

## THE SULPHUR AND CHLORINE CONTENTS OF GRASS AND MAIZE SILAGE

Marija RAJČEVIČ<sup>a)</sup>, Alojz VIDIC<sup>b)</sup>, Pavel KUBA<sup>c)</sup>, Marijan NEČEMER<sup>d)</sup> and Peter KUMP<sup>d)</sup>

<sup>a)</sup> Poslovni sistem Mercator d.d., Dunajska 107, SI-1000 Ljubljana, Slovenia, senior scientist, Ph.D., M.Sc.

<sup>b)</sup> Mesnine dežele Kranjske d.d., Kolodvorska 23, SI-1330 Kočevje, Slovenia, director of agricultural section.

<sup>c)</sup> LITOLAB s.r.o., Chudobin 83, CZ-783 21 Litovel, Czech Republic.

<sup>d)</sup> Jožef Stefan Institute, Jamova 39, SI-1111 Ljubljana, Slovenia, Ph.D.

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### ABSTRACT

In seven defined samples of grass and in six samples of maize silage the contents of sulphur and chlorine were determined by two analytical procedures: both elements with X-ray fluorescence spectrometry (XRF), sulphur separately with inductively coupled plasma – atomic emission spectrometry (ICP-AES) and chlorine according to Volhard (Skoog and West, 1963; Official Journal of the EC, No.L. 155/13, 12/07/71). The average contents of sulphur in grass silage, g kg<sup>-1</sup> DM, were 2.53 g and 1.91 g, in maize 0.87 g and 0.75 g by XRF and ICP-AES, respectively. The differences in the content of sulphur between the two methods were statistically significant ( $P < 0.01$  and  $P < 0.05$ ). The average contents of chlorine in grass silage, g kg<sup>-1</sup> DM, were 7.69 g and 9.09 g, and in maize 2.00 g and 2.55 g by XRF and Volhard, respectively. The differences between the two methods were statistically significant ( $P < 0.01$ ).

Key words: feed / grass silage / maize silage / sulphur / chlorine / prehrana živali / analytical chemistry / X-ray fluorescence spectrometry / atomic emission spectrometry / titration

## VSEBNOST ŽVEPLA IN KLORA V TRAVNI IN KORUZNI SILAŽI

### IZVLEČEK

V sedmih definiranih vzorcih travne in šestih vzorcih koruzne silaže smo po dveh analitskih postopkih določili vsebnost žvepla in klora: oba elementa z rentgensko fluorescenčno spektrometrijo (XRF) in ločeno žveplo z atomsko emisijsko spektrometrijo (ICP-AES), klor pa po Volhardu (Skoog and West, 1963; Official Journal of the EC, No.L. 155/13, 12/07/71). Povprečna vsebnost žvepla v travni silaži, g kg<sup>-1</sup> SS, je bila: 2,53 g in 1,91 g, v koruzni 0,87 g in 0,75 g. Razlika v vsebnosti žvepla med obema metodama je statistično značilna ( $p < 0,01$  in  $p < 0,05$ ). Povprečna vsebnost klora v travni silaži, g kg<sup>-1</sup> SS, je bila: 7,69 g in 9,09 g, v koruzni: 2,00 g in 2,55 g. Razlika med metodama je bila statistično značilna ( $p < 0,01$ ).

Ključne besede: krma / travna silaža / koruzna silaža / žveplo / klor / animal nutrition / analitska kemija / rentgenska fluorescenčna spektrometrija / atomska emisijska spektrometrija / titracija

### INTRODUCTION

The content of mineral matter in fodder is affected by natural growth (weather, soil, water, orographic and biogenic conditions) as well as other factors (agrotechnical measures, harvest and conservation technologies). Some environmental pollutants can affect the concentration of minerals in plants as well.

Natural growth factors differ considerably in Slovenia; they also affect the agrotechnical measures, technologies and harvesting used. It is not strange that the same kinds of fodder

produced in various regions contain different amounts of minerals. Differences can also arise due to various analytical procedures used to determine the same mineral in the same fodder.

