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Effect of dietary mineral phosphorus and phytate on in situ ruminal phytate disappearance from different concentrates in dairy cows

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ABSTRACT

The first objective of this study was to determine the influence of dietary composition on the in situ disappearance of phytate ($InsP_6$) from wheat, corn, soybean meal, and rapeseed meal [solvent-extracted, without (RSM) or with (hRSM) heat treatment] in the rumen of dairy cows. The second objective was to assess the primary degradation products of $InsP_6$ in the rumen. Three diets differing in phosphorus and $InsP_6$ concentration (basal diet = 0.38% P in dry matter; high-P diet = 0.56% P; high-$InsP_6$ diet = 0.39% P) were fed to 3 ruminally fistulated lactating Jersey cows in a 3 × 3 Latin square. Ground concentrates (sieve size = 2 mm) were incubated in polyester bags in the rumen for 2, 4, 8, 16, and 24 h. The bag residues were analyzed for P, $InsP_6$, isomers of lower inositol phosphates ($InsP_5$, $InsP_4$, $InsP_3$), and crude protein. The $InsP_6$ disappeared more rapidly from cereal grains than from oilseed meals; however, after 24 h of incubation ≥95% $InsP_6$ had disappeared from all concentrates except hRSM (57%; diet average). Feeding the high-$InsP_6$ diet increased $InsP_6$ disappearance for oilseed meals, but not for corn and wheat. The predominant $InsP_5$ isomer in all bag residues was Ins(1,2,4,5,6)P_5 followed by Ins(1,2,3,4,5)P_5 and Ins(1,2,3,4,6)P_5. A further $InsP_5$ isomer [Ins(1,3,4,5,6)P_5] was detected in both rapeseed meal bag residues. Feeding the high-$InsP_6$ diet led to lower concentrations of Ins(1,2,4,5,6)P_5 and Ins(1,2,3,4,5)P_5, whereas an interaction between diet, concentrate, and time occurred for Ins(1,2,3,4,6)P_5 and Ins(1,3,4,5,6)P_5. The results confirm the high potential of rumen microorganisms to hydrolyze $InsP_6$; however, increasing the amount of $InsP_6$ in the diet can further enhance $InsP_6$ hydrolysis, which may be relevant when concentrates with slowly degradable $InsP_6$, such as RSM or heat-treated concentrates, are fed to dairy cows. Based on the concentrations of $InsP_5$ isomers, 3 and 6 phytases appear to play a major role in the rumen. Conversely, intrinsic plant phytase activity appears to be less relevant as the percentage of its primary hydrolysis product, Ins(1,2,3,4,5)P_5, changed only slightly upon using wheat known for high intrinsic phytase activity instead of the other concentrates. Additional information regarding the factors influencing the extent of ruminal $InsP_6$ disappearance will require further studies to determine the phytase activity of rumen microorganisms and the characteristics of their respective phytases.
Key words: phytate, rumen, degradation, inositol pentakisphosphates

INTRODUCTION

Phytate, defined as any salt of phytic acid [*myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); **$InsP_6$**], is the major storage form of P in plant seeds. The availability of P bound in $InsP_6$ depends on $InsP_6$ hydrolytic cleavage, which is catalyzed by phytases. Ruminants effectively hydrolyze $InsP_6$ owing to the phytase activity of ruminal microorganisms (Raun et al., 1956; Yanke et al., 1998). Digestibility studies with dairy cows measuring fecal $InsP_6$ excretion confirm high $InsP_6$ hydrolysis ranging from 93 to 99% (Clark et al., 1986; Morse et al., 1992; Ray et al., 2013); however, several factors influence the extent of $InsP_6$ hydrolysis. Differences in $InsP_6$ hydrolysis between feedstuffs (Konishi et al., 1999; Park et al., 1999; Blaabjerg et al., 2010; Brask-Pedersen et al., 2011; Haese et al., 2016) may be due to different localization sites and storage forms in the seeds (Haese et al., 2016). Feedstuff processing methods, such as heat (Konishi et al., 1999; Blaabjerg et al., 2007) or formaldehyde treatment (Park et al., 1999; Martín-Tereso et al., 2009), further affect $InsP_6$ hydrolysis, as can diet composition. Increased dietary $InsP_6$ concentration led to higher ruminal (Ray et al., 2013) and total-tract (Haese et al., 2014) $InsP_6$ disappearance, whereas mineral P addition decreased total-tract $InsP_6$ disappearance in vivo (Haese et al., 2014). Similar effects occurred in vitro upon inorganic

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P addition to the buffer in the system (Godoy and Meschy, 2001; Haese et al., 2016). However, it is difficult to unambiguously determine factors influencing $InsP_6$ hydrolysis through multistudy comparison, as studies may differ in several associated characteristics, such as DMI, diet ingredients, or sampling or analytical methods. Furthermore, factors observed in vitro might not necessarily apply in vivo.

