk 2012, Kawecki, Bieniek 2008, Licznar- Małańczuk

2004, Milošević, Milošević 2012, Skowera et al. 2007, Tao et al. 2008). Snelgar et al. (1992) and Tiyayon and Strik (2004) examined the effect of shading on flowering and fruiting of *Actinidii arguta* Ananasnaja in Oregon (USA). The response of cultivars in terms of the content of macroelements in fruit depending on weather conditions in phenological phases has not been analysed in research publications; therefore it is difficult to refer to literature data. Latocha (2010) found that the content of mineral components in fruit depends mainly on the genetic features of the plant. This research demonstrated that the concentration of Ca, N, P, Mg and Na in fruits of Ukrainian cultivars of actinidia significantly depended (positively or negatively) on the relations between cultivars and meteorological factors in specific phenophases.

CONCLUSIONS

1. The content of mineral components in fruits of cultivars Kijewskaja Krupnopłodnaja, Purpurowa Sadowaja and Sientiabrskaja did not significantly depend on the sums of daily temperatures in any of the phenophases discussed. However, these cultivars responded significantly to the content of phosphorus, nitrogen, magnesium and sodium depending on the sum of precipitation in the first two phenophases.

2. For cv. Figurnaja, the sum of temperatures in the phase from fruit setting to their harvest was a factor significantly influencing the content of magnesium. The content of sodium in fruits of this cultivar was significantly negatively correlated with the sum of temperatures in the phase from the beginning of flowering to fruit setting.

3. The phase between fruit setting and harvest had a favourable effect on the content of calcium and phosphorus in fruits of Ukrainian cultivars of actinidia grown in north-eastern Poland.

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EFFECT OF NITROGEN FERTILIZATION ON THE CONTENT OF TRACE ELEMENTS IN CV. BIANCA GRAPEVINE (*VITIS* SP.)*

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Abstract

The knowledge of interactions between nitrogen and other nutrients and trace elements is the key to improving the uptake of nutrients. A study on a grapevine cultivar called Bianca was carried out in the Garlicki Lamus vineyard located in Garlica Murowana (near Krakow, Poland) in 2010-2011. The plants were treated with three nitrogen doses (0, 50 and 100 kg N ha^{-1}) supplied as ammonium nitrate in a single application at three weeks pre-flowering. Samples of leaf petioles and blades, as well as grapes were taken. After wet microwave digestion in HNO_3, some nutrient elements: B, Cu, Fe, Zn, Mn, Mo and Na, as well as trace elements: Al, Ba, Cd, Cr, Li, Ni, Sr, Ti and V were measured using the ICP-OES technique. Concentrations of microelements in the grapevine tissues were in the optimum (B, Cu, Fe, Zn and Mo) or high (Mn) range of content reported for 'full bloom' plants. N fertilizers enhanced leaf accumulation of trace elements such as Ti and V or depressed the uptake of some elements like B, Mn, Ba, Cd and Sr. Analyzed leaf blades contained higher amounts of Fe, Mn, Al, Ni, Pb, Ti and V than petioles. In contrast, petioles had more B, Zn, Mo, Cd, Ba, Li and Sr. Increased N fertilization diminished Cd and Ti (only at 50 kg N ha^{-1}) in grape must; the reverse was true for Ba and Sr. The vintage strongly influenced grape mineral content. During warmer and wet year 2010, higher amounts Al, Cu, Fe and Ti were measured in fruits. The dry season in 2011 increased the content of Mn, B, Cd, Cr and Ni in fruits.

Key words: leaf analysis, nutrient status, microelements, environmental factors.

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WPŁYW NAWOŻENIA AZOTEM NA ZAWARTOŚĆ PIERWIASTKÓW ŚLADOWYCH W WINOROŚLI ODMIANY BIANCA

Abstrakt

Doświadczenie prowadzono w latach 2010-11 w winnicy Garlicki Lamus położonej w Garlicy Murowanej k. Krakowa. Krzewy winorośli nawożono trzema dawkami azotu (0, 50 i 100 kg N ha^{-1}) w postaci saletry amonowej. Nawóz podawano jednorazowo na 3 tygodnie przed kwitnieniem roślin. Do oznaczeń pobierano próbki liści (osobno blaszki i ogonki) oraz owoce. Po mineralizacji mikrofalowej w HNO_3 oznaczono, z wykorzystaniem spektrometrii ICP-OES, następujące mikroelementy: B, Cu, Fe, Zn, Mn, Mo i Na oraz pierwiastki śladowe: Al, Ba, Cd, Cr, Li, Ni, Sr, Ti i V. Zawartość mikroelementów w liściach była optymalna (B, Cu, Fe, Zn i Mo) lub wysoka (Mn) w porównaniu z liczbami granicznymi dla owocujących winnic. Nawożenie azotem wpłynęło na zwiększenie akumulacji Ti i V w liściach lub ograniczenie zawartości niektórych pierwiastków: B, Mn, Ba, Cd i Sr. Analizowane blaszki liściowe zawierały więcej Fe, Mn, Al, Ni, Pb, Ti i V niż ogonki liściowe. Natomiast ogonki akumulowały większe ilości B, Zn, Mo, Cd, Ba, Li i Sr. Poziom nawożenia azotowego wpłynął na zmniejszenie zawartości Ba i Sr w moszczu winogron. Warunki podczas wegetacji (rocznik) miały istotny wpływ na skład mineralny zarówno liści, jak i owoców. Podczas cieplejszego, ale równocześnie mokrego sezonu 2010 stwierdzono wyższą zawartość Al, Cu, Fe i Ti w owocach. Suchy rok 2011 wpłynął na zwiększenie poziomu Mn, Ba, Cd, Cr i Ni w gronach.

Słowa kluczowe: analiza części wskaźnikowych, status odżywienia, mikroelementy, czynniki środowiskowe.

INTRODUCTION

Nutrition is an important aspect of vineyard management. Nitrogen fertilization increases vegetative growth and crop yield (Bell, Robinson 1999, Spayd et al. 2000, Ekbic et al. 2010). It also influences various parameters in vine production, such as fruit set, fruit quality and the quality of vine (Treeby et al. 2000, Rodrigue-Lovelle, Gaudillčre 2002, Amiri, Fallahi 2007). Excess nitrogen leads to high vigour, increased fruit yield and modified juice composition (i.e. pH and concentrations of organic acids and esters), but may also create favorable conditions for diseases such as bunch stem necrosis and *Botrytis cinerea* bunch rot (Keller et al. 2004).

The fundamentals of nitrogen nutrition in grapevine are well known (Wade et al 2001). Nitrogen is a critical nutrient during grapevine rapid shoot growth in the spring, through bloom and early development of berries. Schaller (2000) indicates that N use efficiency in vineyard is low. The researcher demonstrated that the maximum N demand is reached after bloom, mainly at the end of June/beginning of July. Shifting of the fertilization date from early spring to the phenological stage “3–5 leaves unfolded” resulted in good synchronization between N applied and demanded by the plant. Grapevine has alow nitrogen demand compared with other fruit crops (Van Leeuwena, Seguin 2006, Schreiner et al. 2006). Spayd et al. (2000) showed

that application of 112 kg N ha^{-1} was sufficient to obtain juice N concentrations suitable for healthy yeast fermentation. However, this level of fertilization resulted in excessive vine growth, fruit shading and delayed fruit ripening. Thus, application of no more than 50 kg N ha^{-1} is recommended for most vines varieties (Robinson 1999). However, amounts of N fertilizer differ in respect of the variety, yield, soil type, crop residues and irrigation efficiency. For white wine production, nitrogen supply should be at least moderate to obtain high aroma potential in grapes (Peyrot des Gachons et al. 2005).

A change in one of the soil nutrient levels can interfere with the plant availability and uptake of other nutrients. Therefore, the effects of one nutrient on uptake or use of another, as well as their interactions should be considered in a complete nutrient management program (Fageria, Baligar 2005, Fageria 2009). Nitrogen application in a vineyard can modify absorption of other nutrients by grapevine. High N level can reduce the availability of B, K and Cu. On the other hand, increased N levels create a demand for more magnesium (Bell, Robinson 1999). Application of N-fertilizers to soils can also affect bioavailability of trace elements in soil (Basta et al. 2005). Nitrogen fertilizers induce some direct and/or indirect changes, which affect the dynamics of availability of metals in soils. Mineral N fertilizers containing ammonium can acidify the soil solution and depress the pH of the rhizosphere, thus enhancing the availability of metals (Diatta, Grzebisz 2006).

Plant nutrient analysis is an important tool used to determine the status of plant nutriention and fertilizer requirements. Fallahi et al. (2005) demonstrated that the French Diagnostic Foliar Laboratory at Montpellier relied on the status of leaf tissue status (blade and petiole) sampled twice: at bloom and at the end of *veraison*. In California, petiole tissues alone sampled at bloom time are often used for assessment of the plant nutritional status. Robinson (2000) reported that petiole nitrate-N determination is recommended in the Australian grape industry. However, the author concluded that petiole analysis does not give a good picture of nitrogen status except in very high yielding vineyards. In Poland, petiole or blade sampling are currently used and standards for these methods are adapted from other countries. These analytical techniques and standards may not be appropriate under Polish conditions with a relatively severe climate, considered marginal or unsuitable for growing grapes of European origin for wine production. There is a regional effect on quantities of nutrients grapevine needs (Mackenzie, Christy 2005, Greenough et al. 2005, Pacheco et al. 2010). The uptake of nutrients by plants is strongly influenced by environmental factors (rainfall, temperature and soil condition) and controlled by the plant's metabolic demand (Marchner 1995). Nevertheless, little is known about the uptake of trace elements by grapevine under different environmental condition and management practice. Microelements and other trace elements, for

example copper, chromium, molybdenium, cadmium or mercury, at high concentrations may be toxic to humans (McLaughlin et al. 1999). We lack information on the relationship between leaf and fruit mineral status. Such knowledge could help to choose appropriate sites for vineyards and to understand better the soil and plant interactions. These interactions as well as the specific nutrient needs of particular cultivars would enable us to establish site-specific fertilization plans to promote vineyard sustainability.

As grapevine cultivation becomes increasingly popular in colder regions of Europe, we have decided to assess the influence of different nitrogen fertilization levels on content of micro- and trace elements in leaves and fruits of grapevine in southern Poland.

MATERIAL AND METHODS

Site characteristic

The study was carried out at the Garlicki Lamus vineyard located in Garlica Murowana (near Krakow, Poland, coordinates: 19º56'E oraz 50º08'N) in 2010-2011. The site of the experimental vineyard had long-term average annual precipitation of 576 mm with an average minimum temperature of -2.9ºC (in January) and the maximum temperature of 17.8ºC (in July). On-farm rooted grapevine (*Vitis sp.* L.) shoots of cv Bianca planted in 2007 were used for this investigation. The cultivar is a hybrid of Seyve Villard × Bouvier. This Hungarian variety is moderately vigourous and highly productive. It bears white grapes, with high sugar content and good acidity. As the grapevine is resistant to fungal diseases, the cultivar is recommended for establishing organic vineyards. Grapevines were planted along the north-south orientation, with 3.5 m inter-row and 0.9 in-row spacing, respectively. They were trained as Casenave's horizontal cordon with one arm. Plants were cane-pruned to 10 nodes per 1 meter of canopy, with vertically positioned shoots. The average yield ranged from 2.5 to 4.0 t ha^{-1} in 2010 and 2012, respectively.

The experiment was arranged in a randomized complete-block design with four replications of five vines per block. The plants were treated with three nitrogen doses (0, 50 and 100 kg N ha^{-1}) as ammonium nitrate in a single application at three weeks pre-flowering. Fertilizers were applied within a radius area around each vine. No other macro- and micronutrients were added to the vines in this experiment.

The vineyard soil was characterized as silty clay loam (18% of sand, 43% of silt, 39% of clay) with the pH of about 5.6 and total organic matter content equal 1.68%. The soil available content of phosphorus and magnesium (measured according to the universal method) was in the medium to optimum range (20.8-45.7 mg P dm^{-3}, 56.4-79.0 mg Mg dm^{-3}). The soil avail-

able potassium (128.4-163.7 mg K dm^{-3}) and calcium content (463.7--630.4 mg Ca dm^{-3}) was below the optimum ranges (200-250 mg K dm^{-3} and 1000-2000 mg Ca dm^{-3} of soil, respectively). Average amounts of available soil microelements (extracted with 1 M HCl) were within the optimum range for manganese, copper and zinc. However, soil samples contained less available boron than considered as optimal (0.49 to 0.59 mg B kg compared to recommended 1.3-4.3 mg B kg^{-1} soil). The content of other available soil elements varied from: 837 to 1003 mg Al kg^{-1}, 33.5-35.9 mg Ba kg^{-1}, 0.47--0.57 mg Cd kg^{-1}, 0.79-0.92 mg Cr kg^{-1} and 13.9-15.1 mg Pb kg^{-1}.

Weeds between grapevine plants were controlled by application of glyphosat (Roundup, Monsanto) in mid-June every year. The plant protection was carried out according to the recommendations for commercial vineyards. In the wet year 2010, an incident of *Botrytis cinerea* infection was observed, but it was not severe enough to affect the grapevines.

Tissue analysis

During the subsequent seasons, samples of leaf petioles, leaf blades as well as grapes were taken. Ten leaves per plant were sampled from a cordon on both sides of a vine plant, from the inner and outer canopy layers. Collected samples were taken from recently matured, full-size leaves at the full bloom period in each year, around 15 June in 2010 and 13 June in 2011. Leaves of five vine plants in each block were combined to make a composite sample of 50 leaves. Petioles were separated from blades and their dry matter content was measured. Plant samples were washed in distilled water before forced-air oven drying at 60ºC. Samples were comminuted in a grinder. After wet microwave digestion in HNO_3 (CEM 5-Express microwave), the samples were analysed for the following nutrients: B, Cu, Fe, Zn, Mn, Mo, and trace elements: Al, Ba, Cd, Cr, Li, Ni, Sr, Ti and V, using the ICP-OES technique (Prodigy Teledyne, Leeman Labs. spectrometer).

Grapes were harvested on 15/20 October in 2010 and 2011, respectively. Berries showing average growth and maturation were sampled for each treatment. The grapes were washed in distilled water and stalks were removed after drying at room temperature. Must was made in a laboratory press. The same procedure (wet digestion) was employed for fruit and tissues analyses.

Soil analysis

For determination of soil nutrients, soil samples were collected at 0-20 cm depth. Samples were dried at 60ºC for 48 hours and passed through a 1 mm mesh sieve. The grain-size distribution was analyzed by Casagrande's aerometric method modified by Prószyński (Ostrowska et al. 1991). This procedure is regulated by the PN-R-04032 standard (Polish norm) applied mainly for agricultural soil analysis in Poland. Total organic carbon (TOC) with

wet oxidation followed by titration with ferrous ammonium sulfate was measured by Tiurin's method (OSTROWSKA et al. 1991). Soil pH was determined by adding deionized water at the soil to water ratio equal 1:2. The available microelements (Cu, Fe, Zn, Mn, B) and trace elements (Al, Ba, Cd, Cr, Li, Ni, Sr, Ti and V) were measured in 1 M HCl extractant (OSTROWSKA et al. 1991) using the ICP-OES technique. This soil extractant and procedure are currently used to estimate availability and critical levels for micronutrient cations in Poland.

Climatic condition

In 2010, air temperatures were near the average for Kraków area from April to September (Figs 1a and 1b). Rainfall during the growing season in 2010 was high, especially in May and September. This vintage was warmer and much wetter than the following year. The vintage of 2011 grew in a dry, especially in May, and colder season. In 2011, the average temperature from April to September was 12.9ºC against 14.7ºC in 2010.

Statistical analysis

We tested the data and found some significant interactions between years and N doses and leaf samples. Data were analyzed using Statistica 9.0 software (Statsoft Inc.). All results were subjected to 3-way analysis of variance (MANOVA). The least significant difference (LSD) multiple range test was used to compare means for significant main effects.

RESULTS AND DISCUSSION

Tissue analysis in a vineyard is a very effective tool for monitoring mineral nutrition. In the present work, concentrations of all microelements in the grapevine tissues were in the optimum (B, Cu, Fe, Zn, Mo) or high range (Mn) as compared with the content reported for 'full bloom' plants (Table 1). High Mn levels, especially in leaf blades, indicate low pH values and increased Mn availability in acid soil.

Strong impact of the climatic conditions on mineral acquisition, transport, and finally plant accumulation was observed. Extremely wet but warmer year 2010 favoured higher accumulation of B, Al, Cd, Li, Sr, Ti and V as measured in blades and petioles (Tables 1 and 2). On the contrary, colder but dryer year 2011 stimulated higher content of Fe, Mn, Zn, Mo, Ba, Cr and Ni. The process of absorption and root metabolism is undoubtedly conditioned by ambient temperature. High temperature activates chemical and biological processes in soil and increases solubility of trace elements. According to GREENOUGH et al. (2005), concentrations of trace elements are correlated strongly with Degree Days, indicating that more heat results in increased evaporation, water uptake and higher elemental concentration in

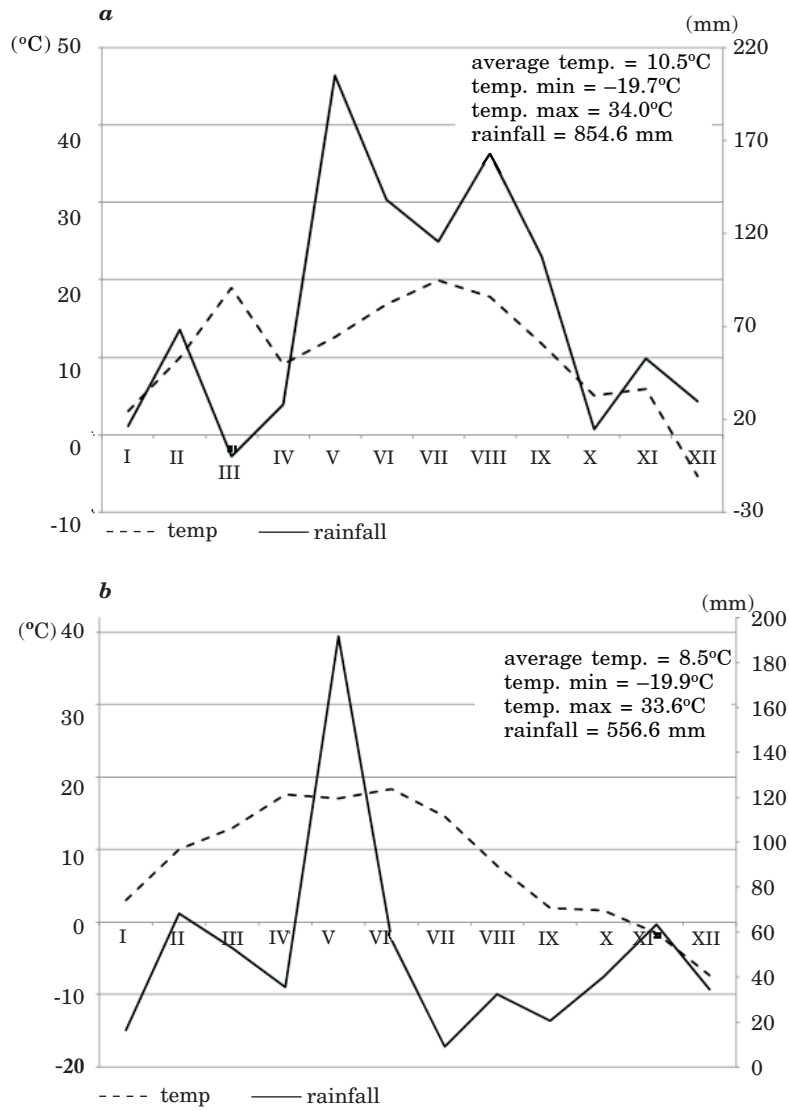

Fig. 1. Climate diagrams for 2010 (*a*) and 2011 (*b*), according to WALTHER and LIETH (1970)

grapevine. Similarly to temperature, rainfall has an effect on the composition of grapevine. Low soil moisture diminishes ions diffusion. On the other hand, the influence of soil moisture on the plant's elemental uptake is dependent on soil nutrient availability. Generally, plants take up trace elements dissolved in the soil solution (KABATA-PENDIAS 2011). In the study of PORRO et al. (2010), the Ca, Mg, S, Mn and B content in leaves and in berries was significantly changed due to water stress. Low levels of these

Table 1

Leaf blade and petiole micronutrient content (mg kg^{-1} d.m.) in grapevine grown under different N fertilization in 2010-2011

Year	N dose (kg ha^{-1})	Leaf sample	B	Cu	Fe	Mn	Zn	Mo
2010	0	blades	42.3	12.4	100.0	156.3	20.7	0.000
	50		31.2	10.3	97.3	111.0	17.3	0.006
	100		30.3	10.5	101.3	144.5	20.7	0.043
	0	petioles	36.0	11.5	27.5	68.1	37.1	0.129
	50		32.4	12.1	30.6	63.6	39.1	0.098
	100		32.7	12.2	27.0	87.6	32.5	0.133
2011	0	blades	29.2	12.0	117.3	396.7	48.7	0.233
	50		29.8	10.6	121.2	289.6	51.9	0.198
	100		29.9	11.4	109.5	370.0	50.9	0.120
	0	petioles	33.2	12.1	30.6	135.7	62.2	0.316
	50		32.9	11.8	30.6	63.1	62.9	0.243
	100		33.0	12.0	30.4	99.0	63.3	0.10
Means	year	2010	34.1	11.5	63.9	105.2	27.9	0.068
		2011	31.4	11.6	73.3	225.7	56.7	0.237
	N dose	0N	35.2	12.0	68.8	189.2	42.2	0.170
		50N	31.6	11.2	69.9	131.8	42.8	0.136
		100N	31.5	11.5	67.1	175.3	41.9	0.151
	leaf sample	blades	32.1	11.2	107.8	244.7	35.0	0.100
		petioles	33.4	12.0	29.4	86.2	49.5	0.205
LSD p=0.05	year (A)		2.16	ns	4.01	24.21	3.00	0.027
	N doses (B)		2.65	ns	ns	29.65	ns	ns
	leaf sample (C)		ns	0.67	4.01	24.21	3.00	0.027
	AxB		3.75	ns	ns	ns	ns	ns
	AxC		ns	ns	5.67	34.23	ns	ns
	BxC		ns	ns	ns	ns	ns	ns
	AxBxC		ns	ns	ns	ns	ns	0.066

elements were found both in leaves and in berries of water-stressed plants. Fallahi et al. (2005) found differences in the Fe blade and petiole concentration between years. The authors explained this phenomenon as an effect of

Table 2

Leaf blade and petiole trace element content (mg kg^{-1} d.m.) in grapevine grown under different N fertilization in 2010-2011

Year	N dose (kg ha^{-1})	Leaf sample	Al	Ba	Cd	Cr	Li	Ni	Pb	Sr	Ti	V
2010	0	blades	33.1	13.7	0.157	0.16	0.067	2.32	1.18	48.6	2.42	0.148
	50		29.9	10.8	0.134	0.11	0.060	2.17	1.38	43.0	2.30	0.125
	100		31.3	15.7	0.139	0.17	0.065	3.15	1.33	52.4	2.59	0.131
	0	petioles	7.73	23.2	0.417	0.07	0.097	0.97	0.67	59.3	0.51	0.082
	50		7.93	18.0	0.285	0.20	0.099	1.26	0.61	59.4	0.36	0.080
	100		9.31	24.5	0.347	0.05	0.126	1.45	0.75	66.6	0.55	0.104
2011	0	blades	24.0	18.5	0.094	0.83	0.040	3.78	1.75	46.9	0.73	0.013
	50		27.7	12.6	0.068	0.72	0.052	1.97	1.50	38.7	1.34	0.025
	100		21.9	14.4	0.094	0.74	0.029	2.69	1.73	34.6	1.46	0.091
	0	petioles	4.60	30.8	0.251	1.04	0.096	2.26	0.49	56.8	0.32	0.049
	50		4.29	21.2	0.121	0.87	0.033	1.46	0.27	48.3	0.28	0.033
	100		3.89	26.4	0.182	1.40	0.048	2.19	0.26	47.0	0.14	0.027
Means	year	2010	19.9	17.7	0.246	0.12	0.086	1.89	0.99	54.9	1.45	0.111
		2011	14.4	20.7	0.135	0.93	0.050	2.39	1.00	45.4	0.71	0.040
	N dose	0N	17.4	21.5	0.230	0.52	0.075	2.33	1.02	52.9	1.00	0.073
		50N	17.5	15.6	0.152	0.47	0.061	1.71	0.94	47.3	1.07	0.066
		100N	16.6	20.3	0.190	0.59	0.067	2.37	1.02	50.2	1.19	0.088
	leaf sample	blades	28.0	14.3	0.114	0.45	0.052	2.68	1.48	44.0	1.81	0.089
		petioles	6.29	24.0	0.267	0.60	0.083	1.60	0.51	56.2	0.36	0.062
LSD p=0.05	year (A)		1.66	2.67	0.0493	0.182	0.0199	0.307	ns	3.51	0.120	0.0139
	N doses (B)		ns	3.26	0.0604	ns	ns	0.376	ns	4.30	0.147	0.0170
	leaf sample (C)		1.66	2.67	0.0493	ns	0.0199	0.307	0.131	3.51	0.120	0.0139
	AxB		ns	ns	ns	ns	ns	0.531	ns	6.08	0.208	ns
	AxC		ns	ns	0.0698	0.257	ns	ns	0.185	ns	0.170	0.0197
	BxC		ns	ns	ns	ns	ns	ns	ns	ns	0.208	ns
	AxBxC		ns	ns	ns	ns	ns	ns	ns	ns	0.294	0.0341

efficient Fe uptake by a more extensive root system created in a year with favourable growth conditions.

In this study, differences in the grape must from the two seasons were found for boron (4.72 and 3.88 mg B kg^{-1} f.w.), copper (0.63 and 0.41 mg Cu kg^{-1} f.w.), iron (2.14 and 1.54), manganese (1.28 and 1.82), aluminium (1.10 and 0.44 mg Al kg^{-1} f.w.), chromium (2.41 and 27.7 µg Cr kg^{-1} f.w.), nickel (58.3 and 56.3) and titanium (36.4 and 28.3 µg Ti kg^{-1} f.w. in the wet 2010 versus the cold but drier 2011, respectively. The content of lead and vanadium in grape juice was under the limit of detection with an ICP spectrometer (Pb <1 ppb, and V <0.07 ppb).

Interactions between nutrients in crop plants are probably among the most important factors affecting yields of plant crops (Marchner 1995). The nitrogen fertilization applied in our experiment significantly influenced the content of microelements and trace elements in leaf blades and petioles. No N-treatments (control with 0 N) resulted in the highest boron and manganese content. In addition, a tendency towards increased copper and molybdenum levels in blades and petioles in control plants was noted. Surprisingly, also the highest cadmium amount was measured for the control in both years and for both petioles and blades. Similar observations albeit on *Brassica* plants were reported by Domagała-Świątkiewicz et al. (2009). N fertilizer containing ammonium significantly decreased the B and Mo content, although the environmental factors considerably modified this tendency. Wolf et al. (1983) showed that increasing N concentration caused increased iron levels in grape tissues. Fallahi et al. (2005) found positive correlation between nitrate-N and Cu blade content. Amiri, Fallahi (2007) showed that nitrogen application did not affect B in grapevine petioles, despite increasing the Mn content. Generally, the lowest content of the trace elements (except Al) was proven for a fertilization treatment with 50 kg N ha^{-1}, which could be attributed to the worst yielding in this treatment and consequently the strongest vegetative growth of grapevine, which in turn might have affected the other patterns of elemental accumulation.

In the present study, significant interaction was determined between climatic conditions during the years of the experiment and N fertilization versus the nickel, strontium and titanium concentrations in tissue samples. In the wet year 2010, an N-dose of 100 kg ha^{-1} significantly increased these elements in blades and petioles in relation to the other treatments. The same trend was observed in 2010 for Mo and Ba in both blades and petioles, and for Mn, Al, Li, Pb and V in grapevine petioles. In the dry 2011, two elements only, that is Cr in petioles and V in blades, increased under the effect of 100 kg N ha^{-1} fertilization (Tables 1 and 2). Rodriguez-Otriz et al. (2006) reported that N fertilizers which contain ammonium can strongly affect accumulation of heavy metals in yield. The acidification of the rhizosphere induced by N supply and by plants (enhanced net excretion of protons or of organic acid) are of particular importance in the acquisition of Fe,

Zn, Mn and Ni in soil (KABATA-PENDIAS 2011). The nickel concentration in leaves of plants grown on uncontaminated soil ranges from 0.05 to 5 mg Ni kg^{-1} d.m. and is the lowest of any element. In our study, the Ni measured content ranged from 0.97 to 3.78 mg kg^{-1} d.m. (Table 2). The content of strontium in edible plants is highly variable and seems to be the highest in vegetables leaves (45-74 mg kg^{-1} f.w.). In cv. Bianca grapevine, the Sr concentration in leaf tissues varied from 34.6 to 66.6 mg kg^{-1} d.m. Strontium occurs in soils as a bivalent cation and is easily taken up by plants (KABATA-PENDIAS, PENDIAS 1999). The solubility of Ti in soils is very limited, and the phytoability of this element is low. The titanium content in food plants ranges from 0.13 to 6.7 mg kg^{-1} d.m. The lowest values are in prepared cereals and fruits (KABATA-PENDIAS 2011). In our study, the Ti concentration in grapevine leaves ranged between 0.14 to 2.59 mg kg^{-1} d.m.

The analysed leaf blades contained higher amounts of iron (107.8 mg), manganese (244.7 mg), nickel (2.68 mg), lead (1.48 mg), titanium (1.81 mg) and vanadium (0.09 mg V kg^{-1} d.w.) as compared to petioles (29.4 mg, 86.2 mg, 1.60 mg, 0.51 mg, 0.36 mg and 0.06 mg V kg^{-1} d.w., respectively) – Tables 1 and 2. In contrast, petioles had more copper (12.0 mg *vs*. 11.2 mg), zinc (49.5 mg *vs.* 35.0 mg), molybdenum (0.205 mg *vs.* 0.100 mg), Cd (0.267 mg *vs.* 0.114 mg), lithium (0.084 mg *vs.* 0.052 mg) and strontium (56.2 mg *vs.* 44.0 mg kg^{-1} d.w. for petioles and blades respectively). A similar effect was obtained ROMERO et al. (2010), who found higher concentration of micronutrients in grapevine leaf blades, except Zn. Also FALLAHI et al. (2005) demonstrated that concentrations of blade Fe and Mn were higher, while blade Zn was lower than in petioles in all of the six examined cultivars. These researchers showed positive correlations between micronutrient concentrations in leaf blades and concentrations of the same elements in petiole tissues.

The chemical composition of grapes depends of many factors, including the cultivar, climatic condition (rainfall, temperature), soil parameters, and vineyard management (VAN LEEUWENA, SEGUIN 2006). Environmental factors such temperature and available water have a significant effect on the nutrient concentration in grapes (COZZOLINO et al. 2010). Trace elements in grapevine fruits are very important, especially for the quality of wine and wine authenticity determination (BAXTER et al. 1997, TAYLOR et al. 2002, GREENOUGH et al. 2005). Generally, the concentration of these elements in fruits and wines is a result of their uptake by plants from soil (GALGANOA et al. 2008). However, several factors besides climatic conditions, such as viticultural techniques and vine production process can modify the chemical composition of wine and alter the relationship between wine and soil composition. In this context, nitrogen nutrition of grapevine is of great importance (BELL, HENSCHKE 2005).

In the present study, the N fertilization influenced composition of elements in grape juice, although the results were inconsistent. The differen-

Table 3

Mean microelement concentration (mg kg^{-1} d.m.) in the must from grapevine grown under different N fertilization in 2010-2011

Year	N dose (kg ha^{-1})	B	Cu	Fe	Mn	Zn	Mo
2010	0	4.50	0.648	2.46	1.40	0.783	0.008
	50	4.70	0.584	1.98	1.14	0.701	0.008
	100	4.98	0.653	1.98	1.31	0.689	0.010
2011	0	3.36	0.415	1.55	1.75	0.888	0.003
	50	4.08	0.325	1.55	1.94	0.898	0.014
	100	4.22	0.477	1.53	1.76	0.877	0.027
Means year	2010	4.72	0.628	2.14	1.28	0.724	0.008
	2011	3.88	0.406	1.54	1.82	0.888	0.015
Means N-dose	0	3.92	0.531	2.00	1.57	0.836	0.005
	50	4.38	0.455	1.77	1.54	0.852	0.011
	100	4.60	0.565	1.76	1.54	0.782	0.018
LSD p=0.05	year (A)	0.80	0.061	0.429	0.342	ns	ns
	N-dose (B)	ns	0.074	ns	ns	ns	ns
	AxB	ns	ns	ns	ns	ns	ns

ces should not be only attributed to environmental factors, but also to the vigour and yielding of grapevine in response to different nitrogen fertilization regimes. While the Cd accumulation in fruits was enhanced by an increased N rate, a reverse tendency was proven for Ba and Sr. Also, the Cu and Ti level in must was differentiated. For plots fertilized with 50 kg N ha^{-1}, the highest Ti and the lowest Cu amounts in grape must were measured (Tables 3 and 4). However, for the other investigated elements, no influence of a nitrogen rate was recorded. The vintage had great impact on the mineral content of grapes. The year 2010 favoured fruit accumulation of Cu, Fe, Al and Ti, whereas the climatic conditions in 2011 increased the content of Mn, Ba and the investigated heavy metals (Cd, Cr and Ni; Table 4). MACKENZIE, CHRISTY (2005) found that grape juice properties including Baumé level and titratable acidity are clearly correlated with several plant available trace elements in soil. Most notable of these were Ca, Sr, Ba, Pb and Si. These authors concluded that soil chemistry had some influence on wine grape composition and such knowledge could be taken advantage of for better vineyard management.

