

# STUDY OF TRACE AND ULTRATRACE ELEMENTS IN SILAGE INTENDED FOR CATTLE NUTRITION

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**Summary:** Grass silage and maize silage are important sources of trace and ultratrace elements in cattle nutrition. Data regarding the function, metabolism and deposition, content in feed and animal requirements, as well as some parts of risk assessment, are still needed. To study the presence of selected elements (Al, As, Ba, Be, Cd, Cr, Co, Cu, B, Fe, Pb, Li, Mn, Hg, Mo, Ni, Se, Ag, Sr, Sn, Sb, V, Tl, Ti and Zn) in silage, the appropriate analytical procedures for their determination were introduced and validated in our laboratory. After closed-vessel microwave digestion, inductively coupled plasma mass spectrometry (ICP-MS) was used to analyse samples. Grass silage and maize silage samples from three important cattle-producing regions of Slovenia were analysed and statistically evaluated. The selected elements were found in both types of silage in a wide range of concentrations, with the exception of Tl in maize silage, where all results were below the limit of detection. Statistically significant differences were found for 9 elements in grass and maize silage from three different regions and for 23 elements when comparing grass silage and maize silage. The data suggest that cattle fed Slovenian grass silage or maize silages should receive routine mineral supplementation, of which the most important trace elements needed are Cu and Se. Cattle fed Slovenian maize silage also should receive supplementary Zn and Co. Based on research in other countries, though not tested here, an I supplement is advisable too.

**Key words:** trace elements; ultratrace elements; grass silage; maize silage; ICP-MS

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## Introduction

Feedstuffs contain a wide range of elements, either naturally occurring, or added on purpose, as well as by adventitious contamination. These mineral elements usually are classified as nutritionally essential major and trace elements, and those regarded as toxic, or with an essential/toxic duality (1). Since 1970, the term 'ultratrace element' has been used for elements with estimated dietary requirements that usually are <1mg kg<sup>-1</sup>, and often <50µg kg<sup>-1</sup> dry matter (DM) (2). In order to distinguish between trace

and ultratrace elements, data on their function, metabolism and deposition, content in feed (feed material and compound feedstuffs) and animal requirements (including allowances and their use in practice), as well as some risk assessments, are still needed (3).

Normal functioning of almost all biochemical processes in the body requires trace elements. They are necessary to maintain body function, to optimize growth and reproduction and to stimulate the immune response; therefore, they determine health status (4). Furthermore, there is evidence that ultratrace elements, given in an appropriate amount, can evoke pharmacological responses in animals (2). The necessary amounts of trace elements are difficult to establish and

most estimates are based on the minimum level required to alleviate the symptoms of deficiency, and not necessarily to promote productivity. When estimating the need for mineral supplements, the quantity and type of feed ingredients and their inherent element content, the processing of the diet, the storage and environmental conditions as well as the inclusion and content of other elements must be considered (4). Most, if not all, elements, including essential trace elements, can cause toxic effects to animals and humans if present at excessive levels (5). The values of trace and ultratrace elements in grass and maize silage, however, remain poorly documented (3, 5–9).

In Slovenia, grass and maize comprise the main fodders used for ensiling. The estimated annual quantity (t/year) of prepared silage in Slovenia is 1.5M for grass silage and 1.2M for maize silage. Silage has been made for more than 100 years, but ensiling was used widely after the 1970s, when ensiling machinery and silos became more accessible to farmers. The use of hay as winter-feed declined gradually as silage making increased. In recent times, silage has become important as summer-feed for cattle and 25% of Slovenian dairy farms now use silage exclusively as a summer forage feed for milking cows (10).

