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ESTIMATION OF MINERAL CONTENT IN FORAGES BY NEAR-INFRARED REFLECTANCE SPECTROSCOPY

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ABSTRACT

In 100 dried samples of forages, harvested three or four times during 1997 and 1999 in different morphological stages the macro- (Ca, P, Mg, K and Na) and trace- (Zn, Mn and Se) mineral contents were determined by chemical methods and by near infrared reflectance spectroscopy (NIRS). The accuracy calibration equations of NIRS for macrominerals Ca, P, Mg and K showed a high degree of accuracy, with an R^2 of 0.963, 0.884, 0.892 and 0.939, respectively. These results suggest that these macrominerals are probably closely associated with the organic components, such as plant cell wall (Ca and Mg), chlorophyll (Mg), phytates, phospholipids, phosphoproteins and nucleic acids (P) and organic acids, such as malate (K). On the contrary, NIRS did not estimate Na content very accurately ($R^2 = 0.52$; coefficient of variability of cross validation (CV_{SECV}) = 35.5%), suggesting that Na is not closely associated with organic functional groups or that the amount of the mineral is below the level of NIRS detection. Similar conlusions are also valid for trace minerals, where the R^2 was 0.642 (SECV = 4.4) for Zn, 0.427 (SECV = 55.6) for Mg and only 0.186 (SECV = 6.5) for Se. The CV_{SECV} for trace minerals always exceeded 20%. Despite the insensibility of NIRS to accurately determine Na and certain trace minerals, the NIRS technique may provide an acceptable prediction of macro mineral contents.

Key words: forages / minerals / macrominerals / trace minerals / analytical chemistry / methods / near infrared reflectance spectroscopy / NIRS

OCENJEVANJE VSEBNOSTI RUDNINSKIH SNOVI V VOLUMINOZNI KRMI Z BLIŽNJO INFRARDEČO REFLEKSIJSKO SPEKTROSKOPIJO

IZVLEČEK

V 100 posušenih vzorcih voluminozne krme, ki smo jo kosili tri ali štirikrat v letih 1997 ain 1999 v različnih morfoloških stadijih, smo določili vsebnost makrorudninskih snovi (Ca, P, Mg, K and Na) in sledovnih elementov (Zn, Mn and Se) z laboratorijskimi metodami in z bližnjo infrardečo refleksijskomisijsko spektroskopijo (NIR). Vsebnost makrorudninske snovi Ca, P, Mg in K smo določili zelo točno, merjeno kot R^2 , ki je znašal 0.963, 0.884, 0.892 in 0.939, za prej naštete rudnine. Rezultati kažejo, da so te makrorudninske snovi močno povezane z organskimi sestavinami, kot so rastlinska celična stena (Ca in Mg), klorofil (Mg), fitati, fosfolipidi, fosfoproteini in nukleinske kisline (P) in organske kisline, kot je jabolčna kislina (K). Nasprotno pa z NIRS-om nismo mogli natančno oceniti vsebnosti Na ($R^2 = 0.52$; koeficient variabilnosti navzkrižnega preverjanja (CV_{SECV}) = 35.5 %), kar napeljuje na možnost, da Na ni povezan z organskimi snovmi ali pa da je vsebnost rudninske snovi pod mejo detekcije NIRSa. Podobno

velja tudi za sledovne elemente Zn, Mn in Se, katerih R² so bili 0.642 za Zn (SECV = 4.4), 0.427 za Mn (SECV = 55.6) in samo 0.186 za Se (SECV = 6.5). Koeficienti variabilnosti standardne napake navzkrižnega preverjanja (CV_{SECV}) so bili pri sledovnih elementih vedno večji od 20 %. Kljub temu da z NIRSom ne moremo zanesljivo določati vsebine nekaterih rudninskih snovi, pa na ta način vseeno lahko grobo ocenimo njihovo vsebnost.