Table 4

Mean trace elements concentration (μg kg^{-1} d.m., except for Al) in grapevine juices under different N fertilization in 2010-2011

Year	N dose (kg ha^{-1})	Al	Ba	Cd	Cr	Ni	Sr	Ti
2010	0	1.12	95.3	6.42	4.16	65.5	225.8	42.6
	50	1.18	63.3	5.16	1.00	47.9	150.5	32.9
	100	1.01	58.3	6.15	2.08	61.8	151.9	33.8
2011	0	0.52	127.1	5.72	28.93	52.4	263.9	11.9
	50	0.40	86.9	7.62	31.19	114.7	188.6	56.3
	100	0.39	76.6	12.83	22.85	121.8	139.2	16.8
Means year	2010	1.10	72.3	5.91	2.41	58.3	176.1	36.4
	2011	0.44	96.9	8.72	27.66	96.3	197.2	28.3
Means N-dose	0	0.82	111.2	6.07	16.54	59.0	244.9	27.3
	50	0.79	75.1	6.39	16.10	81.3	169.5	44.6
	100	0.70	67.5	9.50	12.46	91.8	145.6	25.3
LSD p=0.05	year (A)	0.283	26.6	1.52	0.9	32.6	ns	6.53
	N-dose (B)	ns	32.6	1.96	ns	ns	30.5	8.28
	AxB	ns	ns	2.77	ns	ns	43.6	11.55

CONCLUSIONS

Balanced nutrient supply is one of the most important factors in increasing crop yields and their quality. Plant productivity is directly linked with nutrient availability and uptake. The knowledge of interactions of nutrients and trace elements can help to improve efficiency of nutrient uptake. The present study focused on the effect of nitrogen fertilization on microelements and trace elements in grape plants (leaves and berries). The Cu, Zn, Fe, B and Mo concentrations in the leaves (blades and petioles) of cv. Bianca grapevine were within an optimal range (Mn – high) of content reported for *Vitis vinifera* plants. This experiment showed that on slightly acid soils mineral N fertilizers containing ammonium can enhance the uptake of trace elements such as Ti and V. Nitrogen fertilization depressed the uptake of some elements like B, Mn, Ba, Cd and Sr. The analysed leaf blades contained higher amounts of Fe, Mn, Ni, Pb, Ti and V than petioles. In contrast, petioles had more Zn, Mo, Cd, Li and Sr. Increased N fertilization diminished Ba and Sr must accumulation. Moderate fertilization enhanced Ti and decreased Cu fruit content. The vintage strongly influenced

leaf as well grape mineral content. During the warmer and wetter year 2010, higher amounts of B, Cd, Sr, Ti and V in leaves and Al, Cu, Fe and Ti in grape must were determined. The dry season in 2011 increased the leaf Fe, Mn, Zn, Mo, Ba, Cr and Ni content as well as the amounts of Mn, Ba and heavy metals (Cd, Cr, Ni) in fruits.

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EFFECT OF DIFFERENTIATED PHOSPHORUS AND POTASSIUM FERTILIZATION ON WINTER WHEAT YIELD AND QUALITY

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Abstract

This study was conducted in 2007-2010, on a farm near Śrem (south of Poznań, Poland). A field experiment was set up in a randomized block design with four replications for each combination tested. The effects of differentiated rates of phosphorous and potassium applied together with a fixed level of nitrogen and magnesium fertilization were investigated. During the experiment, the winter wheat grain yields were high and significantly different between both between fertilizer treatments and when compared with the control. Correlation analysis on relationships between grain yield and nutrient content in wheat leaves at the beginning of stem elongation stage (BBCH31) showed significant relationships for phosphorous, calcium, magnesium, zinc and manganese. Regression analysis proved that the content of zinc in leaves at the BBCH31 stage was the main factor which determined winter wheat grain yield. Furthermore, mineral fertilization significantly increased the content of protein and gluten when compared with the control objects, whereas no significant differences were observed among the fertilized objects. Statistically significant relationships were found between leaf content of N, P, Mg, Zn and Mn at BBCH31 and the accumulation of protein and gluten in wheat grain. Protein and gluten in grain depended on the content of magnesium in leaves at the beginning of stem elongation stage. Weather conditions as a factor significantly influenced grain size uniformity while mineral fertilization had no influence on this trait.

Key words: winter wheat, phosphorus and potassium rates, gluten, protein, grain size uniformity.

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WPŁYW ZRÓŻNICOWANEGO NAWOŻENIA FOSFOREM I POTASEM NA PLON I JAKOŚĆ PSZENICY OZIMEJ

Abstrakt

Badania przeprowadzono w latach 2007-2010 w gospodarstwie rolnym w okolicach Śremu. Doświadczenie polowe założono w układzie bloków losowych w czterech powtórzeniach dla każdej kombinacji. Czynnikiem badanym były zróżnicowane dawki fosforu i potasu, przy stałym poziomie nawożenia azotem i magnezem. Plony ziarna pszenicy ozimej w latach badań były wysokie i istotnie różniły się zarówno względem obiektu kontrolnego, jak i wariantów nawożonych. Analiza korelacji między plonem ziarna a zawartością składników w liściach w fazie początku strzelania w źdźbło (BBCH 31) wykazała istotne zależności w przypadku fosforu, wapnia, magnezu, cynku i manganu. Analiza regresji dowiodła, że głównym składnikiem decydującym o plonie ziarna pszenicy była zawartość cynku w liściach w fazie BBCH31. Nawożenie mineralne w porównaniu z wariantem kontrolnym istotnie wpłynęło na zwiększenie również zawartości białka i glutenu, natomiast nie stwierdzono istotnych różnic między obiektami nawożonymi. Wykazano istotną zależność między zawartością N, P, Mg, Zn, Mn w liściach w fazie BBCH 31 a akumulacją białka i glutenu w ziarnie. Zawartość białka i glutenu w ziarnie pszenicy w największym stopniu była determinowana przez zawartość magnezu w liściach na początku strzelania w źdźbło. Czynnikiem istotnie różnicującym wyrównanie ziarniaków były warunki pogodowe, natomiast nawożenie mineralne nie miało wpływu na kształtowanie tej cechy.

Słowa kluczowe: pszenica ozima, dawki potasu i fosforu, gluten, białko, wyrównanie ziarna.

INTRODUCTION

Achievement of the basic goal of fertilization, which is high, stable and good quality yields, requires that phosphorous and potassium should not demonstrate their suppressing effects at any of the plant development stages. Among the nutrients which affect grain quality, nitrogen is most often indicated (Ehdaie, Waines 2001, Shi et al. 2010). Doubtless, application of higher nitrogen rates results in higher yields, although technological parameters of such yields still raise controversies. Besides stimulating yields, high rates of nitrogen can cause worse quality of gluten in grain by increasing the share of low-molecular gliadin (Wooding et al. 2000). For the sake of attaining the producer's and the processor's economic and technological targets, it is important to elaborate such mineral fertilization rates that allow plants to produce high yield, close to the crop's yielding maximum capacity, with good quality characteristics. The risk of yield decline can be minimized by application of mineral fertilization balanced in terms of all nutrient elements (Öborn et al. 2005). The influence of phosphorus and potassium is mainly an aggregate of the functions played by nutrients in mitigating negative effects of biotic and abiotic stresses. Plants provided with sufficient amounts of phosphorus and potassium are less vulnerable to water deficiency, low temperatures and pathogen attacks (Ma et al. 2006). Po-

tassium and phosphorus yield-stimulating functions are different, which stems from their differentiated influence on the plant growth during the vegetation period. These two mineral elements together shape nitrogen management in high-yield cultivation technologies (MARSCHNER 1986). Potassium is an indispensable component during the main stages of protein biosynthesis. Its deficiency leads to a decrease of protein amount produced by a plant, and this effect occurs regardless of the nitrogen nutrition level and accumulation of non-protein nitrogen, the presence of which fosters fungal infections (RICE 2007). Furthermore, potassium deficiency impedes nitrogen uptake and, as a result, the growth of leaf assimilation surface; it also reduces the uptake and transport of nitrates in plants (MARSCHNER et al. 1996). Many researchers have undertaken to evaluate effects of P and K rates on plant yields, but until now information on yield quality traits has been scarce. Relevant literature lacks consistent data concerning the influence of a phosphorus-potassium fertilization level on the technological value of bread wheat cultivated on sites rich in P and K. In this study, a hypothesis was made that differentiated fertilization with P and K before sowing would influence bread wheat yield along with the content of protein and gluten.

The aim of the field study was to evaluate the response of winter wheat in terms of yield volume and quality to optimal and reduced rates of potassium and phosphorus fertilization.

MATERIAL AND METHODS

The study was carried out in 2007-2010, on a farm in Wieszczyczyna, a village near the town of Śrem, Poland, 52º02' N 17º05'E. The research was based on a one-factor experiment conducted on cv. Kris winter wheat. This wheat cultivar belongs to class B of bread wheat varieties. The experiment was part of a long-term research project started in 2000. The trials were set up in a randomized-block design with four replications. The experimental factor was differentiated mineral fertilization with phosphorus and potassium. The experiment was carried out on lessive soil, developed from loam, and lying shallow on glacial till. The soil used in the study was classified as representing quality class IIIb in the Polish soil valuation system. It was rich in available phosphorus (92 mg P kg^{-1} of soil), but the content of available potassium ranged from medium to low (120-80 mg K kg^{-1}) and that of magnesium was medium (37 mg Mg kg^{-1}). The soil reaction was slightly acidic (pH 5.94 1M KCl). Every year, winter wheat was grown after maize. All crop cultivation practices were carried out according to the optimal ones under given agronomic conditions. Taking into account the soil fertility, unit uptake and expected yield of 7 t ha^{-1} during the whole study, an optimal mineral fertilization level (W100) was determined. Every year,

the phosphorus rate was 35 kg ha^{-1}, and the potassium dose was 100 kg ha^{-1} in treatment W100, except in 2007, when the K fertilization level was higher (133 kg K ha^{-1}). Determination of the optimal rate (W100) was performed with the use of NawSald software (IUNG, Puławy, Poland). Based on phosphorus and potassium fertilization levels, which were balanced with regard to nitrogen, the other tested P and K rates were determined by reducing P and K fertilization down to 25% (W25) and 50% (W50) of the optimum balanced treatment (W100). Additionally, control variants were established, where either phosphorus (WKN) or potassium (WPN) was not applied and constant levels of nitrogen and magnesium were maintained. In accordance with the experimental design, fertilization with phosphorus, potassium and magnesium was carried out at the same rate after harvesting preceding crops. Potassium was applied as potassium chloride salt (60% K_2O), phosphorus as single superphosphate and magnesium as kieserite (27% MgO). In the WP1 treatment, phosphorus was applied as Partially Acidulated Phosphate Rock. The WP1 treatment was considered an alternative to single superphosphate as a source of phosphorus. Rock phosphate was also used. It contained 10.2% of phosphorus and its acidification was 50% (i.e. the amount of sulphuric acid used up during the technological processing to obtain the end product was 50% of the amount necessary for production of single superphosphate). Fertilization with nitrogen as ammonium nitrate at a total rate 180 kg N ha^{-1} was carried out four times and divided as specified: (I) before autumn sowing 30 kg (kg N ha^{-1}); (II) before the onset of spring growth 60 kg N ha^{-1}; (III) 3 weeks after application of the second N dose 30 kg N ha^{-1}, and (IV) at the beginning of the earing stage 30 kg N ha^{-1}.

At the beginning of the stem elongation stage (BBCH-31), plants were sampled from all experimental plots for chemical analyses (from 1 linear metre). Plants collected with a harvester from an area of 20 m^2 were used for assessments of grain yield. The plant material was prepared as required for determination of the nutrient content (with atomic absorption spectroscopy) and the following parameters were determined: total protein content (%N x 5.75 – Kjeldahl's method, PN-75A-04018), gluten content (in an Infraanalyser 500) and grain uniformity (standards by the Agency of Agricultural Market).

The results were statistically analyzed with one-factor ANOVA. The years of the experiment were regarded as a random factor and the PK fertilization level was a fixed factor. Multiple regression analysis with choice of the best subset of variables was applied for evaluation of cause and effect relationships between the parameters analyzed. The statistical analyses were performed with the use of Statistica© 10 software.

RESULTS AND DISCUSSION

Grain yield

During the whole experiment, the wheat grain yields were high compared to average winter wheat crops achieved in the last ten years in Poland. The average wheat grain yield obtained from the fertilized treatments was 6.6 t ha^{-1}, 32% higher than from the control (Figure 1). Although the tested wheat cultivar showed a weak response in yield to increasing P and K rates, this did not mean that its nutrient requirements were low, espe-

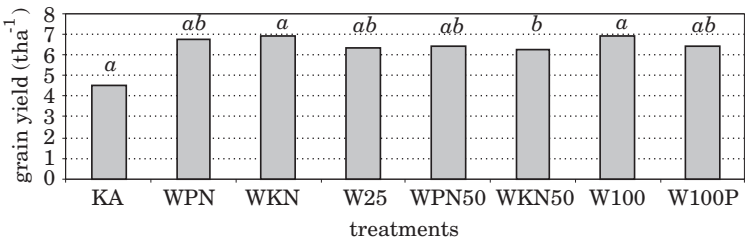

Fig. 1 Yield of grain winter wheat according to phosphorus and potassium fertilization
a,b,c – letters indicate statistical differences between treatments, $p < 0.05$

cially in view of the nutrient uptake (data can be provided by the authors). Wheat responded to a 10-year-long absence of phosphorus and potassium fertilization by a slight yield decrease. Bigger yield appeared in the treatment without phosphorous fertilization (WKN) than without potassium application (WPN). Batten et al. (1984) found that high-yield winter wheat varieties had high requirements with regard to phosphorus, hence this plant should be cultivated on soils rich in phosphorus or else adequately supplemented with high rates of this element. The lack of response of yields produced by wheat as well as other plants to pre-sowing fertilization with phosphorus has been confirmed elsewhere (Gaj 2008, 2010, Rose et al. 2008). Reports on long-term fertilization with phosphorus indicate that a decrease of yield due to absence of phosphorus fertilization becomes demonstrable after longer time (Moskal et. al. 1999, Stępień, Mercik 1999, Kunzova, Hejcman 2010). Potassium is rarely referred to in the world's literature as an element limiting plant production, although it is most often pointed out as the main nutrient shaping the quality of products (Usherwood 1985, Whitehead 2000).

Wheat grain yield significantly depended on plant nutrition at the beginning of stem elongation stage (BBCH 31) – Table 1. Regression analysis showed significant positive influence of leaf content of nitrogen, potassium, magnesium and zinc on wheat grain yield (equation 1). Negative relationship was observed only in the case of copper. The level of plant nutrition with phosphorous, calcium, manganese and iron at that plant development phase had no considerable effect on grain yield.

Table 1

Correlation coefficients between yield of winter wheat, quality parameters and nutrients content in critical state (n=128)

Variable	Nutrient								
	N	P	K	Mg	Ca	Zn	Cu	Mn	Fe
Yield	0.11	0.221*	0.165	0.335*	-0.220*	0.458*	-0.16	0.363*	0.203*
Gluten content	0.211*	0.335*	-0.07	0.379*	-0.08	-0.217*	0.264*	0.216*	0.289*
Protein content	0.238*	0.253*	-0.01	0.283*	-0.07	-0.176*	0.132	0.241*	0.307
Grain uniformity	0.085	-0.433*	0.289*	-0.492*	0.303*	0.266*	-0.183*	-0.209*	-0.301*

*correlation significant at $p < 0.05$

$$(1)\ Y(\text{t ha}^{-1}) = 0.12(Zn) + 6.64(Mg) + 0.43(N) + 0.77(K) - 0.57(Cu) + 1.65$$

$$R^2 = 0.49;\ \ n = 128;\ \ p < 0.000$$

Zinc content in winter wheat leaves at the beginning of stem elongation stage determined wheat grain yield, and explained 36.4% of the variability (equation 2).

$$(2)\ Y(\text{t ha}^{-1}) = 0.195(Zn) + 2.88 \qquad R^2 = 36.4\%;\ n = 128;\ p < 0.000$$

The increase in the zinc content in plants at the beginning of stem elongation by 1 mg kg^{-1} resulted in an increase in the wheat grain yield by an average 195 kg ha^{-1}.

Statistically significant interaction between the experimental factor and years of observations was found. Most advantageous weather conditions for wheat yielding were observed in 2009, whereas the lowest wheat yields were obtained in 2008. The difference in yields between the extreme years was 33%. The irregular distribution of precipitations in the months when the most intensive plant growth takes place (April, May and June) was the main reason for limited nutrient uptake from soil and, consequently, the observed yield reduction.

Evaluation of grain quality

The qualitative cereal analysis comprised three features: protein content, gluten content and grain size uniformity. The amount and quality of protein substances are two of the main criteria of wheat technological usefulness. The winter wheat variety tested in this study, cv. Kris, belongs to the quality group B, which means that its genetically conditioned parameters such as protein or gluten content are on a medium level (between quality wheat and forage wheat) (Gacek 2002). Regardless of the year of observation, treatment-specific differences in the analyzed parameters were mainly demonstrated by the significance of the comparison of the absolute control treatment (no NPK fertilization) with the other treatments (Table 2).

Table 2

Effect of phosphorus and potassium fertilization on technological quality grain of winter wheat

Factors		Feature		
		gluten content (%)	protein (%)	grain uniformity (%)
Years	2007	21.95*d*	10.94*c*	88.91*a*
	2008	28.84*a*	13.10*a*	56.66*d*
	2009	24.33*c*	11.77*b*	74.48*b*
	2010	25.58*b*	12.10*b*	64.69*c*
Treatments	control (KA)	19.86*b*	10.17*b*	78.32*a*
	WNK	26.24*a*	12.38*a*	70.08*a*
	WNP	26.14*a*	12.33*a*	71.44*a*
	W25	25.74*a*	12.01*a*	67.58*a*
	WPN50	25.62*a*	12.11*a*	72.94*a*
	WKN50	26.36*a*	12.39*a*	67.69*a*
	W100	25.59*a*	1203*a*	70.90*a*
	W100P (P as PAPR)	25.84*a*	12.23*a*	72.53*a*
Interaction: year×treatments		*	*	*

* signifficantly different;
a,b,c, - letters indicate statistical differences between treatments, $p < 0.05$

Differentiation of P and K rates had no significant influence on protein and gluten accumulation in grain. The content of gluten indicated a proven linear relationship with the total protein content (Figure 2). Relationships between the total grain protein content and the grain content of gluten are very complex and largely determined by the variety (Grzebisz, Gaj 2009). The content of gluten observed in this study was at the threshold level for bread winter wheat (26%), which corresponds to at least 12.0% accumulation of protein in grain, The content of protein in the control variant was lower than the standard value, and equalled 10.2%. Fertilization is one of the main factors which determine both the volume of wheat yields and the amount of protein and gluten in wheat grain (Flynn et al. 1987, Shewry et al. 1995). The results of Galantini et al. (2000) indicated that fertilization did not increase dry matter, but significantly influenced nutrient accumulation in grain. The results of the present study showed that increasing phosphorus rates had no direct effect on raising the content of protein and gluten in grain, whereas within the locations which had not been fertilized with phosphorus (treatment WNK) or potassium (treatment WNP) for ten years,

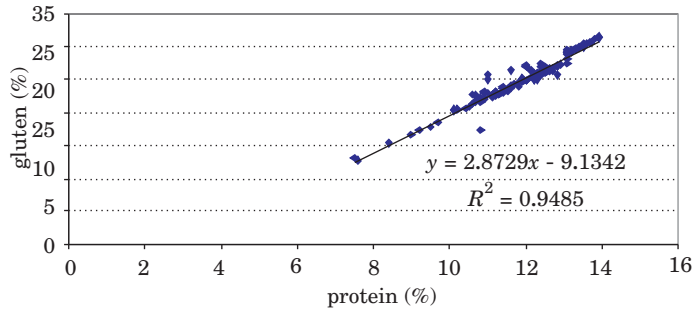

Fig. 2. Gluten content in grain as function of protein content

a tendency was observed for a slight increase in values of the analyzed parameters when compared to the variant with an optimum nitrogen balance (W100). Dick et al. (1985) reported a protein content decrease in barley grain under influence of potassium fertilization. The effect of potassium on the quality traits of plant products is revealed indirectly, through activation of certain enzymes responsible for basic and specific metabolic processes in plants (Szczepaniak 2004). The effect of phosphorus on the content of protein in grains has not been defined unambiguously in literature, hence the issue remains problematic. Available information concerns the volume of yield rather than its quality. It is generally believed, however, that phosphorus slightly affects grain proteins in winter wheat (Cambell 1996, Batten et al. 1999, 1992, Benbella, Paulsen et al. 1998). During the present study, no differences were observed in the analyzed parameters with regard to the form of phosphorus fertilizer applied. The content of protein in grain is directly connected with the overall available nitrogen, both from mineral fertilizers and from mineralization processes in soil. Besides, the wheat grain protein content is strongly connected to temperature, especially at the phase of grain filling (Podolska, Sułek 2003, Budzyński et al. 2004, Shi et al. 2010).

Quality parameters, such as protein and gluten content as well as grain size uniformity, were strongly influenced by the weather conditions. The ANOVA test showed statistical significance of the interaction between the year and the rate of PK fertilization in determination of the tested parameters (Table 2). An average protein content in the fertilized treatments fluctuated within a very narrow range from 12.01 to 12.4. Olson (1984) emphasized that the factors which increased grain yield often caused a decrease of protein content, since yield increase resulted in the dilution effect. According to this author, additional phosphorus or moderate rates of nitrogen can counteract such processes. Berecz (2001) pointed out that the influence of phosphorus fertilization on wheat quality parameters depended largely on the N:P ratio. The issue of interaction between nitrogen and phosphorus is referred to in many publications (Summer, Farina 1986, Kim 2003, Prystupa et al. 2004, Sadras 2006].

The highest amounts of gluten and protein in wheat grain were observed in 2008, which was characterized by low precipitation and high temperature (Table 3). Wheat plants produced relatively less vegetation matter under water stress caused by drought and therefore a larger pool of nitrogen was accumulated in grain. High temperature causes fast hydrolysis of leaf proteins so that the stage of grain filing lasts shorter, which generates lower weight of grain but high grain protein content (CORBELLINI et al. 1998, DANIEL, TRIBOI 2002, FLAGELLA et al. 2010). Also, HAJHEIDARI et al. (2007) observed an increase in the content of gliadins in bread wheat varieties under water stress consistently with an increase in protein content. According to TRIBOĎ et al. (2003), differences in the composition of the protein fraction at plant maturity due to post-anthesis temperatures or drought after the flowering phase, and are primarily caused by differences in the amount of nitrogen accumulated during the grain filling phase. occurs mainly because of differences in the total quality of nitrogen accumulated during grain filling.

Another feature assessed was grain size uniformity. Differentiation of mineral fertilization had no significant effect on variability of this feature, although the effect of weather conditions (Table 2) is noteworthy. In 2008 and 2010, grain uniformity observed on all treatments was below the standard value (< 75%). Poor grain uniformity indicates high sensitivity of the tested wheat variety to weather conditions at the stage of grain filling. Although the quality standards for the content of protein and gluten (the Agency of Agricultural Market norms) were met, poor grain uniformity in 2008 and 2010 disqualified the grain yield for milling.

Table 3

Weather conditions during vegetation of winter wheat

Vegetation season	Months											
	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apr	May	June	July
Temperature (ºC)												
2006/2007	18.1	17.1	11.1	6.6	4.5	4.6	0.9	6.1	10.1	15.2	19.2	18.7
2007/2008	18.7	13.1	7.9	2.6	1.1	2.0	4.0	4.1	8.5	14.2	18.7	19.8
2008/2009	18.6	13.1	9.0	5.5	1.1	-3.3	-0.3	3.8	11.1	13.4	15.9	19.6
2009/2010	19.1	14.7	7.3	6.2	-1.0	-7.0	-1.7	3.6	8.9	12.3	17.6	21.9
Multi-year	17	13.1	8.3	3.2	0.4	-1.2	-0.4	3.2	7.8	13.6	16.6	18.3
Precipitation (mm)												
2006/2007	148	23	35	47	42	86	49	50	6	78	66	112
2007/2008	48	23	15	34	26	65	41	44	5	42	48	126
2008/2009	48	23	15	34	26	72	23	34	38	17	12	46
2009/2010	62	16	61	18	20	25	49	56	19	69	101	86
Mult-year	64	45	40	42	36	30	39	36	51	62	74	64

Plant nutrition and yield quality

According to GRZEBISZ et al. (2003), the basic question in discussions on the effect of fertilization on agricultural produce quality is the assessment of reactions between the plant nutritional status and the quality of plant products. Regression analysis on the relationship between leaf nutrient contents at the BBCH31 stage (in literature referred to as the critical phase; BERGMANN, 1992) and the content of protein (equation 3) and gluten (equation 4) in wheat grain showed positive relationships for nitrogen, magnesium, phosphorus and iron, but negative one for zinc. The equations supported the presumed hypothesis on the effect of phosphorus on grain qaulity.

$$(3)\ Y = 0.67N + 6.62Mg + 4.67P + 0.01Fe - 0.10Zn + 7.75$$

$$R^2 = 0.46;\ n = 128;\ p < 0.000$$

$$(4)\ Y = 2.10N + 20.30Mg + 14.73P + 0.03Fe - 0.35Zn + 13.05$$

$$R^2 = 0.48;\ n = 128;\ p < 0.000$$

The stepwise multiple regression analysis with backward elimination and choice of best variable sub-sets showed that protein and gluten content in grain was determined most strongly by the content of magnesium (equations 5 and 6).

$$(5)\ Y(\%\ \text{protein}) = 6.50(Mg) + 10.90 \quad R^2 = 15.41\%;\ n = 128;\ p < 0.000$$

$$(6)\ Y(\%\ \text{gluten}) = 19.15(Mg) + 22.08 \quad R^2 = 14.74\%;\ n = 128;\ p < 0.000$$

The plant nutritional status at the BBCH31 stage significantly influenced grain size uniformity (Table 1). Regression analysis showed significant positive relationship only for zinc (equation 7**).**

$$(7)\ Y = 1.44Zn - 122.8Mg - 5.73Cu - 0.09Cu + 99.18$$

$$R^2 = 55.08\%;\ n = 128;\ p < 0.000$$

CONCLUSIONS

1. On soils rich in phosphorus and potassium, winter wheat yielding response to different P and K rates was significantly differentiated, which implies that this crop has high demand for nutrient-rich soils.

2. Mineral fertilization significantly increased the protein and gluten content in grain only in comparison with the control, and the differentiation of P and K rates had no effect on the differences between the treatments.

3. Significant relationship was found between the plant nutritional status at the beginning of stem elongation phase (BBCH 31) and bread wheat

quality. Regression analysis showed that the protein and gluten content were most strongly dependent on the content of magnesium in wheat leaves at this plant development stage.

4. Weather conditions constituted a factor which significantly differentiated grain size uniformity, while mineral fertilization had no influence on this feature.

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ACCUMULATION OF POTASSIUM, CALCIUM AND MAGNESIUM BY SELECTED SPECIES OF GRASSLAND LEGUMES AND HERBS

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Abstract

The study was conducted in 1998-2000, in the Olsztyn Lakeland, on permanent grassland communities with at least 5% share of the following leguminous and herbaceous species. *Trifolium pratense*, *Trifolium repens*, *Lotus corniculatus*, *Lathyrus pratensis*, *Lotus uliginosus*, *Vicia cracca*, *Taraxacum officnale*, *Achillea millefolium*, *Plantago lanceolata*, *Alchemilla vulgaris*, *Heracleum sibiricum* and *Cirsium oleraceum*. In total, 444 plant samples were analyzed, including 123 collected on organic soils. The objective of this study was to determine the accumulation of potassium, calcium and magnesium by selected species of grassland legumes and herbs, in view of the abundance of the above elements in soil.

The analyzed organic soils were characterized by low abundance of potassium and moderate abundance of magnesium, whereas the mineral soils had a very low or low potassium content and a very high or high magnesium content. The habitats varied widely with respect to the calcium abundance. The biomass of the analyzed plant species contained high concentrations of potassium, magnesium and calcium. Plants collected from mineral soils contained more potassium and less magnesium than those growing on organic soils. *Taraxacum officinale* and *Achillea millefolium* were rich in potassium, *Achillea millefolium*, *Lotus uliginosus*, *Heracleum sibiricum*, *Vicia cracca*, *Taraxacum officinale* and *Cirsium oleraceum* had a high magnesium content, whereas *Cirsium oleraceum*, *Heracleum sibiricum* and *Alchemilla vulgaris* accumulated the largest amounts of calcium. The ability of dicotyledonous plants to accumulate high concentrations of calcium and magnesium resulted in a low K:(Ca+Mg) ratio.

Key words: grasslands, legumes, herbs, potassium, magnesium, calcium, soil.

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GROMADZENIE POTASU, WAPNIA I MAGNEZU PRZEZ WYBRANE GATUNKI ROŚLIN MOTYLKOWATYCH I ZIÓŁ ŁĄKOWYCH

Abstrakt

Badania przeprowadzono w latach 1998-2000 na terenie Pojezierza Olsztyńskiego. Badaniami objęto zbiorowiska roślinne trwałych użytków zielonych z co najmniej 5% udziałem wybranych gatunków roślin motylkowatych i ziół: *Trifolium pratense*, *Trifolium repens*, *Lotus corniculatus*, *Lathyrus pratensis*, *Lotus uliginosus*, *Vicia cracca*, *Taraxacum officinale*, *Achillea millefolium*, *Plantago lanceolata*, *Alchemilla vulgaris*, *Heracleum sibiricum*, *Cirsium oleraceum*. Łącznie przebadano 444 próby roślinne, w tym 123 pochodzące z gleb organicznych. Celem pracy było określenie zawartości potasu, wapnia i magnezu w wybranych roślinach motylkowatych i ziołach na tle zasobności tych pierwiastków w glebie.

W badanych siedliskach gleb organicznych stwierdzono niską zasobność w potas, średnią w magnez, zaś w glebch mineralnych – bardzo małą i małą zawartość potasu oraz dużą i bardzo dużą magnezu. Stwierdzono również duże zróżnicowanie siedlisk pod względem zasobności w wapń. Roślinność zawierała dużo potasu, magnezu i wapnia, przy czym rośliny pochodzące z siedlisk gleb mineralnych zawierały więcej potasu, a mniej magnezu niż pochodzące z gleb organicznych. Spośród badanych gatunków bogate w potas były *Taraxacum officinale* i *Achillea millefolium*, dużą zawartość magnezu stwierdzono u *Achillea millefolium*, *Lotus uliginosus*, *Heracleum sibiricum*, *Vicia cracca*, *Taraxacum officinale* i *Cirsium oleraceum*, zaś najwięcej wapnia gromadziły *Cirsium oleraceum*, *Heracleum sibiricum* i *Alchemilla vulgaris*. Zdolność roślin dwuliściennych do gromadzenia dużych ilości Ca i Mg wpłynęła na niską wartość stosunku K:Ca+Mg).

Słowa kluczowe: użytki zielone, motylkowate, zioła, potas, magnez, wapń, gleba.

INTRODUCTION

Habitat conditions have a significant effect on the quantitative and qualitative composition of grassland communities comprising numerous species of grasses, legumes, sedges (Cyperaceae), herbs and weeds. The characteristics of grassland habitats are an important consideration since they affect the chemical composition of green forage (TRĄBA, WYŁUPEK 1998, TRZASKOŚ et al. 1998, TRĄBA, WOLAŃSKI 2003, GRZEGORCZYK et al. 2004, 2011). According to TRĄBA (1997), forage obtained from grasslands with a high share of dicotyledonous plant species is a rich source of phosphorus, magnesium, calcium and sodium, but it has a low potassium content. Herbs present in the phytomass used as livestock feed contribute to preventing microelement deficiencies, thus increasing the biological value of feed to adequately meet the nutritional requirements of animals (BENEDYCKI et al. 1999).

The objective of this study was to determine the accumulation of potassium, calcium and magnesium by selected species of grassland legumes and herbs, in view of the abundance of the above elements in soil.

MATERIAL AND METHODS

The study was conducted in 1998-2000, in the Olsztyn Lakeland, on permanent grassland communities with at least 5% share of the following leguminous and herbaceous species. *Trifolium pratense*, *Trifolium repens*, *Lotus corniculatus*, *Lathyrus pratensis*, *Lotus uliginosus*, *Vicia cracca*, *Taraxacum officnale*, *Achillea millefolium*, *Plantago lanceolata*, *Alchemilla vulgaris*, *Heracleum sibiricum* and *Cirsium oleraceum*. In total, 444 plant samples were analyzed, including 123 collected on organic soils (Table 1). The chemical analyses of soil samples were performed by standard methods: potassium - by Egner-Riehm method, magnesium – by Schachtschabel method, calcium – by a universal method proposed by Nowosielski. Plant samples were assayed for potassium and calcium content by flame photometry, and for magnesium content – by atomic absorption spectrometry (AAS).

Table 1

Number of analyzed plant samples

Species	Habitat	
	mineral soil	organic soil
Lathyrus pratensis	29	13
Lotus corniculatus	23	-
Lotus uliginosus	22	29
Trifolium pratense	25	-
Trifolium repens	26	-
Vicia cracca	20	14
Achillea millefolium	33	19
Alchemilla vulgaris	31	17
Cirsium oleraceum	18	31
Heracleum sibiricum	29	-
Plantago lanceolata	32	-
Taraxacum officinale	33	-
Total	321	123

RESULTS AND DISCUSSION

The chemical analyses of samples collected in grassland habitats showed that the average available potassium content of organic and mineral soils was very low or low, at 139.1-300.2 mg kg^{-1} (Figure 1) and 49.7-110.2 mg kg^{-1},

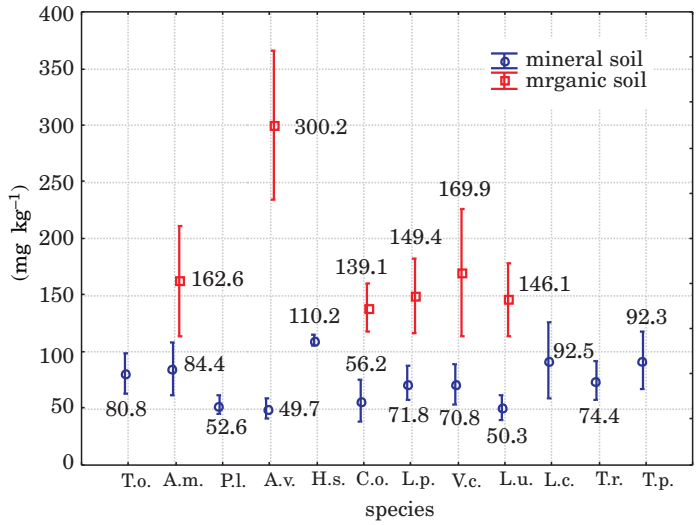

Fig. 1. Potassium content of soil (means and 95.00% confidence interval)

respectively. The communities with *Alchemilla vulgaris* on organic soils and the communities with *Heracleum sibiricum* on mineral soils were most abundant in potassium. Low potassium concentrations noted at many sampling sites corroborate the findings of MICHNA (1997), who reported potassium deficiency in 85.5% of grassland soils in north-eastern Poland.

