In sacco NDF degradability and mineral release from selected forages in the rumen

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ABSTRACT: An *in sacco* technique was used to measure NDF degradability and release of Mg, Ca, Zn, Cu, Fe from six forages – lucerne hay from the 1st cut (LH_1), from the 2nd cut (LH_2), orchard grass (G), grass silage (GS), red clover silage treated with Feedtech (CS_{FT}) and/or with Kofasil (CS_{KO}). The forages differed in the content of NDF and minerals. There were large differences ($P < 0.01$) in element release in the rumen between experimental forages and the particular elements. The rate of release of elements was higher from LH_1, LH_2, CS_{FT}, CS_{KO} than from G and GS. The release of individual elements and NDF over all incubation times is very well expressed by cubic polynomials ($R^2 > 0.9$). Overall, across forages the release of mineral elements ranked as follows: Mg > Fe > Cu > Ca > Zn. The minimum and maximum disappeared portions of individual minerals from forages are as follows: Mg 74.7% from G and > 91.5% from LH_1, Fe 29.7% from G and 99.9% from LH_1 and LH_2, Cu 64.3% from G and 99.8% from LH_1 and LH_2, Ca 28.4% from G and 75.4% from CS_{FT}, Zn 12.0% from G and 38.2% from LH_1, respectively. Calcium and zinc need a longer time for their maximum release. It can be concluded from the results of multiple regression analyses that only the Mg and Ca release is associated depending on NDF and time of observations, with a highly significant influence of forage on Zn and Cu.

Keywords: forage; NDF; mineral element release; rumen; *in sacco* method

Field (1981) referred to the need of further study of solubility of mineral elements contained in forages in the rumen. He stated that their absorption as well as utilization in the organism depended on solubility in the rumen to a large extent. There are large differences in solubility and release rate of individual mineral elements. Ledoux and Marty (1991) reported that more than 60% P, Mg and K were solubilized as early as the forages were washed (in bags) by *in sacco* method. However, only 45% calcium was dissolved out of forages. Other authors (van Eys and Reid, 1987; Emanuele at al., 1991; Flachowsky et al., 1994; Gralak et al., 1997; Třináctý et al., 2000) found a similar tendency and they also described differences between the feeds. Differences in solubility of individual mineral elements can be ascribed to the ability of cell walls to bind certain elements to their surface (cation exchange capacity) that can also be influenced by NDF concentration in feed (Emanuele et al., 1990) or to the content of organic acids in plant structures underlying the ability to bind and retain ions of metals (Ward et al., 1979; Van Soest, 1982).

The aim of this study was to determine the degradation of NDF and release of macro and microelements from six selected forages in the rumen. An additional task was to evaluate the relationship between NDF degradation and the rate of release of individual elements.

MATERIAL AND METHODS

Feeds

To examine the release of macro and microelements six forages were used: lucerne hay from the 1st cut (LH_1) and from the 2nd cut (LH_2), orchard grass (Rela hybrid), grass silage, red clover silage treated with Feedtech (CS_{FT}) and with Kofasil

(CS_{KO}). Feedtech is a chemical and Kofasil is a biological preservation agent.

In sacco method

The release of mineral elements was examined using the *in sacco* method that was performed on three young bulls fitted with a large rumen cannula and housed in individual pens. The animals were fed a diet composed of maize silage, lucerne hay, cereal mix (barley and wheat, 1 : 1) and mineral-vitamin premix. The intake of water was *ad libitum*.

Experimental forages were freeze-dried (except LH_1 and LH_2), ground to pass a 3-mm sieve and weighed into bags (9 × 15 cm, pore size 47 µm) made from nylon cloth UHELON 130T (Hedva, Moravská Třebová, Czech Republic). Three bags were used per each forage, each animal and incubation time. The bags were incubated for 6, 9, 16, 24, 48 and 72 h. Degradation of NDF was studied in the same incubation times. All steps of the *in sacco* method were described by Harazim and Pavelek (1999).