Data on the content of macro- and microelements in forages (Stekar *et al.*, 1987; Stekar *et al.*, 1988; Stekar and Žlindra, 1998; Rajčević *et al.*, 1997a; Rajčević *et al.*, 1997b; Rajčević *et al.*, 1998; Pen and Kapun, 1997) can be found in domestic and foreign scientific literature. Due to the significance of minerals in the nutrition of ruminants our knowledge should be widened by knowing the contents of other macro-elements (Rajčević *et al.*, 2000) and by the use of other analytical procedures for determination of elements. We were interested in the contents of sulphur and chlorine in grass and maize silage in the Kočevje region.

The sulphur and chlorine contents of a sample can be determined by various analytical techniques. These include neutron activation analysis (NAA; Kump *et al.*, 1996), inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma emission-mass spectrometry (ICP-MS; Pöykiö *et al.*, 2000), energy dispersive X-ray fluorescence spectrometry (EDXRF; Kump *et al.*, 1996), wavelength dispersive X-ray fluorescence spectrometry (WDXRF; Ruiz *et al.*, 1991), ion chromatography (IC; Belevi and Monch, 2000), and coulometric titration by Volhard (Atkin and Somerfield, 1994). In our work EDXRF (to be referred as XRF), ICP-AES and the titrimetric technique for chlorine determination were selected according to the facilities available.

Table 1. The content of sulphur in fodder produced in grassland and in maize silage according to various authors

Fodder	Estimated quality	Sulphur content g kg <sup>-1</sup> DM	Author and methods of determination
Grass		1–4	Underwood, 1981
Grass (n = 5)		2.56	Rajčević <i>et al.</i> , 2000; XRF
Hay	Good	0.7	Zeman, 1995
Hay	Bad	0.6	Zeman, 1995
Hay		1.9	Petrikovič <i>et al.</i> , 2000
Hay (n = 6)		1.9	Rajčević <i>et al.</i> , 2000; XRF
Hay (n = 2)		1.8	Gašperlin, 2000; XRF
Grass silage	35 % DM	2.5	Zeman, 1995
Silage	40 % DM	2.0	Zeman, 1995
Silage		0.8	Petrikovič <i>et al.</i> , 2000
Silage (n = 13)	35.7 % DM	2.38	Rajčević <i>et al.</i> , 2000; XRF
Silage (n = 3)		1.6	Gašperlin, 2000; XRF
Maize silage		0.8–1.5	NRC, 1988
Maize silage		1.3	McDowell, 1992
Maize silage	31 % DM	3.3	Zeman, 1995
Maize silage	33 % DM	3.0	Zeman, 1995
Maize silage (n = 7)	33 % DM	0.88	Rajčević <i>et al.</i> , 2000; XRF
Maize silage (n = 3)		0.73	Gašperlin, 2000; XRF

More recent data on the content of sulphur in forage found in domestic and foreign scientific literature are summarized in Tables 1 to 3. Unfortunately all citations do not contain data on number of samples and methods of determination used.

Table 2. The content of chlorine in fodder produced on grassland and in maize silage according to various authors ( $\text{g kg}^{-1}$  DM)