Ključne besede: voluminozna krma / rudninske snovi / makrorudninske snovi / sledovni elementi / analitska kemija / metode / bližnja infrardeča refleksijska spektroskopija / NIRS

INTRODUCTION

Near infrared reflectance spectroscopy (NIRS) is a physical method of analysis that serves both for qualitative and quantitative purposes. NIRS allows the identification of samples or components within a sample, but under the correct conditions it would also allow the quantification of the components within a sample. NIRS shows great potential for rapid (a single scan can be obtained in less than a second) and non-destructive analysis, is environmentally friendly and does not require great skills of instrument operators. NIRS uses reflectance signals resulting from the radiation at different wavelengths in the near infrared region of the electromagnetic spectrum, as measured by the proportion of incident radiation which is diffusely reflected (Deaville and Baker, 1993). The ability of NIRS to determine various parameters of quality in highly variable samples is due to the bending and stretching vibrations of bonds between carbon, nitrogen, hydrogen and oxygen. In the contest of food, bonds involving C-H, O-H, N-H and possibly S-H and C=O are responsible for the majority of the observed absorption (Murray, 1986; Deaville and Baker, 1993).

NIRS has been accepted widely for the determination of crude protein, neutral detergent fibre, acid detergent fibre and *in vitro* digestibilities of animal feeds (Redshaw *et al.*, 1986; Givens *et al.*, 1997; Herrero *et al.*, 1997; Park *et al.*, 1998; Adesogan *et al.*, 1998; Lavrenčič *et al.*, 2001). However, mineral content analyses with NIRS are rarely reported (Clark *et al.*, 1987, 1989; Vasquez de Aldana *et al.*, 1995) and the uncertainty of mineral determination using this technique still remains open. The prediction of trace elements by NIRS has been reported even less frequently (Clark *et al.*, 1989; Vasquez de Aldana *et al.*, 1995). Minerals in agricultural and ecological products in ionic form or in form of salts do not reflect near infrared energy. However, if mineral constituents are associated with organic complexes or are chelated, NIRS could be successfully applied to determine their contents (Clark *et al.*, 1987). Clark *et al.* (1987) and Vasquez de Aldana *et al.* (1995) reported that accurate use of NIRS to determine mineral cation composition in forages appears limited only to major minerals, such as calcium (Ca), phosphorus (P), potassium (K) and magnesium (Mg).

The purpose of the present work was to assess the potential of NIRS to produce accurate, robust calibrations, developed on dried fresh forage spectra, to predict contents for the elements Ca, P, Mg, K, Na, Zn, Mn and Se.

MATERIALS AND METHODS

Six grass species, Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), timothy (*Phleum pratense*), orchardgrass (*Dactylis glomerata*), red fescue (*Festuca rubra*), meadow fescue (*Festuca pratensis*) and red clover (*Trifolium pratense*) were used to quantify the relationship between traditional chemical analysis and NIRS determinations of elemental concentrations. The history of forage samples used in this study is documented elsewhere (Lavrenčič *et al.*, 2001). In brief, the samples were grown in pure stands on Mengeško polje, Slovenia, harvested four times and sampled at different morphological stages during 1997 and 1999. The exceptions were that fescues and orchardgrass were not harvested in 1999, and that in

the third consecutive harvest of both years orchardgrass, red and meadow fescue, perennial ryegrass and timothy were sampled only once in the vegetative stage of growth. Moreover, the fourth harvest (fall harvest) of all samples was performed at vegetative stage of growth. A total number of 100 samples was available, 60 samples obtained in 1997 and 40 samples in 1999.

Immediately after sampling, forages were stored for a week at -21 °C, thawed for one day and dried at temperatures below 50 °C. Air dried samples were then ground to pass a 1 mm screen (Alpine, Augsburg, Germany) and stored at room temperature in a dark room until analysed.

Samples were ashed at 520 °C for eight hours, followed by solubilisation in 25% HCl. Acid was evaporated and the residue was reconstituted in 100 ml distilled water. Calcium, K, Mg, Na, Mn, Zn and Se were determined by atomic absorption spectrophotometry (Perkin Elmer, Norwalk, USA), while P concentration was measured colorimetrically as molybdovanado-phosphoric acid (Varian, Mulgrave, Australia).

Near-infrared spectra of forage samples were obtained with Perkin Elmer Spectrum One NTS FT-NIR Spectrometer (Perkin Elmer, Monza, Italy) equipped with a Perkin Elmer NIRA Sample Spinner, as described elsewhere (Lavrenčič *et al.*, 2001). About 10–20 g of forage contained in a 7.8 mm i.d. Petri disk were scanned at 2 nm intervals over the wavelenght range 1000 to 2500 nm. Calibration equations were calculated using all the samples with Spectrum Quant+ v4.51 software and Principal Component Regression (PCR+) algorithm (Perkin Elmer, Monza, Italy). For all forages the prediction of element contents was obtained.