Chemical analysis

Nutrient content of forages (Table 1) was determined according to the standard STN 46 9072 and cell wall content according to the procedure of van Soest (Lutonská and Pichl, 1983). For mineral analyses 1 g samples of forages and residues were ashed at 550°C (STN 46 9072) and then dissolved in 10 ml of hot HCl (1 : 3) solution. The concentrations of Ca, Mg, Fe, Cu and Zn were measured using atomic absorption spectrophotometry (AAS Solar 9000 Unicam, Cambridge, UK).

Mathematical and statistical processing

The data on the observed losses of NDF and mineral elements were evaluated as follows:
– by calculation of basic variation statistical characteristics
– the influence of forage and time of observations was evaluated by two-way analysis of variance with the fixed effects with one observation per cell (*ij* – subclasses in feed × time), with determination of Tukey's test of nonadditivity of feeds × times
– dependences of the analysed traits on time were evaluated by estimation of parameters of linear functions and polynomials, the most suitable polynomial was polynomial of the 3rd (cubic) degree

Mathematical and statistical processing was done on the basis of statistical methods of Grofik and Fľak (1990) and by help of statistical program pack-

Table 1. Chemical composition of experimental forages

Nutrient	Lucerne		Grass *Dactylis glomerata*	Silage		
					clover	
	1st cut	2nd cut		grass	FeedTech*	Kofasil*
Dry matter (g/kg)	218.6	307.6	171.1	212.8	299.1	314.2
N × 6.25 (g/kg DM)	210.0	191.3	140.7	172.6	211.6	226.8
Crude fibre	281.9	285.5	348.8	331.9	234.2	244.8
NDF	351.7	383.7	597.7	545.5	340.7	361.7
Organic matter	873.7	905.6	899.8	920.7	874.8	867.5
Mg	3.3	1.7	1.5	1.9	3.6	3.7
Ca	15.8	10.4	3.7	4.8	15.3	15.5
Cu (mg/kg DM)	48.1	9.2	10.3	19.5	31.3	16.6
Fe	202.9	109.3	97.4	275.2	1141.5	1330.5
Zn	31.4	34.5	34.6	37.2	52.7	34.2

*preservation agent

age SPSS for Windows, Release 6.0 (1989–1993, licensed for Research Institute of Plant Production in Piešťany).

RESULTS AND DISCUSSION

Forages account for as much as 70% of dry matter proportion in feed rations for cattle. Because of the essential importance of mineral elements, either macro or microelements, it is necessary to know their content in forages and their solubility in the digestive tract as well. Solubility and release from the structure of feed are important preconditions of utilization of mineral elements in animals. There are large differences between feeds in the content of cell walls, and in the representation of individual fractions (cellulose, hemicelluloses and lignin) that influence the degradability of cell walls (Čerešňáková et al., 2000) and release of mineral elements (Ledoux and Martz, 1991; Flachowsky et al., 1994).

Our results suggest possible differences between grasses and legumes in the content of NDF and crude protein as well as in the content of mineral elements (Table 1). Orchard grass contains 1.5 times more NDF than lucerne and clover silage. Grass silage also contains a similarly high amount of NDF.

The release of NDF from feeds during 6 to 72 h of incubation (Table 2) in the rumen indicates differences in degradation between the forages as well as differences in the final maximum release after 72 h incubation. After 6 h incubation we noticed a minimum release of NDF 29% in LH_1 and 19% in LH_2 (1st and 2nd cut), and about 12% only in G and GS. After 72 h in lucerne hay 62%, in grass 69%, in grass and clover silages about 62%. Emanuele and

Table 2. Basic statistical characteristics of the release of selected elements and NDF from forages over all incubation times in the rumen