Analytical methods based on inductively coupled plasma mass spectrometry (ICP-MS) after closed-vessel microwave digestion is the most recent and advanced tool to determine the levels of trace and ultratrace elements in feedstuffs. Its features are low limits of detection (LOD), multi-elemental capabilities, high sensitivity, a wide linear dynamic range, high sample throughput, and the ability to discriminate between isotopes (11). Several articles report the use of different methods for the determination of trace and ultratrace elements in plant material (12–17), cereals (18–25) and different food matrices (11, 26–32), mainly connected with human consumption. To the best of our knowledge, there have been no reports until now on the use of this multi-elemental technique for the determination of trace and ultratrace elements in silage. For this reason, and according to the European Food Safety Authority (EFSA) (3), a procedure for the determination of 25 selected elements in silage was introduced and validated in our laboratory. These elements are: aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), boron (B), iron (Fe),

lead (Pb), lithium (Li), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), tin (Sn), antimony (Sb), vanadium (V), thallium (Tl), titanium (Ti) and zinc (Zn). The validation was performed according to Regulation (EC) No 882/2004 (33) and Decision 2002/657/EC (34). Using the validated procedure, samples of grass and maize silage taken at farms in three important cattle-producing regions of Slovenia were analysed and statistically evaluated.

## Materials and methods

### *Sample collection*

Samples of grass silage and maize silage (n=30 each), grown on Slovenian farms, were collected from three different regions of Slovenia. These three regions: Murska Sobota (16 samples), Maribor (25 samples), and Celje (19 samples), are important cattle-producing areas. Samples were dried in an UFE 500 oven (Mettler, Schwabach, Germany) at 60°C overnight, and then ground and stored in glass containers at temperatures below 14°C until analysis.

### *Reagents and materials*

All solutions were prepared using high purity deionised water obtained by a Milli-Q water purification system (Bedford, MA). Suprapur nitric acid (HNO<sub>3</sub>, 65%) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%) were purchased from Merck (Darmstadt, Germany). Stock standard solution for the calibration of the system VAR-TS-MS and an internal standard solution Var-IS-1, containing 100µg L<sup>-1</sup> of <sup>6</sup>Li, <sup>45</sup>Sc, <sup>115</sup>In, <sup>89</sup>Y, <sup>159</sup>Tb and <sup>209</sup>Bi, were purchased from Inorganic Ventures (Lakewood, VA).

ICP multi-element stock standard solution XVI containing As, Be, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, Tl, V and Zn, with a concentration of each element of 100mg L<sup>-1</sup>, and separate stock standard solutions containing Ba, Al, Ag, B and Sn, with the concentration of each element of 1000mg L<sup>-1</sup>, were purchased from Merck (Darmstadt, Germany). A working standard solution of Hg was prepared from a stock standard solution containing 100mg L<sup>-1</sup> of Hg (Inorganic Ventures, Lakewood, VA) and was added to the working standard solutions separately.

Two standard reference materials ERM-CD 281

**Table 1:** Instrumental operating conditions for ICP-MS

|   | Operating conditions                                |                     |
|---|---|---------------------|
|   | Normal mode   | CRI mode            |
| Nebuliser                                   | Glass concentric, Micromist                         |                     |
| Spray chamber                               | Scott-type, Peltier-cooled (3°C)                    |                     |
| RF power (kW)                               | 1.40  |                     |
| Interface                                   | Nickel sampler and skimmer cones for the CRI 820-MS |                     |
| Plasma gas flow (L min <sup>-1</sup> )      | 18.50   | 17.00               |
| Auxiliary gas flow (L min <sup>-1</sup> )   | 1.85  | 1.70                |
| Sheath gas flow (L min <sup>-1</sup> )      | 0.14  | 0.25                |
| Nebuliser gas flow (L min <sup>-1</sup> )   | 0.94  | 0.90                |
| CRI skimmer gas flow (L min <sup>-1</sup> ) | -   | H <sub>2</sub> , 70 |
| Scan mode                                   | Peak hopping  | Peak hopping        |
| Scans/replicate                             | 20  | 20                  |
| Replicate/sample                            | 5   | 5                   |
| Attenuation mode                            | Automatic   | None                |