The accuracy of the calibration and cross validation process was assessed by the coefficient of determination (\mathbb{R}^2), standard error of calibration (SEC) and standard error of cross validation (SECV) (square roots of the residual mean square). The coefficient of variation (CV) was calculated ($\mathbb{CV}=[SE/mean]\times100$) for cross validation. These are used as a tool to compare calibration and cross validation for different elements, since CV values are affected by the mean from the chemical procedure and SEC or SECV.

RESULTS AND DISCUSSION

The statistical data for the population set used in calibration and cross validation for the macrominerals analysed are presented in Table 1. The Ca and K contents ranged from 1.4 to 21.2 $g \cdot kg^{-1}$ DM and from 13.1 to 43.8 $g \cdot kg^{-1}$ DM, respectively. Inclusion of two plant families, grasses and legumes, can account for the high variability of Ca contents. The reasons for the variability of K contents could be the consequence of plant family, species, variety, maturity, consecutive harvest (season) and prior fertilization practice (Cherney *et al.*, 1998).

Table 1. Macromineral contents $(g \cdot kg^{-1} dry matter)$ and range of values for forage samples determined by chemical analysis (n = 100)

Preglednica 1. Vsebnost makrorudninskih snovi (g·kg⁻¹ suhe snovi) in razpon vrednosti v vzorcih voluminozne krme, določeni s kemično analizo

Element Rudninska snov	Range Razpon	Mean Sredina	SD
Са	1.4-21.2	6.8	4.4
Р	1.6-5.5	3.1	0.9
Mg	0.7–4.8	2.1	0.9
Κ	13.1-43.8	24.9	6.7
Na	0.05-0.45	0.17	0.08

SD = standard deviation / standardni odklon

The ranges of other macrominerals were less variable, with average values of P and Mg similar to those summarised by Stekar (1997). The average Na contents values were lower than those summarized by Stekar (1997) and were similar to those reported by Vasquez de Aldana *et al.* (1995) for natural grasslands.

The results of calibration and cross validation are presented in Table 2, where values of coefficients of determination (R^2), standard errors of calibration (SEC) and cross validation (SECV) and coefficients of variability of cross validation (CV_{SECV}) are presented.

The best performance in the calibration equation was calculated for Ca content (R^2 of 0.963 and SEC 0.90), followed by K (R^2 0.939 and SEC 1.77) and P (R^2 and SECs of 0.884 and 0.34). Clark *et al.*(1987), Ruano-Ramos *et al.* (1999), Vasquez de Aldana *et al.* (1995) and Redshaw *et al.* (1986) reported lower R^2 for Ca (from 0.75 to 0.91) and P (from 0.62 to 0.88) (Table 3). On contrary, R^2 values obtained by Saiga *et al.* (1989) (Table 3) for pure stands of orchardgrass were lower for Ca (0.824), similar for K (0.958) and higher for P (0.968) than our results.

Table 2. Accuracy of the NIRS calibration and cross validation of macrominerals in forage samples (n = 100)

Preglednica 2. Točnost umeritve in navzkrižne preveritve vsebnosti makrorudninskih snovi z NIRSom (n = 100)

Element Rudninska snov	R^2	SEC	SECV	CV _{SECV} , %
Са	0.963	0.90	1.04	15.3
Р	0.884	0.34	0.39	12.4
Mg	0.892	0.29	0.31	14.6
K	0.939	1.77	2.07	8.3
Na	0.520	0.057	0.062	35.5

 R^2 = coefficient of determination / koeficient determinacije; SEC = standard error of calibration / standardna napaka umeritve; SECV = standard error of cross validation / standardna napaka navzkrižne preveritve; CV_{SECV} = coefficient of variation of cross validation / koeficient variabilnosti navzkrižnega preverjanja

The SEC values of macrominerals of the Clark *et al.* (1987) and Vasquez de Aldana *et al.* (1995) studies were similar to those of Redshaw *et al.* (1986) for Ca (1.8) and P (0.2) and were generally lower than those of our study, except for Ca and P which had similar SEC (0.92 and 0.34, respectively) (Table 3).