Forage	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
	NDF				Mg			
LH_1	43.8	12.65	28.9	62.4	91.5	2.51	89.3	94.9
LH_2	39.0	16.77	19.3	63.2	89.1	3.61	83.9	93.7
G	42.2	23.20	12.4	69.3	74.7	16.87	51.0	90.2
GS	37.6	18.65	14.0	62.9	84.6	5.58	75.7	91.6
CS_{FT}	42.6	14.82	22.0	58.5	89.9	5.70	79.8	95.0
CS_{Ko}	45.6	16.68	20.7	64.8	89.9	6.22	79.4	94.8
	Ca				Zn			
LH_1	74.6	6.48	66.8	83.3	38.2	13.11	22.2	57.0
LH_2	69.7	9.01	59.1	82.9	37.2	12.29	21.8	52.9
G	28.4	39.75	–23.6	66.9	12.0	14.90	–15.0	24.4
GS	54.2	20.47	16.3	72.2	25.6	5.67	15.3	31.7
CS_{FT}	75.4	14.34	49.8	86.8	26.9	6.72	20.7	37.5
CS_{Ko}	76.3	14.00	52.1	86.9	19.2	3.14	14.9	23.3
	Cu				Fe			
LH_1	99.8	0.058	99.7	99.8	99.9	0.071	99.7	99.9
LH_2	99.8	0.118	99.6	99.9	99.9	0.021	99.8	99.9
G	64.3	12.55	41.0	75.5	29.7	28.00	–7.8	64.5
GS	79.2	4.35	72.6	83.9	73.6	9.39	56.0	83.3
CS_{FT}	70.0	8.401	61.0	84.6	86.5	11.02	66.5	95.4
CS_{Ko}	66.6	10.22	50.2	75.4	87.9	9.65	71.3	95.8

Table 3. Two-way ANOVA of selected elements and NDF with the test of nonadditivity of group (forage) × time of incubation interactions

Element		Groups (A) $f_a = 5$	Time (B) $f_b = 5$	Error (e) $f_e = 25$	N $f_N = 1$	R $f_r = 24$
NDF	MS	63.2*	1 716.7**	21.73	28.2	21.45
Mg	MS	1 569.1**	192.7**	31.77	622.7**	7.15
Ca	MS	2 142.2**	1 506.8**	203.42	3 112.2**	82.22
Zn	MS	619.0**	482.3**	29.96	25.1	30.16
Cu	MS	872.1**	386.6**	35.88	270.4**	26.11
Fe	MS	237.1**	225.4**	36.16	624.2**	11.66

$F_{0.05}$ (5, 25) = 2.603, $F_{0.01}$ (5, 25) = 3.855, $F_{0.05}$ (1, 24) = 4.260, $F_{0.01}$ (1, 24) = 7.82

*$P < 0.05$, **$P < 0.01$

Staples (1990) gave similar values of NDF release for lucerne after 72 h incubation. Differences in NDF release between forages as well as in dependence on time (Table 3) are significant and they are demonstrated by a linear relation ($P < 0.05$ to $P < 0.01$) (Table 4). Differences in the course of NDF degradation depending on time ($P < 0.05$) in the tested feeds are represented as cubic functions in Table 5 and in Figure 1. During the first 24 h of incubation the release of cell walls was fastest in clover silages CS_{KO} and CS_{SF} ($P < 0.01$), followed by LH_1. The slowest degradation of cell walls was in G and LH_2. We expressed the dependence of NDF degradation on the incubation time by common cubic polynomials ($R^2 = 0.909$ with < 0.01) for all feeds in the experiment (Table 6).

There are different concentrations of macro and microelements in feeds (Table 1), and their release during incubation in the rumen is also different. It is demonstrated in Table 2 by minimum (6 h incubation), maximum (72 h incubation) and mean values from all incubations. The rate and amount of their release is not connected with their original concentration. We found significant differences ($P < 0.01$) in incubation time between forages (Table 3). The release of mineral elements from G

