In sacco macromineral release from selected forages

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ABSTRACT: An in sacco technique was used to measure the release of Mg, Ca, Na, K from six forages – lucerne hay from the 1st and 2nd cut (LH1 and LH2), orchard grass hybrid Rela (GR) and hybrid Niva (GN), grass silage (GS), red clover silage treated with Feedtech (CSFT) and/or with Kofasil (CSKO). The forages differed in the content of macrominerals (Ca 15.82 – 3.66 g/kg DM; Mg 3.68 – 1.46 g/kg DM; Na 0.20 – 3.02 g/kg DM; K 21.57 – 34.79 g/kg DM), and large differences ($P < 0.01$) were also in the element release in the rumen between experimental forages. The lowest DM effective degradability was determined for grass forages (49.5%–51.5%) and forages from legumes had higher degradability (62.9%–67.1%). The extent of disappearance of macroelements was also higher from LH1, CSFT, CSKO than from G and GS. The release of individual elements in all incubation times is expressed very well by cubic polynomials. Maximum disappeared portions of individual minerals from forages are as follows: Ca 86.3% and Mg 93.2% from CSFT, Na 98.7% from GN and K > 98% from all feeds. Potassium solubility is rapid and is not affected by the incubation time.

Keywords: forage; rumen; macroelement release; *in sacco* method

Solubilization and release of mineral elements from feedstuffs are important processes for their utilisation by animals (Khorasani and Armstrong, 1990). According to Playne et al. (1978), the elements not released from feedstuffs in nylon bags after 48 h incubation in the rumen are probably unavailable for absorption to the animal. The location of mineral elements in the forage structure may influence their release (Van Eys and Reid, 1987; Emanuele and Staples, 1990; Gralak et al., 1997; Islam et al., 2001). High proportions (> 60%) of K and Mg were released during short incubation in the rumen. Ca had the lowest disappearance. Flachowsky et al. (1994) reported that Ca release was dependent on the type of feedstuff and incubation time. Disappearance of elements from the bags in the rumen depends on mineral content and fibre content of incubated feeds (Flachowsky and Grün, 1992).

The objective of the present study was to compare the release of selected macrominerals from different types of forages in times of their incubation in the rumen by an *in sacco* method.

MATERIAL AND METHODS

In sacco method

To study the release of mineral macroelements we used the following forages: lucerne hay from the 1st cut (LH1) and 2nd cut (LH2), two hybrids of orchard grass (Rela hybrid GR and Niva hybrid GN), red clover silage treated with the chemical Kofasil (CSKO) and biological conserving agent Feedtech (CSFT) and grass silage (GS). A meadow sward composed of 80% grass (with a dominant portion of *Dactylis glomerata*, and *Festuca pratensis, Poa pratensis*, etc.), 15% herbs (*Taraxacum officinale*) and 5% clover (*Trifolium repens*) was used for ensiling.

An *in sacco* method was used to study the release of mineral elements from forages. Incubation of forages was done in the rumen of 3 young bulls (average live weight 350 kg) with large rumen cannulas (inner diameter 10 cm). The animals were fed a ration consisting of lucerne hay, maize silage, cereal meal and mineral and vitamin feed additive. Access to water was *ad libitum*.

The lyophilized forages (GR, GN, GS, CSKO, CSFT) and LH1, LH2 were ground to pass a 3-mm sieve and weighed (approx. 2.5 g dry matter) into bags made of Uhelon 130T (HEDVA, Moravská Třebová, Czech Republic) with the pore size 47 mm. We used three bags for each forage, incubation and animal. The bags with forages were incubated for 0, 6, 9, 16, 24, 48 and 72 hours. The 0-h variant was only washed without ruminal incubation. The same incubation times were used for the study of DM degradation. We followed the description of *in sacco* method by Harazim and Pavelek (1999).