Fodder	Estimated quality	Chlorine content $\text{g kg}^{-1}$ DM	Author and methods of determination
Grass (intensively supplied pasture)	16.5 % DM	15.5	Kellner and Becker, 1971
Grass (intensively supplied pasture)	<b>20.0 % DM</b>	12.0	Kellner and Becker, 1971
Grass (intensively supplied pasture)	25.0 % DM	5.0	Kellner and Becker, 1971
Grass		5.0	Underwood, 1981
Grass		3–25	McDonald <i>et al.</i> , 1995
Grass (intensively supplied pastures) (n = 5)	14.07 % DM	9.15	Rajčević <i>et al.</i> , 2000; XRF
Hay	best quality	11.29	Kellner and Becker, 1971
Hay	very good	8.12	Kellner and Becker, 1971
Hay	good	7.53	Kellner and Becker, 1971
Hay	middle quality	8.5	Kellner and Becker, 1971
Hay	worse	4.0	Kellner and Becker, 1971
Hay	good	8.5	Zeman, 1995
Hay	bad	9.8	Zeman, 1995
Hay (n = 6)		5.72	Rajčević <i>et al.</i> , 2000; XRF
Hay (n = 3)		0.8	Gašperlin, 2000; XRF
Silage from young grass		17.0	Kellner and Becker, 1971
Silage from young grass	35 % DM	9.4	Zeman, 1995
Silage from young grass	40 % DM	10.0	Zeman, 1995
Silage (n = 13)	33 % DM	7.19	Rajčević <i>et al.</i> , 2000; XRF
Silage (n = 3)		1.1	Gašperlin, 2000; XRF
Maize silage		1.8	McDowell, 1992
Maize silage	31 % DM	2.3	Zeman, 1995
Maize silage	33 % DM	2.4	Zeman, 1995
Maize silage (n = 7)	33 % DM	1.98	Rajčević <i>et al.</i> , 2000; XRF
Maize silage (n = 3)		0.8	Gašperlin, 2000; XRF

Sulphur is an essential constituent of proteins and many other components of the animal body. It is a component of amino acid methionine, B vitamins biotin and thiamine that cannot be synthesised in animal tissues, the hormone insulin, co-enzyme A, and some polysaccharides that

are essential for normal functioning of tissues. Milk contains 0.03 % of sulphur (NRC, 1988), primarily in the form of the amino acids methionine and cystine. There is a strong relation between sulphur and nitrogen in animal and plant cells; hence fodder rich in proteins is rich in sulphur as well. Rumen microbes are important for the usage of sulphur in the rumen (Kandyliis, 1984). They use inorganic sulphur for the synthesis of amino acids (Thomas *et al.*, 1951). In practice, a shortage of sulphur is evident when the ration contains a lot of energy-rich fodder or non-protein nitrogen. Bouchard and Conrad (1973) and NRC (1988) recommended that the ratio of nitrogen to sulphur in a ration should be 12 to 1 (20 % of sulphur in dry matter of a ration); the first mentioned authors thought that such a ratio was important for the maintenance of the highest food consumption. Naylor and Ralston (1991) reported that rations for lactating cows that contained 17 % of crude protein had to have 0.25 % of sulphur in dry matter, so that the ratio of nitrogen to sulphur was 10 to 1. Interactions among sulphur, selenium, copper and molybdeum are known (Suttle, 1974). Interactions between sulphur and selenium cause higher secretion of selenium, while interactions between sulphur and copper cause lower absorption of copper (e.g. a higher content of methionine or sulphate in a ration for sheep from 0.1 to 0.3–0.4 %). A shortage of sulphur in rations for dairy cows results in lower food consumption, lower digestibility (Bouchard and Conrad, 1973), especially of cellulose (Martin *et al.*, 1964, quoted by McDowell, 1992), a lower synthesis rate in the rumen and lower milk production. On the other hand, too high content of sulphur in a ration can diminish food consumption and increase urine excretion.

Generally it can be stated that the content of chlorine in forages corresponds to needs, while wheat contains less chlorine than recommended for dairy cows. More data on the content of chlorine in forages can be found in the literature than data on sulphur. Such data are shown in Table 2. The contents of sulphur and chlorine can be found for various grasses and legumes, but they differ a lot. Some data on grasses that are most frequent in our samples are shown in Table 3.

Chlorine is the most abundant anion in the extra cellular fluid. Its function is to maintain the acid-basic balance, to regulate osmotic pressure, and to transport oxygen and carbon dioxide. It is found in the excretions of digestion. It is very important for the production of hydrochloric acid in the maw. Chlorine can be found in high concentrations in the pancreatic juice, in gall and elsewhere.