Phosphorus is an essential mineral for health maintenance, milk production, and reproduction, although it may contribute to environmental pollution when excreted. To ensure an optimal P supply while avoiding unnecessary excretion, the ruminal availability of $InsP_6$ from different feedstuffs and the factors influencing its hydrolysis are of highest interest.

Therefore, we first aimed to determine the effects of different inorganic P or $InsP_6$ concentrations in the diet of dairy cows on the in situ disappearance of $InsP_6$. For incubation, we used 5 concentrates commonly used in ruminant nutrition: wheat, corn, soybean meal (**SBM**), and 2 rapeseed meals (**RSM**) because of their differing $InsP_6$ concentrations, localizations, and storage forms. We hypothesized that these differences would cause the diet composition to differentially influence $InsP_6$ disappearance from the incubated concentrates. Detailed information on this process may be useful to develop specific recommendations for formulating diets to optimize ruminal $InsP_6$ hydrolysis. Because $InsP_6$ accumulates together with storage proteins in protein storage vacuoles (**PSV**; Gillespie et al., 2005) and can bind proteins in several feedstuffs (Selle et al., 2012), we also analyzed CP concentrations of the bag residues. As strong interactions between protein and $InsP_6$ occur in soybean (Tombs, 1967; O'Dell and de Boland, 1976) and rapeseed proteins (Gillberg and Törnell, 1976), we examined whether ruminal CP disappearance was associated with $InsP_6$ disappearance from concentrates.

Our second aim was to learn about the phytases involved in ruminal $InsP_6$ degradation by determining the main degradation products. Phytases are divided into 3- (E.C. 3.1.3.8), 6- (E.C. 3.1.3.26), and 5-phytases (E.C. 3.1.3.72; Greiner and Konietzny, 2006), referring to the position of the carbon in the *myo*-inositol ring of $InsP_6$ at which dephosphorylation is initiated. Thus, the spectrum of $InsP_5$ isomers in the bag residues provides information about active phytases in the rumen. We analyzed these spectra at all incubation times to determine possible differences in the involved phytases between concentrates and diets.

Table 1. Ingredients and chemical composition of the experimental diets fed to fistulated Jersey cows

Item	Diet[1] Basal	Pi	InsP
Ingredient (%, on DM basis, unless noted)			
Corn silage	24.2	23.9	23.4
Grass silage	32.2	31.9	31.2
Meadow hay	16.1	16.0	15.6
Corn gluten	6.4	6.4	—
Corn starch	4.0	4.0	1.6
Corn grain	—	—	11.7
Dried sugar beet pulp	16.1	16.0	—
Rapeseed meal, solvent extracted	—	—	15.6
Monosodium phosphate	—	0.6	—
Monocalcium phosphate	—	0.8	—
NaCl	0.4	0.1	0.4
$CaCO_3$	0.4	—	0.5
Urea	0.2	0.3	—
DM (g/kg)	394	398	397
Chemical composition (g/kg of DM)			
OM	926	921	928
CP	113	118	120
Total P	3.82	5.59	3.94
$InsP_6$	0.44	0.48	3.45
$InsP_6$-P[2]	0.13	0.13	0.97

[1]Basal = P content of 3.82 g/kg of DM; Pi = inorganic P diet with monosodium and monocalcium phosphate (5.59 g/kg of DM); InsP = exclusively organic P diet (3.94 g of P/kg of DM), with rapeseed meal as the main source of phytate ($InsP_6$).

[2]Phosphorus bound in $InsP_6$.

MATERIALS AND METHODS

Animals and Diets

We used 3 mid-lactation rumen-fistulated Jersey cows for this study with an average DMI of 15 kg/d. The cows were housed in a freestall barn with cubicles covered with rubber mats and chopped straw and milked twice daily at 0500 and 1600 h. The experiment was designed as a 3 × 3 Latin square with 3 cows and 3 diets tested in 3 periods. The P content of the basal diet was 3.82 g/kg of DM (Table 1), which was in accordance with the recommendations of the Gesellschaft für Ernährungsphysiologie (GfE, 2001). Two further diets, differing in P or phytate content, were also prepared. Inorganic P was added as monosodium and monocalcium phosphate to the basal diet (**Pi**; 5.59 g of P/kg of DM). The third diet (3.94 g of P/kg of DM) contained P exclusively of organic origin, with RSM provided as the main source of $InsP_6$ (**InsP**). To achieve similar CP and energy concentrations between the 3 diets, corn gluten, corn starch, and dried sugar beet pulp in diet InsP were substituted with corn grain and RSM.

The diets were prepared every morning as a TMR and filled into weighing troughs (Westfalia Surge, Bönen, Germany) with Calan gates (American Calan, Northwood, NH) at 0800 h. Cows received individual amounts of TMR to ensure adequate energy and protein supply according to their respective milk yield.