Chemical analysis

Dry matter and crude protein content of forages was determined in accordance with the standard STN 46 9072, and cell wall content according to the procedure of Van Soest (Lutonská and Pichl, 1983). For the mineral analysis (STN 46 9072) samples of forages and residues were ashed at 550°C and the ash was dissolved in 10 ml of HCl (1:3). Na, K, Ca and Mg were determined with an AAS Solar 9 000 (Unicam, Cambridge, UK).

Mathematical and statistical processing

The data on mineral elements from experimental observations are expressed in g/kg DM for statistical evaluation. It is a difference from our previous paper (Čerešňáková et al., 2005) where they were given in % of disappearance against the original content).

The data on the observed losses of mineral elements were evaluated as follows:

– by the calculation of basic variation statistical characteristics of observations of the studied traits
– by the two-way analysis of variance with Tukey's test of nonadditivity of group (forage) × time of incubation

As all Tukey's tests of nonadditivity were significant or highly significant, the regressions of the macroelement release over time were analysed for each feed separately.

The statistical methods were realised according to Grofik and Fľak (1990) and SPSS for Windows, Release G, Copyright SPSS, Inc. 1989–1993 (licensed for RIPP, Piešťany).

RESULTS AND DISCUSSION

Solubilization and release of mineral elements from the structure of forage are very important preconditions of their utilization by animals. There are large differences between forages in the cell wall content and their individual fractions (cellulose, hemicellulose and lignin) that affect the degradability of NDF, DM (Gralak et al., 1996; Čerešňáková et al., 2000) and release of mineral elements (Ledoux and Martz, 1991; Flachowsky and Grun, 1992). In our previous study we found that the partial regression coefficients of mineral element release on NDF and time were positively highly significant 0.7247** in Mg and 1.9083** in Ca. (Čerešňáková et al., 2005).

Our results document different contents of NDF, crude fibre and crude protein in grasses and legu-

Table 1. Chemical composition of experimental forages (g/kg DM)

Items	Lucerne		*Dactylis glomerata*		Silages		
	cut		hybrid		grass	red clover	
	first	second	Rela	Niva		Feedtech*	Kofasil*
DM (g/kg)	218.60	307.60	171.10	172.80	212.80	299.10	314.20
N × 6.25	210.00	191.30	140.70	148.40	174.60	211.60	226.80
NDF	351.70	383.70	597.70	565.40	545.50	325.50	361.70
Calcium	15.82	10.40	3.66	3.78	4.76	15.29	15.54
Magnesium	3.30	1.72	1.46	1.54	1.90	3.61	3.68
Sodium	0.28	0.31	1.75	3.02	0.50	0.20	0.39
Potassium	34.79	21.57	31.38	29.98	23.18	28.98	23.18

*Conserving agent

Table 2. Disappearance of minerals after washing (0 h) and after 72 h incubation in the rumen (the percentage of original concentration)

Feeds		DM		Ca		Mg		Na		K	
		0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h
CSFT	$\bar{x}$	33.20	79.20	37.70	84.70	73.30	93.20	32.20	71.50	95.70	98.70
	SD	0.48	0.59	0.21	0.81	0.09	0.36	0.23	1.50	0.01	0.40
CSKO	$\bar{x}$	38.10	81.50	41.90	86.30	73.20	94.70	51.90	89.60	98.20	99.10
	SD	1.02	2.10	0.57	0.80	0.26	0.31	0.61	0.57	0.14	0.67
LH1	$\bar{x}$	42.20	76.40	53.60	69.70	78.00	89.70	50.80	72.00	99.80	99.80
	SD	0.95	4.54	0.23	1.76	0.11	0.60	0.25	2.06	0.001	0.10
LH2	$\bar{x}$	37.60	73.00	33.40	64.60	56.70	88.20	60.80	81.30	99.60	99.80
	SD	1.58	0.82	0.16	1.02	0.10	0.34	0.09	1.40	1.84	0.01
GS	$\bar{x}$	21.20	75.60	34.10	72.20	77.20	91.80	21.20	78.10	98.20	99.70
	SD	2.05	1.94	0.89	2.12	0.31	0.64	3.37	1.67	0.02	0.02
GN	$\bar{x}$	16.40	79.00	1.70	69.00	30.10	90.20	72.60	98.60	98.30	99.80
	SD	0.55	0.28	0.17	4.73	1.05	1.53	0.19	0.22	0.03	0.20
GR	$\bar{x}$	19.70	76.00	2.96	66.90	31.50	88.30	71.00	97.90	98.30	99.80
	SD	1.07	2.11	0.35	5.34	0.94	1.89	0.76	0.33	0.06	0.27