Chlorine and sodium are studied together in the nutrition of cows since the metabolism of chlorine is connected to the metabolism of sodium. Coppock (1986) reported that cows in lactation need 0.20 % of chlorine in dry matter, while the NRC (1988) standards quote 0.25 % of chlorine in dry matter, but this is based on a limited number of data. Cows fed on diets containing 0.10 % of chlorine in dry matter develop subclinical primary hypochloremic, and secondary hypokalemic metabolic alkalosis (Fettman *et al.*, 1984, cit. after NRC, 1988). A shortage of chlorine in fodder results in lower food consumption, lower milk production and decreased body mass in cows.

The content of chlorine in fodder varies a lot and depends on several factors such as plant maturity, the content of chloride in soil, and the content of oxygen in soil, on the kind of plant (NRC, 1988) as well as on the presence of other anions (Butler and Jones, 1973). So, fertilisation of potassium chloride can increase the content of chlorine in plants, and diminish the content of sodium because of antagonism between potassium and sodium (McDowell, 1992).

Table 3. The contents of sulphur and chlorine in some grasses according to various authors

Grass species	State description	Sulphur content g kg <sup>-1</sup> DM	Chlorine content g kg <sup>-1</sup> DM	Author
Perennial ryegrass <i>Lolium perenne</i>	fresh	3.0	-	McDowell, 1992
Perennial ryegrass <i>Lolium perenne</i>	fresh	1.2	1.8	Zeman, 1995
Perennial ryegrass <i>Lolium perenne</i>	fresh	1.2	-	Petrikovič <i>et al.</i> , 2000
Perennial ryegrass <i>Lolium perenne</i>	hay	0.9	-	Petrikovič <i>et al.</i> , 2000
Red fescue <i>Festuca rubra</i>	fresh	1.8–2.0	-	Petrikovič <i>et al.</i> , 2000
Red fescue <i>Festuca rubra</i>	hay	1.1	-	Petrikovič <i>et al.</i> , 2000
Smooth-stalked meadowgrass <i>Poa pratensis</i>	fresh	1.7	-	NRC, 1998
Smooth-stalked meadowgrass <i>Poa pratensis</i>	fresh	2.9	4.0	McDowell, 1992
Smooth-stalked meadowgrass <i>Poa pratensis</i>	1 <sup>st</sup> cut	2.6	6.8	Zeman, 1995
Smooth-stalked meadowgrass <i>Poa pratensis</i>	2 <sup>nd</sup> cut	1.9	8.3	Zeman, 1995
Smooth-stalked meadowgrass <i>Poa pratensis</i>	fresh	7.9	-	Petrikovič <i>et al.</i> , 2000
Smooth-stalked meadowgrass <i>Poa pratensis</i>	hay	1.6	5.3	McDowell, 1992
Timothy <i>Phleum pratense</i>	fresh	1.3	5.7	McDowell, 1992
Timothy <i>Phleum pratense</i>	hay	1.7	5.1	McDowell, 1992
Timothy <i>Phleum pratense</i>	silage	1.3	-	McDowell, 1992
Cocksfoot <i>Dactylis glomerata</i>	fresh	2.1	-	McDowell, 1992

## MATERIAL AND METHODS

In 1999 seven random samples of grass silage and six samples of maize silage were taken for analysis. The analysed fodder was produced under similar soil and weather conditions. Grasslands and fields were intensively manured by bovine and pig slurry and fertilisers when needed. Grasslands were sown with a grass and legume mixture containing 40 % of perennial

ryegrass, 20 % of red fescue, 20 % of smooth-stalked meadowgrass and 20 % of white clover (*Trifolium repens*), but grasslands had already been naturalised.