**Table 2:** Values of elements in samples of grass silage (*n*=30) and maize silage (*n*=30)

|    | Grass silage |      |        |            | Maize silage |       |        |             |
|----|--------------|------|--------|------------|--------------|-------|--------|-------------|
|    | Median       | Mean | S.E.M. | Range      | Median       | Mean  | S.E.M. | Range       |
| Al | 480          | 857  | 172    | 60.7–4204  | 46.0         | 68.0  | 12.3   | 23.0–353    |
| Fe | 468          | 744  | 137    | 113–3489   | 58.3         | 76.9  | 9.3    | 45.7–275    |
| Mn | 71.8         | 79.0 | 5.8    | 26.3–159   | 23.2         | 25.0  | 1.7    | 12.5–49.6   |
| Zn | 27.7         | 30.6 | 1.9    | 20.9–77.5  | 23.1         | 23.9  | 0.9    | 13.3–35.9   |
| Ti | 19.0         | 29.5 | 5.1    | 5.89–143   | 4.09         | 4.69  | 0.48   | 2.17–14.6   |
| Ba | 15.4         | 21.2 | 2.3    | 6.71–62.4  | 2.67         | 2.98  | 0.28   | 1.04–8.29   |
| Sr | 11.7         | 13.2 | 1.1    | 5.96–28.4  | 3.07         | 3.38  | 0.18   | 2.17–6.11   |
| Cu | 7.98         | 8.05 | 0.29   | 5.15–11.1  | 5.36         | 5.53  | 0.15   | 4.27–7.53   |
| B  | 7.13         | 8.04 | 0.65   | 4.02–17.0  | 5.78         | 5.75  | 0.20   | 4.10–8.78   |
| Ni | 2.20         | 2.57 | 0.40   | 0.802–12.2 | 0.395        | 1.32  | 0.52   | 0.179–15.6  |
| Cr | 1.24         | 1.78 | 0.28   | 0.241–7.15 | 0.366        | 0.412 | 0.034  | 0.193–0.848 |
| Mo | 1.02         | 1.17 | 0.14   | 0.272–2.92 | 0.283        | 0.327 | 0.031  | 0.096–0.747 |
| V  | 1.01         | 1.70 | 0.34   | 0.145–9.16 | 0.092        | 0.158 | 0.026  | 0.057–0.723 |
| Li | 605          | 1024 | 203    | 107–5174   | 58.3         | 89.5  | 15.2   | 33.1–458    |
| Pb | 553          | 1060 | 328    | 159–10067  | 135          | 146   | 13.2   | 58.9–384    |
| Co | 270          | 380  | 63     | 74.7–1645  | 24.3         | 44.6  | 7.5    | 14.1–150    |
| As | 210          | 418  | 101    | 60.4–2852  | 51.9         | 53.6  | 6.8    | <7.90–126   |
| Cd | 90.9         | 130  | 19     | 19.8–518   | 59.2         | 68.0  | 7.0    | 22.6–182    |
| Sn | 82.2         | 120  | 20     | 40.3–493   | 47.2         | 49.1  | 2.1    | 27.8–77.7   |
| Se | 52.7         | 65.9 | 7.4    | <32.5–176  | 51.6         | 55.0  | 4.5    | <9.70–65.8  |
| Hg | 33.0         | 40.1 | 8.0    | <7.40–56.1 | 58.2         | 58.2  | -      | <7.40–58.2  |
| Be | 32.2         | 56.0 | 10.2   | 11.3–229   | 9.27         | 9.93  | 0.64   | 5.02–22.0   |
| Tl | 27.9         | 37.6 | 8.3    | <1.90–143  | <1.90        | <1.90 | -      | -           |
| Sb | 22.1         | 27.8 | 2.8    | 11.3–65.2  | 17.6         | 23.2  | 7.3    | <9.8–124    |
| Ag | 11.9         | 13.0 | 1.8    | <1.10–30.6 | 10.6         | 10.3  | 0.9    | <1.10–13.9  |

Notes: Values are reported in mg kg<sup>-1</sup> DM for elements from Al to V and in µg kg<sup>-1</sup> DM for elements from Li to Ag.