mes (Table 1). The plant species showed differences mainly in Ca, Mg and Na concentrations. Mainly the concentration of Ca was much higher in lucerne hay and red clover silages than in GR, GN and GS. Similar results were reported by other authors (Emanuele and Staples, 1990; Flachowsky and Grün, 1992; Gralak et al., 1997). The differences in Na concentrations were found between grass hybrids growing in similar conditions. Legumes were more than 4 times higher in Ca than were grasses. Sodium concentration was higher in GR and GN than in LH1 and LH2.

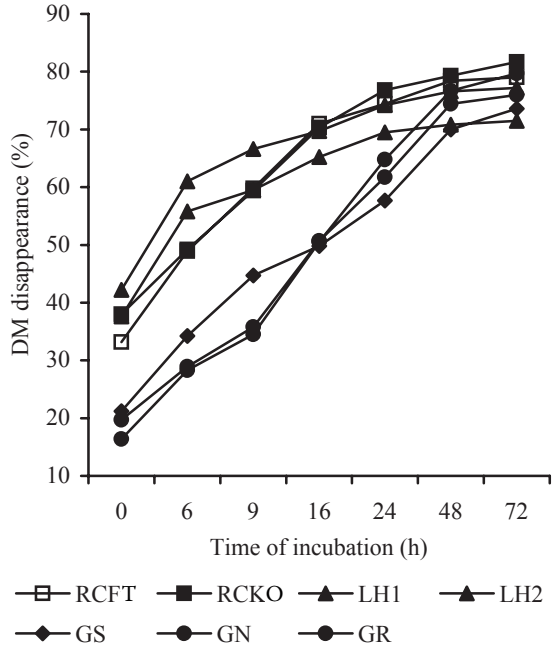

Figure 1. Disappearance of dry matter during the incubation of forages in the rumen by an *in sacco* method

The disappearance of dry matter from individual feeds during the washing process (0 h incubation) was different and was lower from the feeds with a higher content of NDF – grass and grass silage compared to legumes. There were large differences between feeds and elements in the extent of solubilization after washing. The same tendency as in DM disappearance was observed for Ca, Mg, and Na. The lowest solubility was observed for Ca in grasses Rela and Niva. K was the most soluble (>95%). These results are very close to the published results (Van Eys and Reid, 1986; Emanuele and Staples, 1990; Třináctý et al., 2000). It means that K is released rapidly (Table 2).

The disappearance of DM and macrominerals was very different in the particular feeds after 72 h incubation in the rumen. Dry matter disappearance of feeds was from 73% to 79%, the differences were only 2–9% units. The incubation time influenced the rumen dry matter degradability (Figure 1). There were small differences between the feeds in potentially degraded fractions ($a + b$) of DM, larger differences were in the rate of DM degradability (from 0.133/h for RCFT to 0.0458/h for GS) (Table 3).