The R^2 and SEC for Mg content (0.892 and 0.29, respectively) were higher than those reported by Vasquez de Aldana *et al.* (1995) (0.84 and 0.16 for R^2 and SEC, respectively) and Ruano-Ramos *et al.* (1999) (0.80 and 0.15 for R^2 and SEC, respectively) and lower than those reported by Saiga *et al.* (1989) (0.945 and 0.09 for R^2 and SEC, respectively) (Table 3). The variability of the accuracy of calibration could be attributed to the homogeneity of sample sets, because the calibration of NIRS was performed in samples of very different botanic origin in the studies of Vasquez de Aldana *et al.* (1995) and Ruano-Ramos *et al.* (1999), while in the study of Saiga *et al.* (1989) the calibration was performed only on pure orchardgrass samples.

High R^2 values for above mentioned macrominerals indicate that they are closely associated with the organic functional groups, as proposed by Clark *et al.* (1987, 1989). Calcium and Mg are associated with components of plant cell wall. For example Ca is ionically linked with pectins, to form calcium pectate which binds adjacent cells together (Carpita and Gibeaut, 1993). Magnesium is also associated with chlorophyll, while phosphorus occurs in plants mainly in form of organic compounds, such as soluble and insoluble phytates, phospholipids, phosphoproteins and nucleic acids (Spears, 1994). Potassium is associated with organic acids, especially malate, and Clark *et al.* (1987) found some similarities between bands in the potassium malate spectrum and the wavelengths used for the determination of K in grass samples. Organic acids are important plant metabolites and can accumulate in grass up to 8% of dry matter.

Table 3. Macroelement comparisons of R^2 and SEC estimates between published estimates

Element Rudninska snov		Clark <i>et al.</i> , 1987	Vasquez de Aldana <i>et</i> <i>al.</i> , 1995	Redshaw <i>et</i> <i>al.</i> , 1986	Ruano Ramos <i>et</i> <i>al.</i> , 1999	Saiga <i>et al.</i> , 1989
Ca	R^2	0.75-0.86	0.88	0.87	0.91	0.824
Ca	SEC	0.6-1.1	0.92	1.8	0.78	0.26
D	R^2	0.62-0.79	0.70	0.71	0.88	0.968
I S	SEC	0.2-0.3	0.27	0.2	0.22	0.14
Ma	R^2	0.73-0.80	0.84		0.80	0.945
IVI S	SEC	0.2-0.6	0.16		0.15	0.09
V	\mathbb{R}^2	0.83-0.88	0.78		0.87	0.958
ĸ	SEC	1.4–2.6	1.89		1.65	1.40
Na	\mathbb{R}^2	0.24-0.27	0.79			
	SEC	6.0–160.0	0.62			

Preglednica 3. Primerjava med objavljenimi ocenami R² in SEC za makrorudninske snovi

 R^2 = coefficient of determination / koeficient determinacije; SEC = standard error of calibration / standardna napaka umeritve

Noteworthy, the low R^2 (0.520) and SEC (0.57) were observed for Na content (Table 2). Vasquez de Aldana *et al.* (1995) reported higher R^2 (0.79) and SEC (0.62) for Na content in natural grassland forage samples, while Clark *et al.* (1987) obtained lower values (0.27 and 6.0 for crested wheatgrass)(Table 3). In addition, Clark *et al.* (1987) could not produce a calibration equation for tall fescue samples. This could be attributed to the low Na contents of these forages, because the NIRS does not precisely detect compounds with a concentrations lower than 1 g·kg⁻¹ (Van Kempen, 2001). In addition, the calibration obtained by Clark *et al.* (1987) showed large errors, even within single species, due to the extreme range in Na values and perhaps a lack of correlation between Na and organic functional groups sensed by NIRS.

The calibration equations were eventually cross validated (Table 2). Standard errors of cross validation (SECV) for all macrominerals were always higher than those obtained for calibration. The SECV values of Ca, P and K were similar to those of validation set of samples obtained by Vasquez de Aldana *et al.* (1995). The lowest coefficient of variation (CV_{SECV}) was obtained for K, followed by P and Mg. The largest CV_{SECV} was calculated for Na content (35.5%), the value being more than two times lower than that reported by Vasquez de Aldana *et al.* (1995). The performance of Na prediction was significantly lower than all other elements, as can be seen by the low R² and high coefficient of variation (CV_{SECV}). These results agree with those of Clark *et al.* (1987) and suggest that NIRS cannot determine the Na concentration in forages.