**Table 3:** Medians and *p*-values of elements in samples of silage according to region<sup>a</sup>

|    | Grass silage |          |          |                       | Maize silage |          |          |                       |
|----|--------------|----------|----------|-----------------------|--------------|----------|----------|-----------------------|
|    | Median 1     | Median 2 | Median 3 | <i>p</i> <sup>b</sup> | Median 1     | Median 2 | Median 3 | <i>p</i> <sup>b</sup> |
| Al | 1133         | 396      | 480      | 0.209                 | 42.4         | 50.7     | 42.0     | 0.376                 |
| Fe | 972          | 360      | 461      | 0.218                 | 53.9         | 65.8     | 59.4     | 0.383                 |
| Mn | 116          | 74.0     | 50.9     | <0.05                 | 27.4         | 20.7     | 19.7     | 0.107                 |
| Zn | 24.2         | 29.5     | 30.0     | 0.082                 | 21.7         | 23.5     | 26.1     | 0.093                 |
| Ti | 33.2         | 22.7     | 14.5     | <0.05                 | 3.84         | 4.78     | 3.63     | 0.165                 |
| Ba | 24.1         | 16.6     | 15.4     | 0.603                 | 2.80         | 3.71     | 2.13     | <0.05                 |
| Sr | 13.2         | 9.00     | 13.3     | 0.216                 | 3.44         | 3.08     | 2.63     | 0.414                 |
| Cu | 7.46         | 7.26     | 9.62     | 0.107                 | 5.26         | 5.43     | 5.26     | 0.519                 |
| B  | 6.86         | 6.47     | 10.6     | <0.05                 | 5.38         | 6.05     | 5.98     | 0.518                 |
| Ni | 2.33         | 1.87     | 2.13     | 0.985                 | 0.253        | 0.424    | 0.903    | <0.05                 |
| Cr | 2.11         | 1.24     | 1.05     | 0.226                 | 0.339        | 0.355    | 0.450    | 0.476                 |
| Mo | 0.369        | 1.38     | 1.27     | <0.05                 | 0.159        | 0.366    | 0.339    | <0.05                 |
| V  | 2.01         | 0.860    | 1.11     | 0.238                 | 0.076        | 0.096    | 0.099    | 0.184                 |
| Li | 1234         | 494      | 599      | 0.184                 | 54.0         | 75.1     | 58.9     | 0.800                 |
| Pb | 747          | 533      | 594      | 0.485                 | 133          | 143      | 124      | 0.517                 |
| Co | 377          | 180      | 266      | 0.179                 | 24.2         | 23.9     | 33.7     | 0.905                 |
| As | 344          | 174      | 262      | 0.511                 | 46.4         | 52.2     | 51.9     | 0.293                 |
| Cd | 82.7         | 76.3     | 122      | 0.063                 | 51.7         | 61.1     | 72.7     | 0.359                 |
| Sn | 104          | 80.2     | 80.6     | 0.341                 | 46.7         | 47.6     | 49.9     | 0.993                 |
| Se | 47.3         | 48.9     | 56.1     | 0.056                 | 51.6         | <32.5    | 56.5     | 0.084                 |
| Hg | 31.1         | <24.8    | 44.6     | 0.476                 | <7.40        | <7.40    | 58.2     | <0.05                 |
| Be | 58.6         | 28.9     | 35.7     | 0.272                 | 9.09         | 9.22     | 10.2     | 0.349                 |
| Tl | 20.7         | 29.3     | 26.5     | 0.260                 | <1.90        | <1.90    | <1.90    | -                     |
| Sb | 39.9         | 19.9     | 22.5     | <0.05                 | 17.9         | 12.2     | 17.8     | 0.615                 |
| Ag | 13.1         | 8.56     | 15.3     | 0.230                 | 11.4         | 8.51     | 10.6     | <0.05                 |

Notes:

<sup>a</sup>Medians of elements concentrations within regions are reported in mg kg<sup>-1</sup> DM for elements from Al to V and in µg kg<sup>-1</sup> DM for elements from Li to Ag.  
<sup>b</sup>*p*-values for elements in grass silage and maize silage samples from three regions. *p*<0.05, indicate significant differences between three regions.

(rye grass), purchased from IRMM (Geel, Belgium), and SRM 1570a (spinach leaves), purchased from NIST (Gaithersburg, MD, USA), were included in the validation of the analytical procedure.

### *Microwave digestion*

A total of 500mg of dry sample was weighed in a 100mL Teflon vessel and 5mL of 65% HNO<sub>3</sub> and 1mL of 30% H<sub>2</sub>O<sub>2</sub> were added. After being left at laboratory temperature for 60 min, the samples were digested in the closed 12-vessel microwave system CEM MARS 5 (Matthews, NC) at the power of 1600W following a three-step program. In the first 15min, the temperature was raised to 200°C and held at the temperature for the next 20min. After

digestion was completed, the samples were cooled for 20min in a stream of air. After complete cooling, samples were quantitatively transferred to 100mL volumetric flasks and made up with Milli-Q water.