Similar differences between feeds were also determined for Mg and K (Table 2). The range of Ca

Table 3. Parameters of dry matter disappearance during the incubation of forages in the rumen of young bulls

Feeds	*a* (%)	*b* (%)	*a* + *b* (%)	*c* (%/h)	Edg (%)
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
CSFT	13.5 ± 0.379	64.8 ± 0.205	78.3 ± 0.415	0.133 ± 0.0019	63.4 ± 0.368
CSKO	18.8 ± 0.177	61.8 ± 0.160	80.6 ± 0.072	0.118 ± 0.0045	64.9 ± 0.476
LH1	51.3 ± 0.593	26.1 ± 0.194	77.4 ± 0.774	0.0876 ± 0.0005	67.1 ± 0.448
LH2	42.5 ± 0.343	28.6 ± 0.573	71.1 ± 0.412	0.0996 ± 0.0032	62.9 ± 0.349
GS	24.1± 0.265	51.3± 0.349	75.4 ± 0.492	0.0458 ± 0.0008	51.5 ± 0.212
GN	3.1 ± 0.103	77.9 ± 0.137	81.1 ± 0.068	0.0613 ± 0.0006	50.3 ± 0.218
GR	6.1 ± 0.098	71.3 ± 0.236	77.4 ± 0.323	0.0622 ± 0.0007	49.5 ± 0.415

and Na disappearance was much wider. It was from 64.6% to 86.3% for C and from 71.5% to 98.6% for Na. In all feeds more elements were released during 72 h incubation than by washing. The content of K dropped the most in comparison with its original value during 72 h incubation and its disappearance was the highest in all tested feeds (>98.7%). The results in Table 4 show that the content of Mg, K, and partially of Na dropped after washing in experimental forages. An exception was calcium, the content of which relatively increased in CSFT, CSKO, LH1 and LH2. This indicates that these elements are located in different parts of the plant and have different functions. Potassium is located in the cell contents or part of water-soluble plant components and a large portion of slowly released Ca is bound to the cell walls of plants and to bacterial cell walls (Emanuele and Staples, 1990; Flachowsky et al., 1994).

Table 4. Macromineral concentrations in residues of experimental forages before and after washing (g/kg DM)

Feeds	Ca		Mg		Na		K	
	b.w.	a.w.	b.w.	a.w.	b.w.	a.w.	b.w.	a.w.
CSFT	15.29	15.45	3.61	1.54	0.20	0.222	28.98	0.202
CSKO	15.54	15.77	3.68	1.74	0.39	0.331	23.18	0.285
LH1	15.82	13.38	3.30	1.24	0.28	0.206	34.79	0.147
LH2	10.40	11.08	1.72	1.16	0.31	0.194	21.57	0.144
GS	3.66	3.34	1.46	0.559	1.75	0.494	31.38	0.529
GN	3.78	3.14	1.54	0.764	3.02	1.852	29.98	0.204
GR	4.76	2.98	1.91	0.678	0.50	1.105	23.18	0.198

b.w. = before washing, a.w. = after washing

Table 5. Two-way analysis of variance of selected elements with the test of nonadditivity of group (forage) × time of incubation

Element		Groups (A)	Time (B)	Error (e)	*N*	*R*
		f_a = 6	f_b = 6	f_e = 36	f_N = 1	f_r = 35
Ca	MS	133.85**	10.187	4.9930	30.11	4.27000
	F	26.81**	2.04		7.41*	
Mg	MS	0.5021	0.1244	0.0437	0.2071	0.03900
	F	11.50**	2.85*		5.31*	
Na	MS	0.1057	0.1372	0.0566	1.276	0.02170
	F	1.87	2.43		58.76**	
K	MS	0.0394	0.0147	0.0437	0.3439	0.00647
	F	2.49*	0.93		53.16**	

