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RUMINAL PHOSPHORUS RELEASE FROM SELECTED FORAGES DETERMINED BY THE I*N SACCO* TECHNIQUE

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ABSTRACT

This study was focused on measuring the NDF degradability and phosphorus release from forages – lucerne hay from the 1st and 2nd cut (LH_1 and LH_2), orchard grass hybrid Rela (GR) and hybrid Niva (GN), grass silage (GS), red clover silage treated with Feed Tech (RCS_{FT}) or with Kofasil (RCS_{KO}). The forages were different in NDF content (from 325.50 to 597.70 $g.kg^{-1}$DM) and in NDF disappearance ($P \leq 0.01$). The effective NDF degradability ranged from 26% to 39.40% at an outflow rate of $0.06 \cdot h^{-1}$. There were also differences ($P \leq 0.01$) in phosphorus disappearance in the rumen. The release of phosphorus through incubation time has been adequatelly expressed by cubic polynomials regression. A greater percentage of phosphorus was solubilized by washing from GS (92.6 %) followed by RCS_{FT} (84.8 %) and RCS_{KO} (76.5 %). With incubation time the concentration of phosphorus in tested legumes increased in comparison with its concentration after washing (in LH_1 from 1.10 to 4.39 $g.kg^{-1}$ DM, in RCS_{FT} from 0.4 to 2.32 $g.kg^{-1}$ DM for 72 h incubation). This study shows that the *in sacco* method is not an objective one for determination of the kinetics of phosphorus release in the rumen from forages due to re–binding of phosphorus with the nylon-bag residues from the rumen environment.

Key words: forage, rumen, phosphorus disappearance, *in sacco* method

INTRODUCTION

With respect to the necessity of environmental protection, animal nutrition has to adapt to new rules, particularly those concerning the control of animal waste production, not only on nitrogen but also on phosphorus. Ruminants produce 60–70% of phosphorus waste of animal origin (Tamminga, 1992, Bravo and Meschy, 2003). To decrease this pollution, we have to know more about phosphorus utilisation in the rumen, requirements of rumen microbes from animals, and phosphorus availability to rumen microbes (Garton, 1951; Witt and Owens, 1983; Emanuele et al., 1991; Bravo et al., 2000; Kume et al., 2001; Bravo et al., 2003; Meschy and Ramirez-Perez, 2005). Kinetics of phosphorus release in the rumen varies depending on feedstuffs (Emanuele and Staples, 1990; Flachowsky et al., 1994; Bravo and Meschy, 2003; Correa, 2006), and its measurement by the nylon bag technique is an interesting way to describe the phosphorus quality of feedstuffs (Bravo et al., 2000).

The aim of this work was to determine the kinetics of phosphorus release in the rumen from forages with different NDF content and NDF degradability.

MATERIAL AND METHODS

In sacco method

To study the release of mineral macroelements the following forages were used: lucerne (alfalfa) hay from the 1st cut (LH_1) and 2nd cut (LH_2), two hybrids of orchard grass (hybrid Rela (GR) and hybrid Niva (GN), red

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clover silage treated with conserving agents: chemical - Kofasil (RCS_{KO}) and biological - Feed Tech (RCS_{FT}), and grass silage (GS). Meadow sward used for ensiling was composed of 80% grass (with the dominant part *Dactylis glomerata*, and *Festuca pratensis*, *Poa pratensis* etc.), 15% herbs (*Taraxacum officinale*) and 5% clover (*Trifolium repens*).

For the *in sacco* method 3 young bulls (average live weight 350 kg) with large rumen cannula (inner diameter 10 cm) were used. The animals were fed a ration consisting of lucerne hay, maize silage, cereal meal, and mineral and vitamin feed additive. Water was provided *ad libitum*.

The lyophilized forages were ground to pass a 3 mm sieve and were weighed (approx. 2.50 g dry matter) into bags made of Uhelon 130T (HEDVA, Moravska Třebova, Czech Republic) with pore size 47 μm. Three separate bags each for forage, incubation time and animal were used. The bags with forages were incubated for 0, 6, 9, 16, 24, 48 and 72 hours. The 0 h time bags were only washed in water and were not incubated in the rumen. The same incubation times were used for the study of NDF degradability too. The *in sacco* method after Harazim et al. (1999) was followed.

Chemical analyses

Dry matter and crude protein content were determined by STN 46 9072, and cell wall content according to the procedure of van Soest (Lutonská a Pichl, 1983). For mineral analysis (STN 46 9072) samples of forages and *in sacco* residues were ashed at 550˚C and the ash was dissolved in 10 ml of HCl (1:3). The content of phosphorus before and after incubations was determined colorimetrically using the vanadium-molybdate solution.

Mathematical-statistical processing

Obtained data of observed disappearance of phosphorus were evaluated as follows:

- by calculation of basic statistical variation characteristics of observations of the following traits,
- by two way analysis of variance with Tukey´s test of non-additivity of groups (forage) x time of incubation.

All Tukey´s tests of non-additivity for significance and the regressions of phosphorus release over time were analysed for each feed independently.