Dry matter, crude ash and the contents of sulphur and chlorine were determined. Sulphur and chlorine were determined using two analytical procedures: for both elements X-ray fluorescence spectrometry (XRF) (Jenkins *et al.*, 1981; Charalambos, 1984; Kump *et al.*, 1996) and separately sulphur by Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) (Enc. Anal. Sci., 1995; Budič and Klemenc, 2000; JPP SKZUZ, 06/12/1994; Pöykiö *et al.*, 2000). Chlorine was determined as in water soluble chloride of fodder (Official Journal of European Communities, No. L. 155/13, 12/07/71; Skoog and West, 1963). Separate determination of chlorine and sulphur was carried out in the Litolab Institute in Litovel, Czech Republic.

### Preparation and analysis of samples by XRF

The contents of S and Cl were determined by X-ray fluorescence spectrometry, an analytical technique which enables nondestructive multielement determination in the sample. The fundamentals of this technique have been well described in the literature elsewhere (Jenkins *et al.*, 1981, Kump *et al.*, 1996). A radioactive source of Fe-55 was used for excitation of fluorescence radiation and an energy dispersive spectrometer with a Si (Li) detector was used for measurement of the X-ray fluorescence spectra.

Samples under investigation were dried at 105 °C for 3 hours, followed by grinding and homogenisation (mixing). 0.5–1.0 g of powdered sample was pressed into a pellet and analysed by XRF (Rajčević *et al.*, 2000).

### Preparation and analysis of sulphur by ICP-AES

Since samples can be introduced into the plasma only in the form of solutions they should be dissolved. Organic materials (some foods, plants, pharmaceuticals, waste waters) may be decomposed in a microwave oven. Some elements are volatile at higher temperatures so they are decomposed in special closed cups using oxidative reagents, especially nitric acid and hydrogen peroxide. The detection limit for determination of sulphur by ICP-AES was 100 ppb.

### Preparation and analysis of chlorine by the Volhard method

Chlorides are determined by leaching the sample in water and by titration according to Volhard (Skoog and West, 1963; Official Journal of the EC, No.L. 155/13, 12/07/79). This is an indirect flocculative titration. AgNO<sub>3</sub> is added to the solution of the sample. The excess of AgNO<sub>3</sub> (this is the part that is not used in flocculation with chloride from the sample) is titrated with a solution of potassium and ammonium thiocyanate. In the titration the Carres reagent and NH<sub>4</sub> Fe(SO<sub>4</sub>) × 12 (H<sub>2</sub>O) are added to the solution as an indicator. The content of chloride dissolved in water and expressed as NaCl (X) – g kg<sup>-1</sup> is calculated as follows:

$$X = (V_2 - V_1) * C * 58.443 / m, \text{ where} \quad [1]$$

V<sub>2</sub> = the usage of ammonium thiocyanate solution, for blank sample, ml

V<sub>1</sub> = the usage of ammonium thiocyanate solution, for sample, ml

C = exact concentration of ammonium thiocyanate, mol l<sup>-1</sup>

m = mass of sample

The result is expressed as the arithmetic mean of the results of two consecutive trials that could be repeated. It is very accurate – 0.5 g kg<sup>-1</sup> of sample at the content of 10 g kg<sup>-1</sup> and accurateness of 1 g kg<sup>-1</sup> in content over 10 g kg<sup>-1</sup>. The difference between two parallels of the

same sample should not exceed  $0.5 \text{ g kg}^{-1}$  at the content up to  $10 \text{ g kg}^{-1}$  and 5 % at the content over  $10 \text{ g kg}^{-1}$ .

The content of chlorine is calculated from NaCl dissolved in water.

Data were statistically processed by descriptive analysis; differences in the content of sulphur and chlorine between the analytical methods were tested by t-test.