### *Instrumental analysis*

Measurements were performed with a Varian 820-MS system (Mulgrave, Australia) equipped with Varian's ICP-MS Expert software for the system control and data processing.

A Collision Reaction Interface (CRI) was used for measurements of <sup>75</sup>Se and <sup>78</sup>As to reduce common polyatomic interferences. The isotopes <sup>7</sup>Li, <sup>9</sup>Be, <sup>11</sup>B, <sup>27</sup>Al, <sup>47</sup>Ti, <sup>51</sup>V, <sup>53</sup>Cr, <sup>55</sup>Mn, <sup>57</sup>Fe, <sup>59</sup>Co, <sup>60</sup>Ni, <sup>63</sup>Cu, <sup>66</sup>Zn, <sup>88</sup>Sr, <sup>95</sup>Mo, <sup>107</sup>Ag, <sup>111</sup>Cd, <sup>118</sup>Sn, <sup>121</sup>Sb, <sup>137</sup>Ba, <sup>202</sup>Hg, <sup>205</sup>Tl, <sup>206+7+8</sup>Pb were selected as analytical

masses in the ICP-MS normal sensitivity mode. The instrumental settings are given in Table 1.

### *Statistical analysis*

Data from the grass silage and maize silage samples were analysed using the Statistical Package for Social Sciences (SPSS, version 12, November 2003). Arithmetic mean, median and ranges (minimum and maximum) of element values were calculated. If measured values were below the limit of quantification (LOQ), the value equal to LOQ/2 was taken for the calculations (35). Shapiro-Wilk's test was used to test normality. Because the distributions were not normal ( $p < 0.05$ ), the non-parametric Kruskal-Wallis test was used to evaluate differences between silage samples from three different regions. A non-parametric Mann-Whitney test was used to evaluate differences between grass and maize silage. The Spearman's correlation analysis was used to determine correlations between element values.

## **Results and discussion**

### *Analysis of silage samples*

Table 2 summarises the data for the elements in farm samples of grass silage and maize silage ( $n=30$  each). The table shows the median, arithmetic mean, S.E.M. and ranges (minimum–maximum). All results for elemental values, discussed below, are given on a dry matter (DM) basis.

Mean DM in grass- and maize- silage was 47.9% (range 30.6–69.8%) and 38.6% (range 31.3–47.3%), respectively. The selected elements were found in both types of silage, except for Tl in maize silage, where all Tl values were below LOD ( $1.9\mu\text{g kg}^{-1}$ ). Hg (range  $31.1\text{--}56.1\mu\text{g kg}^{-1}$ ) was present in three samples of grass silage and in only one sample of maize silage ( $58.2\mu\text{g kg}^{-1}$ ).

Our results are similar to those reported globally. Our values for Cu, Mo, Zn, Mn, Co, and Se can be compared with values in grass silage from Iceland (8) and with values in grass silage and pasture herbage from Ireland (36).

Cu values ( $\text{mg kg}^{-1}$ ) were similar in Slovenian grass silage and Icelandic forage (mean 8.05, range 5.16–11.1) and (mean 8.0, range 4–16), respectively, but were lower than in Irish grass silage (mean 10.36, range 2.8–39.7) (36). Mean

Cu values in Slovenian and Icelandic grass silage are marginally deficient ( $<10\text{mg kg}^{-1}$ ). Therefore routine supplementation with Cu is advisable.

Mo is a proven Cu-antagonist in cattle. Mo values ( $\text{mg kg}^{-1}$ ) in grass silage showed the biggest differences: Slovenia (mean 1.17, range 0.272–2.92), Iceland (mean 0.23, range 0.0043–2.37) and Ireland (mean 1.48, range 0.1–18.3,  $n=1505$ ). Mo-induced copper deficiency in cattle usually occurs on forages with Mo values  $>5\text{mg kg}^{-1}$  and/or forage Cu:Mo ratios  $<3:1$ . Such values were uncommon in grass silage in Slovenia and Iceland. However, clinical Mo-induced copper deficiency is common in unsupplemented Irish cattle at pasture. Herbage in Irish pasture has higher Mo values (mean 2.49, range 0.1–52.0,  $n=1658$ ) and lower Cu values (mean 9.22, range 1.6–23.7,  $n=1741$ ) than those in Irish grass silage. However, even Irish grass silage had many Mo values  $>5\text{mg kg}^{-1}$  (36). Marginal Cu and elevated Mo in Irish herbage and grass silage mean that Cu supplementation is used routinely in Irish herds (38).