The analyzed plant species were characterized by a high potassium content of phytomass, except for *Lotus uliginosus* growing on organic soils, whose potassium content reached 14.3 g kg^{-1}. Plant species collected from organic soils, more abundant in potassium, contained less potassium than those collected from mineral soils (Figure 2). Particularly high potassium concentrations were noted in *Taraxacum officinale* on mineral soils, which accumulated significantly more potassium than the other analyzed species. The biomass of *Achillea millefolium* was also characterized by a high potassium content. In general, herbs accumulated greater amounts of potassium than legumes, which is why the presence of the former in grassland sward increased the potassium content of forage. The potassium content of grassland vegetation varied over a wide range, from 6.0 to 80.0 g kg^{-1} d.m., depending on species. Herbaceous plants accumulated high amounts of potassium, in excess of 80.0 g kg^{-1} d.m. Forage from permanent grasslands usually provides more potassium than needed by animals. The optimum potassium content of grassland feeds is 17.0 g kg^{-1} d.m., and potassium concentrations above 30.0 g kg^{-1} d.m. are considered undesirable in grassland-based feeding regimes (JANKOWSKA-HUFLEJT et al. 2009).

Organic soils contained significantly more magnesium than mineral soils, and average magnesium abundance at sampling sites ranged between 572.1

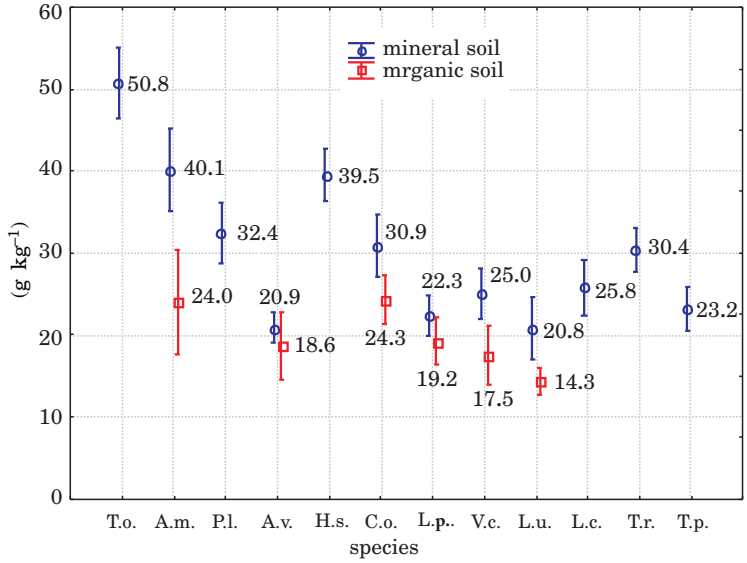

Fig. 2. Potassium content of plants (means and 95.00% confidence interval)

and 711.5 mg kg^{-1} (Figure 3). The average available magnesium content of mineral soils was very high or high, reaching 68.0 to 149.3 mg kg^{-1}. In organic soils, the highest magnesium levels were noted in grasslands communities with *Alchemilla vulgaris* and *Cirsium oleraceum*. The communities with *Achillea millefolium, Lathyrus pratensis, Vicia cracca* and *Lotus uliginosus* were less abundant in magnesium. The differences in magnesium concentrations between organic soil habitats were statistically non-significant, while considerable differences were observed within habitats. In mineral soils, smaller differences in magnesium abundance were noted between plant communities, and higher magnesium levels were reported from the habitats of *Lathyrus pratensis, Vicia cracca, Lotus uliginosus* and *Cirsium oleraceum*. The habitats of *Plantago lanceolata* were characterized by the lowest magnesium content. The above relationships were also described by Trąba and Wolański (2003), and Kitczak (2000).

The magnesium abundance of soil was reflected in the magnesium content of plant dry matter. Plant species collected from organic soils contained significantly more magnesium than those obtained from mineral soils (Figure 4). In general, all analyzed species had a high magnesium content. In the majority of cases, magnesium concentrations in plant dry matter were higher than the optimum magnesium content determined by Falkowski et al. (2000). In organic soils, the largest amounts of magnesium were accumulated by *Achillea millefolium*, *Lotus uliginosus*, *Heracleum sibiricum* and *Vicia cracca.* No significant differences in magnesium abundance were found between those species, while there were considerable differences between

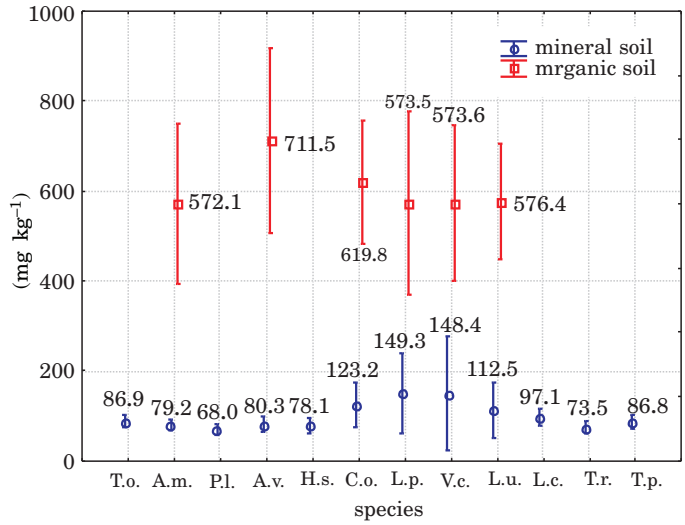

Fig. 3. Magnesium content of soil (means and 95.00% confidence interval)

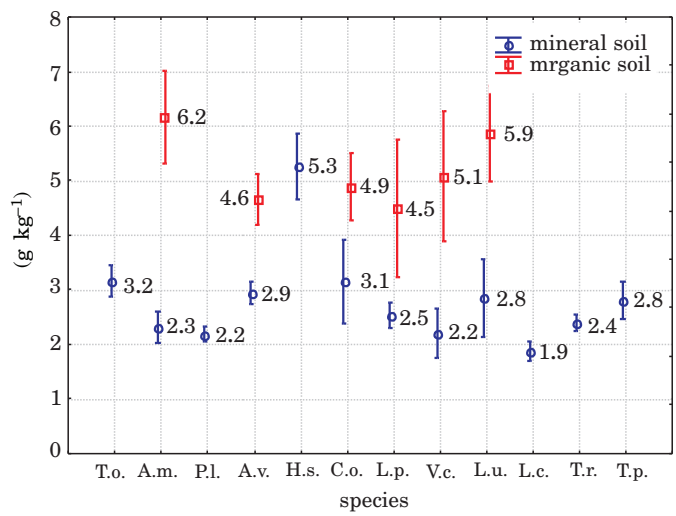

Fig. 4. Magnesium content of plants (means and 95.00% confidence interval)

sampling sites. Smaller differences in the magnesium content of plants were observed on mineral soils. No significant fluctuations in magnesium levels were noted, except for *Cirsium oleraceum* and *Lotus uliginosus*. *Taraxacum officinale* and *Cirsium oleraceum* from mineral soil habitats had a high magnesium content, whereas *Lotus corniculatus* was characterized by the lowest magnesium concentrations. High magnesium levels in the biomass

of common dandelions and cabbage thistles were also reported by ALBERSKI (2004), and KOZŁOWSKI and SWĘDRZYŃSKI (1996).

Substantial differences in the calcium abundance of soil in grassland habitats were noted in the present study (Figure 5). The calcium content of organic and mineral soils varied from 1404.6 to 1895.3 mg kg $^{-1}$, and from 662.5 to 1089.1 mg kg $^{-1}$, respectively. In organic soils, the habitats of *Achillea millefolium* were most abundant in calcium, while the communities with *Lathyrus pratensis* were poorest in calcium. In mineral soils, the highest calcium abundance was noted in the habitats of *Lotus corniculatus* and *Lathyrus pratensis,* whereas the communities with *Plantago lanceolata* and *Alchemilla vulgaris* were less abundant in calcium.

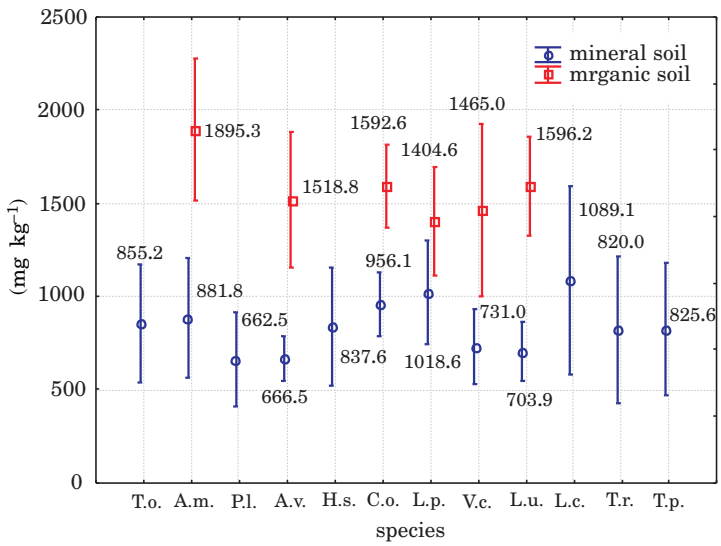

Fig. 5. Calcium content of soil (means and 95.00% confidence interval)

Fodder plants should contain 7 g kg^{-1} calcium. In our study, the average calcium content of plant dry matter was higher than the levels considered optimal for high-quality feeds. The cabbage thistle was characterized by a particularly high calcium content (40.6-42.0 g kg $^{-1}$ d.m.). Regardless of the soil type, this species accumulated significantly higher amounts of calcium than the other studied species (Figure 6). ALBERSKI (2004) also reported high calcium concentrations in *Cirsium oleraceum*. A high calcium content of biomass was noted in *Heracleum sibiricum* (33.6 g kg^{-1}) from mineral soil habitats, and in *Alchemilla vulgaris* (24.0 g kg^{-1}) from organic soil habitats. The remaining species were marked by similar calcium levels, ranging from 14.1 to 20.6 g kg $^{-1}$ d.m. Our results are partially consistent with the findings of TRĄBA (1997). According to the cited author, forage from grasslands

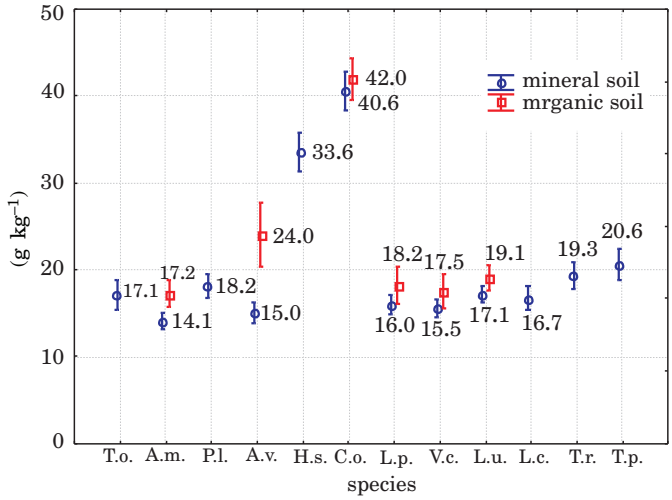

Fig. 6. Calcium content of plants (means and 95.00% confidence interval)

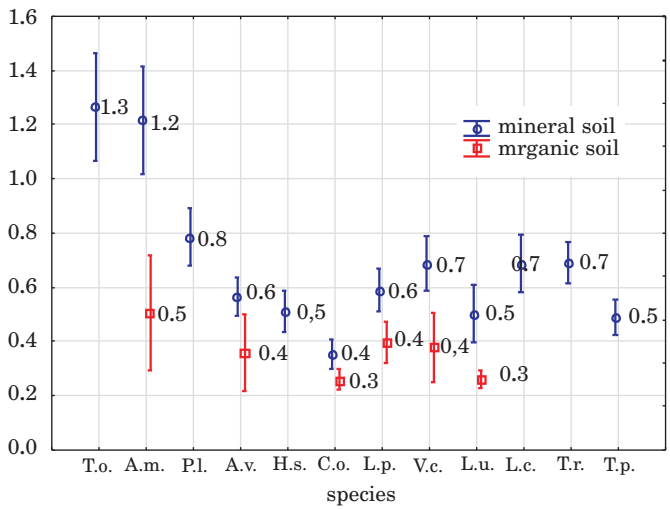

Fig. 7. K: (Ca+Mg) equivalent ratio (means and 95.00% confidence interval)

with a high share of dicotyledonous plants is usually abundant in magnesium and calcium, and poor in potassium. In the present experiment, plants contained also large amounts of potassium.

A synthetic criterion for feed quality assessment is the K: (Ca+Mg) ratio, which should remain within the 1.8-2.2 range. Values higher than 2.2 may be indicative of grass tetany. In the analyzed dicotyledonous plants, the average value of the K: (Ca+Mg) ratio ranged from 0.3 to 1.3, thus being far too low (Figure 7). Dicotyledonous plants accumulate considerable amounts

of calcium and magnesium, and relatively low amounts of potassium, which had a direct effect on the above ratio (TRZASKOŚ et al. 1998). A lower K: (Ca+Mg) ratio was characteristic for PLANTS growing on organic soils. Among the studied species, significantly higher values of the above ratio were noted for *Taraxacum officinale* and *Achillea millefolium*, due to the fact that these species accumulated significantly higher concentrations of potassium than the other species.

CONCLUSIONS

1. The organic soils were characterized by low abundance of potassium and moderate abundance of magnesium, whereas the mineral soils had a very low or low potassium content and a very high or high magnesium content. The habitats varied widely with respect to calcium abundance.

2. The biomass of the analyzed plant species contained high concentrations of potassium, magnesium and calcium. The plants collected from mineral soils contained more potassium and less magnesium than those growing in organic soils.

3. Taraxacum officinale and *Achillea millefolium* were rich in potassium, *Achillea millefolium*, *Lotus uliginosus*, *Heracleum sibiricum*, *Vicia cracca*, *Taraxacum officinale* and *Cirsium oleraceum* had a high magnesium content, whereas *Cirsium oleraceum*, *Heracleum sibiricum* and *Alchemilla vulgaris* accumulated the largest amounts of calcium.

4. The ability of dicotyledonous plants to accumulate high concentrations of calcium and magnesium resulted in a low K: (Ca+Mg) ratio.

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EFFECT OF CHEMICAL CROP PROTECTION ON THE CONTENT OF SOME ELEMENTS IN GRAIN OF SPELT WHEAT (*TRITICUM AESTIVUM* SSP. *SPELTA*)

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Abstract

The aim of the present study was to evaluate the effect of chemical crop protection on the content of N, P, K, Mg, Zn, Cu, Mn, and Fe in grain of 8 spelt wheat cultivars (Franckenkorn, Badengold, Schwabenspelz, Oberkulmer Rotkorn, Ostro, Ceralio, Schwabenkorn, and Spelt I.N.Z.). Chemical protection involved application of a fungicide, two herbicides and a retardant. No plant protection agents were used in the control treatment. The above spelt cultivars were grown in a monoculture on medium heavy mixed rendzina soil. The study was carried out in 2009-2011, at the Bezek Experimental Farm, which belongs to the University of Life Sciences in Lublin.

Among the spelt wheat cultivars compared, grain of cv. Ostro was characterized by the highest content of nitrogen, phosphorus and manganese, whereas grain of cv. Franckenkorn contained the largest amounts of potassium and magnesium. The highest amount of zinc was found in grain of cv. Oberkulmer Rotkorn and that of copper – in grain of cv. Spelt I.N.Z., while grain of cv. Schwabenkorn was found to be the richest in iron. Irrespective of the cultivar, chemical plant protection significantly increased the copper content in spelt grain and simultaneously decreased the magnesium content. The content of N, Mg, Zn, Cu, and Mn in grain decreased in the successive years of the study.

Key words: spelt wheat, cultivars, chemical protection, grain chemical composition.

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WPŁYW CHEMICZNEJ OCHRONY ŁANU NA ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW W ZIARNIE PSZENICY ORKISZ (*TRITICUM AESTIVUM* SSP. *SPELTA*)

Abstrakt

Celem badań była ocena wpływu chemicznej ochrony łanu na zawartości N, P, K, Mg, Zn, Cu, Mn i Fe w ziarnie 8 odmian pszenicy orkisz (Franckenkorn, Badengold, Schwabenspelz, Oberkulmer Rotkorn, Ostro, Ceralio, Schwabenkorn, Spelt I.N.Z.). Ochrona chemiczna polegała na zastosowaniu fungicydu, dwóch herbicydów oraz retardanta. W obiekcie kontrolnym nie stosowano żadnych środków ochrony roślin. Wymienione odmiany orkiszu uprawiano po sobie na średnio ciężkiej rędzinie mieszanej. Badania przeprowadzono w latach 2009-2011 w Gospodarstwie Doświadczalnym Bezek należącym do Uniwersytetu Przyrodniczego w Lublinie.

Spośród porównywanych odmian pszenicy orkisz największą zawartość azotu, fosforu i manganu stwierdzono w ziarnie odmiany Ostro, natomiast najwięcej potasu i magnezu zawierało ziarno odmiany Franckenkorn. Największą zawartość cynku stwierdzono w ziarnie odmiany Oberkulmer Rotkorn, miedzi – w ziarnie odmiany Spelt I.N.Z., zaś żelaza w ziarnie odmiany Schwabenkorn. Niezależnie od odmiany, chemiczna ochrona roślin istotnie wpłynęła na zwiększenie zawartości miedzi w ziarnie orkiszu i jednocześnie zmniejszenie zawartości magnezu. Zawartość N, Mg, Zn, Cu oraz Mn w ziarnie zmniejszała się w kolejnych latach badań.

Słowa kluczowe: pszenica orkisz, odmiany, ochrona chemiczna, skład chemiczny ziarna.

INTRODUCTION

The interest in spelt cultivation is growing continually. It is stimulated by the introduction of eco-friendly technologies and by consumers' growing interest in new products (Sulewska 2004ab, Tyburski, Żuk-Gołaszewska 2005, Sulewska et al. 2008). Spelt is more resistant to diseases and environmental stresses than common wheat (Baumgärtel-Blaschke 1992). On the other hand, it has traits typical of wild wheat varieties, such as a brittle rachis, poor threshing and a significant height, which makes it more vulnerable to lodging. As a result, it is difficult to grow spelt wheat on a large scale (Campbel 1997). Owing to the enclosing glumes, spelt grain does not need to be dressed before sowing (Winzeler, Rüegger 1990). Hence, it can be successfully grown at both conventional and ecological farms (Pałys, Kuraszkiewicz, 2003, Andruszczak et al. 2011, Kwiecińska-Poppe et al. 2011).

For consumers and with respect to the nutritional value of raw material, the content of elements in quality wheat grain is an important trait (Stanisławska-Glubiak et al. 1996, Czuba, 2000, Gembarzewski 2000, Kocoń 2005, Stanisławska-Glubiak, Korzeniowska 2007, Korzeniowska 2008). Spelt wheat is characterized by better biochemical composition of grain compared to common wheat cultivars (Campbell 1997, Sulewska et al. 2008). Generally, it contains more gluten-rich protein and also has more zinc, copper and selenium

(Baumgärtel-Blaschke 1992, Grela 1996, Rachoń, Szumiło 2009). The linoleic acid is predominant in the composition of fatty acids in spelt grain. This grain contains more vitamins from the groups A, E and D. The largest amount of gamma- and alpha-tocopherols is found in the composition of vitamin E and their content is higher than in wheat grain (Tyburski, Żuk-Gołaszewska 2005). Moreover, spelt bread is often tolerated by people who have been found to be allergic to wheat products (Campbell 1997). Spelt grain gives a guarantee that grain of high vigour and high nutritional value is obtained (Tyburski, Żuk-Gołaszewska 2005).

The application of chemical plant protection agents, in particular herbicides, in cereal growing technology has become an indispensable element in modern agriculture. At the same time, this creates a need to control their effects on yield quality, since these compounds may affect plant metabolic processes and cause changes in grain quality (Narkiewicz-Jodko et al. 2002, Mularczyk et al. 2010).

The aim of the present study was to evaluate the effect of chemical crop protection on the content of some macro- and micronutrients in grain of eight spelt wheat cultivars.

MATERIAL AND METHODS

In 2009-2011, a field study was conducted at the Bezek Experimental Farm near Chełm (N: 51º 19; E: 23º 25), which belongs to the Department of Agricultural Ecology of the University of Life Sciences in Lublin. The experiment was established on medium heavy mixed rendzina soil originating from chalk rock, with the grain-size distribution of medium silty loam. This soil is classified as soil quality class IIIb and defective wheat complex. It was characterized by alkaline pH (pH in 1mol KCl – 7.35), a high content of phosphorus (117.8 mg kg^{-1} of soil) and potassium (242.4 mg kg^{-1} of soil) as well as a very low magnesium content (19.0 mg kg^{-1} of soil). The organic carbon content was 24.7 g kg^{-1}. The total rainfall from April to August in 2009, 2010, and 2011 was higher than the long-term average. In particular, the year 2010 was characterized by high rainfall during this period. Worth noticing is very high total rainfall in June 2009, July and August 2010 as well as in July 2011. The mean air temperatures in all the years were higher than the long-term average (Table 1).

The experiment evaluated the effect of chemical crop protection on the content of some macro- and micronutrients in grain of eight spelt wheat cultivars (Franckenkorn, Badengold, Schwabenspelz, Oberkulmer Rotkorn, Ostro, Ceralio, Schwabenkorn, and Spelt I.N.Z.) grown in the same plots year after year. Common wheat was the forecrop for the experiment, while spelt wheat was grown in a monoculture. Tillage was done in accordance

Table 1

Rainfall and air temperatures in April-August in 2009-2011 as compared to the long-term means (1974-2010) according to the Meteorological Station at Bezek

Years	Months					Total
	Apr	May	Jun	Jul	Aug	
	rainfall (mm)					
2009	10.1	86.8	180.5	50.8	46.9	375.1
2010	20.4	72.4	94.4	156.0	141.9	485.1
2011	30.6	40.8	88.5	178.9	38.5	377.3
Mean for 1974-2010	37.9	57.4	76.9	81.6	69.8	323.6
	temperature (°C)					mean
2009	11.2	13.0	16.2	19.9	18.1	15.7
2010	9.0	14.5	17.6	20.8	19.7	16.3
2011	9.9	14.2	18.2	18.8	18.4	15.9
Mean for 1974-2010	7.8	13.5	16.3	18.2	17.6	14.7

with the generally accepted agronomic recommendations. After the harvest of the forecrop, skimming and harrowing were done. Pre-sowing ploughing with harrowing was performed about 3 weeks before spelt sowing. Harrowing was also carried out immediately before sowing. Spelt spikelets were sown in the middle of October at a rate of 350 kg per hectare.

The experiment was set up in a split-plot design with 3 replications and the harvest plot area was 8 m^2. Mineral fertilization was as follows (in kg of nutrient per hectare): N 60 (20+40); P 26.2; K 83. Phosphorus fertilizers in the form of granulated triple superphosphate, potassium fertilizers in the form of 60% potassium salt, and 20 kg N ha^{-1} in the form of ammonium nitrate were applied before spelt sowing. A dose of 40 kg N ha^{-1} was incorporated in the spring at the stem elongation stage (BBCH 32-34).

Chemical plant protection included the application of the following agents: Alert 375 SC (the content of active substances: flusilazole 125 g l^{-1}, a compound from the triazole group; carbendazim 250 g l^{-1}, a compound from the benzimidazole group) applied against diseases at the stem elongation stage (BBCH 32-34) at a rate of 1 l ha^{-1}; Mustang 306 SE (the content of active substances: florasulam 6.25 g l^{-1}, a compound from the triazolopyrimidine group; 2,4-D EHE 300 g l^{-1}, a compound from the phenoxy acid group) used to control dicotyledonous weeds, as well as Attribut 70 WG (the content of active substances: propoxycarbazone 70%, a compound from the sulfonyl-amino-carbonyl-triazolinone group; methyl ester of 2-benzoic acid sodium salt) against monocotyledonous weeds applied at the tillering stage (BBCH 24-29) at rates of 0.4 l ha^{-1} and 60 g ha^{-1}, respectively; Stabilan 750 SL (the active

substance chlormequat chloride 750 g l^{-1}) applied at the stem elongation stage (BBCH 32-34) at a rate of 2 l ha^{-1}. Plots where no chemical plant protection agents were used served as the control treatment.

The elements were determined based on collective samples from three replications using the following methods: total N by Kjeldahl method; P by colorimetry; K by flame photometry; Mg, Cu, Zn, Mn, Fe by atomic absorption spectrometry (AAS). The results were statistically analysed by analysis of variance for three-way classification and least significant differences were calculated using Tukey's confidence half-intervals with a 5% error risk. The calculations were performed using ARStat software developed by the Computing Centre of the University of Life Sciences in Lublin.

RESULTS AND DISCUSSION

The nitrogen content in grain of cv. Badengold was significantly lower than in grain of the cultivars Schwabenspelz, Franckenkorn, Ceralio, Oberkulmer Rotkorn, Schwabenkorn, and Ostro. At the same time, grain of the cultivars Schwabenkorn and Ostro was distinguished by a higher content of nitrogen compared to cv. Spelt I.N.Z. (Table 2). In the study of Pałys and Łabuda (1997), the lowest N content was found in grain of the spelt wheat cultivar Bauländer Spelz (16.52 g kg^{-1}), whereas this content was the highest in grain of cv. Loge (22.75 g kg^{-1}). Stanisławska-Głubiak and Korzeniowska (2011) determined the N content in grain of winter wheat cultivars at a level ranging from 18.5 to 21.1 g kg^{-1}, thus being slightly lower than in the case of the spelt cultivars under evaluation.

Grain of the spelt cultivars Franckenkorn and Ostro was found to have a significantly higher P content in comparison to the cultivars Badengold, Schwabenspelz, and Spelt I.N.Z (Table 2). Pałys and Łabuda (1997) found a higher phosphorus content (from 7.36 to 8.79 g kg^{-1}) in spelt grain. On the other hand, the content of this element in spelt grain determined by Grela (1996), i.e. from 4.16 to 4.39 g kg^{-1}, and by Rachoń and Szumiło (2009), i.e. from 4.20 to 4.60 g kg^{-1}, was similar to the results obtained in the present experiment. Gembarzewski et al. (1995) determined the average P content in winter wheat grain at a level of 3.8 to 4.3 g kg^{-1}. In turn, Kraska (2011) found the P content in grain to be from 3.69 to 5.88 g kg^{-1}.

Grain of the cultivar Franckenkorn was characterized by a significantly higher K content in comparison with cv. Oberkulmer Rotkorn and, at the same time, by a higher Mg content than cv. Badengold. In the studies of Grela (1996) and of Rachoń and Szumiło (2009), the K content in spelt grain was higher (4.39-5.52 g kg^{-1} and 4.30-4.40 g kg^{-1}, respectively,), while the magnesium content was similar to that obtained in the present experiment. Kraska (2007) as well as Kraska and Pałys (2008, 2009) found a distinctly

Table 2

Content of some macronutrients in spelt grain depending on cultivars and different protection levels (means for 2009-2011)

Specification	Cultivars									Mean
Element	protection level	Franken-korn	Badengold	Schwaben-spelz	Oberkulmer Rotkorn	Ostro	Ceralio	Schwaben-korn	Spelt I.N.Z.	
N (g kg^{-1} d.m.)	without protection	22.73	21.07	23.70	24.53	25.00	23.17	24.53	23.23	23.50
	with protection	23.67	21.53	22.30	24.50	26.13	24.40	24.70	22.63	23.73
	mean	23.20	21.30	23.00	24.52	25.57	23.78	24.62	22.93	-
LSD p = 0.05		cultivar 1.686; protection level *ns								
P (g kg^{-1} d.m.)	without protection	4.66	4.38	4.48	4.51	4.79	4.46	4.46	4.43	4.52
	with protection	4.77	4.22	4.17	4.57	4.82	4.49	4.58	4.30	4.49
	mean	4.72	4.30	4.33	4.54	4.81	4.48	4.52	4.37	-
LSD p = 0.05		cultivar 0.346; protection level *ns								
K (g kg^{-1} d.m.)	without protection	3.60	3.55	3.64	3.19	3.48	3.59	3.50	3.42	3.50
	with protection	3.73	3.59	3.41	3.17	3.51	3.46	3.58	3.34	3.47
	mean	3.66	3.57	3.53	3.18	3.50	3.52	3.54	3.38	-
LSD p = 0.05		cultivar 0.434; protection level *ns								
Mg (g kg^{-1} d.m.)	without protection	1.66	1.53	1.65	1.55	1.62	1.56	1.60	1.63	1.60
	with protection	1.71	1.40	1.53	1.58	1.60	1.48	1.49	1.46	1.53
	mean	1.69	1.46	1.59	1.57	1.61	1.52	1.55	1.54	-
LSD p = 0.05		cultivar 0.196; protection level 0.059								

*ns – difference not significant

lower content of N, P, and Mg in wheat and winter triticale grain than that determined in the spelt cultivars under evaluation. In another study (KRASKA 2011), the K content in spring wheat grain (4.02-4.16 g kg^{-1}) was higher, while the magnesium content was lower (1.03-1.18 g kg^{-1}) than in grain of the spelt cultivars compared in our experiment.

The cultivar Oberkulmer Rotkorn was distinguished by a significantly higher zinc content in grain in comparison with the cultivars Badengold, Franckenkorn, Ceralio, and Schwabenspelz (Table 3). In the study of PAŁYS and ŁABUDA (1997), the Zn content in grain of the evaluated spelt wheat cultivars was much lower and ranged from 16.0 to 25.0 mg kg^{-1}. In the present study, the zinc content in grain was in the range from 38.07 to 47.58 mg kg^{-1}, while in the study of RACHOŃ and SZUMIŁO (2009) it ranged from 31.5 to 37.0 mg kg^{-1}. GRELA (1996) found a distinctly higher content of zinc only in grain of cv. Bauländer Spelz (51.30 mg kg^{-1}).

The Cu content in grain of the cultivars Oberkulmer Rotkorn, Franckenkorn and Spelt I.N.Z was significantly higher than in grain of cv. Badengold (Table 3). GRELA (1996) as well as PAŁYS and ŁABUDA (1997) showed a higher Cu content in spelt grain (10.97 mg kg^{-1} and 8.0 mg kg^{-1}, respectively), whereas RACHOŃ and SZUMIŁO (2009) found markedly less Cu (2.85-2.99 mg kg^{-1}). KORZENIOWSKA and STANISŁAWSKA-GLUBIAK (2011) determined the average Cu content in 10 winter wheat cultivars at a level of 2.42-2.59 mg kg^{-1}. GEMBARZEWSKI et al. (1995) report that Polish wheats contain on average 3.1-3.4 mg of copper in 1 kg of grain, thus much less than in the present study. In turn, in the research of KRASKA (2011) the Cu content in spring wheat grain was from 3.06 mg kg^{-1} to 5.68 mg kg^{-1}, depending on the conditions in a particular year.

The highest Mn content was found in grain of the cultivar Ostro, whereas the lowest one in grain of cv. Badengold (Table 3). The Mn content determined in spelt grain by GRELA (1996) ranging from 38.30 mg kg^{-1} (cv. Rouquin) to 64.20 mg kg^{-1} (cv. Bauländer Spelz) was higher, while that obtained by PAŁYS and ŁABUDA (1997) was clearly lower than estimated in the present study. In turn, RACHOŃ and SZUMIŁO (2009) determined the Mn content in grain of the tested spelt wheat lines at a level ranging from 37.0 to 41.6 mg kg^{-1}. The manganese content in grain was similar to that given by GEMBARZEWSKI et al. (1995) for winter wheat grain (35-47 mg kg^{-1}) and markedly higher, except for cv. Badengold, than the one obtained on the same soil by KRASKA (2011) in spring wheat grain (18.38-28.20 mg kg^{-1}).

The genetic factor did not differentiate significantly the Fe content in spelt grain. A trend was only observed towards a lower Fe content in grain of cv. Badengold compared to grain of the other cultivars (Table 3). RACHOŃ and SZUMIŁO (2009) estimated the Fe content in spelt grain at 32.2-33.9 mg kg^{-1}, thus at a similar level to that obtained for cv. Badengold. The highest Fe content determined by PAŁYS and ŁABUDA (1997) in grain of cv. Bauländer Spelz (32 mg kg^{-1}) was lower than the one found in the spelt cultivars

Table 3

Content of some micronutrients in spelt grain depending on cultivars and different protection levels (means for 2009-2011)

Specification		Cultivars								Mean
Element	protection level	Franken-korn	Badengold	Schwaben-spelz	Oberkulmer Rotkorn	Ostro	Ceralio	Schwaben-korn	Spelt I.N.Z.	
Zn (g kg^{-1} d.m.)	without protection	38.02	35.62	40.55	48.52	42.42	39.78	39.90	39.70	40.56
	with protection	38.22	40.52	38.33	46.64	42.28	38.23	43.25	43.74	41.40
	mean	38.12	38.07	39.44	47.58	42.35	39.01	41.58	41.72	-
LSD p = 0.05		cultivar 6.569; protection level *ns								
Cu (g kg^{-1} d.m.)	without protection	5.52	4.06	4.35	5.09	4.63	4.69	4.23	5.58	4.77
	with protection	5.17	4.22	5.35	5.45	5.07	4.85	5.03	5.55	5.09
	mean	5.35	4.14	4.85	5.27	4.85	4.77	4.63	5.57	-
LSD p = 0.05		cultivar 0.973; protection level 0.296								
Mn (g kg^{-1} d.m.)	without protection	34.12	26.52	29.74	32.43	38.18	31.28	29.22	32.25	31.72
	with protection	31.33	27.05	31.77	33.04	36.97	32.42	30.03	32.59	31.90
	mean	32.73	26.78	30.76	32.74	37.58	31.85	29.63	32.42	-
LSD p = 0.05		cultivar 4.576; protection level *ns								
Fe (g kg^{-1} d.m.)	without protection	36.43	35.03	38.40	39.13	37.57	34.37	40.37	40.33	37.70
	with protection	36.63	32.40	33.90	39.70	38.17	36.47	38.70	37.27	36.65
	mean	36.53	33.72	36.15	39.42	37.87	35.42	39.53	38.80	-
LSD p = 0.05		cultivar ns; protection level *ns								

*ns – difference not significant

compared. In turn, KRASKA (2011) assessed the Fe content in spring wheat grain at a level ranging from 23.12 to 42.60 mg kg^{-1}.

Differences in the content of individual elements in spelt grain determined by various authors could have resulted from the genetic traits of the compared cultivars and different climatic, soil and agronomic conditions under which the particular cultivars were grown.

The copper content in spelt grain from the treatments where chemical crop protection was used was significantly higher than in grain from the plots where no plant protection agents were applied (Table 3). A reverse relationship was found with respect to magnesium, whose content in grain from the plots without chemical protection was significantly higher than in grain obtained from the chemically protected plot (Table 2). At the same time, the applied chemical plant protection agents did not have any significant effect on the content of N P, K, Zn, Mn, and Fe in grain. Nevertheless, under the influence of chemical crop protection, there was a tendency towards a lower content of P, K, and Fe in grain compared to the treatment without chemical protection (Tables 2, 3). On the other hand, however, a reverse trend was found with regard to the content of N, Zn, and Mn. BRZOZOWSKA and BRZOZOWSKI (2002), KRASKA (2007) as well as KRASKA and PAŁYS (2008, 2009) found no effect of herbicide application on the content of N, P, K, Mg in winter wheat and winter triticale grain. In the study of ANDRUSZCZAK et al. (2009), however, the applied herbicides decreased the N content in winter wheat grain.