## RESULTS AND DISCUSSION

The accuracy of the results obtained was tested by the standard reference materials CRM 129 (Elements in hay powder) and CRM 101 (Elements in Spruce needles, Community Bureau of Reference, Brussels). The estimated accuracy of the results for S, Cl, K and Ca varies from 0.16 to 5.88 %, which was within the estimated uncertainty of the results, as shown in next table:

Table 4. The results of tested standard reference materials

Element	CRM 101 c, %		
	Results obtained	Certified value	Uncertainty
S	$0.1540 \pm 0.015$	$0.1700 \pm 0.0040$	2.35
Cl	$0.0696 \pm 0.073$	$0.0688 \pm 0.0040$	5.88
Ca	$0.4140 \pm 0.036$	$0.4280 \pm 0.0080$	1.87
CRM 129 c, %			
S	$0.332 \pm 0.029$	$0.316 \pm 0.004$	1.27
K	$2.96 \pm 0.24$	$3.38 \pm 0.08$	2.37
Ca	$0.574 \pm 0.036$	$0.640 \pm 0.001$	0.16

The estimated limit of detection for S and Cl in these samples was around 200 ppm.

Tables 5 and 6 show the results of analyses of grass and maize silages according to the two analytical methods and some statistical indicators.

Table 5. Contents of sulphur and chlorine in grass silage, determined by two analytical procedures ( $\text{g kg}^{-1}$  DM)

No. of samples	Dry matter $\text{g kg}^{-1}$	Crude ash	Sulphur		Chlorine	
			XRF	ICP-AES	XRF	Volhard method
1.	246.0	105	2.20	1.63	7.09	8.82
2.	336.5	156	2.29	1.90	7.05	9.18
3.	261.0	103	1.84	1.50	6.13	7.26
4.	327.0	251	3.18	1.94	8.36	8.40
5.	430.2	160	2.74	2.05	7.45	9.18
6.	248.6	150	3.42	2.52	9.86	11.28
7.	256.2	111	2.03	1.80	7.87	9.54
Mean	300.8	148	2.53	1.91	7.69	9.09
Median	261.0	150	2.29	1.90	7.45	9.18
Standard deviation	68.3	51.74	0.60	0.33	1.19	1.22
Coefficient of variability, %	22.7	34.49	23.68	17.26	15.44	13.41
t			4.65**		-5.50**	

\* =  $P < 0.05$ , \*\* =  $P < 0.01$

Table 5 shows that the dry matter found in our samples of grass silage varies from 246 g kg<sup>-1</sup> to 430.2 g kg<sup>-1</sup>, the mean value being 300.8 g kg<sup>-1</sup>. In 4 of 7 samples the dry matter was less than the mean. The mass of silage is not always of the same composition and quality because it is cut in diverse stages of growth and wither. The same variability in dry matter was noticed in previous years (Rajčević *et al.*, 1997a). Stekar and Žlindra (1998) reported a mean value of dry matter of 384.74 g kg<sup>-1</sup> (n = 17) in silage from the same (Kočevje) region, with variations ranging between 227 and 617.19 g kg<sup>-1</sup>. Pen and Kapun (1998) reported that grass silage produced in north-easteren and central Slovenia from 1990 to 1996 contained 378.6 g kg<sup>-1</sup> (n = 584) dry matter.

The mean value for the content of sulphur that was determined according to XRF method was 2.53 g kg<sup>-1</sup> dry matter, the coefficient of variability being 23.68 %. The lowest content of sulphur was found in sample 3 (1.84 g) and the highest in sample 6 (3.42 g). In the previous study the mean value for sulphur (n = 13) was 2.38 g kg<sup>-1</sup> dry matter in grass silage from the same region (Rajčević *et al.*, 2000).

The mean value for sulphur determined according to the ICP-AES method was 1.91 g kg<sup>-1</sup> dry matter, which was 25 % less than the XRF method. The median equalled the mean value. The values for all 7 samples were within the normal distribution. Analytical results covered a range of  $\pm 7$  %.

The difference between the methods used for determination of the content of sulphur in grass silage was about 25 % and was statistically significant (P < 0.01, t = 4.65).

The XRF method used to determine chlorine in the grass silage gave a value of 7.69 g kg<sup>-1</sup> dry matter. The coefficient of variability was 15.44 %. The values of all 7 samples were within the normal distribution. In our previous determinations the mean value of chlorine in grass silage (n = 13) was 7.19 g kg<sup>-1</sup> dry matter (Rajčević *et al.*, 2000).