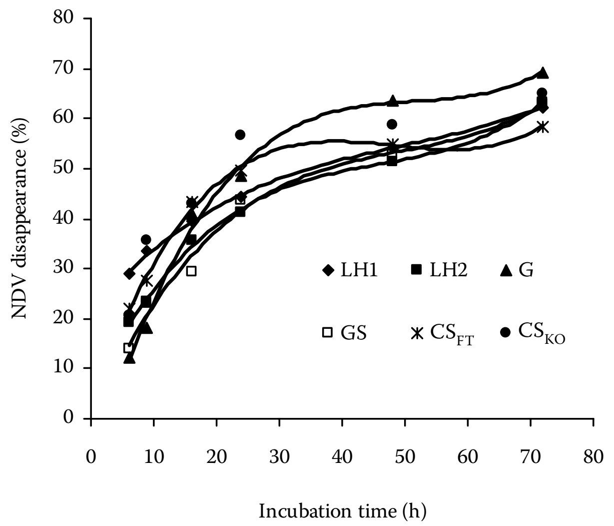

Figure 1. Cubic polynomials of NDF release in relation to incubation time (see Table 5)

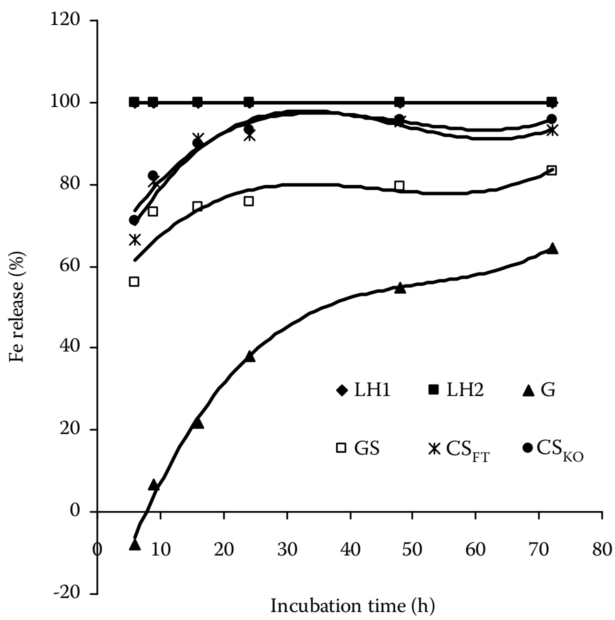

Figure 2. Cubic polynomials of Mg release in relation to incubation time (see Table 5)

Table 4. Parameter estimates of the linear function $y = b_0 + b_1t$ of selected elements and NDF release from forages in the time of ruminal incubation

Forage	b_0	b_1	R^2	b_0	b_1	R^2	b_0	b_1	R^2
	NDF			Mg			Ca		
LH_1	29.85	0.4792	0.957**	92.52	–0.0354	0.132	76.05	–0.0486	0.037
LH_2	20.74	0.6259	0.928**	88.61	0.017	0.015	70.76	–0.0363	0.011
G	18.40	0.8157	0.824*	57.32	0.5276	0.652	–8.81	1.2753	0.686*
GS	17.65	0.6855	0.900**	78.99	0.1928	0.729*	37.28	0.5794	0.534
CS_{FT}	28.25	0.4932	0.738*	85.52	0.1488	0.454	64.39	0.3764	0.459
CS_{Ko}	30.56	0.549	0.723*	84.89	0.1735	0.518	65.02	0.3855	0.505
	Zn			Cu			Fe		
LH_1	23.95	0.4896	0.930**	99.85	–0.0020	0.777*	99.98	–0.0026	0.894**
LH_2	24.29	0.4420	0.862**	99.81	–0.0012	0.073	99.89	–5E-07	0.000
G	–0.357	0.4239	0.540	53.87	0.3588	0.545	0.28	1.0082	0.864**
GS	37.28	0.5794	0.534	74.92	0.1452	0.724*	65.55	0.276	0.576
CS_{FT}	64.39	0.3764	0.459	60.77	0.3162	0.942**	78.22	0.2847	0.445
CS_{Ko}	65.02	0.3855	0.505	57.76	0.3044	0.592	79.79	0.0280	0.560

$R^2_{0.05}$ (1, 4) = 0.658, $R^2_{0.01}$ (1, 4) = 0.841

*$P < 0.05$, **$P < 0.01$

and GS was the lowest compared with other forages (Table 2). Cubic functions of the release of individual elements from feeds in dependence on incubation time have high values of R^2, however, not all of them are statistically significant (Tables 5 and 6).