Statistical methods were realised after Grofik and Fľak (1990) and with SPSS for Windows, Release G, Copyright © SPSS, Inc. 1989-1993 (licensed for Research Institute for Plant Production, Piešťany).

RESULTS AND DISCUSSION

Available phosphorus is the proportion of dietary phosphorus that is solubilised during digestion of feeds. Solubility and release of phosphorus from the structure of forage is a very important prerequisite for its utilization in animals (Sauvant et al., 1999). Forages are characterized by different cell wall content and NDF degradability. Release of some elements is closely related to the NDF degradation in the rumen of cattle. Bravo et al. (2000) found a significant association between solubility of phosphorus (rapidly degraded fraction) and crude fibre content in cereals, cereal by-products and meals. However, no significant correlation was seen between the NDF degradability and phosphorus release (r = 0.266) during incubation of forages in the rumen.

Our results revealed that NDF content in grasses is much higher than in legumes and that all tested forages are low in phosphorus content (Table 1).

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

Item	Lucerne cut		*Dactylis glomerata* Hybrid		Silage		
					Grass	Red clover	
	first	second	Rela	Niva		Feed Tech*	Kofasil*
DM (g/kg)	218.6	307.6	171.1	172.8	212.8	299.1	314.2
N x 6.25	210.0	191.3	140.7	148.4	174.6	211.6	226.8
NDF	351.7	383.7	597.7	565.4	545.5	325.5	361.7
Phosphorus	2.58	1.79	2.68	2.46	3.11	2.64	2.58

*Conserving agent

Disappearance of NDF during incubation from 0 h to 72 h (Table 2) in the rumen showed differences among forages ($P \leq 0.01$). The NDF of legumes was more degraded at shorter times (6 h and 9 h), and NDF of the GN and GR was more degraded at 24 h to 72 h. The significant differences between forages ($P \leq 0.01$) were also stated in the phosphorus release (Table 3).

In spite of the lower NDF content in lucerne (LH_1 and LH_2) than in grass samples, the solubility of phosphorus during the washing process (0 h incubation) was high and comparable in all forage samples (Table 4). The concentration of phosphorus was much higher in the lucerne (LH_1, LH_2) after 72 h of incubation than in non-incubated samples (Table 4). Phosphorus concentration changed very significantly ($P \leq 0.01$) with the time of incubation (Table 3) in feed residues and fluctuated with time alternately between local maximum and local minimum (Fig. 1). The changes of phosphorus concentrations in the forages on incubation time were expressed by cubic polynomials, which are significant or

Table 2: NDF disappearance during incubation of forages in the rumen and NDF effective degradability (%) at outflow rates 0.04·h^{-1} vs. 0.06·h^{-1}

Forage	Incubation time (h)							$Edg_{0.04}$	$Edg_{0.06}$
	0 h	6 h	9 h	16 h	24 h	48 h	72 h		
LH_1	17.3	28.7	33.5	39.6	44.3	52.6	54.7	36.9	33.3
LH_2	9.2	19.3	22.9	33.1	41.2	51.5	53.9	33.6	28.6
RCS_{FT}	4.8	22.6	30.2	41.7	49.5	59.0	62.8	39.7	34.0
RCS_{KO}	19.9	20.7	33.1	49.2	56.7	58.7	64.8	44.7	39.4
GN	-0.5	11.2	18.1	40.1	53.2	69.6	72.0	38.4	29.8
GR	1.9	12.4	21.3	38.7	50.0	66.2	69.3	37.7	29.8
GS	-0.7	11.5	22.7	28.6	40.8	59.8	63.9	33.1	26.0

Table 3: Two-way analysis of variance of phosphorus and NDF with the test of non-additivity of group (forage) x time of incubation

Item		Group (A)	Time (B)	Error (e)	N	R
		$f_a = 6$	$f_b = 6$	$f_e = 36$	$f_N = 1$	$f_r = 35$
NDF	MS	10846.5	20595.3	1362.6	6630. 7	1212.1
	F	7.96**	15.11**		5.47*	
Phosphorus	MS	2.274	2.1167	0.2711	3.278	0.1852
	F	8.38**	7.81**		17.70**	