Table 6. The contents of sulphur and chlorine in maize silage, determined by two analytical procedures (g kg<sup>-1</sup> DM)

No. of samples	Dry matter g kg <sup>-1</sup>	Crude ash	Sulphur		Chlorine	
			XRF	ICP-AES	XRF	Volhard method
1.	354.0	34.2	0.82	0.58	1.85	2.28
2.	321.3	38.9	0.80	0.70	1.74	2.04
3.	328.0	33.6	0.55	0.61	1.57	2.28
4.	332.8	34.1	0.87	0.79	1.22	1.50
5.	294.6	43.2	0.81	0.69	1.77	2.64
6.	352.5	76.7	1.37	1.14	3.87	4.56
Mean	330.5	43.4	0.87	0.75	2.00	2.55
Median	330.0	36.6	0.82	0.70	1.76	2.28
Standard deviation	22.0	16.7	0.27	0.20	0.94	1.05
Coefficient of variability, %	6.7	38.5	30.99	26.67	47.0	39.62
t			2.63*		-5.49**	

\* = P < 0.05, \*\* = P < 0.01

The mean value for chlorine determined by the Volhard method was 9.09 g kg<sup>-1</sup> dry matter, which was 1.40 g (18 %) more than by the XRF method. The coefficient of variability, which



was 13.41 %, was lower than the XRF method. The median 9.18 g was closer to the mean value than with the XRF method. The analytical results covered a range of  $\pm 8$  %.

The difference in the content of chlorine in grass silage determined by two methods was 18 % and was statistically significant at the level  $P < 0.01$ ,  $t = -5.50$ .

The content of dry matter in the studied samples of maize silage varied between 294.6 and 354 g kg<sup>-1</sup>. Similar indicators were obtained in the previous years (Rajčević *et al.*, 1997b). The ensiling mass consisted of several maize hybrids and not all reached the same stage of ripeness at ensiling. The mean value was 330.5 g kg<sup>-1</sup>. Pen and Kapun (1997) quoted 328.5 g kg<sup>-1</sup> dry matter ( $n = 1144$ ) as a mean value for maize silage produced in Slovenia between the years 1990 and 1996.

The mean value for sulphur in maize silage was 0.87 g kg<sup>-1</sup> dry matter according to the XRF method, the median was 0.82, and the coefficient of variability 30.99 %. The values of all samples were within the normal distribution. Analytical results covered a range of  $\pm 7$  %.

The ICP-AES method gave 0.75 g kg<sup>-1</sup> sulphur in kg dry matter and was 0.12 g lower (14 %) than the XRF method. The coefficient of variability was 26.67 %, which was 4.32 % less than according to the XRF method. Values for all samples were within the normal distribution. Analytical results covered a range of  $\pm 7$  %.

The difference between the two methods of determining the sulphur content in the maize silage was 0.12 g (14 %) and was statistically significant at the level  $P < 0.05$ ,  $t = 2.63$ .

When the XRF method was used to determine chlorine in the maize silage, the mean value was 2.0 g kg<sup>-1</sup> dry matter, while the median was lower (1.76 g). The coefficient of variability was 47 %. Values for all samples were within the normal distribution. Sample 6 deviated most regarding the content of sulphur as well as chlorine (3.87 g). Also a high content of ash was determined in this sample (74.33 g kg<sup>-1</sup> dry matter), which might be the result of the presence of soil in the silage and therefore of both analytes, which can be proved by data on the content of ash insoluble in hydrochloric acid.

The Volhard method gave somewhat higher content of chlorine in maize silage, with the mean value of 2.55 g kg<sup>-1</sup> dry matter, i.e. 0.55 g more (27 %) than determined by the XRF method. The median was 2.28 g kg<sup>-1</sup> dry matter. Samples 4 (1.50 g) and 6 (4.56 g) deviated most from the mean value and median. The coefficient of variability was 39.62 % and was 7.38 % lower than by the XRF method. Analytical results covered a range of  $\pm 8$  %.