The course of Mg release from the particular feeds in time is shown in Figure 2 by cubic polynomials that have high statistical significance ($P <$ 0.01) in all forages. Although the average release of Mg from all forages was higher than 90%, Figure 2 shows evident differences between feeds, mainly between G, GS and legumes. The release from legumes was higher than from grasses ($P < 0.01$). The values of Mg release after 72 h incubation in the rumen are comparable with results given by Emanuele et al. (1991) for lucerne and some grass species. Ledoux and Martz (1990) also reported a higher solubility of Mg from lucerne than from grasses. We found a certain similarity in the release of Mg and Zn in all studied forages. Mg release was on average the highest in all forages and on the contrary, Zn release was the lowest in all forages, from 12% to 38%.

After 6-hour incubation of orchard grass (G) in the rumen we found increased concentrations of Ca and Zn in the undegraded residue, and % of release achieved negative values (Figures 3 and 4). Emanuele and Staples (1990) found a similar tendency in Ca and Zn in grass (*Pennisetum purpureum* Schum.) that had a high content of NDF (593 g/kg DM) similarly like our orchard grass. Lower values of Ca release from forages can be explained by its function in the plant in which it is important for the stability of cell walls and membranes. McManus et al. (1979) confirmed that Ca accounted for 34% and 64% of all minerals found in the cell walls of grasses and legumes, respectively. Therefore the amount of released Ca does not increase before the increase of cell wall degradation. Emanuele and Staples (1990) ascribed the low and slow Zn release from grass and lucerne also to its linkage to cell walls and they concluded that Zn could also be a part of plant proteins. Kabaija and Smith (1988) explained the low water solubility and release of Zn from plants by its bond to various complexes from which it was released in the rumen gradually. We found out that a shorter incubation time (24 h) would be necessary for the release of the maximum of Ca from LH_1 and LH_2 than from G and GS (72 h). The fastest release of Ca and the highest amount of released Ca were determined in CS_{FT} and CS_{KO} out of which more

Table 5. Parameter estimation of cubic polynomials $y = b_0 + b_1t + b_2t^2 + b_3t^3$ of selected elements and NDF release from forages in the time of ruminal incubation

Forage	b_0	b_1	b_2	b_3	R^2	b_0	b_1	b_2	b_3	R^2
	NDF					Mg				
LH_1	21.76	1.4239	–0.0229	0.0002	0.998**	83.77	1.1308	–0.0343	0.0003	0.995**
LH_2	5.437	2.5026	–0.0494	0.0004	0.997**	75.65	1.6498	–0.0447	0.0003	0.991*
G	–10.64	4.1557	–0.0791	0.0005	0.992*	27.71	4.2681	–0.093	0.0006	0.984*
GS	0.0387	2.7503	–0.0505	0.0003	0.988*	72.96	0.9182	–0.0185	0.0001	0.899
CS_{FT}	3.467	3.4474	–0.0743	0.0005	0.995**	72.80	1.6442	–0.0367	0.0002	0.944
CS_{Ko}	1.864	4.0308	–0.0901	0.0006	0.975*	70.38	1.9402	–0.0459	0.0003	0.977*
	Ca					Zn				
LH_1	53.03	2.852	–0.0793	0.0006	0.992*	12.16	1.9179	–0.0369	0.0003	1.000**
LH_2	37.86	4.14554	–0.1157	0.0009	0.999**	9.510	2.1931	–0.0436	0.0003	0.998**
G	–77.7	9.3697	–0.1989	0.0013	0.979*	–32.59	4.3575	–0.1027	0.0007	0.931
GS	–6.979	6.0706	–0.1469	0.0011	0.904	10.52	1.4267	–0.0342	0.000	0.863
CS_{FT}	31.28	4.3386	–0.1003	0.0007	0.941	19.98	0.1328	0.0051	5E-05	0.998**
CS_{Ko}	32.80	4.2689	–0.0994	0.0007	0.970*	12.97	0.4471	–0.0078	5E-05	0.978*
	Cu					Fe				
LH_1	99.79	0.0049	–0.0002	9.5E-07	0.908	99.92	0.003	–0.0001	5.7E-07	0.982*
LH_2	99.85	–0.0153	0.0008	-8E-06	0.853	99.94	0.0062	0.0002	–2E-06	0.333
G	28.20	3.5266	–0.0841	0.0006	0.881	-30.57	4.5417	–0.0831	0.0005	0.997**
GS	69.33	0.7494	–0.0127	6.9E-05	0.976*	49.56	2.292	–0.0552	0.0004	0.796
CS_{FT}	54.66	1.2254	–0.0302	0.0003	0.998**	53.06	3.2924	–0.076	0.0005	0.916
CS_{Ko}	35.94	2.9372	–0.0675	0.0005	0.990*	59.48	2.7044	–0.0611	0.0004	0.964