In each successive year of spelt monoculture cropping, the content of N, Mg, Zn, Cu, and Mn in grain decreased significantly (Table 4). This could be attributable to the deterioration of the soil chemical properties as a result of spelt monocropping. WESOŁOWSKI and KWIATKOWSKI (2000) as well as WOŹNIAK (2004) draw attention to the fact that an increasing proportion of cereals in

Table 4

Chemical composition of spelt grain during the study years

Specification	Year			LSD $p = 0.05$
	2009	2010	2011	
N content in grain (g kg^{-1} d.m.)	26.36	22.86	21.62	0.766
P content in grain (g kg^{-1} d.m.)	4.63	4.64	4.26	0.157
K content in grain (g kg^{-1} d.m.)	2.83	3.89	3.74	0.197
Mg content in grain (g kg^{-1} d.m.)	1.69	1.59	1.41	0.089
Zn content in grain (mg kg^{-1} d.m.)	58.03	39.28	25.64	2.983
Cu content in grain (mg kg^{-1} d.m.)	6.99	4.63	3.16	0.442
Mn content in grain (mg kg^{-1} d.m.)	43.71	28.89	22.83	2.078
Fe content in grain (mg kg^{-1} d.m.)	45.18	32.83	33.53	2.696

a crop structure leads to adverse soil changes and raise the infection rate with stem base diseases over time, which in turn affects both grain yield and grain quality. The P content in grain in 2009 and 2010 was significantly higher than in 2011, whereas the K content in the first year of the study was lower than in the next two years. In turn, the Fe content was higher in 2009 than in 2010-2011 (Table 4).

CONCLUSIONS

1. The evaluated spelt wheat cultivars differed in the content of macro- and microelements in grain. Among the cultivars compared, grain of the cultivar Ostro was characterized by the highest content of N and P, while grain of cv. Franckenkorn had the highest content of K and Mg. Grain of cv. Oberkulmer Rotkorn contained the largest amount of Zn; grain of cv. Spelt I.N.Z had the highest amount of Cu, whereas grain of the cultivars Ostro and Schwabenkorn was found to have the highest amount of Mn and Fe, respectively.

2. Chemical crop protection involving the application of two herbicides, a fungicide, and a growth regulator significantly increased the copper content in spelt grain compared to the treatment in which no chemical plant protection agents were used, but it had a negative impact on the magnesium content.

3. In the last year of the study, the content of all the investigated elements in grain, except for potassium, was lower than in the first year of investigation.

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DIETARY CHROMIUM(III) PROPIONATE COMPLEX SUPPLEMENTATION AFFECTS TISSUE MINERAL LEVELS IN RATS FED HIGH-FRUCTOSE DIET

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Abstract

Chromium(III) plays an important role in carbohydrate and lipid metabolism, thus supplements containing this element are broadly advertised as efficient agents improving blood glucose levels or even reducing body mass. However, their hypoglycemic potential depends on the chemical form, bioavailability, dosage and the duration of treatment. Chromium(III) supplementation is generally considered safe although some data point to interaction of this ion with other elements. Thus, the aim of this study was to evaluate the effect of chromium(III) supplementation on tissue mineral content in rats fed high-fructose diets. Nine-week old male Wistar rats were fed *ad libitum* with a standard diet (control) or a high-fructose diet to induce insulin resistance. Next, supplementary dosages of chromium(III) propionate complex (1.0 and 5.0 mg Cr kg^{-1} b.w. day^{-1}) were introduced and rats were fed those diets for 4 weeks.

It has been found that supplementary chromium(III) did not affect tissue calcium, iron and zinc levels, but significantly increased hepatic magnesium and copper level, while decreasing splenic copper level in rats fed high-fructose diets. Higher chromium(III) dosages increased content of this element in kidneys.

In conclusion, short-term supplementation of chromium(III) propionate complex affects mineral homeostasis in the tissues of rats fed high-fructose diets; however, the mechanisms of such interactions are only partially known.

Key words: chromium(III) propionate, supplementation, minerals, high-fructose diet.

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WPŁYW SUPLEMENTACJI DIETY PROPIONIANEM CHROMU(III) NA POZIOMY TKANKOWE PIERWIASTKÓW U SZCZURÓW KARMIONYCH DIETĄ WYSOKOFRUKTOZOWĄ

Abstrakt

Chrom(III) odgrywa znaczącą rolę w metabolizmie węglowodanów i lipidów, co sprawiło, że suplementy zawierające ten pierwiastek są szeroko reklamowane jako skuteczne środki poprawiające poziom glukozy we krwi, a nawet obniżające masę ciała. Aczkolwiek potencjał hipoglikemiczny suplementów chromu(III) zależy od ich formy chemicznej, biodostępności, dawki oraz czasu podawania. Ogólnie suplementacja chromem(III) uważana jest za bezpieczną, chociaż nieliczne dane wskazują na możliwość interakcji chromu(III) z innymi pierwiastkami. Z tego względu celem badań była ocena wpływu suplementacji Cr(III) na tkankowe poziomy pierwiastków u szczurów karmionych dietą wysokofruktozową. Szczury Wistar w wieku 9 tygodni karmiono *ad libitum* dietą kontrolną lub dietą wysokofruktozową w celu wywołania insulinooporności. Następnie do diet dodano dawki kompleksu chromu(III) z kwasem propionowym (1,0 i 5,0 mg Cr kg^{-1} m.c. na dzień) i karmiono szczury tymi dietami przez 4 tygodnie.

Stwierdzono, że dawki chromu(III) nie wpływały na tkankowe poziomy wapnia, żelaza i cynku, ale znacząco podwyższały poziom magnezu i miedzi w wątrobie, jednocześnie obniżając poziom miedzi w śledzionie u szczurów karmionych dietą wysokofruktozową. Ponadto wyższe dawki chromu(III) wpływały na wzrost zawartości tego pierwiastka w nerkach.

Podsumowując, krótkotrwała supplementacja kompleksem chromu(III) z kwasem propionowym wpływa na homeostazę pierwiastków u szczurów karmionych dietą wysokofruktozową, jednakże mechanizm tych interakcji jest poznany tylko częściowo.

Słowa kluczowe: propionian chromu(III), suplementacja, składniki mineralne, dieta wysokofruktozowa.

INTRODUCTION

Several minerals, owing to their function as cofactors of enzymatic processes involved in the metabolism of carbohydrates and lipids, play a crucial role in insulin resistance and diabetes. Some (zinc and magnesium) participate in insulin production, secretion and action on the cellular level (BARBAGALO et al. 2003, EMDIN et al. 1980). Also disturbances in carbohydrate metabolism, mainly high blood glucose level, can affect mineral indices in the body. Insulin resistance or diabetes affect absorption, excretion and tissue levels of some elements. One of these elements is chromium (III), which has been shown to play an important role in metabolic disorders associated with insulin resistance and hyperglycemia, including type 2 diabetes mellitus (VINCENT 2000). This role probably involves potentiation of insulin signaling and consequently various Cr(III) compounds have been introduced to dietary supplements for the treatment of diabetes and its complications. However, their hypoglycemic potential depends on the chemical form, bioavailability, dosage and duration of treatment.

The use of Cr(III) compounds as dietary supplements requires examination of its safety for animals and humans. In our latest publication (Staniek et al. 2010) we provided experimental evidence that the chromium(III) propionate complex (CrProp) is of low acute toxicity in rat. In another study a high-fructose diet was used to induce insulin resistance in laboratory animals. It was shown (Król, Krejpcio 2010) that supplemental CrProp given orally at dosages of 1 and 5 mg kg^{-1} b.w. day^{-1} for 8 weeks to Wistar rats fed high-fructose diets is able to ameliorate insulin resistance symptoms, at the same time not causing toxic effects. However, supplementary Cr(III), administered at excessive dosages or over a long period of time, can accumulate in internal organs and affect mineral homeostasis in animals and humans (Campbell et al. 1997, Lamson, Plaza 2002, Król, Krejpcio 2010, 2011).

The aim of this study was to evaluate the effect of 4-week CrProp supplementation on mineral homeostasis in rats fed high-fructose diets.

MATERIAL AND METHODS

The experiment was performed on 9-week old male Wistar rats (n=32) divided into 4 groups (8 animals each). The control group was fed *ad libitum* a standard AIN-93M diet (0.1 mg Cr kg^{-1} b.w. day^{-1}), while three groups were fed high-fructose diets (60% w/w) for 4 weeks to induce insulin resistance. Then supplementary Cr(III) (in the form of chromium(III) propionate, CrProp) dosages (1.0 and 5.0 mg Cr kg^{-1} b.w. day^{-1}) were introduced to those diets. Two groups of rats were fed those diets for another 4 weeks, while the other two with control and high-fructose diets with a standard Cr(III) level. After 4 weeks, at the end of study, rats were sacrificed to collect blood and internal organs for biochemical analyses. Organs were washed in saline, weighed and stored at -20ºC until analyzed. All the procedures used in this study were approved by the Animal Bioethics Committee of Poznan, Poland (Approval # 37/2007). Tissue samples were digested by wet mineralization in a microwave system (MARS-5, CEM). The content of Ca, Mg, Fe, Zn and Cu in the liver, kidneys and spleen was determined by the flame atomic absorption spectrometry method (AAS-3, Carl-Zeiss, Germany), while Cr content in organs was assayed using a graphite furnace AAS technique (AAS-5, Jenoptic). The accuracy of quantitative determinations of these elements was assured by simultaneous analysis of the certified reference material (Pig Kidney BCR® No 186, Brussels, fortified with Cr standard).

All results are presented as means ± standard deviation. Significance of differences of means was calculated using the one-way ANOVA and Tukey's tests. Means were considered statistically different at $p < 0.05$. All calculations were made using the Statistica (ver. 7.0) program (StatSoft, Inc., Tulsa, USA).

RESULTS AND DISSCUSION

Insulin resistance and diabetes are states that alter the metabolism of minerals, mainly due to imbalances in carbohydrates metabolism and oxidative stress. In this study, insulin resistance was induced by feeding rats a high-fructose diet. This state was assessed by comparing the HOMA – Insulin Resistance index in the control group and high-fructose diet fed group (data not shown). The effect of high-fructose diet on tissue mineral content is presented in Tables 1, 2 and 3. It has been shown that a high-fructose diet given for 8 weeks affected mineral accumulation in the rat organs. In particular, rats fed for 8 weeks a high-fructose diet had a decreased hepatic Cu level (by 13%), while splenic Cu level increased (by 27%). Additionally, a fructose diet slightly reduced the kidney Fe content. The tissular Ca, Mg, Zn and Cr concentrations remained constant in rats fed high-fructose diets with a standard Cr level.

Table 1

Content of minerals in liver of experimental rats

Mineral (mg kg^{-1} d.m.)	Experimental group			
	C	H-FRU	H-FRU + 1 Cr	H-FRU + 5 Cr
Mg	156 ± 34	146 ± 28	153 ± 37	148 ± 34
Ca	707 ± 58^{a}	739 ± 51^{ab}	837 ± 85^{b}	833 ± 87^{b}
Fe	316 ± 37	351 ± 50	296 ± 57	348 ± 38
Zn	100 ± 9	103 ± 10	94.8 ± 5	106 ± 10.7
Cu	22.9 ± 2.5^{b}	19.9 ± 2.7^{a}	22.5 ± 2.9^{b}	23.7 ± 2.1^{b}
Cr	0.71 ± 0.18	0.48 ± 0.06	0.66 ± 0.18	0.68 ± 0.19

Abbreviations: C – control group, H-FRU – high-fructose diet fed group with 0.1 mg Cr kg^{-1} b.m. day^{-1}, H-FRU + 1 Cr – high-fructose diet fed group with 1 mg Cr kg^{-1} b.m. day^{-1}, H-FRU + 5 Cr – high-fructose diet fed group with 5 mg Cr kg^{-1} b.m. day^{-1}; means in a row with different letters differ significantly ($p<0.05$)

Chromium(III) is an element involved in carbohydrate metabolism. An increasing body of evidence supports the hypothesis that Cr is necessary to the proper functioning of the insulin receptor; however, the mechanism by which it improves blood glucose or lipid levels is still being investigated. Despite several advantages of Cr supplementation in diabetics, there are some concerns about its safety. There are some examples of research on humans, in which the authors suggested that ingestion of a high Cr supplemental dosage (above 1200 mg Cr per day) might cause dermatitis (Fowler 2000) or renal impairment (Cerulli et al. 1999). Other adverse effects have

Table 2

Content of minerals in kidney of experimental rats

Mineral (mg kg^{-1} d.m.)	Experimental group			
	C	H-FRU	H-FRU + 1 Cr	H-FRU + 5 Cr
Mg	230 ± 37	193 ± 52	156 ± 31	171 ± 32
Ca	764 ± 89	826 ± 58	934 ± 62	887 ± 53
Fe	368 ± 58	337 ± 59	339 ± 37	359 ± 35
Zn	81.2 ± 7.3	82.6 ± 8.8	79.9± 4.9	80.4 ± 8.2
Cu	39.4 ± 6.8	35.5 ± 6.2	30.8 ± 2.8	33.3 ± 3.1
Cr	0.57 ± 0.23^{a}	0.66 ± 0.36^{ab}	0.86 ± 0.18^{b}	1.35 ± 0.33^{c}

Abbreviations: cf. Table 1

Table 3

Content of minerals in spleen of experimental rats

Mineral (mg kg^{-1} d.m.)	Experimental group			
	C	H-FRU	H-FRU + 1 Cr	H-FRU + 5 Cr
Mg	145 ± 42	134 ± 38	138 ± 32	136 ± 35
Ca	612 ± 73	564 ± 63	639 ± 73	662 ± 71
Fe	2571 ± 638	2398 ± 310	2744 ± 638	2624 ± 441
Zn	41.1 ± 4.2	42.3 ± 7.2	48.1 ± 6.3	43.3 ± 6.0
Cu	8.71 ± 1.81^{ab}	11.1 ± 2.09^{b}	5.63 ± 0.81^{a}	6.62 ± 0.80^{a}
Cr	5.30 ± 1.22	4.90 ± 1.41	5.11 ± 1.37	4.92 ± 1.39

Abbreviations: cf. Table 1

not been observed. On the other hand, another aspect that should be taken into account during Cr(III) supplementation is its possible interaction with other minerals.

This study investigated the effect of 4-week CrProp supplementation on tissue content of minerals in rats fed high-fructose diets (Tables 1, 2 and 3). Supplementary CrProp (1 and 5 mg kg^{-1} b.w. day^{-1}) did not affect tissue Ca, Fe and Zn levels, but significantly increased hepatic Mg level (by 13%) and hepatic Cu content (by 13 and 19%, respectively), while decreasing splenic Cu (by 40 and 50%, respectively) in rats fed high-fructose diets. Moreover, CrProp given in a higher dosage (5.0 mg kg^{-1} b.w. day^{-1}) doubled the Cr content in the kidneys.

The Cr(III) – Fe interaction seems most likely, since these elements have the same transport protein, i.e. transferrin. Some studies have confirmed a relationship between an increased dietary Cr(III) intake and iron

stores, especially if high Cr(III) dosages were used (LAMPSON, 2002, CAMPBELL et al. 1997). For example, in our previous study, we confirmed that 8-week CrProp supplementation (in dosages of 1 and 5 mg kg^{-1} b.m.) decreased the Fe kidney concentration (KRÓL, KREJPCIO 2010). Similarly, in a study by CLODFELDER et al. (2005) a reduced kidney Fe level was observed in obese type 2 diabetic rats after supplementation of 1000 µg Cr kg^{-1} b.w. day^{-1}. Similarly, the same dosages used in another experiment normalized an increased liver Fe content in rats fed high-fat diets and injected with streptozotocin (KRÓL, KREJPCIO 2011). However, DOGUCAN et al. (2009) did not report any changes in tissue Fe concentration in a similar animal model of type 2 diabetes after 10 weeks of chromium(III) histidinate supplementation. In that study, Fe levels in analyzed tissues did not change, probably due to short supplementation period. To the authors' best knowledge such interaction has not been noticed in human studies, when Cr(III) compounds were given in dosages of 200 to 1000 µg Cr day^{-1} for at least 2 months (CAMPBELL et al. 1997, 2002, VOLPE et al. 2001, KRÓL et al. 2011).

The mechanism of Cr(III) interaction with other elements is unknown although such a relationship has been reported in animal studies. RHEE et al. (2004) proved that in diabetic prone BHE/cdb rats Cr deficiency increased Zn content in the liver, while decreasing Cu tissue content and enhancing Mg and Fe accumulation in the liver.

In the study by DOGUCAN et al. (2009) mentioned above, Cr(III) supplementation decreased Cu and increased Zn content in the liver and kidneys of fat-fed and streptozotocin-treated type II diabetic rats. These data correspond well with results of ŚCIBOR, ZAPOROWSKA (2007), who found that an aqueous solution containing 0.42 mg Cr kg^{-1} b.w. day^{-1} given for 12 weeks increased Zn content in the kidneys of healthy Wistar rats. Additionally, there was no influence on Cr, Fe or Cu levels in either the liver or kidneys of these animals. In another study (SAHIN et al. 1999), pregnant rabbits supplemented with chromium(III) picolinate had a decreased Cu concentration in their liver and kidneys, while Zn levels in those organs increased.

In human subjects, there were no changes in Zn or Cu indices after Cr(III) supplementation for a period of up to 12 weeks in patients with type 2 diabetes as well as moderately obese subjects (ANDERSON et al. 2001, VOLPE et al. 2001, KRÓL et al. 2011).

CONCLUSION

On the basis of the results, it can concluded that CrProp supplementation disturbs mineral homeostasis in the rats' organs. In particular, CrProp given for 4 weeks in dosages of 1 and 5 mg kg b.w. day^{-1} affects Mg, Cu and Cr levels, although it does not influence tissue Ca, Fe and Zn content

in rats fed high-fructose diets. Based on the results, it should be said that the most important issue in Cr(III) supplementation and its interaction with other elements in the body is the dosage used and the duration of supplementation.

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INFLUENCE OF MAGNESIUM ADDED TO DIET OF PULAWSKA BREED FATTENERS ON PHYSICAL AND CHEMICAL PROPERTIES OF MEAT

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Abstract

Magnesium is a macronutrient involved in numerous physiological and biochemical processes in an animal organism. This study aimed at evaluating the influence of Mg supplementation of feed for fatteners on physical, chemical and sensory properties of their meat. The experiment was conducted on 30 Pulawska breed fatteners divided into two groups: experimental and control. The former group was daily supplemented with 1 g $MgCl_2 \cdot 6H_2O$ for 30 days before slaughter. After slaughter (when fatteners had gained 105 kg ± 0.4 kg of body weight), samples of ham muscle (*musculus adductor femoris*) were taken for determinations of the meat quality.

Magnesium supplementation had a positive effect on pH_1 and pH_{24} of meat. Decreased free water percentage, re-emission and green colour share, accompanied by an increase in the red colour proportion in ham meat, occurred owing to magnesium (these differences were important statistically). Another finding was a higher level of hematin in meat from the experimental animal group. As for the general chemical composition of meat from the experimental group, dry matter and glycogen content increased. No effect of magnesium supplementation was observed in the sensory quality assessment of meat. Magnesium added to feed caused slower decomposition of muscular glycogen, thus preventing a substantial decrease in the meat pH after slaughter. The meat of fatteners from the experimental group was characterized by superior technological indices.

Key words: magnesium, meat quality, fatteners.

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WPŁYW DODATKU MAGNEZU ZASTOSOWANEGO W ŻYWIENIU TUCZNIKÓW RASY PUŁAWSKIEJ NA WŁAŚCIWOŚCI FIZYCZNE I CHEMICZNE MIĘSA

Abstrakt

Magnez jest makroelementem biorącym udział w wielu procesach fizjologicznych i biochemicznych w organizmie zwierząt. Celem pracy było określenie wpływu dodatku Mg w paszy podawanej tucznikom na wskaźniki fizyczne, chemiczne oraz ocenę sensoryczną mięsa. Badaniami objęto 30 szt. tuczników rasy puławskiej, które podzielono na dwie grupy: eksperymentalną i kontrolną. Grupa eksperymentalna otrzymywała przez 30 dni przed ubojem dodatek magnezu w ilości 1g $MgCl_2 \cdot 6H_2O$ dziennie. Po uboju tuczników (po osiągnięciu masy ciała 105 kg ± 0,4 kg) pobrano próbki z mięśnia szynki (*musculus adductor femoris*) do badań cech jakości mięsa.

Dodatek magnezu wpłynął pozytywnie na pH_1 i pH_{24} mięsa. Pod wpływem magnezu nastąpiło zmniejszenie procentowej zawartości wody luźnej, reemisji oraz udziału barwy zielonej, natomiast stwierdzono wyższy udział barwy czerwonej w mięsie szynki (różnice istotne statystycznie). Wykazano także większą zawartość hematyny w mięsie zwierząt z grupy doświadczalnej. W podstawowym składzie chemicznym mięsa z grupy eksperymentalnej wzrosła ilość suchej masy i zawartość glikogenu. Nie zaobserwowano wpływu dodatku Mg na ocenę sensoryczną mięsa. Dodatek magnezu do paszy spowodował spowolnienie rozkładu glikogenu mięśniowego, co zapobiegło znacznemu spadkowi pH mięsa po uboju. Stwierdzono, że mięso tuczników z grupy doświadczalnej miało korzystniejsze wskaźniki technologiczne.

Słowa kluczowe: magnez, jakość mięsa, tuczniki.

INTRODUCTION

Magnesium is a macronutrient present in bodies of mammals. It is involved in many physiological and biochemical processes in an animalorganism, and participates in about 300 enzymatic reactions by playing the role of an activator of numerous enzymes. Magnesium has a positive effect on the colour and water absorption capacity of meat. It also inhibits lipid oxidation in stored pork. Supplemented magnesium decreases post-slaughter glycolysis evoked by stress, thus preventing a considerable pH decrease (APPLE et al. 2000, D'SOUZA et al. 1999, 2000, ROSENVOLD, ANDERSEN 2003).

The aim of this study was to assess the effect of Mg in feed given to fatteners on the nutritional value and selected meat quality traits.

MATERIAL AND METHODS

The experiment comprised 30 Pulawska breed fatteners divided into two groups: experimental (E) and control (C). Animals were fed a complete mixture according to Standards of Swine Feeding (12.5 MJ of ME and 150 g protein). The experimental group was supplemented with an active form of

magnesium (Mg) (absorption 98%) at the amount of 1 g $MgCl_2 \cdot 6H_2O$ daily added to feed for 30 days before slaughter. Animals were slaughtered at the body weight of 100 kg. The evaluation of ham meat (*musculus adductor femoris*) was carried out using normalized samples taken from right halves. The following were determined: pH (45 min. and 24 h after the slaughter) applying a pH STARTER CPU device, percentage of free water according to POHJA and NIINIVAARA'S method (1957), pork colour using a leukometer, fat content in meat with the Soxhlet method (according to PN-ISO 1444:2000.), dry matter (ASM) according to the drier method (PN-ISO 1442:2000), ash with the combustion method (according to PN-ISO 936:2000), total protein with Kjeldahl method (according to PN-75/A-04018). The total content of hematin dyes was determined according to HORNSEY'S method (1956). The shear force of meat was measured using a versatile SZ type device that is analogous to a Warner-Bratzler apparatus. Meat samples were measured after thermal processing (70°C for 1 h) by recording the force necessary to break muscle fibers (kG). Meat columns measuring 5 x 1 x 1 cm (height x width x length) and with a cutting section surface area of 1 cm^2 were subject to the cutting test. Muscle fibers were arranged perpendicularly to the cutting surface. Mean value for a sample was calculated from taking 5 replicates. Meat samples for the glycogen level determination were collected directly after slaughter and stored during the transport in dry ice at about -20°C. Next, glycogen was determined on the basis of the quantity of glucose released during glycogen hydrolysis according to Bertrand method (PN-67/A--86430) by isolating the animal polysaccharide from tissues (muscles) and hydrolyzing glycogen for the final evaluation of its level (in g 100 g^{-1} of tissue). The glycogen isolation, its hydrolysis to glucose and determination of the analyzed polysaccharide content were performed as proposed by GOOD et al. (1933). The sensory assessment of ham was performed by means of the point scoring method, which evaluated the scent, tenderness, juiciness and tastiness (BARYŁKO-PIKIELNA 1975).

The content of minerals were analyzed by flame atomic absorption spectrometry – AAS-PSAA Varian AA280 FS (*Official methods*...2000).

Statistical analysis of the results included calculations of mean values (x) and standard deviation (SD). The significance of differences between the control and experimental mean values was verified applying a single-factor variance analysis of Duncan's test with the aid of Statistica 6.0 software.

RESULTS AND DISCUSSION

Physical parameters of muscle tissues from the experimental group of Pulawska breed hogs, supplemented with magnesium in a diet, differed substantially from the control group, which did not receive extra magnesi-

um (Table 1). Magnesium supplementation prevented a considerable pH decrease: pH_{24} equalled 5.83 compared to 5.72 for the control group. The element caused some decrease in the percentage of free water content and ham re-emission, as well as shares of green and blue colours. On the other hand, the share of red colour in meat significantly increased: 46.55% in the experimental versus 44.90% in the control group. A higher hematin content was also found in meat from the experimental animals. It helped to stabilize the intensity of meat's natural colour. The shear force of ham muscles attained a lower value for experimental fatteners, administered magnesium, than for the control group.

Table 1

Influence of magnesium supplementation administered to Pulawska breed fatteners on physical properties of ham

Specification	Experimental group		Control group	
	x	SD	x	SD
pH_1	6.32	0.45	6.20	0.50
pH_{24}	5.83	0.56	5.72	0.51
% of free water	17.25^A	1.45	22.86^A	1.62
% of re-emission	24.32^a	1.09	25.20^a	0.83
Share of colours (%):				
r_L red	46.55^B	0.77	44.90^B	0.88
g_L green	27.03^C	0.73	28.24^C	0.64
b_L blue	26.40	0.82	26.85	0.59
Hematin (μg g^{-1})	54.21	8.76	50.11	9.12
Shear force (N)	53.95	6.49	59.70	7.88

Mean values marked the same capitals in a row differ significantly at $p \leq 0.01$.
Mean values marked the same small letters in a row differ significantly at $p \leq 0.05$.

The content of dry matter and the level of glycogen increased in meat of swine from the experimental group (Table 2). All the above changes in meat of the experimental animals occurred owing to less intensive glycogenolysis and glycolysis processes, the fact that was confirmed by partial inhibition of glycogen decomposition in meat, and which induced a slight increase of the pH_1 and pH_{24} values in ham from the experimental swine as compared to the control group. The results of the sensory assessment of ham (Table 3) indicate its high consumption value: all the evaluated parameters scored above 4 points. No significant differences between the experimental and control group in meat's minerals composition was found (Table 4).

Among many traits which characterize meat quality, its pH value, which determines other physical features, provides some important information (Koćwin-Podsiadła et al. 2006). Stasiak and Kamyk (2001) reported some dependences between pH value and re-emission % (W) and percentage of free

Table 2

Influence of magnesium supplementation administered to Pulawska breed fatteners on chemical properties of ham

Specification	Experimental group		Control group	
	x	SD	x	SD
Dry matter (%)	22.39	0.75	21.97	0.95
Crude ash (%)	1.15	0.05	1.14	0.04
Total protein (%)	20.20	0.96	19.91	1.04
Crude fat (%)	0.69	0.04	0.65	0.03
Glycogen (g 100 g^{-1} of tissue)	0.85^{A}	0.08	0.70^{A}	0.11

Mean values marked the same capitals in a row differ significantly at $p \leq 0.01$.

Table 3

Results from sensory assessment of ham (in points)

Specification	Experimental group	Control group
Scent:		
intensity	4.3	4.3
desirability	4.4	4.3
Tenderness	4.1	4.0
Juiciness	4.0	4.0
Tastiness		
intensity	4.2	4.1
desirability	4.3	4.3

water. Strzyżewski et al. (2008) found similar tendencies. Analogous correlations were also observed for muscle tissue samples studied in the present study and involving Pulawska breed swine, both from the control and experimental group. Lower pH values in ham from the examined swine breed corresponded to higher re-emission (W) and free water coefficients (%); the achieved physical parameters were characteristic for normal quality meat: $pH_1 \geq 6.0$; $pH_{24} \geq 5.5$ (Przybylski et al. 2012). And inversely, lower re-emission (W) and free water coefficients (%) corresponded to higher pH values of the fattener's ham, which proves that glycogenolysis and glycolysis were slower. As the present results indicate, magnesium – by inhibiting glycogenolysis and glycolysis – slows down the decrease in the ham pH_1 and pH_2 , which improves the indices of re-emission (W) and free water content, and this ensures better meat quality. The achieved indices of re-emission (W) and percentage of colours provided evidence for stabilization of meat's natural colour after magnesium supplementation. D'Souza et al. (1999, 2000) reported similar results achieving higher pH values for meat after the

Table 4

Mineral composition of ham (mg kg^{-1})

Specification	Experimental group		Control group	
	x	SD	x	SD
Ca	64.18	9.13	65.17	7.41
K	3327.28	28.99	3378.19	37.25
Na	426.21	19.57	418.56	22.47
Mg	266.18	25.32	254.77	28.34
Zn	25.42	1.95	25.07	2.45
Fe	22.45	2.34	21.32	2.11
Cu	0.67	0.11	0.71	0.17

slaughter, lower percentage of free water content, and meat colour stabilization. Some of the references cited in this paper and dealing with the influence of magnesium on the fattener's meat quality do not reported positive impact of this element on pork (water holding capacity, lightness, colour); in some cases, the effect depended on the duration of magnesium supplementation and age of slaughtered animals (FREDERICK et al. 2004, 2006). Retarded decrease of the glycogen content by delaying the progress of glycogenolysis in meat owing to magnesium supplementation could support discussions on physical changes in ham reported both in this article and in the in literature. Magnesium supplementation induced some changes which slowed down glycogen decomposition, manifested as a slower pH_1 and pH_{24} decrease in meat from the experimental animals compared to the control group.

CONCLUSIONS

1. Magnesium added to a diet for 30 days before slaughter slowed down muscle glycogen decomposition and prevented a substantial pH decrease in meat after slaughter, which contributed to stabilization of the colour and improvement of meat quality.

2. Meat of the experimental fatteners was characterized by lower indices of re-emission, percentage of free water content and shear force.

3. No significant influence of magnesium supplementation on the chemical composition of ham was recorded.

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CONTENT OF TOTAL CARBON AND AVAILABLE FORMS OF PHOSPHORUS, POTASSIUM AND MAGNESIUM IN SOIL DEPENDING ON THE SULPHUR RATE AND FORM

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Abstract

The reduction of sulphur emissions achieved over the last 20 years has led to sulphur deficit in soil, which decreases crop yields and deteriorated yield quality. Sulphur fertilisation affects both plants and physicochemical soil properties. The total carbon content in soil affects the capacity and quality of the sorption complex, which in turn determines the buffer capacity. The content of available forms of phosphorus, potassium and magnesium in soil has strong influence on the soil fertility. The uptake with yield and the acidification of soil, which intensifies the processes of retardation and nutrient leaching, result in depletion of those nutrients in soil.

In 2005-2007, an experiment was carried out at the Experiment Station of the Faculty of Agriculture and Biotechnology, the University of Technology and Life Sciences in Bydgoszcz, to assess the effect of sulphur fertilisation on the content of total carbon and available forms of phosphorus, potassium and magnesium in soil. Sulphur fertilisation was applied in the ionic form, i.e. sodium sulphate (VI), and in the elemental form. The rates were 0, 20, 40, 60 kg S ha^{-1}. The results demonstrated that increasing sulphur rates considerably decreased the content of available forms of phosphorus, potassium and magnesium in soil. A significant increase was also found in the total carbon content in soil after fertilisation with 20 and 40 S kg ha^{-1}. Interestingly, the organic carbon content in soil clearly depended on the form of applied sulphur: sulphate (VI) or elemental.

Key words: sulphur, fertilization, total carbon, phosphorus, potassium, magnesium.

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ZAWARTOŚĆ WĘGLA OGÓŁEM ORAZ PRZYSWAJALNYCH FORM FOSFORU, POTASU I MAGNEZU W GLEBIE W ZALEŻNOŚCI OD DAWKI I FORMY SIARKI

Abstrakt

Redukcja emisji siarki w ostatnim 20-leciu doprowadziła do deficytu tego pierwiastka w glebie, co spowodowało zmniejszenie plonów i pogorszenie ich jakości. Nawożenie siarką wpływa nie tylko na rośliny, ale również oddziałuje na właściwości fizykochemiczne gleb. Zawartość węgla ogółem w glebie decyduje o pojemności i jakości kompleksu sorpcyjnego, od którego zależą zdolności buforowe. Zawartość przyswajalnych form fosforu, potasu i magnezu w glebie w znacznym stopniu decyduje o jej żyzności. Wynoszenie z plonem oraz zakwaszenie gleb, które intensyfikuje procesy uwsteczniania i wymywania składników pokarmowych, powoduje zubożenie gleb w te składniki.

W latach 2005-2007 na terenie Stacji Badawczej Wydziału Rolnictwa i Biotechnologii UTP w Bydgoszczy realizowano badania, których celem była ocena wpływu nawożenia siarką na zawartość węgla ogółem i przyswajalnych form fosforu, potasu oraz magnezu w glebie. Nawożenie siarką stosowano w formie jonowej – siarczan (VI) sodu oraz w formie elementarnej: 0, 20, 40, 60 S kg ha^{-1}. Wykazano, iż wzrastające dawki siarki powodowały znaczące zmniejszenie zawartości przyswajalnych form fosforu, potasu i magnezu w glebie. Stwierdzono także istotny wzrost zawartości węgla ogółem w glebie po nawożeniu 20 i 40 kg S ha^{-1}. Warto zaznaczyć, że zawartość węgla organicznego w glebie była wyraźnie uzależniona od formy siarki stosowanej w badaniach – siarczanowej (VI) lub elementarnej.

Słowa kluczowe: siarka, nawożenie, węgiel organiczny, fosfor, potas, magnez.