Se values ( $\mu\text{g kg}^{-1}$ ) in Slovenian grass silage (mean 65.9, range 32.5–176) agree with those reported by Pick et al. (9) (mean 65.3, range 17.6–180). These means are slightly higher than the mean (50.2) reported previously by Žust et al. (6) but were considerably lower than in Irish grass silage (mean 93, range 2–232,  $n=1507$ ) and Irish pasture herbage (mean 93, range 1–250,  $n=1459$ ), reported by Rogers & Murphy (36). Mean Se values ( $\mu\text{g kg}^{-1}$ ) in maize silage (55.0 in our case, 28.6 according to Pick et al. (9) and 38.7 in the study by Žust et al. (6)) were even lower than in grass silage.

Our data confirm that the content of Se in Slovenian grass silage and maize silage is low and does not meet the requirements of ruminants. The data also confirm the conclusion of Pick et al. (9), which stated the need to update the spreadsheets from the German Agricultural Society (DLG), since most of the results were  $<90\text{--}130\mu\text{g kg}^{-1}$ , the recommended value in both cases. Indeed, the optimum Se value recommended in cattle diets in Ireland is higher still ( $>200\mu\text{g kg}^{-1}$ ) (37). From those data, cattle fed Slovenian forages need a substantial Se supplement.

Mean Zn values ( $\text{mg kg}^{-1}$ ) in grass silage in Slovenia, Iceland and Ireland (36) were 30.6 (range 20.9–77.5), 35 (range 14.1–85.0) and 29.7 (range 10–94), respectively. Zn values for Irish pasture herbage were very similar to those in Irish grass silage (mean 30.8, range 13–84,  $n=928$ ) (36).



Some values in all countries were  $<25\text{mg kg}^{-1}$  (the minimum Zn value recommended for cattle feed) but the means were all normal and the risk of simple Zn deficiency arising on such feeds is low. Rogers (38) reported that he had failed to confirm simple Zn deficiency in Irish herds, but had confirmed a few cases of secondary Zn deficiency, induced by excessive Ca values in feed.

Mean Mn values ( $\text{mg kg}^{-1}$ ) in grass silage in Slovenia, Iceland and Ireland (36) were 79.0 (range 26.3–159), 125 (range 40–550) and 103.5 (range 2–477), respectively. Values in Irish pasture herbage were 119.8 (range 10–693,  $n=1872$ ) (36). As the mean values greatly exceeded the minimum Mn level recommended in cattle feed ( $25\text{mg kg}^{-1}$ ) and there were few values in Ireland below that, the risk of simple Mn deficiency in cattle is very low in all three countries. Rogers (38) reported that he had failed to confirm simple clinical Mn deficiency in Irish herds, but an Irish colleague (Dr. John Mee, Moorepark Dairy Research Centre) had seen a few cases of achondroplastic/dwarf calves attributed to it.

Mean Co values ( $\mu\text{g kg}^{-1}$ ) in grass silage in Slovenia and Iceland were 380 (range 74.7–1645) and 317 (range 41–2010), respectively. These values exceeded the minimum Co recommended for cattle feed ( $100\mu\text{g kg}^{-1}$ ). However, because soil Co values greatly exceed values in forage tissue, the elevated Co values probably reflect soil contamination.

Evidence for significant soil contamination when harvesting grass silage comes from the massive differences between Co, Al and Fe values in grass silage versus maize silage. Co in grass silage was circa 9-times higher than in maize silage (mean 44.6, median 24.3, range 14.1–150). Al values ( $\text{mg kg}^{-1}$ ) usually found in pasture are  $<100$  but can exceed 1000 under unfavorable conditions, because of soil contamination. Al values in grain products are usually 5–68 (3). Al values in Slovenian grass silage (mean 857, median 480, range 60.7–4204) was circa 13-times higher than in maize silage (mean 68.0, median 46.0, range 23.0–353) and Fe in grass silage (mean 744, median 468, range 113–3489) was circa 10-times higher than in maize silage (mean 76.9, median 58.3, range 45.7–275). Though cattle on Slovenian grass silage may not need a Co supplement, those on maize silage probably need one because the mean values in maize silage were  $<50\%$  of the minimum Co recommended for cattle feed ( $100\mu\text{g kg}^{-1}$ ).