$R^2_{0.05}$ (3, 2) = 0.966, $R^2_{0.01}$ (3, 2) = 0.994

*P < 0.05, **P < 0.01

than 80% Ca were released within 24 h. The highest amount of Zn was released from LH_1 and LH_2, however, it reached the maximum value (> 50%) after 72 h only (Table 2, Figure 4).

Rooke et al. (1983) placed Cu among Mg, K, Na and Ca, Zn; P as far as the rate of release from lucerne silage is concerned. Based on their own results and on results of other authors Emanuele and Staples (1990) stated that Cu release depended on a forage species. We obtained similar results in our experiment, with large differences between forages (P < 0.01) (Table 3) for the shortest incubation time (i.e. 6 h) as far as the release is concerned – more than 95% Cu from LH_2 and only 40% from G were released (Table 2, Figure 5). Equally different was the course of Cu release from experimental forages between 6 h and 72 h incubation (P < 0.01). The lowest values of Cu release were found in G and CS_{KO} (Table 2).

The release of Fe from feeds expressed by cubic polynomials (Table 5) is shown in Figure 6. Differences between feeds are highly significant (P < 0.01), with the highest values for LH_2 and LH_2 and the lowest ones for G. The course of Fe release in grass is very similar to Cu, Zn, Ca and Mg (Figures 2, 3, 4, 5). Similarly to Zn the percentage of Fe release also decreases to negative values after the first 6 hours of incubation. In both elements it can be caused by adhesion of bacterial cells, rich in these elements, to the cell walls of forages and it increases their concentration in the undegraded feed residue. Joblin and Lee (1990) reported high concentrations

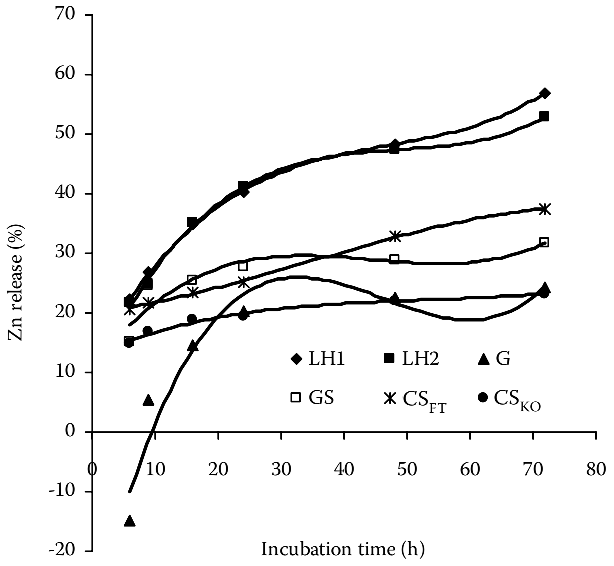

Figure 3. Cubic polynomials of Ca release in relation to incubation time (see Table 5)