INTRODUCTION

Sulphur is an essential nutrient in plants. Being incorporated into some organic compounds, it functions as a building block of matter (Bednarek et al. 2008). Until the early 1990s, it had been thought that the amount of sulphur introduced into soil with fertilisers and atmospheric pollution satisfied the requirements of crops for this nutrient (Szulc et al. 2004). Later, however, some economic changes and eco-friendly actions resulted in a very high reduction of sulphur emissions to the atmosphere, causing sulphur deficit in soil (Motowicka-Terelak, Terelak 2000). Sulphur deficit or excess in soil can limit the yielding of crops (Mcgrath et al. 1996, Kaczor et al. 2004) and the soil capacity for meeting the sulphur requirements of plants. Soil is the key source of sulphur for plants, which absorb it from soil as sulphate (VI). At present, over half of the soils in Poland are poor in available sulphur forms (Kozłowska-Strawska, Kaczor 2004). Sulphur fertilisation affects both the plant growth and development and the physicochemical properties of soil (Klikocka 2004, Kaczor, Łaszcz-Zakorczmenna 2009, Skwierawska, Zawartka 2009). In view of the above, this research has been launched to evaluate the effect of two sulphur forms and their rates on the content of total carbon and available forms of phosphorus, potassium and magnesium in soil.

MATERIAL AND METHODS

The research was performed in 2005-2007, at the Experiment Station of the Faculty of Agriculture, located in Wierzchucinek near Bydgoszcz. The experiment was set up as a two-factor multiple experiment (3) in a split-plot design with 3 replications. It was run on Haplic Luvisol soil, assessed as good rye complex of agricultural usability in the Polish soil valuation system. The following crops were grown:

experiment 1: spring barley in 2005, narrow-leaf lupine in 2006 and mustard in 2007;
experiment 2: narrow-leaf lupine in 2005, mustard in 2006 and spring barley in 2007;
experiment 3: mustard in 2005, spring barley in 2006 and narrow-leaf lupine in 2007.

The experimental factors consisted of:

factor I – sulphur form: S elemental sulphur and sodium sulphate (VI);
factor II – sulphur rate: 0, 20, 40, 60 S kg ha^{-1}.

In 2007, after the harvest of crops, soil from the arable layer (0-20 cm) was sampled and the following were determined: the content of total carbon with Tiurin method, available phosphorus and potassium form with Egner-Riehm method (DL) and available forms of magnesium with Schachtschabel method.

The results were statistically verified with the analysis of variance at the significance of α=0.05 and the boundary differences were estimated with Tukey's test.

RESULTS AND DISCUSSION

Literature reports imply an invariably positive relationship between the content of sulphur and humus in soil, being the basic source of that element for plants (Spychaj-Fabisiak et al. 2004, Szulc et al. 2004). The present results have demonstrated that sulphur fertilisation at the rates of 20 and 40 S kg ha^{-1} resulted in a significant increase in the content of total carbon in soil: by 6.2% and 1.7%, respectively, as compared to the control (Table 1). Interestingly, the lowest sulphur rate (20 S kg ha^{-1}) significantly increased (by 3.2% to 9.2%) the amount of total sulphur in soil of all the experiments. Significant increases in the content of that sulphur form in soil were noted following the application of 40 S kg ha^{-1} (experiment II) and 60 S kg ha^{-1} (experiment III), which suggests that low sulphur rates (20-40 kg ha^{-1}) are essential for humification processes in soil to run properly. The research demonstrated that the form of sulphur significantly modified the amount of

Table 1

Content of total carbon in soil (g kg^{-1})

Experiment	Form of sulphur	Doses of sulphur (kg S ha^{-1})				Mean
		0	20	40	60	
I	Na_2SO_4	5.82	6.00	5.76	5.46	5.76
	S elemental	5.46	5.64	5.40	5.34	5.46
	mean	5.64	5.82	5.58	5.40	
$LSD_{0.05}$	I – 0.073, II – 0.140, I in II – n. s., II in I – n. s.					
II	Na_2SO_4	5.64	6.18	6.06	5.78	5.92
	S elemental	5.88	6.00	5.94	5.74	5.89
	mean	5.76	6.09	6.00	5.76	
$LSD_{0.05}$	I – n. s., II – 0.167, I in II – n. s., II in I – n. s.					
III	Na_2SO_4	6.24	6.96	6.36	6.60	6.54
	S elemental	6.84	7.32	6.96	7.02	7.04
	mean	6.54	7.14	6.66	6.81	
$LSD_{0.05}$	I – 0.094, II – 0.181, I in II – n. s., II in I – n. s.					
Mean	Na_2SO_4	5.90	6.38	6.06	5.95	6.07
	S elemental	6.06	6.32	6.10	6.03	6.13
	mean	5.98	6.35	6.08	5.99	
$LSD_{0.05}$	I – 0.046, II – 0.089, I in II – n. s., II in I – n. s.					

total carbon in soil and the difference in the mean values was 0.06 units. KLIKOCKA (2004) showed that sulphur rates of 25 and 50 S kg ha^{-1} did not modify the amount of total carbon in soil.

According to KOZŁOWSKA-STRAWSKA (2007), excessive amounts of sulphur introduced into soil result in a decrease in the value of soil pH, which can stimulate changes in the content of available forms of nutrients. The effect of the content of available forms of phosphorus in soil on its reaction is common knowledge. In the present research, it was found that sulphur fertilisation, irrespective of the form, limited the availability of soil phosphorus to plants (Table 2). As for the control, there was a significant decrease in the content of P available in soil, ranging from 2.54 mg P kg^{-1} of soil at the rate of 20 kg S ha^{-1} to 4.18 mg P kg^{-1} of soil at the rate of 60 kg S ha^{-1}. The form of sulphur applied did not differentiate much the content of available phosphorus forms in soil.

The content of available forms of potassium in soil, depending on the sulphur fertilisation, ranged from 175.3 mg K kg^{-1} to 217.1 mg K kg^{-1} (Table 3). The form of sulphur, whether sulphate or elemental sulphur, did

Table 2

Content of available phosphorus in soil (g kg^{-1})

Experiment	Form of sulphur	Doses of sulphur (kg S ha^{-1})				Mean
		0	20	40	60	
I	Na_2SO_4	66.16	64.27	64.64	61.58	64.12
	S elemental	64.49	63.55	63.08	63.28	63.60
	mean	65.32	63.91	63.86	62.43	
$LSD_{0.05}$	I – n. s., II – 2.326, I in II – n. s., II in I – n. s.					
II	Na_2SO_4	62.79	59.29	56.68	58.28	29.26
	S elemental	63.80	59.22	57.55	58.55	59.58
	mean	63.30	59.26	56.72	58.42	
$LSD_{0.05}$	I – n. s., II – 2.935, I in II – n. s., II in I – n. s.					
III	Na_2SO_4	79.74	76.98	75.02	74.78	76.63
	S elemental	79.81	78.26	76.85	75.29	77.55
	mean	79.78	77.62	75.94	75.04	
$LSD_{0.05}$	I – n. s., II – 2.481, I in II – n. s., II in I – n. s.					
Mean	Na_2SO_4	69.56	66.85	65.45	64.88	66.68
	S elemental	69.37	67.01	65.57	65.71	66.91
	mean	69.47	66.93	65.51	65.29	
$LSD_{0.05}$	I – n. s., II – 1.301, I in II – n. s., II in I – n. s.					

not differentiate significantly the content of available potassium in soil. The rates of sulphur increasing from 20 through 40 to 60 kg ha^{-1} resulted in a significant decrease in the content of available potassium in soil, as compared with the control, by 7.5%, 3.6% and 3.7%, respectively. Identical relationships were observed in experiments II and III.

The relevant literature reports show that sulphur intensifies the processes of leaching of alkaline-forming nutrients deep down into the soil profile (Motowicka-Terelak, Terelak 1994, Spychaj-Fabisiak 2000, Skwierawska et al. 2006). These processes can lead to the depletion of soluble forms of potassium and magnesium in soil (Murawska et al. 1999, Spychaj-Fabisiak 1999). Kaczor and Łaszcz-Zakorczmenna (2009) claim that a decrease in the content of available forms of potassium and magnesium in soil induced by sulphur fertilisation was caused by a higher uptake of these elements by crops.

The present study has demonstrated that sulphur fertilisation, depending on the rate, decreased significantly the content of available forms of magnesium in soil from 1.1% to 3.6% (Table 4). According to the mean values, no significant effect of the sulphur fertiliser form on the content of

Table 3

Content of available potassium in soil (g kg^{-1})

Experiment	Form of sulphur	Doses of sulphur (kg S ha^{-1})				Mean
		0	20	40	60	
I	Na_2SO_4	177.8	180.2	182.7	175.3	179.0
	S elemental	175.3	175.3	177.6	177.9	176.5
	mean	176.5	177.8	180.2	176.6	
$LSD_{0.05}$	I – n. s., II – n. s., I in II – n. s., II in I – n. s.					
II	Na_2SO_4	195.1	182.8	180.4	182.7	185.2
	S elemental	197.5	180.4	182.8	180.2	185.2
	mean	196.3	181.6	181.6	181.58	
$LSD_{0.05}$	I – n. s., II – 6.29, I in II – n. s., II in I – n. s.					
III	Na_2SO_4	216.9	209.0	207.0	207.0	210.0
	S elemental	217.1	209.4	209.5	206.9	210.4
	mean	217.0	209.2	208.3	210.0	
$LSD_{0.05}$	I – n. s., II – 5.62, I in II – n. s., II in I – n. s.					
Mean	Na_2SO_4	196.6	190.7	190.0	188.3	191.4
	S elemental	196.6	188.3	190.0	188.3	190.8
	mean	196.6	189.5	190.0	188.3	
$LSD_{0.05}$	I – n. s., II – 4.81, I in II – n. s., II in I – n. s.					

available Mg in soil was determined, which was analogous to phosphorus and magnesium. However, a significant effect was attributed to the sulphur form on magnesium content in soil, and the values reported ranged from 0.73 Mg mg kg^{-1} of soil to 1.66 Mg mg kg^{-1} of soil.

CONCLUSIONS

1. The sulphur rates of 20 kg S ha^{-1} as well as 40 kg S ha^{-1} increased significantly the content of total carbon in soil.

2. Sulphur demonstrated an unfavourable effect on the content of available forms of phosphorus, potassium and magnesium in soil.

3. The sulphur form, sulphate or elemental, did not affect the content of organic carbon in soil or the availability of phosphorus, potassium and magnesium.

Table 4

Content of available magnesium in soil (g kg^{-1})

Experiment	Form of sulphur	Doses of sulphur (kg S ha^{-1})				Mean
		0	20	40	60	
I	Na_2SO_4	44.89	43.11	42.67	42.67	43.34
	S elemental	45.33	46.67	44.89	43.11	45.00
	mean	45.11	44.89	43.78	42.89	
$LSD_{0.05}$	I – 0.466, II – 0.894, I in II – n. s., II in I – n. s.					
II	Na_2SO_4	43.11	42.67	43.11	41.34	42.56
	S elemental	41.78	40.89	41.34	40.45	41.12
	mean	42.44	41.78	42.22	40.90	
$LSD_{0.05}$	I – 0.442, II – 0.849, I in II – n. s., II in I – n. s.					
III	Na_2SO_4	52.89	53.33	52.00	52.89	52.78
	S elemental	53.78	52.08	51.17	51.17	52.05
	mean	53.34	52.70	51.58	52.03	
$LSD_{0.05}$	I – 0.725, II – 1.391, I in II – n. s., II in I – n. s.					
Mean	Na_2SO_4	46.96	46.37	45.93	45.63	46.22
	S elemental	46.96	46.55	45.80	44.91	46.06
	mean	46.96	46.46	45.86	45.27	
$LSD_{0.05}$	I – n. s., II – 0.449, I in II – n. s., II in I – n. s.					

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MINERAL COMPOSITION AND BIOAVAILABILITY OF CALCIUM AND PHOSPHORUS FROM ACID WHEY CONCENTRATED BY VARIOUS MEMBRANE PROCESSES*

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Abstract

This study has been undertaken to investigate the effect of different membrane separation processes (nanofiltration, nanofiltration with diafiltration, and ultrafiltration) on the content of macro- and microelements in acid whey from tvarog cheese (white cheese) production and on bioavailability of calcium and phosphorus in rats' diets with 20 or 40% content of spray dried whey. The use of nanofiltration and ultrafiltration processes in whey concentration did not cause differences in the content of Ca, Mg, P, Fe, nor Zn in the end product. Compared to nanofiltration and ultrafiltration, the introduction of diafiltration to the nanofiltration process was observed to reduce the content of Ca from over 14 to less than 10 mg g^{-1}, and that of phosphorus from 8.3 and 7.4 to 5.9 mg g^{-1}, although the biggest reduction was noted in the content of monovalent Na and K. The changes in the mineral composition of whey did not affect coefficients of Ca bioavailability in diets for rats. By substituting Ca in a standard mineral mixture with a 20% addition of whey concentrate, coefficients of the apparent absorption of this element were increased from 36.4% to 42.8-44.6%, and coefficients of its apparent retention from 33% to 38.5-41.7%. The 40% addition of whey concentrates lowered Ca bioavailability coefficients compared to the diets with the 20% whey concentrate content, but not in respect of the control diet. The application of nanofiltration with diafiltration for whey concentration deteriorated phosphorus ab-

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sorption from the diet with 40% of whey, which was indicated by decreased values of the apparent retention coefficient and absolute retention of P in the body of rats within 5 days, i.e. from 35.2 to 18.5% and from 98.8 to 64.5 mg, respectively, compared to the control diet.

Key words: acid whey, calcium, phosphorus, bioavailability.

SKŁAD MINERALNY I BIODOSTĘPNOŚĆ WAPNIA I FOSFORU Z SERWATKI KWASOWEJ KONCENTROWANEJ PRZEZ SEPARACJĘ MEMBRANOWĄ

Abstrakt

W pracy analizowano wpływ różnych procesów separacji membranowej (nanofiltracji, nanofiltracji z diafiltracją i ultrafiltracji) na zawartość makro- i mikroelementów w kwasowej serwatce potwarogowej oraz biodostępność wapnia i fosforu w dietach szczurów z 20 lub 40% zawartością suszonej serwatki. Zastosowanie nanoflitracji i ultrafiltracji w procesie zagęszczania serwatki nie zróżnicowało zawartości Ca, Mg, P, Fe i Zn w produkcie końcowym. W porównaniu z nanofiltracją i ultrafiltracją, zastosowanie diafiltracji przy nanofiltracji zredukowało zawartość Ca z ponad 14 do poniżej 10 mg g^{-1}, a fosforu z 8.3 i 7.4 do 5.9 mg g^{-1}, a najbardziej zmalała zawartość jednowartościowych Na i K. Odnotowane zmiany w składzie mineralnym serwatki nie wpłynęły na wskaźniki biodostępności Ca w dietach szczurów. Zastąpienie Ca ze standardowej mieszanki mineralnej 20% dodatkiem koncentratu serwatki kwasowej wpłynęło na zwiększenie współczynników absorpcji pozornej tego pierwiastka z 36.4% do 42.8-44.6%, a współczynnika retencji pozornej z 33% do 38.5--41.7%. Dodatek 40% pogorszył wskaźniki biodostępności Ca w stosunku do diet z 20% udziałem koncentratu serwatki, jednakże nie w stosunku do diety kontrolnej. Zastosowanie nanoflitracji z diafiltracją do zagęszczania serwatki pogorszyło wykorzystanie fosforu z diety zawierającej 40% serwatki, skutkujące obniżeniem współczynnika retencji pozornej z 35.2 do 18.5% oraz bezwzględnej retencji P w ciele szczurów z 98.8 do 64.5 mg w ciągu 5 dni, w stosunku do diety kontrolnej.

Słowa kluczowe: serwatka kwasowa, wapń, fosfor, biodostępność.

INTRODUCTION

Processed acid whey subjected may become a food component providing low-molecular milk proteins as well as macro- and microelements to diet. Concentration of whey through membrane filtration and dehydration facilitates the use of its valuable ingredients, thus improving nutritive and sensory properties of food products, including bread. Supplementation of wheat and wheat-rye bread with a dry whey commercial concentrate increases concentrations of minerals as well as the content and biological value of protein (Wronkowska et al. 2012). Enrichment of food products with calcium is especially desirable, although the bioavailability of this element from dairy products is very high (Greger et al. 1987).

Recently, valuable components have been separated from highly hydrated materials with the use of membrane techniques. The concentration proc-

ess affects the content and ratios between mineral components. The biggest changes in the composition of whey solids occur after separation processes which employ ultra- and nanofiltration membranes. Ultrafiltration, usually performed at 20-25 kDa cut-off value, is used in order to increase the concentration of whey proteins while simultaneously reducing lactose and the content of mineral compounds (YORGUN et al. 2008, DUSHKOVA, DINKOV 2009). Therefore, ultrafiltrated whey concentrates contain an increased amount of proteins of high nutritive value (SMITHERS 2008). Nanofiltration enables partial demineralization of whey and removal of both salt and lactic acid from acid whey (NGUYEN et al. 2003, SUÁREZ et al. 2006). This process improves the composition of whey concentrate by partial removal of monovalent ions responsible for salty taste and low nutritive value. Depending on the molecular cut-off value of a membrane, the pH of whey as well as its processing characteristics, the total content of minerals in whey solids can be reduced by 30-70% (RICE et al. 2005, SUÁREZ et al. 2006). Nanofiltrated whey concentrate contains an increased amount of Ca^{2+}, Mg^{2+} and phosphorus, whereas the concentration of monovalent ions like in NaCl, which negatively affect the quality of food products, is reduced (NGUYEN et al. 2003). This effect is enhanced if nanofiltration is combined with diafiltration, but in this case some lactose is lost due to dilution (KELLY, KELLY 1995, SUÁREZ et al. 2006). Nonetheless, little information is available on effects of particular membrane techniques on the mineral composition of whey and bioavailability of the most important components, i.e. bioelements.

In view of the above, the objective of this study has been to determine the effect of different processes of membrane separation (nanofiltration, nanofiltration with diafiltration, and ultrafiltration) on the content of macro- and microelements in experimental concentrates of acid whey from tvarog cheese (white cheese) production and on the bioavailability of elements vital for health, including calcium and phosphorus.

MATERIAL AND METHODS

Material

Whey after lactic fermentation and acidic coagulation of milk proteins during tvarog manufacture was used as raw material for production of liquid concentrate with a high content of non-denaturized proteins. Physical membrane separation methods were used: nanofiltration (N), nanofiltration with diafiltration (ND), ultrafiltration (U). The retentates were dehydrated by spray drying (A/S Niro Atomizer Type P-6.3, Denmark) at the inlet/outlet temp. of 190/86°C. Powdered dried concentrates were used in this study.

Membrane separation processes

Acid whey (pH 4.4) was cooled to 5ºC immediately after production. Membrane separation processes were performed using a pilot scale system with 18 tubular membranes in series forming a separation area of 0.85 m^2. The flow rate through the module was maintained from 26.1 L min^{-1} to 34.8 L min^{-1} depending on the membrane type and pressure values. An AFC 30 (PCI) membrane capable to retain 75% $CaCl_2$ was used in the nanofiltration (N) and nanofiltration with diafiltration (ND) experiments. Each process was carried out in a batch mode under the pressure of 1.7-3.2 MPa, achieving the concentration of total solids in the retentate of approximately 18-20%. In the ND separations, the retentate from regular N concentration was diluted with demineralized water added in the amount equal to the volume of extracted permeate. The diluted solution was then concentrated in a replicated N process. A EM006 type membrane (6 kDa cut-off) was applied in ultrafiltration (U) experiments. The process was carried out at the pressure of 1.21-1.32 MPa. The resulting retentates were used as the material for further experiments.

Analysis of the content of elements

The content of elements was determined using the atomic absorption spectroscopy (AAS) method. Samples were wet-mineralized in a mixture of nitric and perchloric acids (3:1). Potassium was assayed with the photometric flame method and phosphorus was investigated with the colorimetric molybdate method with hydroquinone and sodium sulphate (IV). For the validation of calcium determinations, solution of lanthanum chloride was added to all samples in the amounts providing 0.5% concentration of La^{3+}.

Evaluation of Ca and P bioavailability

The bioavailability of Ca and P, indicated by coefficients of apparent absorption and retention, was analyzed using 48 Wistar rats from a 4-week experiment that evaluated physiological effects of diets with 20% or 40% content of acid whey concentrates. In a balance experiment, each group included 8 rats kept in individual cages. The body weight of the rats reached 320 g. The diet for rats was a modified standard AIN-93 diet (Reeves 1997) (Table 1). In order to balance the Ca content in diets in respect of the Ca content in whey concentrates, the quantity of this element was regulated by adjusting the content of the mineral mix. In the diets with 20% of whey concentrates, the content of the mineral mix was ca 5 g kg, and in those containing 40% of whey concentrates, the added mix dose was higher (Table 1). The content of P in the control diet was 2 g kg^{-1}, while in the experimental diets it was increased through addition of whey concentrates.

In the fourth week of the experiment, a daily collection of faeces and urine was continued for 5 days from rats kept in balance cages. The collect-

Table 1

Composition of experimental diets containing spray dried acid whey concentrates after nanofiltration (N), nanofiltration with diafiltration (ND) and ultrafiltration (U)

Component (%)	Diet						
	C	N_{20}	ND_{20}	U_{20}	N_{40}	ND_{40}	U_{40}
Casein	20.0	17.6	17.4	15.8	15.2	14.8	11.6
DL-methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Saccharose	10.0	-	-	-	-	-	-
Acid whey	-	20	20	20	40	40	40
Soybean oil	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Mineral mix	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Maize starch	52.2	44.6	44.8	46.4	27.0	27.4	30.6
Ca source (g k^{-1})							
Mineral mix	5	2.94	1.97	2.98	5.87	3.93	5.77
Acid whey	-	2	3	2	-	1.0	-
Total	5.0	4.94	4.97	4.98	5.87	4. 93	5.77

ed samples were averaged and determined for the content of Ca and P by calculating quantities of these elements in diets and in excreted faeces for 5 days. The coefficient of apparent absorption was calculated as the difference between intake of minerals and their quantity excreted with faeces. The coefficient of apparent retention was calculated as the difference between the quantity of minerals absorbed (digested) with a diet and excreted with urine and expressed in absolute (as mg/5 $days^{-1}$) or relative (%) values.

Statistical analysis

Results of the physiological response of the treated animals are expressed as means and pooled standard error (SEM). Statistical comparisons were done transversely among different dietary groups. Data were analyzed by one-way ANOVA with one factor (diet) or by two-way ANOVA with two factors (diet and dose). If significance was observed ($P<0.05$), Duncan's multiple range test was used to identify differences in the effect of individual diets. Calculations were made with Statistica 6.0 software (StatSoft Corporation, Kraków, Poland).

RESULTS AND DISCUSSION

The mineral composition of the acid whey concentrates produced experimentally differed depending on the applied method of membrane filtration applied prior to the dehydration of the product (Table 2). Similar results of the analyzed micro- and macroelements were achieved using nanofiltration and ultrafiltration. Significantly lower concentrations of most of the elements were noticed in the acid whey concentrate obtained by the coupled process of nanofiltration and diafiltration. At a lower content of Ca and P in this product (ND), the quantitative ratio between these elements was 1.7:1. In turn, in the concentrate after nanofiltration (N), this ration was 1.8:1, whereas in the concentrate after ultrafiltration (U) it was close to 2.0:1 (Table 2). The coupling of diafiltration with nanofiltration caused a decrease in the content of monovalent elements (Na, K), and in that of the microelement Zn. Such an effect of diafiltration used for acid whey concentration was also reported by ROMAN et al. (2009, 2012).

Table 2

Mineral composition of the spray dried acid whey

	Type of spray dried acid whey		
	N	ND	U
Ca (mg g^{-1})	14.68	9.83	14.42
P (mg g^{-1})	8.25	5.86	7.38
Na (mg g^{-1})	2.36	0.64	3.06
K (mg g^{-1})	8.16	2.48	9.17
Mg (mg g^{-1})	1.58	1.16	1.42
Zn (µg g^{-1})	51.2	40.9	47.8
Fe (µg g^{-1})	23.2	25.7	20.9

Explanations see Table 1

Bioavailability of dietary supplements, including minerals, can be defined as the proportion of the administered substance capable of being absorbed and available for use or storage (SRINIVASAN 2001). The bioavailability of calcium from foods, including dairy products, is influenced by many factors, including its intake and content of other dietary components, which either facilitate or inhibit transport through intestinal walls (GUEGUEN, POINTILLART 2000).

During the balance test, an unexpected decrease was observed in the intake of some diets (U_{20} and ND_{40}), which resulted in a significantly lower Ca intake when compared to the control group (Table 3). Simultaneously, differences were noted in Ca excretion with faeces proportionally to its in-

Table 3

Calcium balance in an in vivo experiment

Group	Ca intake (mg/5 days)	Ca fecal (mg/5 days)	Ca urinal (mg/5 days)	Ca absorption		Ca retention	
				(mg)	(%)	(mg)	(%)
Control	438.5	278.9	14.83	159.6	36.4	144.8	33.0
2-way Anova							
N20	429.8^{ab}	243.0	13.23	186.8*	43.9*	171.6*	40.9*
ND20	417.0^{ab}	238.5*	17.85	178.5*	42.8*	160.6*	38.5*
U20	378.3^{b}*	209.8*	11.17	168.5*	44.6*	157.3*	41.7*
N40	463.4^{a}	259.4	21.99*	204.0*	44.3*	180.0*	39.5*
ND40	381.7^{b}*	215.3*	29.60*	166.4*	43.4*	136.8*	35.7
U40	442.6^{a}	260.6	22.18*	182.0*	40.8	159.8*	35.8
SEM	8.817	6.446	1.568	4.626	0.797	4.602	0.890
Acid whey (W)							
N	446.6^{a}	251.2	17.61^{b}	195.4	44.1	177.8^{a}	40.2
ND	399.4^{b}	226.9	23.72^{a}	172.5	43.1	148.8^{b}	37.1
U	410.5_{b}	235.2	16.68^{b}	175.3	42.7	158.6^{ab}	38.8
P values	0.039	0.245	0.046	0.089	0.780	0.028	0.343
Dosage (D)							
20	408.4	230.4	14.08^{b}	178.0	43.8	163.9	40.4
40	429.2	245.1	24.59^{a}	184.1	42.8	159.5	37.0
P values	0.170	0.222	<0.001	0.488	0.591	0.609	0.061
P (W × D)	0.032	0.052	0.865	0.347	0.483	0.258	0.566

SEM – Standard error of the mean (SD for all rats divided by square root of rat number, $n = 48$);
^{a,b} Data with different superscripts in the same column differ significantly at $P<0.05$ (two-way ANOVA followed by Duncan's multiple range test);
*Data significantly different from the control group at $P<0.05$ (*t*-test procedure).

take, but excretion with urine was different. In all the groups of rats receiving diets with whey concentrates, the absorption of Ca from the gastrointestinal tract was higher than in the control group. The difference appeared in the quantity of absorbed Ca and/or value of apparent absorption coefficient. The two-factor analysis demonstrated alike coefficients of Ca absorption from the diet, irrespective of the dose/type of the applied whey concentrate. The inclusion of whey concentrates to diets affected a significant increase in coefficients of Ca retention in rat bodies against the control, especially at dose 20%. The two-factor statistical analysis demonstrated that the application of the studied whey concentrates in the diet had a similar effect on Ca reten-

tion coefficients. Additionally, a higher content of Ca in diets (40%), reduced apparent retention to an extent close to statistical significance (p=0.061). This indicates that the 40% dose of the analyzed concentrates does not yield a beneficial effect in Ca bioavailability.

The present experimental results corresponded to findings of other authors, who demonstrated that calcium bioavailability from organic sources, milk in particular, was superior to that of mineral calcium (Ranhotra et al. 1997, Toba et al. 1999). Some reports also showed that an increasing content of Ca in a diet was accompanied by its reduced retention (Buchowski, Miller 1991, Schaafsma 1997). Such a tendency occurred in our experiment when the rats were fed a diet with a 40% content of N and U concentrates, and the content of calcium exceeded the level of 5 g kg^{-1} recommended in standard diets for rats (Reeves 1997). In the reported experiment, coefficients of apparent absorption and apparent retention of Ca accounting for 40.8--44.6% and 35.7-41.7%, respectively, were high. Their values corresponded with results of other experiments with similar content and sources of Ca in diets (Howe, Beecher 1981, Campos et al. 1998, Kłobukowski et al. 2006).

The intake of phosphorus was significantly higher in the groups of animals fed the diets containing whey concentrates than in the control group (Table 4). This was due to an additional source of P in respect of the P level in the standard mineral mix (Table 1). Simultaneously, it was demonstrated that P excretion with faeces was significantly higher and, to a large extent, proportional to its content in the diet. The increased content of P in diets as a result of whey concentrate supplementation was the reason for a significant increase in phosphorus absorption from the gastrointestinal tract and in coefficients of apparent phosphorus digestibility. The significance of differences in absorption coefficients versus the control group was not confirmed for all the treatments except 40% whey supplementation in the ND and U groups. Apart from the ND_{40} group, absolute increase was noted in the content of phosphorus retained in the rat's body, yet the coefficient of its apparent retention was observed to decrease. Results of the two-factor statistical analysis demonstrated that the coefficients of apparent digestibility of P were alike, irrespective of differences in its intake and the applied membrane separation method. No statistically significant differences were noted in the value of phosphorus apparent retention coefficient as affected by the method of whey concentrate production.

Dairy products are claimed to be a rich source of easily available phosphorus and to assure an optimal ratio between calcium and phosphorus, taking into account demands of human and animal bodies (Tsuchita et al. 1995). Hence, in the present study, despite a higher level of phosphorus in diets owing to added whey concentrate, the coefficients of apparent digestibility of P were high and ranged from 57 to 60%, compared to the value of 54% achieved in the control diet. In contrast, significantly lower values were noted for coefficients of apparent retention of P from diets containing whey

Table 4

Phosphorus balance in an *in vivo* experiment

Group	P intake (mg/5 days)	P fecal (mg/5 days)	P urinal (mg/5 days)	P absorption		P retention	
				(mg)	(%)	(mg)	(%)
Control	227.6	105.5	42.29	122.1	53.7	79.8	35.2
2-way Anova							
N20	347.1*	139.6*	118.5*	207.5*	60.1*	89.0*	25.9*
ND20	312.8*	124.8*	103.2*	188.0*	59.9*	84.4*	27.0
U20	296.7*	117.9	94.25*	178.8	60.3*	84.5*	28.5
N40	439.8*	176.9*	162.2*	262.9*	60.0*	100.7*	23.0*
ND40	344.3*	148.7*	131.1*	195.6*	56.6	64.5*	18.5*
U40	375.1*	159.3*	127.8*	215.8*	57.3	88.0*	23.2*
SEM	10.250	5.206	5.001	6.733	0.827	4.289	1.181
Acid whey (W)							
N	393.5^a	158.2	140.4^a	235.3^a	60.1	96.9	24.5
ND	328.5^b	136.7	117.2^b	191.8^b	58.3	74.6	22.7
U	335.9^b	138.6	111.0^b	197.3^b	58.8	86.3	25.8
P values	<0.001	0.072	0.003	0.002	0.682	0.16	0.528
Dosage (D)							
20	318.9^b	127.4^b	105.3^b	191.5^b	60.1	86.2	27.1a
40	386.4^a	161.6*a*	140.4^a	224.8^a	58.0	84.4	21.6b
P values	0.000	0.000	0.000	0.002	0.221	0.836	0.019
P (W × D)	0.122	0.651	0.602	0.252	0.700	0.287	0.603

SEM – Standard error of the mean (SD for all rats divided by square root of rat number, $n = 48$);
^{a,b} Data with different superscripts in the same column differ significantly at $P<0.05$ (two-way ANOVA followed by Duncan's multiple range test);
*Data significantly different from the control group at $P<0.05$ (*t*-test procedure).

concentrates (18.5-28.5 vs 35.2%), which resulted from a higher supply of P with these diets. A similar tendency was observed by Campos et al. (1998), who reported that despite a larger uptake of phosphorus at the intestinal level, the phosphorus balance decreased as a result of increased urinary excretion. The same tendency was signalled in the case of calcium, showing that an increasing Ca content in a diet resulted in its diminished uptake (Buchowski, Miller 1991, Schaafsma 1997). The absolute retention of P from the diets containing whey concentrates, except for ND_{40} group, was comparable or even higher than in the control group. The inferior phosphorus retention from the concentrate after nanofiltration coupled with diafiltration

was probably due to the diminishing amount of monovalent elements, sodium and potassium, in the end product. Elevated intake of both of these elements in a diet may increase urinal excretion of other elements, in particular calcium (KAUP, GREGOR 1990). In the present study, the decrease in the Na and K content had no beneficial effect on the bioavailability of the analyzed elements. This could have resulted from the fact that the content of P in the diets containing whey concentrates exceeded the value of 3 g kg^{-1}, which is an accepted standard value of nutritional demands of young rats (REEVES 1997).

CONCLUSION

1. The application of nanofiltration and ultrafiltration processes did not cause differences in the mineral composition of acid whey concentrates, whereas the application of nanofiltration with diafiltration reduced contents of Ca and as well as these of Na and K in the finished product, compared to the concentrate after nanofiltration and ultrafiltration.

2. Differences in the chemical composition of acid whey concentrates had no effect on the coefficients of calcium bioavailability in diets for rats, but lowered phosphorus absorption from the acid whey concentrate produced by nanofiltration with diafiltration.

3. Supplementation of rats' diets with dry acid whey concentrates at a level of 40% led to inferior coefficients of the Ca and P bioavailability, mostly due to the increased intake of these elements in excess of the nutritional demands of the rats.

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ANTIHYPERTENSIVE DRUGS AFFECT POTENTIAL BIOAVAILABILITY OF MINERALS FROM SHELLED PEA

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Abstract

Interaction of antihypertensive drugs with minerals can occur during digestion in the digestive tract of patients. The aim of this study was to estimate the influence of hypotensive drugs on the bioavailability of magnesium, iron, zinc and copper from shelled pea during *in vitro* enzymatic digestion.

The degree of release of magnesium, iron, zinc and copper from shelled pea was determined with and without (the control) addition of hypotensive drugs. Four antihypertensive drugs in standard doses (one tablet per sample) were analysed: Metocard (β-blocker), Cardilopin (Ca-antagonist), Apo-perindox (angiotensin-converting-enzyme inhibitor ACE-I) and Indapen (diuretic). The samples were subjected to enzymatic digestion under *in vitro* conditions. The content of minerals in shelled pea before and after enzymatic digestion was determined by flame atomic absorption spectrometry (AAS).

It was found that Indapen (indapamide) significantly decreased the release of magnesium, iron and zinc from shelled pea. The degree of release of magnesium was lower in samples with Metocard (metoprolol) than in the control. The release of copper was significantly reduced by Cardilopin (amlodipine).

Indapamide, metoprolol and amlodypine decreased the release of minerals from pea *in vitro* enzymatic digestion of shelled pea.

Key words: food-drug interaction, mineral bioavailability.