$F_{0.05}$ (6, 36) = 2.364; $F_{0.01}$ (6, 36) = 3.351; $F_{0.05}$ (1, 35) = 4.120; $F_{0.01}$ (1, 35) = 7.42

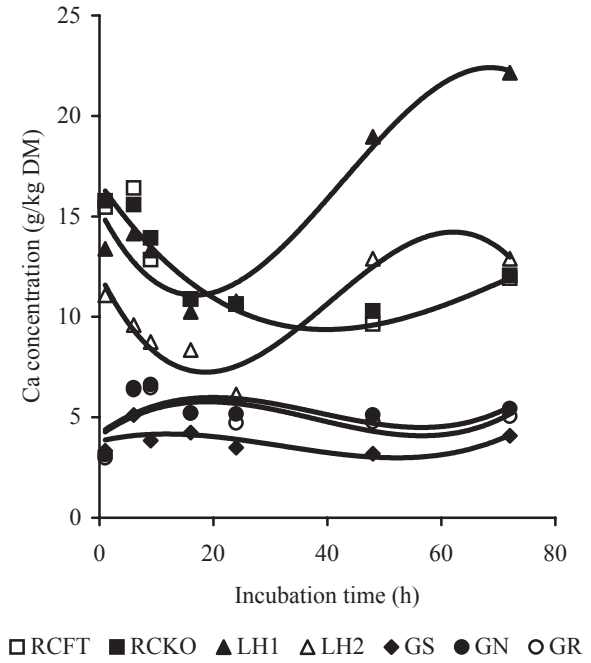

Figure 2. The changes in calcium concentration during the incubation of forages in the rumen (see Table 6)

The effect of the type of forage on the release of individual macroelements is highly significant ($P < 0.01$) during incubation in the rumen, with the exception of Na (Table 5). The time of incubation affected significantly also the release of Mg ($P < 0.05$). In all macroelements significant or highly significant tests of nonadditivity of forage × time of incubation were observed.

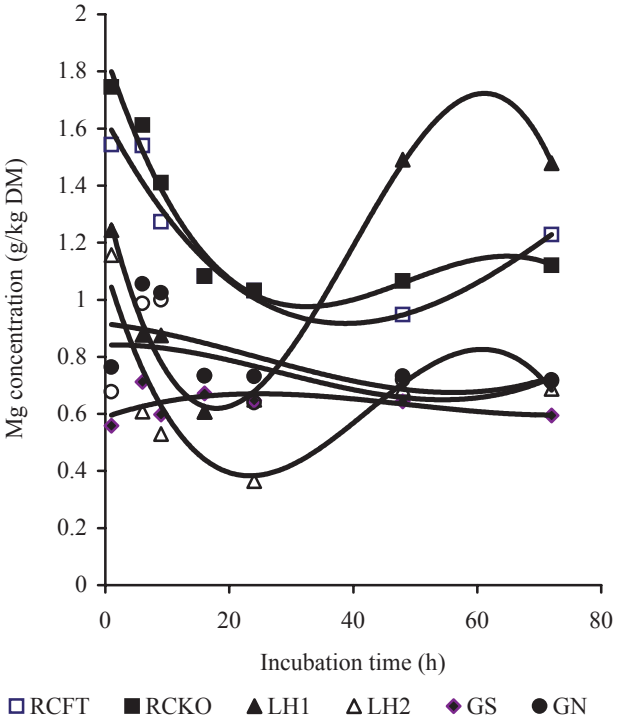

Figure 3. The changes in magnesium concentration during the incubation of forages in the rumen (see Table 6)

The course of Ca release in the times of incubation (Figure 2) is different between legumes and grass forages. The course is expressed by cubic polynomials with high regression coefficients (R^2 = 0.903 – 0.930) for legumes, CSKO, LH1 and LH2 (Table 6). The cation exchange capacity of the cell walls has a significant effect especially on Ca release (binding) and on the availability of Ca. Similar results were also reported for other feeds (Flachowsky and Grun, 1992; Correa, 2006). After the shortest incubation (already 6 h) of bags in the rumen the concentration of Ca increased in all feeds. Ca concentration in the residues of grass samples was nearly constant after the next incubations. On the contrary in the legumes, Ca concentration dropped at first and after longer incubation times (>24 h) it increased mainly in LH1 and LH2. Microorganisms have similar effects like the plant fibre that can readily bind the cations of the elements. This increase in Ca concentration in LH1 and LH2 can be explained by the adhesion of microorganisms to the undegraded cell walls of residues in bags.