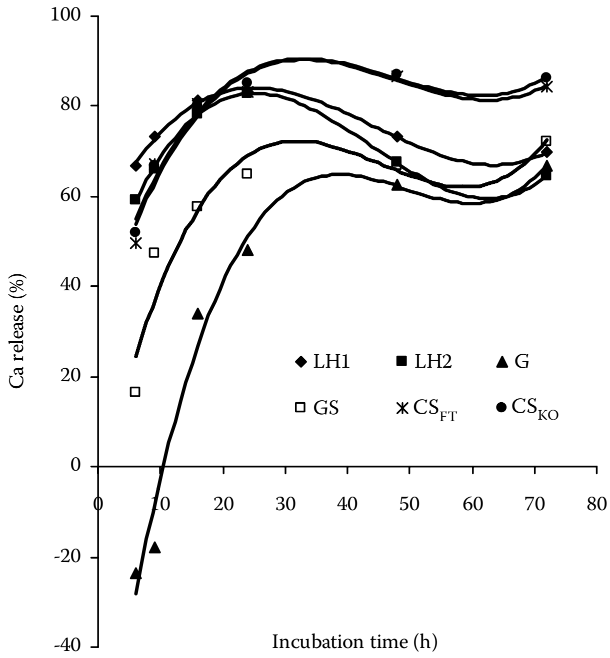

Figure 4. Cubic polynomials of Zn release in relation to incubation time (see Table 5)

of Zn, Fe as well as Cu in the fraction of bacteria during the study of element distribution in rumen ingesta among its other fractions. The release of Fe rose with the length of incubation only, with increasing NDF degradation. In orchard grass the Fe release is also low with regard to the highest content of cell walls and their slowest degradation.

Table 7 shows the results of multiple regression analyses of mineral elements on NDF, time and code of forage species. The latter independent

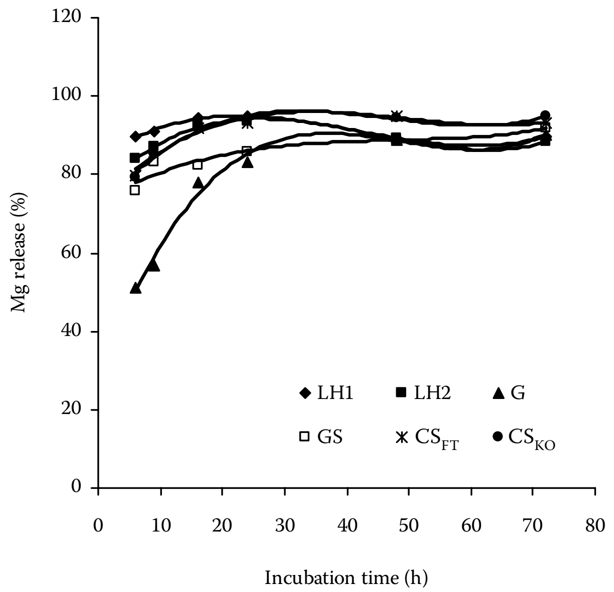

Figure 5. Cubic polynomials of Cu release in relation to incubation time (see Table 5)

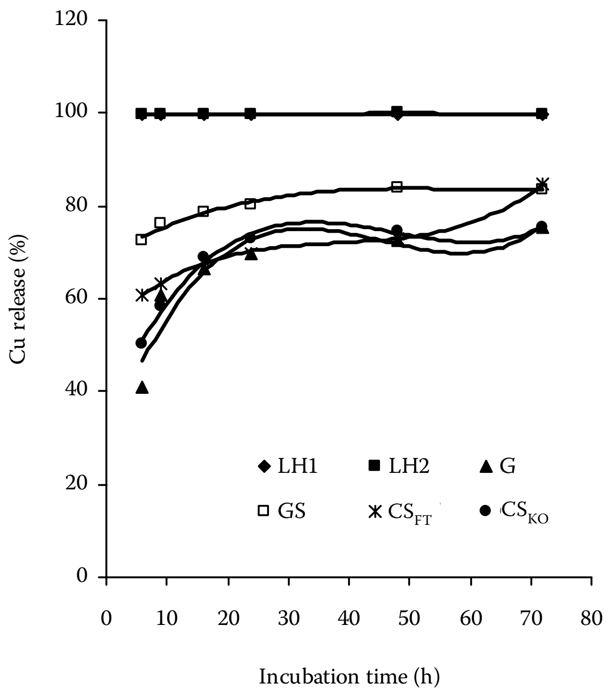

Figure 6. Cubic polynomials of Fe release in relation to incubation time (see Table 5)