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WPŁYW WYBRANYCH LEKÓW HIPOTENSYJNYCH NA BIODOSTĘPNOŚĆ SKŁADNIKÓW MINERALNYCH Z GROCHU ŁUSKANEGO W PROCESIE TRAWIENIA ENZYMATYCZNEGO *IN VITRO*

Abstrakt

Podczas trawienia może dojść do interakcji między lekami hipotensyjnymi a składnikami mineralnymi w przewodzie pokarmowym pacjentów. Celem pracy było określenie wpływu leków hipotensyjnych na biodostępność magnezu, żelaza, cynku i miedzi z grochu łuskanego w warunkach trawienia enzymatycznego *in vitro*.

Stopień uwolnienia magnezu, żelaza, cynku i miedzi z grochu łuskanego oceniano w próbkach z dodatkiem i bez dodatku (próba kontrolna) leków hipotensyjnych. Analizowano cztery leki hipotensyjne w dawkach standardowych (jedna tabletka w próbie): metocard (β-bloker), cardilopin (antagonista wapnia), apo-perindox (inhibitor konwertazy angiotensyny (ACE-I)) i indapen (diuretyk). Próbki poddano trawieniu enzymatycznemu w warunkach in vitro. Zawartość składników mineralnych w grochu łuskanym przed i po trawieniu enzymatycznym określono za pomocą spektrofotometrii atomowo-absorpcyjnej (AAS).

Stwierdzono, że indapen (indapamid) istotnie zmniejszył uwalnianie magnezu, żelaza i cynku z grochu łuskanego. Stopień uwalniania magnezu był niższy w próbkach z metocardem (metoprolol) niż w próbkach kontrolnych. Uwalnianie miedzi było istotnie mniejsze pod wpływem cardilopinu (amlodypina).

Indapamid, metoprolol i amlodypina zmieniają stopień uwolnienia składników mineralnych z grochu łuskanego w procesie trawienia enzymatycznego *in vitro*.

Słowa kluczowe: interakcje żywność-lek, biodostępność składników mineralnych.

INTRODUCTION

Disorders in the mineral budget of patients with hypertension have been observed in several studies (Kesteloot et al. 2011, Suliburska et al. 2011b). It is known that many nutritional and non-nutritional factors influence absorption of minerals from food, their excretion and metabolism. Both *in vitro* and *in vivo* studies show that bioavailability of minerals depends on the food content of various antinutrients, such as oxalic acid, phytates, dietary fibres and polyphenols, which act as mineral binders or chelators (Oatway et al. 2001, Sandberg 2002, Skibniewska 2002). The degree of mineral release from food products also depends on the processing technology (Suliburska et al. 2009a)

Experimental results show that drugs can also affect the mineral status in patients. Hypotensive drugs influence specially the levels of magnesium, potassium, sodium and calcium in the organism. Treatment with ACE-I and loop diuretics results in a decrease in the serum concentration of magnesium and high losses of this element with urine. However, it has also been shown that lisinopril saves magnesium in patients with congestive heart failure (Oladapo, Falase 2000). It was found that thiazides had a beneficial effect on calcium metabolism in elderly individuals (Ott et al. 2008). Golic

et al. (1998) found that treatment of hypertensive patients with captopril or enalapril may result in zinc deficiency.

Interaction of antihypertensive drugs with minerals can occur during digestion in the digestive tract of patients. The aim of this study was to estimate the influence of selected hypotensive drugs on the bioavailability of magnesium, iron, zinc and copper from shelled pea during *in vitro* enzymatic digestion.

MATERIAL AND METHODS

Food sample

The experimental material was shelled pea, purchased on the local market (the city of Poznan, 2010). Food samples were ground in an electrical mill under laboratory conditions and passed through sieves to divide into fractions with particles having the maximum diameter of less than 2 mm. Samples were dried at 105ºC.

Drugs

In the experiment, four antihypertensive drugs were used: Metocard (β-blocker), Cardilopin (Ca-antagonist), Apo-perindox (ACE-I) and Indapen (diuretic). Characteristics of the drugs are shown in Table 1.

Table 1

Characteristics of the drugs

Drug	Active substance	Dose of active substance (mg/1 tablet)	Antihypertensive - drug class
Metocard	metoprolol	47.5	β-blocker
Cardilopin	amlodipine	10.0	Ca-antagonist
Apo-Perindox	perindopril	3.34	ACE inhibitor
Indapen	indapamide	1.5	diuretic

Enzymatic digestion

Samples were divided into five groups: the control, Metocard, Cardilopin, Apo-perindox and Indapen. The control samples comprised only the product without any drugs. One tablet of a given drug was added the other samples.

In vitro enzymatic digestion was performed according to Skibniewska et al. (2010). In the experiment, one dose of each drug (equivalent of 1 tablet) was analyzed. Each tablet of a given drug was crushed in a mortar and

mixed with a sample (2 g) of finely ground shelled pea in conical beakers, filled with deionised water (20 ml) and shaken for 10 min. In order to create suitable conditions for pepsin activity, pH was brought to 2 using 0.1 M HCl aqueous solution (Suprapure, Merck). Afterwards, pepsin solution (0.5 ml 100 ml^{-1}) was added to the homogenate. Next, the samples were placed in a thermostat shaker (37°C) for 2 hours. During the incubation, pH was maintained or corrected by addition of 6 M HCl aqueous solution whenever necessary. After 2 hours, the digested samples were treated with 6% $NaHCO_3$ aqueous solution (Extrapure, Merck) to bring pH to 6.8-7.0, subjected to pancreatin solution (10 ml/40ml of homogenate) and placed in a thermostatic shaker (37°C) for 4 hours. Afterwards, the digested samples were centrifuged for 15 min (4000 rpm min^{-1}) and clear solution was quantitatively transferred to quartz crucibles, where it was treated with a mixture of concentrated nitric (65% w/w) and perchloric (70% w/w) acids (2:1 v/v) (Suprapure, Merck). The samples were placed in a thermostatic block and heated until complete mineralization.

Control samples were also prepared, in which the product was digested without any addition of the drugs. For each drug, a reagent sample was made, which contained one tablet of a given drug and reagents. All samples were subjected to enzymatic digestion.

In order to determine the total content of minerals in native products, food samples (2 g) were ashed in a muffle furnace at 450°C until complete mineralization and then dissolved in 1N nitric acid. All samples were analyzed in triplicate.

Determination of minerals

The content of minerals in native, *in vitro* digested food products (with and without drugs) was determined by atomic absorption spectrometry (AAS-3, Zeiss spectrometer) with air-acetylene flame, after diluting each sample adequately with deionized water (for Fe, Zn, Cu) or with LaCl3 (0.3% solution, for Mg). The methods were validated by simultaneous assays of the reference material (Soya Bean Flour, INCT-SBF-4), at the accuracy 93.1%, 97.2%, 94.5% and 103.1% for Mg, Fe, Zn and Cu, respectively. The content of minerals in food products was expressed in mg 100 g^{-1} dry mass, while the degree of release for a mineral (its potential bioavailability) was expressed as a percentage of the mineral released vs its total content.

Statistical analysis

The experimental results were given as means ± SD of three parallel measurements. The statistical analysis was carried out using Statistica 7.0 software and Anova was performed at the significance level α=0.05.

RESULTS AND DISCUSSION

Table 2 shows the total content of minerals in shelled pea. This table also presents the amount of minerals (mg) released from 100 g of products (the control sample). This index may reflect their potential bioavailability.

Table 2

Content of minerals in shelled pea and amount released in digestion process (mg 100 g^{-1} d.w.).

Parameters	Mg	Fe	Zn	Cu
Content	90.2±0.6	3.37± 0.03	5.66± 0.03	0.67±0.01
Amount released	85.6±0.5	1.07±0.03	2.75±0.01	0.60±0.01

Table 3 presents the degree of release of minerals in samples with or without the drugs. In some cases, the analysed drugs affected the degree of release of minerals from pea. It was found that Indapen caused a markedly lower release of magnesium, iron and zinc from shelled pea when compared with the control sample. The amount of available copper was significantly lower in samples with Cardilopin than in samples without any drugs. Moreover, Metocard markedly reduced the release of magnesium from the product. Apo-perindox did not affect the release of analysed minerals from shelled pea.

Like all chemical compounds, pharmaceuticals may interact with nutrients. These interactions can lead to reduced or increased release of minerals from food and may affect the bioavailability of minerals.

ACE inhibitors, e.g. captopril and enalapril, have functional groups such as sulphydryl groups or carboxyl groups, whose capacity for binding zinc determines the mineral status of the organism (Golik et al. 1998). Angiotensin converting enzyme inhibitors bind metal ions (iron, copper and zinc) and through this mechanism drugs may interfere with metal-catalyzed reactions (free radical generation) or metal absorption and excretion (Fernandes et al. 1996, Leary et al. 1992). In our previous study, we observed some interaction between perindopril and magnesium and iron (Suliburska et al. 2011a). Contrary to that finding, in the present experiment, perindopril did not markedly affect the release of minerals from shelled pea.

In this study, metaprolol decreased the concentration of magnesium in the supernatant after digestion. No information was found in literature which would indicate direct interaction between metoprolol and magnesium or other elements. However, another β-blocker – carvedilol – is a metal chelator and exhibits antioxidant activity (Oettl et al. 2001). It was also found that propranolol (a β-blocker) and verapamil (a Ca-antagonist) have significant inhibitory impact on peroxidation in tissues in the presence of iron ions (Aruoma et al. 1991).

Table 3

Influence of antihypertensive drugs on release of minerals during digestion

Samples	Mg (%)		Fe (%)		Zn (%)		Cu (%)	
Control	94.9±3.5^{b}	-	31.6±1.3^{b}	-	48.6±3.2^{b}	-	90.0±3.2^{b}	-
Metocard (β-bloker)	57.9±0.8^{a}	(-)39.0*	31.6±1.6^{b}	(-)0.2*	49.1±0.2^{b}	(+)1.0*	85.2±3.2^{ab}	(-)5.3*
Cardilopin (Ca-antagonist)	96.1±0.4^{b}	(+)1.3*	39.0±0.6^{b}	(+)3.3*	49.6±0.1^{b}	(+)1.9*	77.2±2.1^{a}	(-)14.3*
Apo-Perindox (ACE-inhibitor)	93.4±0.4^{b}	(-)1.6*	33.4±0.4^{b}	(+)5.6*	51.8±0.1^{b}	(+)6.6*	96.0±1.1^{b}	(+)6.7*
Indapen (diuretic)	43.1±0.3^{a}	(-)54.6*	16.1±0.2^{a}	(-)49.0*	36.4±0.5^{a}	(-)25.2*	88.5±1.8^{b}	(-)1.6*

*+/ – degree of released minerals compared with control sample
a,b - significant differences; $p<0.05$

Several studies have shown that administration of indapamide is associated with hyponatremia and other electrolytic disorders, especially hypomanesemia and depressed zinc in the organism (Khedun et al. 1995, Pak 2000, Yong et al. 2011). It is known that indapamid forms complexes with copper when the conditions are suitable (Radi 2003). Under the *in vitro* digestion performed in this study, indapamid did not result in the release of copper, but decreased the release of the other minerals, i.e. magnesium, zinc and iron. In our previous study, indapamid affected potential bioavailability of copper from buckwheat (Suliburska et al. 2011a). Moreover, in another experiment we found out that amlodipin and indapamid induced an evident increase in the activity of pepsin (Suliburska et al. 2009a). Higher activity of digestive enzymes can influence the release of minerals from complexes with other components in shelled pea. Both Cardilopin (amlodipine) and Indapen (indapamid) significantly decreased the release of some minerals from the product.

These differences, observed in our previous and the present experiments, may have been caused by the fact that buckwheat groats and pea differ significantly in terms of their composition. Pea contains much more proteins and fiber but less fat than buckwheat groats (Periago et al. 1998, Christa, Soral-Śmietana 2008). Individual ingredients in a product may interact with drugs, thus affecting the release of minerals. Moreover, the differences in the results from our experiments on buckwheat and pea may be due to their effect on pH. Buckwheat is acidic and this can enhance the effects of indapamine and amlodipine on pepsin. In contrast, pea is alkaline and the effect of these drugs on pepsin in its presence could be weakened.

CONCLUSIONS

1. Indapamide, amlodipine and metoprolol affected the release of magnesium, iron, zinc and copper from shelled pea in vitro enzymatic digestion.

2. Hypotensive drugs may diminish the potential bioavailability of minerals.

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PLASMA AND CANCEROUS TISSUE CONCENTRATIONS OF Zn, Cu AND Mn IN PATIENTS UNDERGOING SURGICAL TREATMENT FOR GASTROINTESTINAL CANCER

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Abstract

Gastrointestinal cancers have a complex and multifactorial etiology and their immediate cause remains to be discovered. Elements such as zinc, copper or manganese, which are important components of antioxidant enzymes, may affect malignant processes.

The objective of this study was to determine zinc, copper and manganese concentrations in the blood, cancerous and healthy control tissue of patients operated on for gastrointestinal malignancies as well as to assess the effect of surgical removal of the tumour on the concentration of these elements.

The study included 68 patients who underwent surgery for gastrointestinal cancers. Patients were divided into three groups according to tumour location: group I – oesophageal cancer, group II – gastric cancer and group III – colorectal cancer. Study material consisted of venous blood samples obtained from patients before the surgery and on the seventh day after the surgery; tissue samples were taken during the surgery.

The study has demonstrated that the malignant disease process causes changes in trace element status both in plasma and in cancerous tissues. Copper concentrations were elevated both in patients' plasma and in cancerous tissues, while a decrease was observed in zinc and manganese concentrations in comparison with control tissue. The surgery affected levels of these elements to various degrees corresponding to the location of removed tumour.

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Key words: zinc, copper, manganese, antioxidants, oesophageal cancer, gastric cancer, colorectal cancer, surgical treatment.

STĘŻENIE Zn, Cu I Mn W OSOCZU I TKANCE NOWOTWOROWEJ PACJENTÓW LECZONYCH OPERACYJNIE Z POWODU NOWOTWORÓW ZŁOŚLIWYCH PRZEWODU POKARMOWEGO

Abstrakt

Nowotwory przewodu pokarmowego mają złożoną i wieloczynnikową etiologię, a ich bezpośrednia przyczyna nie jest znana. Pierwiastki, takie jak: cynk, miedź i mangan, mogą wpływać na procesy nowotworowe, gdyż są ważnymi elementami składowymi enzymów antyoksydacyjnych.

Celem badań było oznaczenie stężenia cynku, miedzi i manganu we krwi oraz w tkance nowotworowej i kontrolnej pacjentów operowanych z powodu nowotworów przewodu pokarmowego, a także ocena wpływu operacyjnego usunięcia nowotworu na badane stężenia pierwiastków.

Badaniem objęto 68 chorych operowanych z powodu nowotworów złośliwych przewodu pokarmowego. Chorych podzielono na 3 grupy, biorąc pod uwagę umiejscowienie nowotworu: I – rak przełyku, II – rak żołądka, III – rak jelita grubego.

Materiał do badań stanowiła krew żylna pobierana od chorych przed zabiegiem operacyjnym oraz w 7. dobie po operacji, a także wycinki tkanek pobierane podczas zabiegu operacyjnego.

Badania dowiodły, że proces nowotworowy powoduje zmiany w statusie pierwiastków śladowych zarówno w osoczu, jak i w tkance nowotworowej. Stężenie miedzi w osoczu chorych wzrastało, podobnie jak i w tkance nowotworowej, natomiast stężenie cynku i manganu malało w porównaniu z kontrolą. Zabieg operacyjny wpływał w różnym zakresie na stężenia pierwiastków, co było zależne od umiejscowienia usuwanej zmiany nowotworowej.

Słowa kluczowe: cynk, miedź, mangan, antyoksydanty, rak przełyku, rak żołądka, rak jelita grubego, leczenie operacyjne.

INTRODUCTION

Gastrointestinal cancers have a complex and multifactorial etiology and their immediate cause remains to be discovered. Nowadays, neoplasms are believed to be induced by DNA damage resulting in mutations and abnormalities of cell division. Previous studies have demonstrated that inflammatory states and cancerous processes are associated with the so-called oxidative stress, or imbalance between the generation of reactive oxygen species and antioxidant concentrations as well as the response of defense enzymes (Bartosz 2006**,** Zyska, Kucharzewski 2007).

Such chemical elements as for instance zinc, copper or manganese, constitute important components of antioxidant enzymes; they are involved in DNA biosynthesis and stimulate the immune system; consequently, they may affect cancerous processes (Puzanowska-Tarasiewicz et al. 2009).

Zinc has anticancer properties; its antiproliferative and proapoptotic effect has been demonstrated in *in vitro* studies. The antioxidant effect of zinc is also relevant. Zinc ions protect protein sulfhydryl groups against oxidation. Its antioxidant effect is also associated with the induction of metallothioneins, i.e. proteins with the capacity to remove reactive oxygen species (Knychalska-Karwan et al. 1999, Zaborowska et al. 2005, Bartosz 2006). An essential component of Cu/Zn superoxide dismutase, zinc is involved in the elimination of free radicals.

Copper may impair DNA synthesis by displacing zinc necessary to the process. Deficiency of this element also affects the elements of antioxidant defense system in a direct as well as indirect manner (Olszewski et al. 2003, Yaman et al. 2007, Hadi et al. 2010).

Copper is a cofactor in Cu/Zn superoxide dismutase (CuZnSOD) crucial to the process of removing free radicals. Low copper concentrations cause decreased activity of CuZnSOD, CAT, GPx, ceruloplasmin and glutathione (Berger et al. 2001, Skrzycki et al. 2008). Copper may be a diagnostic factor in the cancerous process – persistent lack of changes in serum copper concentrations is a good prognostic factor while elevated copper levels may be a sign of the recurrence of malignancy (Dobrowolski et al. 2000, Daradó et al. 2005).

A component of superoxide dismutase (Mn-SOD), manganese is involved in the process of eliminating free radicals generated during metabolic changes in cells (Nozoe et al. 2003, Kot, Zaręba 2005).

The objective of the study was to determine zinc, copper and manganese concentrations in the blood, cancerous and healthy control tissues of patients operated on for gastrointestinal malignancies as well as to assess the effect of surgical removal of the tumour on the concentration of these elements.

MATERIALS AND METHODS

The study included 68 patients operated on for gastrointestinal malignancies at the Second General, Gastroenetrology and Digestive Neoplasm Surgery Clinic in the teaching clinical hospital SPSK1 in Lublin.

Control blood samples were collected from 21 healthy people undergoing regular checkups.

The study was approved by the Bioethics Committee at the Medical University in Lublin (decision no. KE-0254/222/2007).

Patients were divided into three groups according to tumour location: group I – oesophageal cancer (10 subjects), group II – gastric cancer (35 subjects) and group III – colorectal cancer (23 subjects).

Blood samples were collected before the surgery and on the seventh day after the surgery. Two tissue samples were also obtained from each subject during the surgical treatment: one from cancerous tissue and a control sample from a most distant margin of healthy tissue.

Concentrations of zinc (Zn), copper (Cu) and manganese (Mn) were determined in the plasma and in tissue homogenates by the atomic absorption spectroscopy method (AAS) with the use of a Pye-Unicam atomic absorption spectrophotometer.

Samples of plasma or tissue (0.5 g) were placed in quartz crucibles and dried at 80-90°C for twelve hours. Next, they were ashed in a muffle furnace at 450°C. The ash was dissolved in a 5% HCl solution, placed in volumetric flasks and filled up to a defined volume with double-distilled water. Quantifications were made at wavelengths specific to particular elements, i.e. 213.856 nm for Zn; 324.754 nm for Cu; 257.610 nm for Mn. Plasma concentrations of the elements were given in μmol l^{-1}, and tissue concentrations in μg g^{-1} protein.

The software package Statistica v. 7.1 was used to perform statistical analysis of the results of the study.

RESULTS AND DISCUSSION

Plasma copper concentrations in the subjects ranged between 7.66 and 11.53 μmol l^{-1} and they were statistically lower than in the control group (15.40 μmol l^{-1}) both prior to and after the surgery. Zinc concentrations before the surgery were similar to those on the seventh day after the surgery; statistically significant differences were observed in groups II and III (Table 1). Moreover, statistically significant differences ($p<0.05$) were observed in zinc concentrations in all the three groups of patients in relation to the tumour location.

Similar results have been obtained in the study by Drozda et al. (2008), where characteristically lower zinc concentrations were detected in the plasma of patients suffering from sigmoid colon cancer or rectal cancer compared with the control group.

Zn concentrations in cancerous tissues ranged between 1.39 and 2.79 μg g^{-1} protein and were lower than in control tissues (1.43-3.22 μg g^{-1} protein). No appreciable differences were observed in cases of oesophageal cancer, while Zn levels in cancerous tissues were lower than in control tissues in colorectal cancer in a statistically significant way (Table 2). This confirms the results obtained by Reddy et al. (2000, 2003) for gastric and kidney malignancies. Lower zinc concentrations in laryngeal cancer were also detected by Niedzielska et al. (2000).

Table 1

Plasma Zn concentration in patients undregoing surgery for oesophageal (group I), gastric (II) and colorectal cancer (III)

Groups		Plasma Zn concentration (μmol l^{-1})					
		$\bar{x}$	SD	range of variability	S_1	S_2	S_3
Before	I	7.93	1.39	6.10 - 10.0	0.001	0.647	0.000
	II	10.23	0.71	9.20 - 11.50	0.000	0.000	
	III	11.53	0.95	10.10 - 13.10	0.008	0.008	
7 day after	I	7.66	0.84	6.90 - 9.20	0.001	0.647	0.000
	II	11.53	1.18	10.00 - 13.80	0.003	0.000	
	III	10.23	1.45	7.70 - 12.30	0.002	0.008	
K		15.40	3.80	6.80 - 19.10			

S_1 – level of statistical significance when comparing Zn concentration in groups (I, II, III) with the control group;
S_2 – level of statistical significance when comparing plasma Zn concentration in groups (I, II, III) before the surgery and on the 7th day after the surgery;
S_3 – level of statistical significance plasma Zn concentration of patients in relation to tumour location.

Table 2

Mean Zn concentration in tissues of patients undregoing surgery for oesophageal (group I), gastric (II) and colorectal cancer (III)

Groups		Zn concentration in cancerous tissue (μg g^{-1} protein)				
		$\bar{x}$	SD	range of variability	S_1	S_2
Cancerous tissue	I	1.39	0.27	1.03 - 1.81	0.789	0.024
	II	2.79	1.93	1.06 - 10.10	0.232	
	III	1.61	0.93	0.45 - 3.82	0.001	
Control tissue	I	1.43	0.56	0.77 - 2.53	0.789	
	II	2.99	1.79	0.94 - 7.36	0.232	
	III	3.22	1.91	0.83 - 8.00	0.001	

S_1 – level of statistical significance when comparing Zn concentration in cancerous and control tissue;
S_2 – level of statistical significance of Zn concentration in cancerous tissue relation to tumour location.

As a component of Cu/Zn superoxide dismutase, zinc is involved in the process of inactivation of reactive oxygen species. Plasma zinc concentrations change when metabolic activity of the body is increased; a decline is observed in malignancies in various locations (NIEDZIELSKA et al. 2000, DROZDA et al. 2008). Low zinc levels both in plasma and in cancerous tissues may be linked to partial depletion of antioxidant barrier in oxidative stress, i.e. cancer.

Plasma copper concentrations in subjects were within the normal range except for group I, in which the average plasma copper concentration before the surgery was markedly lower and amounted to 44.7 μmol l^{-1}. Plasma copper concentrations in patients suffering from gastrointestinal cancers were lower (13.7-15.9 μmol l^{-1}) compared with the control group (17.1 μmol l^{-1}). Plasma copper concentrations (44.7 μmol l^{-1}) were nearly three-fold as high as in the control group only in patients suffering from oesophageal cancer.

A comparison of copper concentrations before and after the surgery demonstrated that there was a statistically significant decrease after surgical treatment of oesophageal cancer. Statistically significant differences between copper concentrations in all the three groups were observed with regard to tumour location (Table 3).

Table 3

Plasma Cu concentration in patients undregoing surgery for oesophageal (group I), gastric (II) and colorectal cancer (III)

Groups		Plasma Cu concentration (μmol l^{-1})					
		$\bar{x}$	SD	range of variability	S_1	S_2	S_3
Before	I	44.7	9.7	26.8 - 57.2	0.000	0.000	0.000
	II	13.8	1.3	11.8 - 15.7	0.035	0.880	
	III	15.9	0.8	14.8 - 17.3	0.400	0.128	
Day	I	15.7	0.8	14.2 - 16.8	0.328	0.000	0.017
	II	13.7	2.2	9.9 - 17.3	0.034	0.880	
	III	15.2	1.23	13.8 - 17.3	0.209	0.128	
K		17.1	4.7	10.6 - 23.5			

S_1 – level of statistical significance when comparing Cu concentration in groups (I, II, III) with the control group;
S_2 – level of statistical significance when comparing plasma Cu concentration in groups (I, II, III) before the surgery and on the 7th day after the surgery;
S_3 – level of statistical significance plasma Cu concentration in relation to tumour location

Copper concentrations in cancerous tissues ranged between 10.3 and 36.7 μg g^{-1} protein, and in control tissue between 6.3 and 22.6 μg g^{-1} protein. In the cancerous tissue of the oesophagus, copper levels were twice as

low as in control tissues, while in the other cancers, tissue copper concentrations were higher. The differences were statistically significant ($p<0.05$). Statistically significant differences between copper levels were also observed in regard to tumour location (Table 4).

Table 4

Mean Cu concentration in tissues of patients undregoing surgery for oesophageal (group I), gastric (II) and colorectal cancer (III)

Groups		Cu concentration in cancerous tissue (μg g^{-1} protein)				
		$\bar{x}$	SD	range of variability	S_1	S_2
Cancerous tissue	I	11.6	3.8	6.4 - 17.1	0.000	0.000
	II	36.7	16.9	8.9 - 68.3	0.000	
	III	10.3	3.0	4.2 - 15.4	0.001	
Control tissue	I	22.6	7.3	10.4 - 39.8	0.000	
	II	21.8	7.4	7.5 - 37.4	0.000	
	III	6.3	1.8	3.1 - 11.2	0.001	

S_1 – level of statistical significance when comparing Cu concentration in cancerous and control tissue;
S_2 – level of statistical significance Cu concentration in cancerous tissue in relation to tumour location.

The study carried out by Witkowski et al. (1993) and Niedzielska et al. (2000, 2004) has also demonstrated that copper concentrations were lowered in the cancerous tissue of the larynx and oesophageal. However, the study conducted by Daradó et al. (2005) has shown that copper levels were higher in cancerous tissues than in healthy tissues in the large bowel and, as observed by Yaman et al. (2007), also in the stomach.

Low plasma copper concentrations and high tissue copper concentrations may be associated with the repositioning of copper from blood to tissues as a result of malignant processes. Elevated plasma copper concentrations correlating with its low concentrations in cancerous tissues in oesophageal cancer remain to be explained.

Plasma manganese concentrations in subjects suffering from cancer ranged between 1.27 and 1.81 μmol l^{-1} and were lower than its concentrations in the control group (3.03 μmol l^{-1}), which was statistically significant.

Mn levels before the surgery did not significantly differ from its levels on the seventh day after the surgical treatment, but there was a statistically significant ($p<0.05$) increase in manganese concentrations on the seventh day after the surgery in comparison to its values before the surgery in group II. Moreover, statistically significant differences in manganese levels prior to the surgery between groups I, II and III were observed (Table 5).

Table 5

Plasma Mn concentration in patients undregoing surgery for oesophageal (group I), gastric (II) and colorectal cancer (III)

Groups		Plasma Mn concentration (μmol l^{-1})					
		$\overline{x}$	SD	range of variability	S_1	S_2	S_3
Before	I	1.64	0.51	0.90 - 2.20	0.000	0.538	0.042
	II	1.27	0.33	0.80 - 1.80	0.000	0.001	
	III	1.50	0.33	0.90 - 1.80	0.000	0.928	
7 day after	I	1.50	0.34	0.90 - 1.80	0.000	0.538	0.110
	II	1.81	0.57	0.90 - 2.70	0.000	0.001	
	III	1.51	0.34	0.90 - 1.80	0.000	0.928	
K		3.03	3.30	2.50 - 3.50			

S_1 – level of statistical significance when comparing Mn concentration in groups (I, II, III) with the control group;
S_2 – level of statistical significance when comparing plasma Mn concentration in groups (I, II, III) before the surgery and on the 7^{th} day after the surgery;
S_3 – level of statistical significance plasma Mn concentration in relation to tumour location.

Manganese concentrations in cancerous tissues ranged between 0.23 and 1.72 μg g^{-1} protein and were similar to its concentrations in control tissues (0.30-1.33 μg g^{-1}). Statistically significant differences were observed only in manganese concentrations in cancerous tissues depending on the location of cancer (Table 6).

Table 6

Mean Mn concentration in tissues of patients undregoing surgery for oesophageal (group I), gastric (II) and colorectal cancer (III)

Groups		Mn concentration in cancerous tissue (μg g^{-1} protein)				
		$\overline{x}$	SD	range of variability	S_1	S_2
Cancerous tissue	I	0.23	0.08	0.15 - 0.36	0.201	0.000
	II	0.89	0.65	0.33 - 3.33	0.210	
	III	1.72	0.72	0.65 - 3.25	0.066	
Control tissue	I	0.30	0.14	0.16 - 0.58	0.201	
	II	1.06	0.94	0.29 - 4.50	0.210	
	III	1.33	0.70	0.68 - 3.46	0.066	

S_1 – level of statistical significance when comparing Mn concentration in cancerous and control tissue;
S_2 – level of statistical significance Mn concentration in cancerous tissue in relation to tumour location.

As a component of superoxide dismutase (MnSOD), manganese is involved in the process of eliminating free radicals generated in metabolic changes. Its low plasma levels may be linked to low MnSOD activity in the course of malignant disease processes caused by intense oxidative stress and overproduction of free radicals.

CONCLUSIONS

1. Malignant disease processes cause changes in trace element status both in plasma and in cancerous tissue.

2. Copper concentrations are elevated in plasma and in cancerous tissue of the patients, while zinc and manganese concentrations are low compared with the control group.

3. Surgery affects concentrations of the elements to various degrees, depending on the location of removed cancer (oesophageal cancer, gastric cancer, colorectal cancer).

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REVIEW PAPERS

SELENIUM IN MEDICINE AND TREATMENT

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Abstract

Selenium, one of non-metals, has attracted great interest among many researchers over the last years. Properties of selenium were first mentioned back in the 12th century. Selenium exists in two forms, organic and inorganic one. But whatever the form of supplementation, it is an essential micronutrient conditioning many vital functions. Large-scale research has shown that it has many important properties, including antioxidant ones, for living organisms. It is incorporated in many enzymes and proteins. Numerous studies on this element have demonstrated its beneficial effects, mainly on the cardiovascular and nervous systems. It also contributes to reduction in the incidence of many neoplastic diseases. However, despite numerous desirable effects of this element in the human body, it should be remembered that selenium is also a toxic substance with a narrow therapeutic index. Its excessive consumption contributes to the development of a condition called selenosis. The recommended dose of selenium, depending on the patient's age, ranges from 25 to 70 μg 24 h^{-1}. However, selenium in excess of 700 μg 24 h^{-1} shows strong toxicity. Therefore, adequate selenium supplementation is crucial. Nonetheless, despite numerous studies on selenium and its biological role, this trace element still raises many unresolved questions.

Key words: selenium, qualities of selenium, selenium deficiency, excess of selenium.

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Abstrakt

Selen, który zaliczany jest do grupy niemetali, na przełomie ostatnich lat stał się pierwiastkiem budzącym zainteresowanie wielu badaczy. Właściwości selenu były opisywane już w XII wieku. Selen występuje w dwóch formach, organicznej oraz nieorganicznej. Jednak bez względu na przyjmowaną formę, jest niezbędnym mikroelementem warunkującym wiele funkcji życiowych. W badaniach wykazano, iż ma on niezwykle istotne dla organizmu żywego właściwości, m.in. antyoksydacyjne. Wchodzi w skład wielu enzymów i białek. Liczne badania nad tym pierwiastkiem udowodniły jego korzystny wpływ m.in. na układ sercowo-naczyniowy i nerwowy. Przyczynia się on także do zmniejszenia występowania wielu nowotworów. Jednak pomimo wielu zalet tego pierwiastka należy pamiętać o tym, iż jest również substancją toksyczną, o wąskim indeksie terapeutycznym. Jego nadmierne spożycie przyczynia się do rozwoju choroby określanej jako selenoza. Zalecane dawki selenu, w zależności o wieku pacjenta, wahają się od 25 do 70 μg 24 h^{-1}, natomiast w dawce powyżej 700 μg 24 h^{-1} wykazuje on silne właściwości toksyczne. Dlatego niezwykle ważna jest odpowiednia suplementacja selenu, bowiem pomimo licznych badań, nadal pozostaje on nie do końca poznanym pierwiastkiem.

Słowa kluczowe: selen, właściwości selenu, niedobór selenu, nadmiar selenu.

HISTORY OF SELENIUM

Selenium was discovered in 1817 by a Swedish scientist J.J. Berzelius, who was analysing slime in a chamber that served for production of sulphuric acid. Initially, it was misidentified as well-known tellurium. However, detailed analysis showed that it was a completely different element, unknown up to that moment. Because of its frequent co-occurrence with tellurium (from the Greek name Tellus – earth), it was called selenium (from Greek Selene – moon).

But the earliest descriptions regarding proprieties of selenium and its toxicity date back to the 12th century (Reid et al. 2004).

The Venetian merchant and traveller Marco Polo, travelling along the Silk Route, reached China. There, in a mountainous area, he found a selenium-rich plant, which – when eaten by cattle – caused their hooves to drop off. In his diaries, he described different states presenting toxicity of this element such as hair loss, tooth loss or sialorrhea. However, despite the unfavourable influence of selenium on living organisms, trace amounts of selenium are essential for maintaining proper functions of the human body. In 1973, selenium was found to have antioxidant properties because of its presence in the active centre of glutathione peroxidase. This finding generated an increased interest in the role of this element in living organisms as well as in its side effects resulting from excess or deficiency of the element (Mckenzie 2000, Zagrodzki 2000a, b).

Bioavailability of selenium depends on many factors, but mainly on its form, which conditions its assimilation. It may occur in an inorganic form as selenites (Me_2SeO_3) or selenates (Me_2SeO_4) and in an organic form as selenomethionine (SeMet) and selenocysteine (SeCys). It is the best absorbed in the organic form as well as in the presence of vitamin A, D and E (Haratake 2007, Holben, Smith 1999, Kabata-Pendias, Pendias 1999, Zaporowska 2002).

The recommended dose of selenium (RDA – Recommended Dietary Allowances) is 25 µg/p.d. for children, 50 µg/p.d. for women and 70 µg/p.d. for men (Boyle, Holben 2004, *Panel on Dietary*…1996, 2000, Scholl, Reilly 2000, Zaporowska 1997).