In some studies (8), samples with Fe values  $>1000\text{mg kg}^{-1}$  were considered to be contaminated

by soil and these samples were excluded from further processing. As for the other elements, the processes of harvesting herbage for conservation as hay or silage often result in soil being picked up with crops, and the elevated values of elements may reflect spurious contamination (5). This would explain the wide ranges of selected elements present in the analysed samples and the differences between mean and median values. However, in our opinion, the exclusion of these samples would not provide an accurate reflection of the values of elements in silage fed to animals on Slovenian farms.

Values for I in Slovenian grass silage and maize silage are not included in this paper. However, in humans and animals, I deficiency is the most prevalent trace element deficiency globally (4, 8). It can be associated with common clinical problems in cows (infertility, late abortion, high postnatal calf mortality (goiter in calves, stillbirth, weak calf syndrome), retained placenta, etc) (37–40). Mean I values ( $\mu\text{g kg}^{-1}$ ) in Irish grass silage and pasture herbage were very similar (36), namely, 269 (range 4–980,  $n=627$ ) and 261 (range 5–1000,  $n=777$ ), respectively. National Advisory Recommendations on minimal I requirement of cattle and on optimum I supplementation levels differ greatly between countries. In Ireland, a routine supplement of 30–60mg I/cow/day is advised from circa 1 month prepartum to circa 4 months postpartum. If I deficiency is suspected as a cause of late abortions in cows at pasture, 30–60mg I/cow/d (via the trough water supply) is advised (37). This far exceeds the I intake from Irish forage (circa 2.6–4.2mg/cow/day assuming a mean forage DM input of 10–16kg/cow/day and a mean forage I value of  $265\mu\text{g kg}^{-1}$ ). This approach may also be advisable in Slovenia.

Li et al. reported values of Zn, Cu, Cr, Cd, Pb and As in maize silage fed to Wisconsin dairy herds (7).

Zn values ( $\text{mg kg}^{-1}$ ) in maize silage in Slovenia and Wisconsin (mean 23.9, median 23.1, range 13.3–35.9) versus (mean 27, median 24, range 17–77) were similar but the range in Slovenia was narrower than in Wisconsin. Maize silage in both surveys was marginal in Zn because the minimum Zn recommended for cattle feed is  $25\text{mg kg}^{-1}$  DM.

Mean Cu values in Slovenia ( $5.53\text{mg kg}^{-1}$ , range 4.27–7.53mg  $\text{kg}^{-1}$ ) were slightly higher than in Wisconsin ( $4.0\text{mg kg}^{-1}$ , range 2–6.4mg  $\text{kg}^{-1}$ ). Maize silage in both surveys was very low in Cu because the minimum Cu recommended for cattle feed is  $10\text{mg kg}^{-1}$  DM.

Mn values in Slovenian silages ( $\text{mg kg}^{-1}$ ) are

comparable with values reported previously (3). The reported values of Mn in grass and maize silage were 98 and 28, respectively. Our mean Mn values were slightly lower: 79 in grass silage and 25 in maize silage.

Except for Cu, the maximum values of all investigated elements were 2–10 times lower in Slovenian maize silage than in Wisconsin maize silage.

Cr values ( $\text{mg kg}^{-1}$ ) in Slovenian maize silage (mean 0.412, range 0.194–0.848) were slightly lower than in Wisconsin (0.519, range 0.207–1.702). Mean Cd values ( $\mu\text{g kg}^{-1}$ ) in Slovenia and Wisconsin, 62 and 68, respectively, were very similar. However, mean Pb value ( $\mu\text{g kg}^{-1}$ ) in Slovenia was half of that in Wisconsin (146 versus 260, respectively), and mean As ( $\mu\text{g kg}^{-1}$ ) was much less than in Wisconsin (53.6 versus 201, respectively).