Table 6. Linear and cubic functions of selected elements and NDF release from forages in the time of ruminal incubation

Element	Linear function			Cubic function				
	b_0	b_1	R^2	b_0	b_1	b_2	b_3	R^2
NDV	24.24	0.6081	0.783**	3.653	3.0518	–0.0611	0.0004	0.909**
Mg	81.65	0.1707	0.181**	67.21	1.9252	–0.0455	0.0003	0.346**
Ca	50.78	0.4219	0.153*	11.71	5.1742	–0.1234	0.0009	0.319**
Zn	17.26	0.3172	0.322**	5.427	1.7458	–0.0367	0.0003	0.380**
Cu	74.50	0.1869	0.073	64.63	1.4047	–0.0323	0.0002	0.097
Fe	70.62	0.3077	0.072	55.23	2.1379	–0.0459	0.0003	0.097

$R^2_{0.05}$ (1, 34) = 0.108, $R^2_{0.05}$(3, 32) = 0.213, $R^2_{0.01}$ (1, 34) = 0.180, $R^2_{0.05}$(3, 32) = 0.295

*$P < 0.05$, **$P < 0.01$

Table 7. Multiple regression analyses of mineral elements on NDF, time and forage

Element		Constant b_0	NDF b_1	Time b_2	Forage b_3	R^2
Mg	b_i	65.0779++	0.7247++	–0.2700+	–0.2855	0.5106++
	sb_i	4.6121	0.1563	0.1072	0.6903	
Ca	b_i	3.2563	1.9083++	–0.7385	0.3614	0.4765++
	sb_i	12.8467	0.4353	0.2986	1.9228	
Zn	b_i	21.1839++	0.2393	0.1170	–3.4009++	0.5298++
	sb_i	6.3048	0.2136	0.1465	0.9437	
Cu	b_i	91.1824++	0.32304	–0.0140	–7.0554++	0.6110++
	sb_i	7.1046	0.2407	0.1651	1.0634	
Fe	b_i	53.3767++	1.0271	–0.3169	–2.1881	0.1633
	sb_i	17.2273	0.5838	0.4004	2.5785	

$R^2_{0.05}$ (3,32) = 0.213, $R^2_{0.01}$ (3,32) = 0.295

variable – code of forage type – was considered for adjusting multiple linear regression functions on this variable, which follow from significant or highly significant differences between forage species that were determined by two-way analyses of variance (Table 3). It follows from Table 7 that all coefficients of determination up to Zn were highly statistically significant. Except Ca the constants b_0 were all highly statistically significant. The code of forage species was highly significant only in Zn and Cu. In multiple regression functions we do not consider the term of forage species × time interaction, which corresponds to the nonadditivity factor (N) in two-way analyses of variance. It can be concluded from partial regression coefficients of mineral elements on NDF release and time that there are positive highly significant regressions on NDF 0.7247** in Mg and 1.9083** in Ca and negative significant regressions on time in these elements, i.e. –0.2700* in Mg and –0.7385* in Ca. It can be concluded from reported results of multiple regression analyses that only Mg and Ca are associated depending on NDF and time of observations, with a highly significant influence of forage species on Zn and Cu.

The *in sacco* technique is useful for determination of differences between forages in the release of mineral elements in the rumen. The results indicate that the rumen is a major site of Ca and Mg release from these forages because there are highly significant regressions on NDF degradation. The low release of Zn in the rumen from all species of forages and Cu from orchard grass must be taken into account with Zn and Cu requirements of animals.

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