The threshold between the due and a toxic dose is small. Daily consumption of selenium below 0.1 mg kg^{-1} b.w. may induce symptoms of its deficiency, whereas quantities above 1.0 mg kg^{-1} b.w. may have toxic effects on the human organism.

An essential and safe dose of selenium in the human organism is from 55 to 200 µg 24 h^{-1}, depending on the place of residence. Many authors underline that consumption of selenium in the above doses has a preventive effect in the course of different neoplastic diseases (Drake 2006). Doses of selenium above 700 µg 24 h^{-1} are defined as toxic for the human organism (Seńczuk 1994, Więckowski 1995).

Recommended daily consumption of selenium is different depending on the geographical region. The recommended dose of selenium in Great Britain is 75 µg a day for men and 60 µg a day for women. In the United States, the supply of selenium slightly exceeds due doses and is 90 µg a day, although in Finland the intake of selenium is even higher: 125 µg a day (Bergqvist et al. 2003, Mckenzie et al. 1998).

Therefore, adequate Se supplementation is very important. Protein products like milk, meat, fish, sea food and also cereal products or nuts are the main dietary sources of selenium (B'Hymer, Caruso 2006, Cabrera et al. 1996, Haratake et al. 2007, Pappa et al. 2006, Rayman 2000, Ventura et al. 2007).

Supply of this element can occur indirectly, owing to soil fertilization with selenium or animal fodder enrichment. Other forms of Se supplementation are direct ones, that is together with vitamins and other microelements. Some researchers suggest superiority of the organic form of selenium over the inorganic one (Leeson et al. 2008, Rezanka, Sigler 2008, Slavik et al. 2008, Taylor et al. 2009).

However, irrespective of the form, selenium is an indispensable microelement, needed to maintain normal life functions.

PROPERTIES OF SELENIUM

Selenium belongs to non-metals. It is similar to sulphur in its proprieties. It occurs in three allotropic forms as silver-grey fragile metal, red amorphous body and glassy grey and pink solid body. In nature, it exists in two forms: organic and inorganic. The organic form is more easily accessible to the human body than the inorganic one.

Dihydrogen selenide, selenium dioxide, selenous acid (IV), selenic acid (VI), sodium selenite (IV), sodium selenate (VI) are the most common inorganic forms, although for living organisms the most important organic selenium compounds are selenocysteine, methylselenocysteine, selenomethionine, methylselenomethionine, selenocystyne, selenourea, selenoniocholine, selenobetaine (Pyrzyńska 1996, Wesołowski, Ulewicz 2000).

Selenium is widespread in nature. Penetrating from the atmosphere to oceans, seas and lakes, it is absorbed by plants and then, through the food chain, it reaches animals and humans. Plants can store Se in amino acids (Masłowska et al. 1998, Wachowicz 1993, Wesołowski, Ulewicz 2000).

Selenium supplied with food or through the respiratory system is attached to erythrocytes and plasma proteins: albumins and globulins, and then it is distributed to tissues. It is mainly eliminated with urine and some amounts are also removed from the body with sweat and exhaled air (Wesołowski, Ulewicz 2000).

The best-known metabolic pathway of selenium compounds delivered with food is reduction of selenites (IV) to hydrogen selenide H_2Se and then its methylation. In a non-enzymatic route: selenite (IV) reacts with glutathione (GSH) and is transformed into selenodiglutathione (GS - Se - SG), which in the presence of glutathione reductase undergoes transformation to selenoglutathione (GS - SeH). Hydrogen selenide, an indirect metabolite of the reaction, is then ethylated and eliminated with urine. It can also be used for synthesis of selenoproteins (Daraga, Szymańska 2003).

The main function of selenium is its inclusion in numerous proteins and enzymes. Selenium-dependent enzymes are glutathione peroxidase, selenoproteine P, selenoproteins as well as tetraiodothyronine 5'-deiodinase (Bruk, Hill 1994). It has been discovered that over half of selenium in the blood serum occurs as selenoprotein P. Selenoprotein P is the protein transporting and storing selenium; it has the ability to bind to special biological receptors and it shows antiradical activity (Bruk, Hill 1994, Taylor 1995, Van Cauwenbergh et al. 2004).

Selenoproteins W occur mainly in muscles and in the spleen**,** heart and brain (Vandeland et al. 1993).

Selenium influences activities of selenium-dependent proteins, which are indispensable to the proper functioning of immunological system, mainly lymphocytes T (Mckenzie et al. 1998)

DEFICIENCY AND EXCESS OF SELENIUM

Medical literature contains much information on the role of selenium in the human organism. The physiological role of selenium in a human body, demand for this element, its influence on the development of various diseases and pathological conditions have been studied.

It has been shown that development of tumours, fully manifested AIDS symptoms in patients with the HIV virus or pathologies of the cardiovascular system are more common in patients with low values of selenium (Allovena et al. 1995, Combs et al. 1997, Combs, Gray 1998, Combs 2005, Reilly 1998, Taylor 1995, Zimmerman 2007).

Numerous studies confirm that after absorption in the alimentary tract selenium is distributed with red blood cells and its highest concentration occurs in the thyroid. It is the main component of thyronine 5'–deiodinase, an enzyme catalyzing conversion of thyroxine T4 into its active form of 3,3',5-triiodothyronine (T3). Thus, selenium deficiency causes decrease in triiodothyronine in the blood and symptoms of hypothyreosis, which manifests itself through dry skin, hypersensitivity to cold, wrong heart functioning and disturbed fat metabolism (obesity) (Behne, Kyriakopulos 2001, Berry et al. 1991, Floriańczyk 1996, Hordyjewska, Pasternak 2004, Seńczuk 1994).

Therefore, production of triiodothyronine, an active thyroid hormone, in the presence of the enzyme deiodinase, of which selenocysteine is the main component, depends on the proper level of selenium in the human organism (Duntas 2006).

Duntas et al. (2003) tried to resolve the problem whether the selenium concentration in blood can affect autoimmune thyroiditis. Patients with autoimmune thyroiditis were divided into two groups. For 6 months, one group was given selenometionine in the dose of 200 mg as well as L-thyroxine and the other group received placebo. In the first group, a decrease in the count of antibodies against thyroidal peroxidase was observed: by about 46% after 3 months and about 55.5% after 6 months. It the second group, the analogous decrease was 21% after 3 months and 27% after 6 months. Combination of selenomethionine with L-thyroxine was proven to produce beneficial effects in treatment of the autoimmune thyroid disease.

A low level of thyroid hormones unfavourably influences the functioning of nervous tissue, decreases intellectual efficiency and causes emotional disorders such as depression. A low level of selenium has also been implied in Alzheimer's disease. It is mainly connected with the harmful influence of free radicals on mitochondria (Rayman 2000).

Selenium plays an unusual role in the proper functioning of the cardiovascular system. It is connected mainly with its antioxidant function. The protective role of this element against prooxidants results from its presence in the active centre of antioxidant enzymes like glutathione peroxidase (GSH-

-Px) (Hartikainen 2005, Mckenzie et al. 2002, Navarro-Alarcon et al. 2000, Van Cauwenbergh et al. 2004).

Atherosclerotic changes as a side effect of the vascular endothelium dysfunction are the background of all cardiovascular disorders. High concentration of LDL fraction, excessive activity of shear stress, arterial hypertension, diabetes, hyperhomocysteinemia, anoxaemia, free radicals and also mechanical damage have adverse effects on the endothelium (Naruszewicz, Zapolska-Downar 2006).

More intensive oxidation processes as well as elevated generation of free radicals induced by enzymes such as NADPH oxidase, xanthine oxidase, cyclooxygenase as well as nitrogen oxide synthase in sites affected by arteriosclerosis intensify the destructive changes in the endothelium (Skoczynska 2006).

Positive effects of selenium use were noticed by Huang et al. (2002) while analysing the relation of Se supplementation with changes in blood vessels. Examinations were conducted on Wistar rats with selenium deficiency. They were fed with selenium for 13 weeks. A significantly lower blood selenium concentration, decrease in glutathione peroxidase activity as well as decrease in the HDL and plasma prostacyclins concentrations were stated in the group of selenium-deficient rats in comparison with the control. However, such parameters as the level of lipid peroxides, cholesterol LDL fraction, total cholesterol as well as the thromboxane concentration significantly increased. The lumen of the aorta in selenium-deficient rats was observed with scanning electron microscopy and numerous damages to the endothelium cells were found. They were swollen, contained a vacuole in the cytoplasm and demonstrated lack of integrity in the most severely damaged places. Selenium and selenoproteins were reported to play an extremely important role in the cytoprotective activity against the unfavourable influence of cholesterol on endothelium cells. This is highly important because maintenance of the proper structure of vessels is absolutely necessary for preventing atherosclerotic changes (Huang et al. 2002).

According to other researchers, the unfavourable influence of cholesterol on the endothelium of vessels remains an important clinical problem. Selenium and selenoproteins are hoped to be factors preventing arteriosclerosis development (Wu, Huang 2006).

The human organism possesses many mechanisms controlling free radical formation. Glutathione peroxidase (the GSH - the Px) is an important element of the defence system (Kohrle et al. 2000).

Peroxidase occurs in 4 isoforms. The first form is classic peroxidase (GPx), which is found in the cytosol and participates in reactions of hydrogen peroxide and organic hydroxides reduction. However, it cannot reduce lipid hydroxides. The second form is glutathione peroxidase (PH - GPx), which occurs in the cytosol, too, and is partly attached to the cellular membranes.

It can reduce phospholipid hydroxides. The third isoform is glutathione peroxidase (GPx), also called extracellular peroxidase, located in the plasma. It catalyzes reactions of lipid hydroxides and hydrogen peroxide reduction. The fourth isoform of peroxidase, most recently discovered, is gastric peroxidase (GI - GPx) (JAESCHKE et al. 2002, SAITO et al. 1999).

Glutathione peroxidase is a tetrameric protein of the molecular mass of 84 kDa. Each of the four subunits contains selenocysteine in its catalytic centre. The highest activity of peroxidase was found in the liver, blood and lungs, and the lowest appeared in the brain and eye lenses. Glutathione peroxidase is part of an enzymatic system connected with glutathione (COHEN 1993, FLOHE 1988).

Glutathione peroxidase (GSH - Px) is an antioxidant enzyme, which in the presence of reduced glutathion (GSH) catalyzes reduction of hydrogen peroxide (H_2O_2) to water or organic peroxides (ROOH) to appropriate alcohols (ROH), thus participating in the antioxidant defence of the cardiovascular system.

Glutathione peroxidase breaks the route of phospholipase A2, preventing the liberation of phospholipids of arachidic acid, which in the presence of cyclooxygenase and lipooxygenase could be metabolized to prostaglandins. Thus, the production of inflammatory process mediators, which affect adversely the cardiovascular system, is limited.

Activation of inflammatory potential within a cell can be also modified by products of lipid peroxidation. They modify the physical proprieties of cellular membranes by enhancing their permeability to hydrogen ions. The multiradical process of lipid oxidation is partly dependent on excessive generation of reactive forms of oxygen. Lipid peroxidation can be initiated by hydroxyl, alloxil, alkyl radicals, ozone, nitrogen oxide and dioxide, sulphur dioxide or hypochlorite. Glutathione peroxidase acts protectively on the endothelium of vessels by reducing the peroxynitrite anion (BARTOSZ 2003, SHEVCHUK et al. 2002, SKOCZYNSKA 2006).

Beneficial influence of glutathione peroxidase was also noted in relation to cholesterol hydroxides or its esters or oxysterols. Oxysterols show apoptotic activity on cells of vessels in smooth muscles. Studies conducted on rats state that oxysterol-induced apoptosis is inhibited by increased activity of selenium-dependent enzymes (TANG et al. 2005).

Therefore, maintenance of a proper level of selenium in blood serum is essential for the correct functioning of the antioxidant enzyme glutathione peroxidase.

Numerous studies concerning evaluation of selenium influence on the occurrence of cardiovascular system pathology were conducted. TANGUY et al. (2004) in experiments conducted on Wistar rats examined whether selenium consumption influences the degree of necrosis of the heart muscle caused by transient ischaemia. Animals were divided into two groups. For 10 weeks,

one group received a high dose of selenium (1.5 mg Se kg^{-1}) and the other group – a low selenium dose (0.05 mg Se kg^{-1}). The rats' coronary arteries were ligated for 30 minutes in order to cause ischaemia. The conclusions of the experiment were as follows: infarct size in the group of animals receiving the higher selenium dose was about 25% smaller in comparison with the group receiving the lower selenium supplementation, and the ratio of reduced glutathione to its oxidised form was more beneficial in the first group.

Selenium attracts attention of researchers all around the world. A German research team has tried to estimate the influence of the blood serum selenium concentration in patients with stable angina pectoris or severe coronary syndrome on distant prognosis. Six-year-long observations of a group of 1,700 persons enabled the researchers to conclude that the blood serum selenium level in patients who died because of cardiovascular disorders was low. Therefore, a low level of selenium was stated to correlate with cardiovascular future (LUBOS et al. 2010).

The influence of selenium supplementation on glutathione peroxidase and thioredoxin reductase was analyzed in an experiment carried out on Wistar rats. Male rats were fed a diet including 0, 50, 240 and 1000 mg of sodium selenate kg^{-1} b.w. Then, a 22.5-minute heart muscle ischaemia incident followed by 45 minute-long reperfusion were induced. The examinations resulted in the following conclusions: the heart muscle of animals with selenium deficiency was more vulnerable to damage in comparison with the control group. However, supplementation with higher selenium doses caused intensification of the activity of glutathione peroxidase and thioredoxin reductase as well as acceleration of repair processes in the ischaemic heart muscle. Decrease in the enzymatic activity was closely correlated with impairment of repair functions in the heart after ischaemia and reperfusion (VENARDOS et al. 2004).

Disturbed balance between production and removal of reactive oxygen forms leads to development of oxidative stress, which plays an essential role in pathogenesis of numerous illnesses. Its intensification is noted in myocardial infarction or ischaemia after reperfusion. Glutathione peroxidase participates in reduction of the unfavourable influence of stress on cardiomyocytes. Venardos et al. examined influence of selenium supplementation on mRNA expressions of glutathione peroxidase and thioredoxin reductase in the rat ischaemic myocardium. In the experiment, rats were fed with various doses of selenium for 5 weeks. Then, RNA was extracted by the PCR quantitative method. Selenium rich diet was found to contribute to increase in activities of the examined enzymes of the antioxidant system in comparison with the control group, which did not receive selenium supplementation. Therefore, selenium improves the tolerance to ischaemia after reperfusion by modulating mRNA expression of thioredoxin and peroxidase (VENARDOS et al. 2005).

The relationship between selenium deficiency and the range of ischaemic heart disease has been studied in rat models.

Arterial hypertension, stable coronary disease, myocardial infarction or arteriosclerosis are still a serious health problem. They affect more and more young persons, limiting their professional activity. Therefore, it seems to be essential to perform studies to explain causes of these states.

Smoking cigarettes is undoubtedly one of the risk factors of ischaemic heart disease development. The relationship between low blood selenium concentration and the risk of infarct was estimated by researchers from New Zealand. Examinations were carried out in reference to tobacco smoking as a risk factor of heart diseases. And the conclusion of the study was that a decrease in the blood selenium level with simultaneous smoking is a risk factor of ischaemic heart disease (Beaglehole et al. 2001).

However, not all investigations confirm a strong relationship between blood selenium concentration and a risk of myocardial infarction occurrence.

Another health problem implicated as resulting from a low blood serum selenium level is Keshan and Kashin-Back diseases, which cause changes in the osteoarticular system leading to cartilage degeneration within upper and lower limbs (Li et al. 1985, Nelson et al. 1996, Xu et al. 1991, Zhai et al. 1990).

Keshan disease is defined as a childhood cardiomyopathy. First, the symptoms of the disease were observed in Keshan, a province in China, where the selenium concentration in local soils was extremely low. The disease afflicts children and women at child-bearing age. It attacks the heart muscle, leading to an increase in the heart size and occurrence of numerous necrotic focuses. Against the background of these changes, the development of congestive heart failure occurs (Xia 1994, Zaporowska 2002).

Low selenium levels contribute to the development of another disease, Friedreich disease (FRDA), i.e. hereditary ataxia. Foyer found many histological similarities between Friedrich disease and Keshan disease (Fryer 2002).

The crucial role in the pathogenesis of ataxia is attributed to the mitochondrion. As a result of mutations in the first intron of FXN gene, encoding a mitochondrial protein called frataxin, an excessive number of repetition of three nucleotides GAA occurs. Consequently, the absence of frataxin causes iron accumulation in mitochondria and thereby more intensive oxidative stress (Cooper, Schapira 2003, Lodi et al. 2006).

Thus, disturbances in the functioning of the mitochondrial respiratory chain, oxidative damage and accumulation of iron play an important role in the pathogenesis of ataxia (Cooper, Schapira 2007). Friedrich ataxia, a degenerative disease, apart from producing a devastating effect on the nervous system, affects the heart (González-Cabo et al. 1997).

Another important issue is the role of selenium in preventing neoplasic diseases. Selenium as an antioxidant, reduces lipids and limits DNA and RNA peroxidation processes, thus having a protective effect against genetic damage. Being a component of numerous redox enzymes, cytochrome b and c, selenium is involved in metabolic processes at the cellular level. All types of macromolecular damage resulting from oxidative stress contribute to cancer development. The protective effect of selenium is not only limited to the action of the antioxidant enzyme glutathione peroxidase. Other selenoproteins, such as selenomethionine or selenocysteine, have protective properties. Selenium has beneficial influence on health during many stages of carcinogenesis, both at both initiation and during tumour growth, by blocking the synthesis of DNA in cancer cells (Bartosz 2003, El-Bayoumy 1994, Masłowska et al. 1998). Selenomethionine and selenocysteine are components available in many foodstuffs such as cereals, broccoli and garlic.

While analysing the causal relationship between selenium and cancer, Whanger noted significant relationships. The level of blood plasma selenium in patients with malignant cancer is significantly lower than in the healthy control group. However, inconsistent results were provided by tests determining the selenium level in nails and its relationship with the incidence of cancer (Whanger 2004).

Consuming selenium-rich foods significantly reduces the risk of cancer. Beneficial effects of a diet rich in broccoli and therefore in selenium have been reported. This was confirmed by an experiment in which rats with a chemically induced colon cancer were fed with selenium-rich diet. A 50% decrease in incidence of irregular crypts compared with the control group was stated. Consumption of selenium-enriched plant food also reduced the incidence of breast cancer (Finley 2003, Finley et al. 2005).

Reactive oxygen species, which contribute to the development of oxidative stress, can be generated as a result of external factors (UV light, ionising radiation, etc.) as well as internal ones (defensive reactions of the immune system). Their increased concentration in an organism activates a series of reactions leading to pathological changes in the structure of DNA and mutations. Thus, it seems very important to know all the available forms of defence and reduce the incidence of cancer.

Numerous reports on the effects of antioxidants on the development of cancer can be found in the literature.

The Nutritional Prevention of Cancer Trial group tried to determine whether selenium supplementation affects the incidence of prostate cancer. Cases of men whose initial plasma selenium concentration was low and the PSA ratio (prostate specific antigen) lower or equal to 4 ng/mL were analysed in details. The conclusion of that study was that selenium supplementation has a beneficial effect on decreasing the incidence of prostate cancer in the examined group (Duffield-Lillico et al. 2003).

Similar conclusions were drawn by researchers from Canada. They determined the impact of mineral supplementation including selenium and antioxidant vitamins on the incidence of prostate cancer in SU.VI.MAX study. Men were divided into two groups, with normal and elevated PSA. In the group of men with normal baseline PSA receiving selenium-enriched diet, statistically significant reduction in the incidence of prostate cancer was found. However, in men with elevated PSA no such relationship was verified. Moreover, they were observed to present an increased incidence of cancer. Thus, the earlier hypothesis that consumption of antioxidant vitamins and minerals, including selenium, reduced the risk of prostate cancer has been confirmed (Mayer et al. 2005).

The study conducted in Holland on 59 thousand men born between 1955 and 1969 evaluated the level of selenium in segments of nails. After 6 years of observations, it was found out that a higher selenium intake may reduce the risk of prostate cancer (Van Den Brandt et al. 2003).

However, not all studies verify the correlation between selenium intake and incidence of prostate cancer. Peters et al., when examining a group of about 35 thousand men, tried to find out if there was a relationship between selenium and vitamin E intake and reduced incidence of prostate cancer. They found out that vitamin E and selenium supplementation is not related to the risk of cancer (Peters et al. 2008).

Neoplastic prostatic hyperplasia is a serious clinical problem. But begin prostate hyperplasia, which is characterized by the following symptoms: hiperuresia, nocturia, inability to stop micturition, dysuria, etc, is another serious and common disorder. Therefore, it seems worth investigating the problem whether selenium supplementation may have beneficial effects on the human health in the case of lower urinary tract pathology. Rohrmann et al. (2004) determined the protective effect of selenium in begin prostatic hyperplasia. The study covered a group of 2,497 aged over 60, who demonstrated symptoms of begin prostatic hypertrophy. Before the test, concentrations of vitamins A, C and E, carotenoids and selenium had been determined. The control group were men without dysuric symptoms. The results demonstrated that the concentration of selenium was much lower in the group with the lower urinary tract pathology than in the control group. In contrast, men with a higher selenium concentration showed the symptoms of dysuria significantly less often. Thus, selenium was claimed to exert protective effects on prostate cancer as well as beneficial effects on benign prostatic hyperplasia patients (Rohrmann et al. 2004).

Clinical problems discussed above are more common in men over the age of 50. But in the group below 50 years of age, infertility is frequently a clinical problem. Many external factors, environmental or nutritional ones, lead to a reduction in the number of sperm cells in semen. Selenium supplementation was found to have positive influence on sperm motility and consequently on sexual functions. Research conducted by Ammar-L Keskes

on a group of 54 infertile men was designed to determine the effect of selenium and vitamin E supplementation on the improvement of sperm parameters. Vitamin E in a dose of 400 mg and selenium in a dose of 200 μg were given to 28 men for 3 months. The marker of lipid peroxidation, i.e. concentration of malondialdehyde (MDA), was determined in the study. Selenium and vitamin E supplementation caused a significant decrease in the MDA level and thus improved sperm motility. The authors emphasize the protective effect of selenium on semen quality, indicating a high potential of this element use in infertility treatment (KESKES-AMMAR et al. 2003).

Similar conclusions were reached by Safarinejad MR et al. while examining 468 men with infertility, who were given 200 μg of selenium and 600 mg of N-acetylcysteine for 26 weeks. Supplementation with selenium and N-acetylcysteine positively correlated with improvement of sperm quality (SAFARINEJAD, SAFARINEJAD 2009).

Thus, selenium supplementation may be a significant factor in improving sperm condition and decreasing infertility among men.

By analogy to prostate cancer in men, breast cancer is an extremely serious and dangerous disease in women. It is the most frequently occurring cancer among women. Genetic predisposition as well as hormonal and environmental factors underlie tumour development. Many carcinogens have direct impact on the tumour transformation, which is then modulated by hormones. Due to a high incidence of breast cancer, particularly among women over 50 years of age, it becomes essential to search for any factors which could prevent the disease. Japanese researchers assessed the impact of eating selenium-enriched vegetables and tried to determine the role of selenium in chemoprevention of cancer. Japanese radishes grown on a medium enriched in selenium were given to rats with artificially induced breast cancer (by administration of 10 mg or 14 mg DMBA 7,12-dimethylbenz (α) anthracene). The experiment showed that the incidence of breast cancer was statistically significantly lower in the group eating the selenium-enriched diet than in the control group. They suggest that consumption of selenium-enriched products can be an effective diet component in the prevention of breast cancer (YAMANOSHITA et al. 2007).

Selenium is hoped to be a dietary component that can prevent development of tumours. Many studies have shown positive correlations between selenium-enriched diet and reduction in the frequency of tumour occurrence. However, not all examinations have yielded consistent results.

Influence of selenium supplementation on the prevention of basocellular cancer of skin was studied by a team of scientists from New York. About 1,300 persons from the eastern part of the United States participated in randomized examination. They were given selenium in the dose of 200 μg a day. The results showed that selenium supplementation was ineffective in prevention of basocellular cancer. Moreover, it was demonstrated to increase the risk of planoepithelial cancer (DUFFIELD-LILLICO et al. 2003).

Beneficial effects of selenium supplementation can result from its detoxification role. It reduces toxicity of xenobiotics and heavy metals by affecting their metabolism in a living organism. Glutathion and co-operating enzymes e.g. glutathione peroxidase, in which selenocysteine is the catalytic centre, play an unusually important role in cells (ŁUKASIEWICZ-HUSSIAN 2003).

Selenium beneficially influences the metabolism of cadmium, reducing its immunotoxicity (BOSCOLO et al. 2004). It also reduces the toxicity of mercury in the human organism by forming quite stable chemical bonds with this heavy metal (BUKOWSKA 2003).

The protective influence of selenium against ionization and UV radiation in alleviation of the side-effects of chemotherapy and nitrosoamine poisonings has also been reported (MCKANZIE 2000, MICKE et al. 2004). Research also shows that selenium is an unusually important vestigial element in many cases of diseases.

However, it should be remembered that it is also a toxic substance of a narrow therapeutic index. First records about the toxic influence of selenium can be found in the diaries of Marco Polo written in the 12th century. Numerous investigations have shown that excessive consumption of selenium has unfavourable influence on the human organism, causing selenosis. Selenium toxicity symptoms, as J.K. MacFarquhar et al. stated, are diarrhoea (78%), fatigue (75%), hair loss (72%), pain of joints (70%), change of colour of nails and their fragility (61%) as well as nausea (58%) (MACFARQUHAR et al. 2010, ZAWIERTA et al. 1997). Full-blown selenosis is most common in Venezuela, Colombia, United States and in China (LEE et al. 1996, ZIMMERLI et al. 1997).

The mechanism of the toxic activity of selenium is connected with the competent cooperation of the element with sulphur, causing disorder of its metabolism. In the synthesis of mercapto acids selenium from selenite (IV) can displace sulphur and enter into reactions with thiol groups. Large quantities of selenium disturb the metabolism of methionine, catalyze oxidation of hydrosulphurs and cause inhibition in protein synthesis (THOMSON 2004, VILLA et al. 1999). The excess of selenium interferes with processes of catecholamine amine alkylation and stimulates the synthesis of toxic alkyloselenic compounds (MASŁOWSKA, JANIAK 1991).

Despite numerous intensive investigations on selenium, in many aspects the element remains unknown. Many metabolic routes as well as a range of its action within cells await examination. Therefore, elucidation of the selenium physiology and pathology is a challenge for many researchers.

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HEALTH-PROMOTING PROPERTIES OF SELECTED MILK COMPONENTS

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Abstract

The human diet should be a rich source of nutrients, energy and tissue-building materials. Bovine milk is one of the few food products of animal origin that meet the above requirements. It contains proteins rich in readily available amino acids, fatty acids, vitamins, micronutrients and macronutrients, such as calcium, magnesium, phosphorus, sodium, iodine, potassium, chlorine and small quantities of iron.

This study discusses the positive effects of bovine milk on human health, resulting from its composition and high nutritive value. We have reviewed numerous publications and reports indicating that milk contains readily available amino acids, unsaturated fatty acids which are vital components of the human diet, as well as macronutrients and micronutrients that regulate biochemical processes in the body. Particular attention has been paid to the anti-carcinogenic, antioxidant, anti-sclerotic, anti-inflammatory and antibacterial properties of milk, which is also known to lower blood pressure and strengthen the immune system.

The health benefits delivered by selected minerals contained in milk are also described. Calcium content largely determines the nutritional value and heat stability of milk, as well as its suitability for cheese production. Milk calcium is easily absorbed, and it is characterized by a high level of physiological activity due to a favorable calcium to phosphorus ratio of 1.2:1. Milk contains 0.75 g-1.10 g dm^{-3} of phosphorus, and phosphorus concentrations are generally stable and independent of the nutritional regime of cows. The magnesium content of milk is determined in the range of 100 to 150 mg dm^{-3}. In milk, magnesium is found in the form of soluble compounds (75% of total Mg) as well as colloidal compounds (phosphates, citrates). Magnesium concentrations are correlated with the calcium content of milk. The magnesium to calcium ratio determine milk's heat stability. Milk contains 1.35 to 1.55 g dm^{-3} of fully ionized potassium. The sodium content of milk is

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determined at 350-600 mg dm^{-3}, and chlorine levels are noted in the range of 0.80-1.40 g dm^{-3}. Sodium chloride stabilizes the osmotic pressure of milk (including lactose). Milk contains trace quantities of iron (0.42 to 0.45 mg kg^{-1}).

Key words: milk, proteins, fatty acids, macronutrients, micronutrients, amino acids, human diet.

PROZDROWOTNA WARTOŚĆ WYBRANYCH SKŁADNIKÓW MLEKA

Abstrakt

Dieta człowieka powinna zawierać produkty bogate w składniki odżywcze, budulcowe i energetyczne. Jednym z nielicznych surowców pochodzenia zwierzęcego spełniającym wymienione warunki jest mleko krowie. W swoim składzie zawiera białka bogate w łatwo przyswajalne aminokwasy, kwasy tłuszczowe, witaminy oraz mikro- i makroelementy, takie jak wapń, magnez, fosfor, sód, jod, potas, chlor oraz w niedużej ilości żelazo.

Celem pracy było wykazanie wartości odżywczej mleka w aspekcie jego bogatego składu i pozytywnego wpływu na zdrowie człowieka. Na bazie literatury wykazano, że mleko zawierając łatwo przyswajalne aminokwasy, niezbędne dla człowieka nienasycone kwasy tłuszczowe oraz makro- i mikroelementy pozytywnie wpływa na wiele przemian biochemicznych w organizmie. Zwrócono szczególną uwagę na udokumentowanie właściwości antynowotworowych, antyoksydacyjnych i przeciwmiażdżycowych mleka, a także działanie przeciwzapalne, antybakteryjne, obniżające ciśnienie krwi i wzmacniające układ odpornościowy.

W pracy podano także znaczenie dla zdrowia człowieka wybranych pierwiastków, tj. wapnia, fosforu, magnezu i żelaza. Podano, że wapń decyduje o wartości odżywczej mleka, jego stabilności cieplnej i przydatności do produkcji serów. Jednocześnie wykazano, że charakteryzuje się dobrą przyswajalnością i aktywnością fizjologiczną ze względu na korzystny dla organizmu człowieka stosunek do fosforu, wynoszący 1,2:1. Ponadto ukazano zawartość w mleku niektórych pierwiastków w kontekście aspektów warunkujących te zależności. Określono, że poziom fosforu w mleku jest stały i niezależny od żywienia, w granicach 0,75-1,10 g dm^{-3}. Zawartość magnezu w mleku wynosi od 100 do 150 mg dm^{-3}. Pierwiastek ten występuje w formie związków rozpuszczalnych (75% ogólnej ilości Mg) oraz koloidalnej (fosforany, cytryniany). Ilość magnezu jest skorelowana z ilością wapnia w mleku. Stosunek ten decyduje o stabilności cieplnej mleka. Zawartość potasu w mleku waha się od 1,35 do 1,55 g dm^{-3} w formie całkowicie zjonizowanej. Zawartość sodu w mleku wynosi 350-600 mg dm^{-3}, a chloru 0,80-1,40 dm^{-3}. Rola chlorku sodowego polega na utrzymaniu ciśnienia osmotycznego mleka (wraz z laktozą) na stałym poziomie. Mleko zawiera niewiele żelaza (od 0,42 do 0,45 mg kg^{-1}).

Słowa kluczowe: mleko, białka, kwasy tłuszczowe, makroelementy, mikroelementy, aminokwasy, dieta człowieka.

INTRODUCTION

According to current scientific knowledge, the human diet should incorporate around 60 nutrients that are essential for growth, development and functioning of the body. Human breast milk and bovine milk are nutritionally the richest foods of animal origin (Przybojewska, Rafalski 2003). For adult

humans, milk and dairy products are a vital source of energy, tissue-building nutrients and components that play a regulatory role in the body (SÉVERIN, WENSHUI 2005). As demonstrated by research studies, the bioactive components found in milk prevent obesity and cancer, and they offer a variety of health benefits for consumers (PARODI 1997, BARR 2003, ZEMEL 2003). Humans are quite special in this respect as no other mammals consume milk regularly after reaching adulthood (ARAKAWA et al. 1999).

The biological value of milk is determined by the content of bioactive components that offer health benefits (REKLEWSKA et al. 2005, WONG et al. 2006). Recent years have witnessed a growing interest in functional foods, including milk (WARD, GERMAN 2004). Milk contains biologically active substances, including proteins, peptides, amino acids, sugars, vitamins, enzymes, sterols, phospholipids and fatty acids which regulate biochemical processes in the human body. Milk is also a source of polyunsaturated fatty acids (PUFA), which have anti-carcinogenic, antioxidant, anti-sclerotic, anti-inflammatory and antibacterial properties. PUFAs effectively lower blood pressure and reinforce the immune system (BARŁOWSKA et al. 2005, STANTON et al. 2005, KRÓL et al. 2006, WONG et al. 2006).

Milk provides a plethora of bioactive ingredients for incorporation into functional food products. This has come at a time when consumers want more from food than just basic nutrition, including the prevention of lifestyle diseases through a healthy diet. Using milk as a model system could prove invaluable for developing designer foods or even 'designer milk', a concept that has been discussed in a review by SABIKHI (2007). However, it should be noted that many of the physiological effects observed for the bioactive components of milk have only been proven *in vitro* or in animal models and have yet to be proven in humans. Another major challenge faced by food scientists and manufacturers alike is the cost-effective large-scale production of milk bioactive ingredients. For example, while the potential for milk proteins and peptides as ingredients in functional food products has been well documented on a vast scale, their large-scale production and commercialization are still limited. Major efforts are now underway to develop methods to ensure optimal activity of these agents in food systems and their subsequent utilization in the body. One group has recently cloned the 11-residue antimicrobial peptide from bovine lactoferrin (BL-11) and the 12-residue hypotensive peptide from αs1-casein (C-12) in the dairy starter culture *Streptococcus thermophilus*, which is utilized in the production of yoghurt and various cheeses (RENYE, SOMKUTI 2008). Multiple repeats of the hypocholesterolemic peptide IIAEK, derived from bovine milk β-lactoglobulin, have been introduced into the five variable regions of soybean proglycinin A1aB1b and when expressed in *E. coli* have demonstrated the large-scale production of a small peptide of fewer than 10 amino acids (PRAK et al. 2006, PRAK, UTSUMI, 2009). The potent antihypertensive peptide derived from ovalbumin, RPLKPW (novokinin), has recently been incorporated into the soybean through transgenesis (YAMADA et al. 2008). Through such appro-

aches, milk bioactive peptide sequences may even be incorporated into food proteins of non-dairy origin. However, using molecular genetic approaches to increase the bioavailability or production of therapeutic peptides is perhaps an unrealistic goal, considering the strict regulations governing the use of genetically modified organisms in the food industry in certain parts of the world. The existing modern technologies applicable for the isolation of bioactive native proteins and peptides from milk are beyond the scope of this review but have been discussed in detail by KORHONEN and PIHLANTO (2003, 2007).