The changes in Mg concentration during incubation times in individual feeds are represented in Figure 3. The curves are very different for each group of feeds. They are expressed by cubic polynomials and the regression coefficients for legumes are significant (R^2 = 0.926 – 0.985). Longer rumen incubation (>24 h) increased Mg concentration in the residues. Emanule and Staples (1990) concluded that the incubation time of 12–30 h is required for the maximum Mg release from lucerne and a longer time (24–36 h) from grasses. Our results are similar. The increase in Mg concentration (like in Ca) in undegraded residues could be caused by the binding of rumen microbes to the cell walls of forages. Mainly gram-negative bacterial walls contain Mg in their outer membrane (Mackie and Therion, 1984).

The expression of Na release by the cubic polynomials for all tested forages is shown in Table 6 and the curves of concentration changes in time show the differences between them (Figure 4). The concentration of Na in the residues fluctuated in the times of incubation. Sodium cations from the rumen content and attached microbes could in-

Table 6. Parameter estimation of cubic polynomials $y = b_0 + b_1t + b_2t^2 + b_3t^3$ of element release from selected feeds in the times of ruminal incubation and inflection points (t_i, y_i)

Forage	b_0	b_1	b_2	b_3	R^2	t_i	y_i
Ca							
CSFT	16.6816	–0.4157	0.0070	–3E-05	0.852	77.78	12.58
CSKO	16.9005	–0.4450	0.0086	–5E005	0.922*	57.33	10.23
LH1	15.3549	–0.5568	0.0207	–0.0002	0.930*	34.50	12.57
LH2	12.1543	–0.5862	0.0205	–0.0002	0.903*	34.17	8.07
GS	3.8092	0.0659	–0.0034	3.6E-05	0.373	31.48	3.64
GN	4.1848	0.2050	–0.0070	6.1E-05	0.269	38.25	5.20
GR	4.0793	0.2018	–0.0071	6.3E-05	0.260	37.57	4.98
Mg							
CSFT	1.6352	–0.0412	0.0007	–3E-06	0.926*	77.78	1.25
CSKO	1.8639	–0.0657	0.0015	–1E-05	0.966**	50.00	1.08
LH1	1.3461	–0.0898	0.0032	–3E-05	0.985**	35.56	0.85
LH2	1.1137	–0.0718	0.0021	–2E-05	0.797	35.00	0.32
GS	0.5888	0.0074	0.0002	1.4E-06	0.311	–47.62	0.54
GN	0.9158	–0.0025	–0.0001	1.9E-06	0.344	17.54	0.85
GR	0.8413	0.0007	–0.0002	2.6E-06	0.217	25.64	0.77
Na							
CSFT	0.2667	–0.0073	0.0003	–3E-06	0.493	33.33	0.25
CSKO	0.3118	–0.0129	0.0003	–2E-06	0.740	50.00	0.17
LH1	0.2488	–0.0068	0.0003	–3E-06	0.656	33.33	0.24
LH2	0.1955	–0.0053	0.0002	–2E-06	0.786	33.33	0.17
GS	0.4605	–0.0015	–7E-05	1.2E-06	0.663	19.44	0.41
GN	1.5592	–0.1383	0.0037	–3E-05	0.696	41.11	0.04
GR	0.9983	–0.0737	0.0019	–1E-05	0.812	63.33	1.41
K							
CSFT	0.1906	0.0009	–5E-05	5.9E-07	0.598	28.25	0.19
CSKO	0.2686	0.0071	0.0002	–1E-06	0.671	66.67	1.33
LH1	0.1539	0.0028	2.7E-05	–7E-07	0.947*	12.88	0.19
LH2	0.1265	0.0034	–7E-05	4.3E-07	0.689	54.26	0.178
GS	0.4477	0.0325	0.0009	7E-06	0.587	–42.86	0.16
GN	0.3372	0.0204	–0.0009	8.3E-06	0.500	36.14	0.29
GR	0.3264	0.0258	–0.0011	1.0E-05	0.503	36,67	0.29

t_i = time, y_i = concentration of element in g/kg DM
$R^2_{0.05}$ (3, 3) = 0.902, $R^2_{0.01}$(3, 3) = 0.966; *P < 0.05, **P < 0.01

crease the Na concentration of residues of LH1, LH2 and CSFT.