Ni mean values ( $\text{mg kg}^{-1}$ ) in Slovenian maize silage (mean 1.32, range 0.179–15.6) agree well with 1.28  $\text{mg kg}^{-1}$  reported by Van Paemel et al. (3).

Until now, data were lacking for the values of elements in feed materials that were considered to be non-essential for animals. Forage and grains are generally rich Li sources, but the Li values vary with the soil on which the plants are grown (3). The range of B value of terrestrial plants is 2–95  $\text{mg kg}^{-1}$ . Reported Sr values of hay were 9.4  $\text{mg kg}^{-1}$ , but there are no data available for grass silage. The accumulation by terrestrial plants of Ag from soils is low, even from soils fertilised with Ag-containing sewage sludge. Generally, land plants have Ag values of 60  $\mu\text{g kg}^{-1}$ . Sn values in pastures were reported to range from 0.3–0.4  $\text{mg kg}^{-1}$ . However, no data are available on V values in feed materials in the main literature sources (3). Therefore, we cannot compare our results for these elements.

### *Regional differences between elements and differences between grass silage and maize silages*

Analysis of variance found significant differences in 9 element values in silage between three regions in Slovenia (Table 3). There were significant regional differences ( $p < 0.05$ ) between B, Ti, Mn, Mo and Sb in grass silage samples and between Ni, Mo, Ba and Hg in maize silage samples. Median Mo values in grass silage and Ni in maize silage showed the biggest regional differences. Significant differences between grass silage and maize silage were found

for most of the elements, except for Ag and Hg.

## Conclusions

Marginal or low values were found for some essential trace elements in grass silage, and especially in maize silage in Slovenia. We suggest that cattle fed grass silage or maize silages should receive routine mineral supplementation, of which the most important trace elements needed are Cu and Se. Cattle fed maize silage should also receive supplementary Zn and Co. Though we did not test for I values, the research and conclusions from other countries suggests that I supplementation is also advisable.

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## ŠTUDIJA ELEMENTOV V SLEDOVIH V SILAŽI ZA PREHRANO GOVEDI

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**Povzetek:** Travna in koruzna silaža sta v prehrani goved pomemben vir elementov v sledovih. Podatki o bioloških vlogah, metabolizmu in odlaganju teh elementov v živalskih tkivih, vsebnostih v krmi, potrebah živali in ocenah tveganja se še vedno zbirajo. Za preučevanje vsebnosti izbranih elementov v silažah (Al, As, Ba, Be, Cd, Cr, Co, Cu, B, Fe, Pb, Li, Mn, Hg, Mo, Ni, Se, Ag, Sr, Sn, Sb, V, Ti, Ti in Zn) smo vpeljali in validirali ustrezne analizne postopke. Zaradi nizkih vsebnosti teh elementov v silažah smo uporabili najsodobnejšo metodo indukcijsko sklopljene plazme z masno detekcijo (ICP-MS). Za pripravo vzorcev smo uporabili razklop v zaprtem mikrovalovnem sistemu z dušikovo kislino in vodikovim peroksidom. Vzorce silaž, odvzetih v treh pomembnih govedorejskih regijah v Sloveniji, smo analizirali in dobljene podatke statistično obdelali. Izbrani elementi so prisotni v obeh vrstah silaž v širokih koncentracijskih območjih, razen Ti v koruzni silaži, kjer so vsi rezultati pod mejo določanja. Statistično značilne razlike za travno in koruzno silažo iz treh različnih regij smo ugotovili za 9 elementov. Pri primerjavi vzorcev travnih in koruznih silaž pa so se statistično značilne razlike pokazale za 23 elementov. Rezultati študije kažejo, da bi moralo govedo, hranjeno s slovensko travno in koruzno silažo, dobivati tudi ustrezne mineralne dodatke. Najpomembnejša elementa, ki jih je potrebno dodajati, sta Cu in Se. Govedo krmljeno s slovensko koruzno silažo, bi potrebovalo tudi dodatke Zn in Co. Glede na raziskave v drugih državah, bi bilo potrebno dodajati tudi I.

**Ključne besede:** elementi v sledovih; travna silaža; koruzna silaža; ICP-MS