This study discusses the nutritive value and the positive health effects of bovine milk based on a review of scientific evidence.

Milk tolerance

Most consumers tolerate milk and its nutritional components, but some adult humans are unable to digest milk due to lactose intolerance. This defect results from an absence of lactase, an enzyme produced by the small intestine which hydrolyzes lactose into glucose and galactose. Nearly 70% of the worldwide population is lactose intolerant. The above applies to approximately 5% Caucasians, whereas other ethnic groups are much more severely affected (around 90% Chinese adults, 85% Aboriginal Australians, more than 45% indigenous Africans). In Poland, lactose intolerance affects 1.5% infants and children and 20-25% adults. The latest population research advocates the production of lactose-free milk and recommends that lactose-intolerant people should limit their milk consumption (JACKSON, SAVAIANO 2001, KEITH et al. 2011).

Nutritional value of milk

Milk is one of the most nutritionally complete foods in the human diet. Its high nutritive value can be attributed to its unique chemical composition which supports optimal digestion and absorption. The nutritional value of milk is determined by its energy value, digestibility, assimilability and biological properties. Milk dry matter contains nutrients that are vital for the growth and healthy functioning of the human body: lactose, protein, fat, minerals and vitamins. The energy content of milk is a sum of individual energy contributing components – 49% fat, 40% milk sugar and 11% proteins. The composition of milk varies subject to the breed of dairy cows, their nutritional regime, successive lactation, lactation stage, season and herd management practices (WALSTRA et al. 1999).

Proteins and peptides

The nutritive value and processing suitability of milk are determined mainly by its protein content. The major protein fractions in milk are casein and whey proteins, including immunoglobulins, α-lactalbumin, β-lactoglobulin, lactoferrin and transferrin (SEVERIN, WENSHUI 2005) – Table 1.

Table 1

Concentration and biological activity of major bovine milk proteins (Séverin, Wenshui 2005, Walstra, Jenness 1984, Yamauchi 1992, Korhonen et al. 1998)

Protein	Concentration (g L^{-1})	Function
Total caseins	26.0	ion carrier (ca, po4, fe, zn, cu), precursor of bioactive peptides
α-casein	13.0	precursor of bioactive peptides
β-casein	9.3	precursor of bioactive peptides
κ-casein	3.3	precursor of bioactive peptides
Total whey protein	6.3	anti-carcinogenic, weight management
β-Lactoglobulin	3.2	retinol carrier, binding fatty acids, possible antioxidant
α-Lactalbumin	1.2	lactose synthesis in mammary gland, ca carrier, immunomodulation
Immunoglobulins (A, M, and G)	0.7	immune protection
Serum albumin	0.4	
Lactoferrin	0.1	antimicrobial, antioxidant, immunomodulation, iron absorption
Lactoperoxidase	0.003	antimicrobial
Lysozyme	0.0004	antimicrobial, synergistic effect with immunoglobulins and lactoferrin
Miscellaneous	0.8	
Proteoseepeptone	1.2	function unknown; precursor of bioactive protein and peptide in vitro
Glycomacropeptide	1.2	antiviral, bifidogenic

Milk proteins contain a full range of exogenous amino acids, whose concentrations exceed the recommended daily protein intake for adults formulated by the Food and Agriculture Organization of the United Nations (FAO) in 1990. The protein content of milk varies from 2.6% to 5.3%, depending on genetic and environmental factors (Miciński et al. 2008). Protein levels in milk largely determine cheese yield per 100 kg of milk. Casein accounts for 78% of all milk proteins, and the casein content of milk ranges from 2.2% to 3.6% (Grega 1999). There are five main casein fractions: αs1-casein, αs2-casein, β-casein, γ-casein, and κ-casein (Pikul 2004). Bovine milk proteins are also functional food components that deliver nutritional and health benefits (Pikul 2004). In the group of six milk protein fractions, two polymorphic forms, β-lactoglobulin and κ-casein, are genetic markers for quantitative trait loci which determine the chemical composition of milk and cheese yield (Aleandri et al. 1990, Litwińczuk et al. 2006).

Milk also contains whey proteins, including β-lactoglobulin (2-4 g dm^{-3}) and α-lactalbumin (1.0-1.5 g dm^{-3}) (Michalski, Januel 2006). They are a rich source of lysine, methionine and cysteine, as well as essential amino acids such as threonine and tryptophan. All bovine milk proteins, including casein, are an abundant source of lysine, an essential amino acid which is found in very small quantities in cereals. The consumption of milk, hard cheese or cottage cheese with bread supplements lysine-deficient wheat proteins with this essential amino acid. As a result, cereal proteins become wholesome building blocks that are absorbed by the body and used to build new tissue (Zmarlicki 2006). Whey amino acids are commonly used in the production of food supplements for athletes, elderly consumers and recovering patients. As foreign antigens, bovine milk proteins may cause an allergic reaction in humans (Zwierzchowski et al. 2011). Their anti-mutagenic and anti-carcinogenic properties have been demonstrated in an *in vitro* study (Zmarlicki 2006).

The medicinal properties and uses of milk proteins have been extensively studied (Madureira et al. 2007, Zimecki, Kruzel 2007). Research results show that whey, containing lactoferrin, β-lactoglobulin and serum albumin, contributes to suppressing the growth of neoplastic cells (Parodi 2007) and accelerates their apoptosis (Svanborg et al. 2003). The utilization of whey as a functional food ingredient for weight management is attracting much interest (Luhovyy et al. 2007). The special membrane structure surrounding milk micro-lipid droplets, composed of a lipid bilayer and proteins, termed the milk fat globule membrane (MFGM), has also shown potential as a therapeutic agent against many pathological conditions (Spitsberg 2005). One of the proteins isolated from MFGM, referred to as the fatty acid binding protein (FABP), has been shown to inhibit some breast cancer cell lines (Spitsberg, Gorewit 1997a, 2002). In addition, the onco-suppressor protein BRCA1 has also been found in both bovine and human MFGM (Spitsberg, Gorewit 1997b). Wang et al. (2001) also demonstrated that glycoproteins of MFGM were capable of inhibiting the infection of *Helicobacter pylori* in a BALB/cA mouse model, and the hemagglutination and adhesion of *Helicobacter pylori* in HeLa S3 monolayers.

Bioactive peptides which exert numerous physiological responses can also be generated from milk proteins in the gastrointestinal tract. Such bioactive peptides are latent or encrypted within native protein precursors, thus proteolysis is required for their release (Gobbetti et al. 2004). Bioactive peptides have been generated from most of the major proteins in both bovine and human milk (Séverin, Wenshui 2005). Bioactive milk peptides were first described in 1950, when Mellander (1950) reported that ingestion of casein-derived phosphorylated peptides led to enhanced vitamin D-independent calcification in rachitic infants. While bioactive peptides can be generated from a variety of foods, milk proteins are generally regarded as a very rich source of those peptides and, as a result, have become fundamental constituents of several commercially available functional food products and ingredients (Table 2).

Table 2

Compositions and concentrations of oligosaccharides in bovine milk, bovine colostrum and human milk (Mehr, Kelly 2006, Kunz, Rudloff 200), Gopal, Gill 2000, Nakamura, Urashima 2004

Oligosaccharide	Bovine milk (g L^{-1})	Bovine colostrum (g L^{-1})	Human milk (g L^{-1})
Lactose	40 - 50	40 - 50	55 - 70
Neutral oligosaccharides			
Lacto-N-tetraose	trace	-	0.5 - 1.5
Lacto-N-fucopentaose I	-	-	1.2 - 1.5
Lacto-N-fucopentaose II	-	-	0.3 - 1.0
Lacto-N-fucopentaose III	-	-	0.01 - 0.2
Lacto-N-difucohexaose I	-	-	0.1 - 0.2
Lacto-N-novopentaose		NR*	
N-acetylgalactosaminylglucose		NR*	
N-acetylgalactosyl-lactose		NR*	
α-3'-galactosyl-lactose		NR*	
β-3'-galactosyl-lactose		NR*	
6'-galactosyl-lactose		NR*	
N-acetyl-lactoseamine		NR*	
N-acetyl-galactosaminyl-lactose			
NeuAc(a2e6)lactose	0.03 - 0.06	0.019	0.3 - 0.5
NeuAc(a2e3)lactose		0.095	0.1 - 0.3
N-glycoylneuraminyl-lactose			
NeuAc-lacto-N-tetraose a	trace	-	0.03 - 0.2
NeuAc-lacto-N-tetraose c	trace	-	0.1 - 0.6
NeuAc2-lacto-N-tetraose	trace	-	0.2 - 0.6
6-Sialyl-lactosamine		0.047	
3-Sialyl-galactosyl-lactose		trace (3 µmol L^{-1})	
Disialyl-lactose		0.028	
Sialyl-lactose-1-phosphate		trace (3 µmol L^{-1})	
Sialyl-lactose-6-phosphate		trace (1 µmol L^{-1})	
3-Glycolyl-neuraminyl-lactose		trace (2 µmol L^{-1})	
6-Glycolyl-neuraminyl-lactose		NR	
GlcNAcb(1-3)Galb(1-4)	-	-	-
GlcNAcb(1-3)Galb(1-4)Glc	-	-	-

* NR – oligosaccharides detected and structurally characterized, but concentration not reported

Bioactive peptides from milk can be divided into the following categories based on their physiological effect on the body or the protein from which they have been derived: antihypertensive, antithrombotic, opioid, casein phosphopeptides (CPPs), antimicrobial, cytomodulatory, immunomodulatory, and miscellaneous peptides (HAYES et al. 2007).

Antithrombotic peptides that interfere with the formation of thrombi have also been identified in milk (ZIMECKI, KRUZEL 2007). Enzymatic hydrolysis of κ-casein has resulted in some of the most antithrombotic peptides to date from food sources. Thrombosis is a pathological condition that results in clot or thrombus formation in arteries, veins or the ventricles of the heart. It is interesting that comparisons can be drawn between the mechanisms involved in milk clotting, defined by the interaction of κ-casein with chymosin. and the mechanisms of blood clotting, defined by the interaction of fibrinogen with thrombin (RUTHERFURD, GILL 2000).

The so-called opioid peptides can also be found in bovine milk**.** Opioid peptides show pharmacological similarities to opium. Caseins (αs1-, αs2,- β- and κ-) and whey proteins are potential sources of opioid peptides. However, the major opioid peptides are fragments of β-casein, called β-casomorphins (CLARE, SWAISGOOD 2000). Similar proteins have also been reported from human β-casein fractions. On the other hand, all κ-casein fragments, known as casoxins, behave as opioid antagonists (SÉVERIN, WENSHUI 2005). Opioid peptides have also been found encrypted within the primary sequence of whey proteins such as lactoferrin, β-lactoglobulin, and bovine serum albumin (BELEM et al. 1999). β-casomorphins are resistant to the action of gastrointestinal enzymes and have been associated with the following activities: antihypertensive, immunomodulatory, antidepressant, anti-secretory and anti-diarrheal (PIHLANTO 2001). Opioid peptides are thought to be biologically very potent; potentially, micromolar amounts may be sufficient to exert physiological effects (MEISEL, FITZGERALD 2000).

Milk is a rich source of antimicrobial proteins and peptides, capable of exerting antimicrobial activities comparable to antibiotics. This effect is due to the synergistic activity of naturally occurring peptides and defense proteins besides immunoglobulins, such as lactoferrin, lactoperoxidase and lysozyme and is greater than any individual contribution (CLARE et al. 2003, SÉVERIN, WENSHUI 2005). The potent properties of these agents can be reflected by the example of bovine lactoferrin, which has displayed strong antiviral activity against HIV and the human cytomegalovirus, the latter of which is thought to act synergistically in patients with acquired immunodeficiency syndrome (FLORIS et al. 2003).

Milk fat

Milk fat is a concentrated source of energy (48% of the total energy value of milk), responsible for the pleasant flavor of milk. Owing to its high digestibility and nutritional value (up to 99%), bovine milk fat plays an im-

portant role in human nutrition, although its fatty acid profile is less than ideal (Barłowska, Litwińczuk 2009).

Fat is not a homogenous substance, and it comprises fat globules which are dispersed in the aqueous phase of milk to form an emulsion. Fat globules contain triacylglycerols that account for 98% of milk fat. Their membrane consists of glycoproteins, 1.1% phospholipids, monoacylglycerols (0.16-0.38%), diacylglycerols (0.28-0.59%), free fatty acids (0.1-0.4%), sterols (0.42%), carotenoids and fat-soluble vitamins (Górska et al. 2006).

Bovine milk fat is characterized by one of the most complex structures among all natural fats. According to various authors, it contains from 400 (Jensen 2002) to around 500 fatty acids (Reklewska, Bernatowicz 2003), of which only 15 have more than a 1% share of the total fat content, but their combined composition (by weight) accounts for approximately 95% of the total fatty acid profile. Around 36 fatty acids and their isomers have more than a 0.1% share of the fatty acids profile in milk. The remaining fatty acids are found in trace quantities (Parodi 2004).

Fatty acids – functional components of milk, whose properties are determined by the length of the hydrocarbon chain and the number of unsaturated bonds, have a decisive impact on fat quality. They are synthesized by ruminal microflora from acetate, β-hydrobutyrate, triacylglycerols, lipoproteins and, in smaller quantities, from sterols, phospholipids and free fatty acids. The nutritive value of milk is also determined by the presence of minerals essential for human health (Bodkowski et al. 2004, Parodi 2004, Barłowska et al. 2005).

Fatty acids which are chain compounds with 4 to 16 carbon atoms are synthesized by the glandular tissue of the udder, whereas fatty acids with a longer chain are produced in the blood plasma. The diet of dairy cows can by manipulated to affect the content of milk fat (Barłowska, Litwińczuk 2009).

Bovine milk contains approximately 70% saturated fatty acids and 30% unsaturated fatty acids. The latter are composed of monounsaturated fatty acids (MUFAs) in around 83% and polyunsaturated fatty acids in 17% (Brzóska et al. 1999).

The unique value of bovine milk fat can be attributed to short-chain and medium-chain fatty acids (14% share of total fatty acids), which provide a source of energy for muscles, heart, liver, kidneys, blood platelets and the nervous system. The above fatty acids do not increase blood lipid levels and they do not contribute to the risk of obesity (Barłowska, Litwińczuk 2009). Butyric acid plays an important role in the prevention and treatment of colorectal cancer. It stilts the development of neoplastic cells by inhibiting DNA synthesis in their nuclei. Short-chain fatty acids contribute to the treatment of colorectal diseases, including inflammatory bowel disease and ulcerative colitis (Przybojewska, Rafalski 2003).

Long-chain fatty acids contain 16 and more carbon atoms, and they have an estimated 56-68% share of the total fatty acid profile in bovine milk

(Pisulewski 2000, Barłowska et al. 2006). This group of fatty acids comprises mostly palmitic acid (C16:0 – 25-30% of total fatty acids) and stearic acid (C18:0) (Pisulewski 2000, Kolanowski 2007). Lauric acid (C12:0) and myristic acid (C14:0) contribute to the risk of cardiovascular disease (Sundram et al. 1994). Stearic acid (C18:0) has a neutral effect. This unsaturated fatty acid is easily converted into oleic acid (C18:1, an unsaturated fatty acid) in the body, and it has been shown to lower blood cholesterol levels. Similarly as lauric acid and myristic acid, also palmitic acid increases the levels of low-density lipoproteins (LDL) and total cholesterol, it contributes to platelet aggregation and the risk of arterial thrombosis. Excessive consumption of palmitic acid increases the risk of cardiovascular disease and atherosclerotic heart disease (Wilke, Clandinin 2005).

The remaining bovine milk fatty acids contain one or more double bonds. Monoene fatty acids have an estimated 30% share of the fatty acid profile, including oleic acid with a 25% share. According to research, the above fatty acids effectively counteract atherosclerosis (Reklewska et al. 2005).

Milk fat contains approximately 3% essential fatty acids (EFAs) which are not synthesized by the body and therefore have to be supplied with food. In the process of dehydrogenation and chain elongation, EFAs are converted into polyunsaturated fatty acids (PUFAs) which contain 2 to 6 double unsaturated bonds. There are two distinct PUFA families, omega-3 (*n*-3) and omega 6 (*n*-6). α-linolenic acid and linoleic acid are heads of the respective families. The proportions of omega-6 to omega-3 fatty acids consumed in the diet should be balanced, and the ideal *n*-6/*n*-3 fatty acid ratio is 4-10:1. Essential fatty acids that deliver the greatest health benefits are C18:2 (linoleic acid) (*n*-3), C18:3 (α-linolenic acid) (*n*-3) and the resulting long-chain fatty acids (containing more than 18 carbon atoms and more than 3 unsaturated bonds), including arachidonic acid (C20:4, *n*-6), eicosapentaenoic acid (C20:5, *n*-3) and docosahexanoic acid (C22:6, *n*-3) (Simopoulos 2002, Dymnicka et al. 2005, Miciński et al. 2012).

The discussed fatty acids are essential components in the diet of infants and children in the first months of life. They play an important role in the development of the central nervous system and the retina. EFAs are bioactive components that lower cholesterol levels and reduce the risk of atherosclerosis. Those prostaglandin precursors are found in cell membrane phospholipids, and they have crucial cellular functions (Reklewska, Bernatowicz 2003).

Oleic acid (*n*-9 family) is one of the most functionally important fatty acids that delivers a variety of health benefits. Oleic acid inhibits the uptake of cholesterol from ingested foods, lowers LDL levels, reduces blood viscosity and lowers blood pressure. Other fatty acids with health-promoting effects include linoleic acid (*n*-6 family) and the resulting arachidonic acid (prostaglandin and leukotriene precursor), as well as fatty acids of the *n*-3 family (eicosapentaenoic acid and docosahexanoic acid). Cell membrane phos-

pholipids contain *n*-6 and *n*-3 polyunsaturated fatty acids. When released from phospholipids, they become a substrate for the synthesis of eicosanoids, including prostaglandins (PG), prostacyclins (PGI), thromboxanes (TXA), leukotrienes (LT) and lipoxins (Turley, Strain 1993, Simopoulos 2002).

The health effects delivered by polyunsaturated fatty acids can be largely attributed to the activity of eicosanoids, which regulate cardiovascular function, blood pressure, coagulation, plasma triacylglycerol concentrations, immune response, inflammatory processes, proliferation and development of neoplastic cells, hormone and neurotransmitter activity, gene expression, renal function and pain sensation. Eicosanoids prevent ischemic heart disease, they boost immunity, transport lipids, including cholesterol, and lower cholesterol levels in peripheral blood (Kolanowski 2007, Kowalski et al. 2010, Zwierzchowski et al. 2011).

A deficiency of unsaturated fatty acids can adversely affect growth and development, including weight loss and lower daily gains; it may promote the pathogenesis of many diseases, impair cholesterol transport, increase capillary brittleness and reduce the contractility of the cardiac muscle. Adequate amounts of unsaturated fatty acids in the diet of pregnant women contribute to increasing the birth weight of infants, support the development of the baby's central nervous system, and reduce the risk of allergies and atopic ailments. Unsaturated fatty acids are considered essential for neural and retinal development in newborn babies (Barłowska, Litwińczuk 2009).

Polyunsaturated fatty acids include conjugated dienes of linoleic acid (CLA) whose double bonds may exhibit cis- and trans-type configuration. CLA is an intermediate product of biohydrogenation of polyunsaturated fatty acids by *Butyrivibrio fibrisolvens* bacteria in the rumen (Barłowska, Litwińczuk 2009). In a study investigating the CLA content of butter, Bauman et al. (2000) isolated bonds with three configuration types: cis-trans, trans-trans and cis-cis. In the above experiment, the CLA content of milk fat was determined at 5.30 g kg^{-1}, and the identified forms had a 85.8%, 9.4% and 4.8% share of total CLA, respectively. According to Parodi (2004), the CLA content of milk fat may exceed 30.00 g kg^{-1}, with a predominance of biologically active isomer cis-9, trans-11. Bovine milk products contain 2.90-11.30 g CLA kg^{-1} of fat, and cis-9, trans-11 CLA has a 73-93% share of this group of fatty acids. Cheese is the richest source of CLA.

CLA has many important functional properties – it impairs the growth of skin, breast, colorectal and gastric cancer cells. The cis-9, trans-11 isomer shows the highest levels of biological activity, whereas the trans-10, cis-12 isomer is believed to prevent the development of obesity (Bawa 2003, Wang, Jones 2004). CLA helps prevent osteoporosis, reduces blood sugar levels, boosts immune system function, lowers total cholesterol and LDL cholesterol levels, and improves the LDL/HDL ratio in the blood plasma, thus contributing to the prevention of ischemic heart disease and atherosclerosis (Reklewska, Bernatowicz 2003).

Cholesterol accounts for 0.2-0.4% of total milk lipids. Average cholesterol concentrations are noted at 12 mg in milk with a 3.5% fat content, whereas butter contains 240 mg cholesterol per 100 g fat. In milk lipids, around 90% of cholesterol occurs in free form, whereas the remaining cholesterol is esterified with linoleic acid (18:2), palmitic acid (16:0) and oleic acid (18:1) (Żegarska 1998). Low density lipoproteins (LDL), high-density lipoproteins (HDL) and very low-density lipoproteins (VLDL) account for 60%, 30% and 10% of total cholesterol in milk lipids, respectively. Saturated fatty acids have varied effects on cholesterol concentrations. Fatty acids which contain 4 to 10 carbon atoms and stearic acid reduce blood cholesterol levels. Lauric acid (C 12:0) and myristic acid (C 14:0) increase the risk of cardiovascular disease, and so does palmitic acid, but only in elderly people (Żegarska 1998, Parodi 2004).

Milk appears to protect against diabetes. The ingredient responsible is trans-palmitoleic acid, found in milk, cheese, yoghurt and butter, which cannot be synthesized in the human body and has to be supplied by food. On the other hand, eating dairy products may contribute to excessive weight gain and lead to the development of obesity-related diseases, including diabetes, particularly in elderly people (Barłowska, Litwińczuk 2009).

While milk fat serves as a source of energy for the neonate of each species, it is also a source of bioactive agents which can influence all aspects of physiology from the immune system to the CNS. In addition, its components exert both antibacterial and antiviral activities. In the future transgenesis may provide an approach towards altering the fatty acid composition of milk. The expression of a rat stearoyl-CoA desaturase gene under the control of the bovine β-lactoglobulin promoter in transgenic dairy goats altered the fatty acid composition of milk, resulting in a less saturated and more monounsaturated profile (Reh et al. 2004). However, milk fat, unlike other components, is more pliable in that its content can be manipulated through the diet. The ability to manipulate the content of certain fatty acids, such as CLA, has enabled scientists to directly enhance the therapeutic properties of milk and dairy products (Mills et al. 2011).

Oligosaccharides

Infants and young children digest milk (lactose) easily since they possess specific bacterial flora. The majority of adults, however, lose this ability. Researchers from the University of California in Davis and from the Utah State University (USA) have analyzed the genomes of intestinal bacteria in children and adults. They demonstrated that human milk oligosaccharides (HMOs) are resistant to enzymatic hydrolysis, but they are degraded by some *Bifibobacterium longum* strains. *Bifibobacterium longum* subsp. *infantis* dominates in breastfed infants, and *Bifibobacterium longum* subsp. *longum,* specialized for plant-derived carbon metabolism, is found in adults (Mills et al. 2011).

The origin of *lactobacilli* and *bifidobacteria* that colonize the neonatal gut had in the past been attributed to contamination of the infant with maternal microorganisms upon passage through the birth canal. However, recent studies provided clear evidence that human milk is a direct source of both *lactobacilli* (Martin et al. 2007) and *bifidobacteria* (Martin et al. 2009). Martín et al. (2006) successfully tracked a *Lactobacillus salivarius* isolate, which was shown to have potential probiotic properties, from the feces of a one-month-old breast-fed infant to the breast milk of the respective mother through the use of DNA finger-printing. Moreover, in a related study, an analysis of the probiotic potential of another three *Lactobacillus* isolates from breast milk indicated that each strain had probiotic properties comparable to those of strains commonly used in commercial probiotic products (Martín et al. 2005).

A total of 40 oligosaccharides identified in bovine milk are used to produce infant formulas. Most of those oligosaccharides are composed of short oligomeric chains (Tao et al. 2008). Fructo-oligosaccharides and galacto--oligosaccharides (FOS and GOS) are highly effective in promoting the growth of *bifidobacteria* and suppressing the development of pathogenic bacteria in the gastrointestinal tract of newborns and adults (Clostridia and *Escherichia coli*) (Boehm et al. 2005, Fanaro et al. 2005).

FOS are found in vegetables and fruit, such as onions, asparaguses, artichokes and tomatoes (Crittenden, Playne 1996). GOS are produced through the enzymatic conversion of lactose contained in bovine milk (Montserrat, Santamaria-Orlean 2001). In 1905, Tissier (1905) demonstrated that *Bifidobacterium bifidum* is the predominant bacterial species in the intestines of breastfed infants. Gyorgy et al. (1974) isolated glycans from milk, which enabled to identify all oligosaccharides (Ninoneuvo et al. 2006) – the most important bioactive components of milk (Table 2). Also recent research findings indicate that breastfeeding provides protection against pathogens, and that the use of infant formulas supplemented with the GOS/FOS mixture is an adequate alternative (Bakkar-Zierikzee et al. 2005, Knol et al. 2005). The incidence of respiratory inflammation ($p = 0.01$), atopic dermatitis ($p = 0.01$), fever ($p = 0.00001$) and antibiotic use ($p = 0.05$) has been shown to be lower among two-year-olds who were fed formulas supplemented with GOS in the amount of 8 g dm^{-3} for the first six months of their life (Moro et al. 2006, Van Hoffen et al. 2009).

It has been estimated that human milk contains 7-12 g dm^{-3} oligosaccharides (Boehm, Stahl 2007), about 5-10% of the lactose concentration. Milk has a high and relatively stable lactose content (4.5-5.2%), and it also contains trace amounts of glucose and galactose. Lactose is fully synthesized from blood glucose by mammary gland cells. Glucose enhances calcium and phosphorus absorption from food, and it effectively prevents rickets (Litwińczuk et al. 2006).

A comparison of the oligosaccharide content of human milk with milk from other animal species indicates that human milk is unique in terms of the complexity and content of oligosaccharides (KUNZ et al. 2000, BOEHM, STAHL 2007). HMOs reach the maximum concentration in the colostrum (above 20 g dm^{-3}) after which they remain at a stable level in mature milk (approx. 12-14 g dm^{-3}) (COPPA et al. 1999). Bovine milk, on the other hand, contains very low levels of oligosaccharides, around 1 g dm^{-3} (MONTREUIL 1960). In addition to lactose, bovine milk contains trace quantities of glucose and galactose. Glucose promotes the absorption of calcium and phosphorus from milk, thus preventing rickets in children (KOZIKOWSKI, PRZYBYŁOWICZ 1994, LITWIŃCZUK et al. 2004).

Interestingly, milk oligosaccharides have been shown to play a significant role in the induction the inflammatory immune response. Inflammation is a complex multi-step process providing a non-specific defense mechanism against tissue injury. During the inflammatory process, injured tissue cells release chemical signals called inflammatory mediators that activate the endothelium of nearby capillaries. This activation results in the release of cell surface adhesion molecules referred to as selectins, which are glycoprotein in nature, and essential for the formation of plateletneutrophil complexes – PNC (KANNAGI 2002, LEY 2003, RHEE et al. 2003). These heterogeneous cell aggregates represent a large subpopulation of neutrophils with a greater capacity for phagocytosis and increased production of reactive oxygen species – ROS (PETERS et al. 1999).

Calcium and vitamins in milk

Milk lipids are also abundant in vitamins A, D, E and K. One liter of milk covers 25% of the daily recommended intake of β-carotene and vitamin A, and 10% of RDI values for vitamins D and E (PEŁCZYŃSKA 1996, SCHOROEDER et al. 2003). The vitamin A content of bovine milk lipids ranges from approx. 0.60 to approx. 2.00 mg in 100 g of fat, and it is affected by the cow's feeding period (ŻEGARSKA 1998). Vitamins A, E and β-carotene boost the immune system, and they play a significant role in growth, reproduction and vision.

As demonstrated by ZEMEL (2005), calcium found in bovine milk helps us stay slim. It has been suggested that dietary calcium exerts this effect on weight through the calcitrophic hormones, parathyroid hormone and 1,25 (OH) 2 D (ZEMEL 2004). These hormones have been shown to respond to low-calcium diets and exert coordinated regulatory effects on human adipocyte lipogenic and lipolytic systems (ZEMEL 2004). Dairy products may supply 580 mg calcium daily. For optimal results, high calcium intake should be accompanied by an increase in blood vitamin D levels. At high milk consumption, vitamin D concentrations may reach 30 nanograms per milliliter. Researchers from the Ben-Gurion University in Beer-Shera, the USA and

Germany have found that increased calcium intake and high blood levels of vitamin D contribute to weight loss.

Dietary calcium sources, including milk, exert markedly greater effects in attenuating weight than supplemental calcium (ZEMEL 2005). In a study by ZEMEL (2004), 32 obese adults were maintained on balanced calorie-deficit diets – 500 kcal/d and 400–500 mg Ca/d (group I) and 800 mg Ca/d (group II), or a diet high in dairy products (3-4 servings of milk, yogurt, or cheese daily; total calcium intake of 1.20-1.30 g/d). Over the 24-wk trial, group I subjects lost 5.4% of their body mass, compared with 8.6% and 10.9% in groups II and III, respectively (differences significant at $p \leq 0.01$).

The greater effect observed for dairy calcium has been attributed to additional bioactive components which have been assigned to the whey portion of milk (CAUSEY, ZEMEL 2003, ZEMEL 2005). ACE-inhibitory (Angiotensin Converting Enzyme) activity has been postulated as increasing the fat-reducing effects of dairy calcium, since angiotensin-II regulates, in part, adipocyte lipogenesis (PFEUFFER, SCHREZENMEIR 2007). Moreover, branched-chain amino acids (BCAA, leucine, isoleucine and valine) have also been implicated in the process, since in addition to protein synthesis, these amino acids also play specific metabolic roles as energy substrates and in the regulation of muscle protein synthesis (LAYMAN 2003). As already discussed in this review, MCFAs (Medium Chain Fatty Acids) have been shown to have a positive effect on weight reduction. However, not all studies have concluded that dietary calcium or dairy calcium has a positive effect on obesity, and the controversy relating to the results of clinical trials has been discussed in the following reviews (BARBA, RUSSO 2006).

Longer term trials are required to thoroughly investigate the effects of dietary calcium as changes in fat mass may be too small to detect in short periods of time. In addition, several other dietary factors must be considered such as total energy intake, dietary protein content and vitamin D status. Moreover, nutrigenomics has been proving that different populations and different individuals demonstrate variable responses to the same dietary components. BARBA and RUSSO (2006) have suggested that the complex composition of dairy foods may be responsible for this biological variability. Overall, the evidence suggests that dairy foods have an important role to play in weight regulation. In the future, a better understanding of the mechanisms involved in dairy calcium and weight loss will enable reliable recommendations for dairy calcium consumption which will potentially help reduce the development of obesity in many individuals.

Drinking milk after exercise has positive effects in both children and adults. Milk supplies carbohydrates, electrolytes, calcium and vitamin D. Casein and whey contained in milk encourage muscle gain and regeneration. Eating large quantities of dairy products may help achieve longevity. A study involving 4000 human subjects has shown that individuals eating adequate amounts of dairy products (cheese, milk, butter), beginning in early child-

hood, are less likely to have a stroke later in life. Surprisingly, although many dairy products contain fat and cholesterol, their consumption by children does not increase the risk of heart diseases in adults, and it reduces mortality rates by 1/4. Therefore, children should drink a glass of milk every day, eat yoghurt and cheese, and switch to skim milk and milk products at a later age (BARŁOWSKA, LITWIŃCZUK 2009).

Minerals in milk

Milk is a rich source of minerals, mainly calcium, phosphorus, sodium, potassium, chlorine, iodine, magnesium and, in small quantities, iron. Calcium is the key mineral component in milk. Its content largely determines the nutritional value and heat stability of milk, as well as its suitability for cheese production. The average calcium content of bovine milk falls in the range of 0.60-1.20 g dm^{-3} (milk calcium is easily absorbed and is characterized by a high level of physiological activity owing to a favorable calcium to phosphorus ratio of 1.2:1). Milk contains 0.75-1.10 g dm^{-3} of phosphorus, and phosphorus concentrations are generally stable and independent of the nutritional regime of cows. The magnesium content of milk is determined in the range of 100 to 150 mg dm^{-3} In milk, magnesium is found in the form of soluble compounds (75% of total Mg) as well as colloidal compounds (phosphates, citrates). Magnesium concentrations are correlated with the calcium content of milk. Magnesium levels and the magnesium to calcium ratio determine milk's heat stability (NAJERA et al. 2003, KRÓL et al. 2006, LITWIŃCZUK et al. 2006).

Milk has a high content of potassium, sodium and chlorine. It contains 1.35 to 1.55 g dm^{-3} of fully ionized potassium. The sodium content of milk is determined at 350-600 mg dm^{-3}, and chlorine levels are noted in the range of 0.80-1.40 g dm^{-3} (KRÓL et al. 2006). Sodium chloride stabilizes the osmotic pressure of milk (including lactose). A drop in lactose levels resulting from, for example, mastitis, increases the rate of NaCl diffusion from blood. Milk contains trace quantities of iron – 0.42 to 0.45 mg kg^{-1} (GÓRSKA, OPRZĄDEK 2004).

Summary

As a rich source of minerals and nutrients, milk is one of the key components of the human diet. Milk proteins contain readily available amino acids such as lysine, methionine and tryptophan. Milk lipids are abundant in essential fatty acids, and they are characterized by an optimal ratio of omega-3 to omega-6 fatty acids. Bovine milk is also rich in readily available macronutrients and micronutrients (calcium, phosphorus, chlorine, magnesium) which support an array of critical biochemical functions in the human body.

Milk is the natural food which has evolved under selective pressure to meet the nutritional needs of mammalian offspring. Today scientists, members of the Milk Genomics Consortium, are going beyond an analysis of individual milk components to examine the genomics of milk, i.e. the genes that code the composition of milk (GERMAN et al. 2006), with the expectation that such an approach will help describe the principal biological defi-

nition of mammalian nutrition. The future for milk research therefore looks brighter than ever and no doubt will continue in this direction as scientists learn more about milk, yet highly organized food which should serve as a model system for creating superior functional food products (MILLS et al. 2011) .

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