Ranges, means and standard deviations for Zn, Mn and Se are presented in Table 4, to show the content variations of the forages.

Zinc and Mn contents were highly variable, with values between 4.4 and 38.1 mg·kg⁻¹ DM for Zn, between 16.7 and 311.0 mg·kg⁻¹ DM for Mn, whilst Se varied between 16.9 and 48.8 μ g·kg⁻¹ DM. Only the values of Se were considered to be within the normal ranges of forages, as reported by Mayland and Wilkinson (1996), while the lower value of Zn was lower and the higher value of Mn greatly exceded the upper limit of 15 and 100 mg of Zn and Mn, respectively, reported by Mayland and Wilkinson (1996).

- Table 4. Trace mineral content and ranges (per kg of dry matter) of forage samples determined by laboratory chemical analysis (n = 100)
- Preglednica 4. Vsebnost mikrorudninskih snovi (na kg suhe snovi) in razpon vrednosti v vzorcih voluminozne krme, določeni s kemično analizo (n = 100)

Element Rudninska snov	Range Razpon	Mean Sredina	SD
Zn, mg	4.4-38.1	21.3	6.5
Mn, mg	16.7-311.0	75.9	66.6
Se, µg [‡]	16.9-48.8	28.7	6.2

n = 60; SD = standard deviation / standardni odklon

The statistics for calibration showed generally lower accuracy for trace elements (Table 5) than for macrominerals (Table 2). For all the trace elements the R^2 was smaller than 65% with increased SECs. The highest R^2 was observed for Zn (0.642) which had also lowest SEC (4.1) and SECV (4.4) compared with Mn (0.427, 51.5 and 55.6 for R^2 , SEC and SECV, respectively) and Se (0.186, 5.6 and 6.5 for R^2 , SEC and SECV, respectively).

- Table 5. Accuracy of the NIRS calibration and cross validation of trace elements in forage samples (n = 100)
- Preglednica 5. Točnost umeritve in navzkrižne preveritve vsebnosti mikrorudninskih snovi z NIRSom (n = 100)

Element Rudninska snov	R^2	SEC	SECV	CV _{SECV} , %
Zn	0.642	4.1	4.4	20.4
Mn	0.427	51.5	55.6	73.2
Se [‡]	0.186	5.6	6.5	22.7

 R^2 = coefficient of determination / koeficient determinacije; SEC = standard error of calibration / standardna napaka umeritve; SECV = standard error of cross validation / standardna napaka navzkrižne preveritve; CV_{SECV} = coefficient of variation of cross validation / koeficient variabilnosti navzkrižnega preverjanja; ${}^{\ddagger}n = 60$

In the Table 6 the microelement comparisons of R^2 and SEC between published estimates are presented.

Table 6.Microelement comparisons of R^2 and SEC between published estimatesPreglednica 6.Primerjava med objavljenimi ocenami R^2 in SEC za mikrorudninske snovi

Element Rudninska snov		Clark et al., 1987	Vasquez de Aldana et al., 1995	Clark et al., 1989
Zn	R^2	0.44-0.61	0.72	
	SEC	2.3-4.3	3.8	
Mn	\mathbb{R}^2	0.24-0.50	0.74	
	SEC	6.0–19.0	50	
Se	R^2			0.24
	SEC			0.3

 R^2 = coefficient of determination / koeficient determinacije; SEC = standard error of calibration / standardna napaka umeritve

The R^2 values were similar to those of Clark *et al.* (1987), who reported R^2 in the range of 0.44 to 0.61 for Zn and 0.24 and 0.50 for Mn. (Table 6). Comparing Zn and Mn calibration and validation statistics with those of Vasquez de Aldana *et al.* (1995) we obtained lower R^2 with greater SECs and SECVs, especially for Mn, which had R^2 of 0.74 in the study of the above mentioned authors (Table 6).