The concentration of the most soluble potassium markedly dropped in the residues of CSFT, CSKO and GS till the 16th hour of incubation and then it increased. The course of the curves (Figure 5) of K release from the other forages is very different, but all are expressed by cubic polynomials (Table 6) with significant regression coefficients R^2 = 0.947 for LH1. The potassium concentration did not reach the concentration in the original feeds (Flachowsky et al., 1994; Eys and Reid, 1987; Emanuele et al., 1991).

From the results it can be concluded that the elements which are released by washing and during the first time of incubation in the rumen represent those

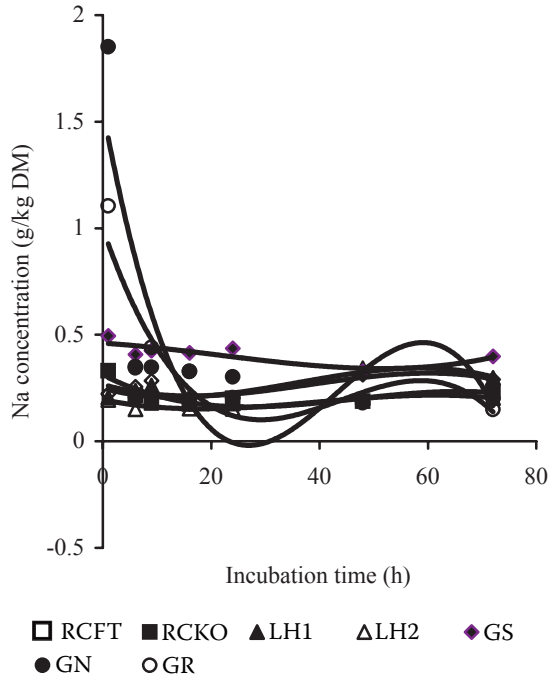

Figure 4. The changes in sodium concentration during the incubation of forages in the rumen (see Table 6)

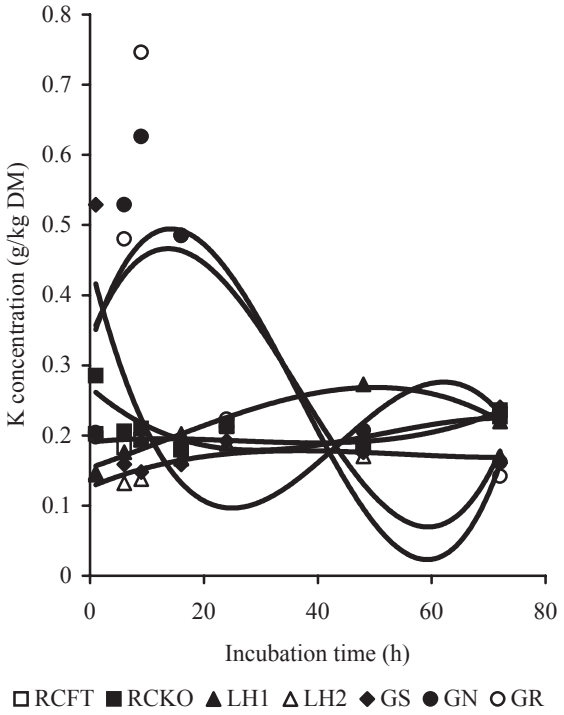

Figure 5. The changes in potassium concentration during the incubation of forages in the rumen (see Table 6)

potentially available to rumen microorganisms and absorption from the rumen. The in sacco method can be used for the determination of differences between feeds and element release in the ruminal environment. But it is not suitable for making deductions about mineral availability to ruminants.

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