The difference between the two methods of determining the content of chlorine in maize silage was statistically significant at the level  $P < 0.01$ ,  $t = -5.49$ .

For maize as well as grass silage the determination of chlorine showed a lower variability using the second method.

It was observed during the analysis of S and Cl in silages that the most important step in the analysis is sample preparation. The grinding, mixing and pulverization were not perfect due to larger pieces of very tough grass and corn stalk observed in the sample.

## CONCLUSION

Differences between the methods used for determination of the content of sulphur and chlorine in silages were statistically significant. The content of sulphur in grass and maize silage was higher when determined by the XRF method; in grass silage the difference between the methods regarding the content of sulphur was statistically significant at the level  $P < 0.01$  and in maize silage at the level  $P < 0.05$ .

The determination of chlorine content showed the opposite findings. We found lower contents of chlorine according to the XRF method than by the Volhard method in samples of both silages, and that differences were statistically significant at the level  $P < 0.01$ .

The results of our study have shown that it is worthwhile to quote the method used when the content of a mineral is reported for a certain feed.

The obtained results appointed that rightness of the methods used to determine sulphur and chlorine in samples of fresh fodder should be well considered.

### POVZETEK

Na območju Kočevja smo naključno odvzeli 7 vzorcev travne in 6 vzorcev koruzne silaže. Krma je pridelana v podobnih talnih in podnebnih razmerah. Površine gnojijo z govejo in prašičjo gnojovko ter mineralnimi gnojili. Po dveh različnih analitskih postopkih smo določili vsebnost žvepla in klora: najprej žveplo in klor z rentgensko fluorescenčno spektrometrijo (XRF), nato pa žveplo z atomsko emisijsko spektrometrijo (ICP-AES-Inductively Coupled Plasma-Atomic Emission Spectrometry) (JPP SKZUZ, 6.12. 1994), klor pa z vodnim izluževanjem vzorca in s titracijo s srebrovim nitratom po Volhardu (Skoog in West, 1963; Official Journal of the EU, No. L. 155/13, 12/07/71). Vsebnost žvepla in klora je bila po XRF metodi naslednja: travna silaža ( $\text{g kg}^{-1}$  suhe snovi): žveplo  $2,53 \pm 0,60$ , KV = 23,68 %, klor  $7,69 \pm 1,19$ , KV 15,44 %. Koruzna silaža je po XRF metodi vsebovala v kg suhe snovi žvepla  $0,87 \pm 0,27$ g, KV = 30,99 %, klora pa  $2,00 \pm 0,94$  g, KV = 47 %. Po ICP-AES metodi smo v travni silaži določili žvepla  $1,91 \pm 0,33$  g, KV = 17,26 %, v koruzni silaži pa  $0,75 \pm 0,20$  g, KV = 26,67 %. Po Volhardu je bilo klora v travni silaži  $9,09 \pm 1,22$  g, KV = 13,14 %, v koruzni silaži pa  $2,55 \pm 1,05$  g, KV = 39,62 %. Razlika v vsebnosti žvepla v travni silaži med metodama je statistično značilna na ravni  $p < 0,01$ ,  $t = 4,65$ , razlika v vsebnosti klora pa prav tako na ravni  $p < 0,01$ ,  $t = -5,50$ . V koruzni silaži je razlika v vsebnosti žvepla med metodama statistično značilna na ravni  $p < 0,05$ ,  $t = 2,63$ , razlika v vsebnosti klora pa na ravni  $p < 0,01$ ,  $t = -5,49$ . Tako pri travni kot koruzni silaži smo med določitvami ugotovili manjšo variabilnost po drugi metodi. Glede na dobljene rezultate menimo, da bi kazalo metode, uporabljene za določitev žvepla in klora v vzorcih voluminoznih krmil, temeljito preučiti. Vsekakor pa je pri navedbi vsebnosti določenega elementa v nekem krmilu priporočljivo navesti uporabljeno metodo.

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