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

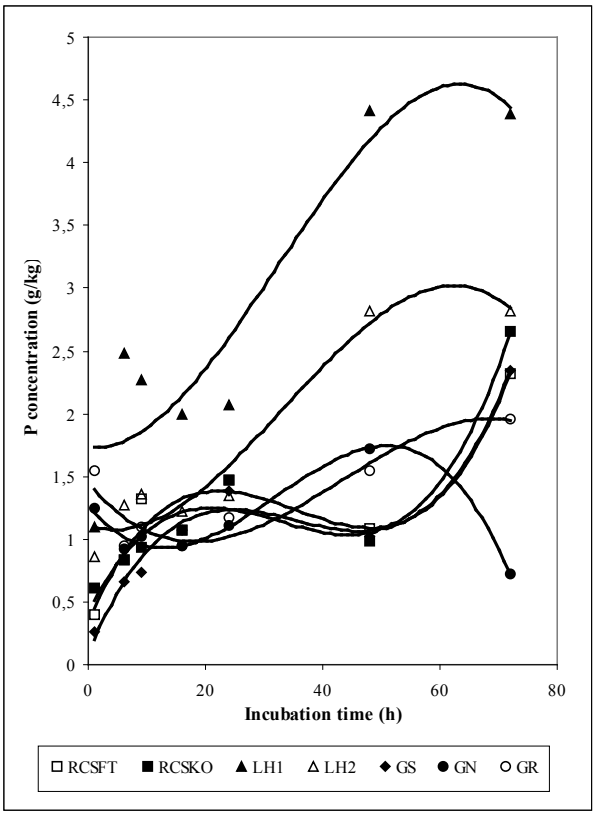
Table 4: Influence of incubation time in the rumen on phosphorus concentration (g·kg^{-1} DM) in undegraded forage residues

Forage	before washing	after washing	Incubation time (h)					
			6h	9h	16h	24h	48h	72h
LH_1	2.283	1.099	2.486	2.275	1.995	2.072	4.416	4.394
LH_2	1.799	0.856	1.278	1.361	1.224	1.348	2.821	2.821
RCS_{FT}	2.637	0.400	0.835	1.317	1.075	1.467	1.089	2.320
RCS_{KO}	2.579	0.606	0.830	0.933	1.075	1.467	0.985	2.658
GN	2.456	1.252	0.918	1.018	1.047	1.106	1.723	0.719
GR	2.677	1.552	0.942	1.093	0.947	1.177	1.544	1.957
GS	3.615	0.267	0.656	0.731	1.074	1.379	1.021	2.349

Table 5: Parameter estimation of cubic polynomials of phosphorus concentration as a function of incubation time (h) ($y = b_0 + b_1t + b_2t^2 + b_3t^3$)

Feed	b_0	b_1	b_2	b_3	R^2
	phosphorus				
RCS_{FT}	0.3384	0.1107	-0.0036	3.4E-05	0.937*
RCS_{KO}	0.4205	0.0936	-0.0033	3.4E-05	0.966**
LH_1	1.7377	-0.0063	0.0024	-2E-05	0.848
LH_2	1.122	-0.0169	0.0020	-2E-05	0.940*
GN	1.2705	-0.0577	0.0028	-3E-05	0.975**
GR	1.4491	-0.0567	0.0020	-2E-05	0.872
GS	0.0934	0.1128	-0.0035	3.3E-05	0.938*

$R^2_{0.05}(3, 3) = 0.902$, $R^2_{0.01}(3, 3) = 0.966$ *P ≤ 0.05, ** P ≤ 0.01



(Legend: P – phosphorus, RCSFT - clover silage treated with Feed Tech, RCSKO - clover silage treated with Kofasil, LH1 – lucerne hay 1st cut, LH2 lucerne hay 2nd cut, GS – grass silage, GN – grass Niva, GR – grass Rela)

Fig. 1: Changes of phosphorus concentration during incubation of forage residues in the rumen with incubation time (see Table 5)

highly significant ($P \leq 0.01$) except for the LH_1 and GR (Table 5). Due to this fact the phosphorus degradability curve was not calculated after Ǩrskov (1979).

Although rumen is regarded as the major site for phosphorus release from forages (Emanuele et al., 1991), the concentration of phosphorus increased during prolonged incubation of forages in the rumen (Flachowsky et al., 1994; Eys and Reid, 1987; Emanuele et al., 1991; Bravo et al., 2000). This phenomenon has been explained by many investigators (Emanuel and Staples, 1990; Flachowsky and Grün, 1992; Bravo et al., 2000) by the adhesion of microbes (that are rich in phosphorus content) on the cell walls of undegraded feed residues. This can explain higher concentration of phosphorus in the residues of LH_1, LH_2, RCS_{FT} and RCS_{KO} than other forages. NDF disappearance from legumes was during the longer incubation period, which is slower than from grasses (Table 2). Usually, the microbes pass through the bags and can not be removed by washing (Varvikko and Lindberg, 1985). Similar problems were observed during studies on nitrogen degradation in forages with higher cell wall content (Zander et al., 1989; Gralak et al., 1990). Microbial contamination of residues in bags after 48h of ruminal incubation can be as high as 26.75% of residue dry matter (Gralak et al., 1990). Even if the rumen is considered to be the major site for phosphorus release from forages, the major site of phosphorus absorption is the small intestine. Hence for the phosphorus availability it is also important to what extent phosphorus is released in the abomasum and intestinal sites (Pfeffer et al. 1970).

Bravo et al. (2000) warned of phosphorus contamination that becomes quantitatively important for feedstuffs low in phosphorus content such as forages and with high microbial phosphorus content such as cellulolytic bacteria. In this study, no significant correlation was observed between the NDF degradability and phosphorus release from forages. This work shows

that the *in sacco* method is not an objective one for determination of the kinetics of phosphorus release in the rumen from forages, due to re-binding of phosphorus with the bag residues from the rumen environment.

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