Coefficient of variation (CV_{SECV}) of Zn and Mn concentrations (Table 5) were high and exceeded 20% in the case of Zn, while that of Mn was greater than 70%, being generally greater than those reported by Vasquez de Aldana *et al.* (1995). Similarly to the present results in the study of Vasquez de Aldana *et al.* (1995) Mn had also greater CV_{SECV} than Zn. Clark *et al.* (1989) determined Se concentration more accurately with R² of 0.24 and SEC of 0.3 (Table 6). However, their calibration was done only on samples of a single species, tall fescue, which were much more homogeneous than samples used in our study.

The statistical analyses calculated in this paper and those reported in the literature underline the difficulty to obtain accurate and precise calibration equations to predict trace mineral contents of forages with NIRS technique. This could be due to the concentrations of trace elements in the samples, considerably lower than the detection limit of 1 g·kg⁻¹ reported for NIRS (Van Kempen, 2001). Moreover, it must also be considered that trace elements are not completely associated with organic functional groups in forages, preventing NIRS technique to detect them.

CONCLUSIONS

The results of this study indicated that prediction of macromineral and, especially, trace elements contents with NIRS is not comparable with that reported for other organic class compounds, such as crude protein, crude fiber and ether extract (eg. Lavrenčič *et al.*, 2001). However, on the basis of the results obtained, NIRS can be considered suitable for the estimation of macromineral concentrations in forages, with the high accuracy for Ca and K concentrations and somewhat lower accuracy for P and Mg concentrations. For Na, Zn, Mn and Se concentrations, NIRS was not very successful for predictions. However, it seems reasonable that NIRS could be somewhat sensitive to the presence of these minerals within samples of simpler botanical composition (Clark *et al.*, 1989). If exact Na and trace element concentrations are required, the NIRS is not the method to use. However, NIRS may provide an acceptable monitoring technique for certain minerals in forage samples and in many instances the mineral values obtained from NIRS can be considered as an acceptable first approximation.

POVZETEK

Z raziskavo smo želeli preučiti, kako točno lahko z bližnjo infrardečo refleksijsko spektroskopijo (NIRS) ocenimo vsebnost makro- in mikro-rudninskih snovi v vzorcih voluminozne krme, ki smo jih kosili večkrat letno v različnih morfoloških stadijih razvoja. Reflektirano bližnjo infrardečo svetlobo z vzorcev smo merili v 2 nm intervalih v razponu valovnih dolžin med 1000 in 2500 nm. Dobljene spektralne podatke smo z algoritmom regresije poglavitnih elementov (principal component regression – PCR) prilagodili vsebnostim makro- in mikrorudninskih snovi. Med procesom umerjanja smo izvedli tudi popolno navzkrižno preverjanje, kar nam je omogočilo določitev umeritvene enačbe, ki je imela največji koeficient determinacije (R²) in najmanjše standardne napake umeritve (standard error of calibration – SEC) in navzkrižnega preverjanja (standard error of cross validation – SECV). Rezultati te raziskave so pokazali, da lahko z NIRS predvidimo vsebnosti makroelementov, kot so Ca, P, K in Mg, z veliko stopnjo točnosti (koeficienti determinacije so bili v razponu od 0,884 za P do

0,963 za Ca), medtem ko so bili determinacijski koeficienti za Na in tri mikroelemente (Zn, Mn in Se) veliko manjši (od 0,186 za Se do 0,642 za Zn), ter s koeficienti variabilnosti SECV (CV_{SECV}), ki so bili vedno večji od 20 %. Ti rezultati kažejo, da z NIRS lahko točno določamo le tiste makrorudninske snovi, ki so povezane z organskimi sestavinami, kot so rastlinska celična stena (Ca in Mg), klorofil (Mg), fitati, fosfolipidi, fosfoproteini in nukleinske kisline (P) in organske kisline, kot je jabolčna kislina (K). Nasprotno pa vsebnosti Na, Zn, Mn in Se niso povezane z organskimi snovmi, ali pa je vsebnost rudninskih snovi pod mejo detekcije NIRSa. Kljub temu da z NIRSom ne moremo zanesljivo določati vsebnosti nekaterih rudninskih snovi, zastopanih v vzorcih v manjših količinah, pa lahko na ta način vseeno opravimo grobo oceno njihove vsebnosti.

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