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Effect of exogenous phytase on degradation of inositol phosphate in dairy cows

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ABSTRACT

The effect of exogenous phytase on inositol phosphate degradation in the rumen of dairy cows was investigated in a 4 × 4 Latin square design. Four lactating Danish Holstein cows fitted with ruminal, duodenal, and ileal cannulas were offered a total mixed ration (TMR) with a high content of inositol phosphate and supplemented with 1 of 4 concentrations of phytase [none, low, medium, or high, corresponding to 23, 2,023, 3,982, and 6,015 phytase units/kg of dry matter (DM)]. Exogenous phytase lead to a higher rumen pool of phytase. Inositol phosphate content in digesta samples from rumen, duodenum, ileum, and feces was almost entirely composed of *myo*-inositol hexakisphosphate ($InsP_6$), indicating that degradation of this compound is the rate-limiting step in inositol phosphate degradation in the digestive tract. Ruminal and total-tract degradations of $InsP_6$ were higher when exogenous phytase was added to the TMR. Degradation of $InsP_6$ occurred mainly before the duodenum. The ruminal degradability of $InsP_6$ was increased with increasing dietary concentrations of phytase: 86.4, 93.7, 94.5, and 96.3% for none, low, medium, or high, respectively. A comparison of the $InsP_6$ content in individual feedstuffs and in samples of the TMR revealed that the exogenous phytase started degrading the inositol phosphate when feeds and phytase were mixed, and thus the $InsP_6$ phosphorus ($InsP_6$-P) content in the TMR was found to decrease with higher doses of phytase (1.69, 1.51, 1.39, and 1.25 g/kg of DM for the none, low, medium, and high phytase doses, respectively). It was not possible to distinguish between the degradation of inositol phosphate occurring in the TMR and in the rumen. Exogenous phytase had no effect on total P intake or flow of total P to the duodenum and ileum, whereas exogenous phytase increased flow of microbial P to the duodenum and total fecal P excretion. None of the investigated rumen variables (pH, degradability of neutral detergent fiber, and rumen kinetics for neutral detergent fiber) were affected by treatment. Rumen and total-tract degradations of inositol phosphate were increased when exogenous phytase was added to the TMR, which offers the potential for reducing P excretion through reduced dietary P.
Key words: inositol phosphate, phytase, phosphorus availability, cattle

INTRODUCTION

Phytate, the salt of phytic acid (*myo*-inositol hexakisphosphate, $\mathbf{InsP_6}$), is the main storage form of P in cereals, legumes, and oilseeds (Nelson et al., 1968; Godoy et al., 2005), and the P associated with $InsP_6$ ($\mathbf{InsP_6}$**-P**) can constitute 60 to 80% of the total P content in cereal grains and 35 to 50% in byproducts such as soybean meal and rapeseed cake (Eeckhout and De Paepe, 1994; Viveros et al., 2000). Forages have a much lower content of $InsP_6$, and only traces have been found in alfalfa and different grasses (Nelson et al., 1976), as most forages merely consist of stems and leaves. The enzyme phytase catalyzes the hydrolysis of $InsP_6$ and produces lower inositol phosphates: *myo*-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates ($\mathbf{InsP_5}$ to $\mathbf{InsP_1}$) (Irving, 1980; Wodzinski and Ullah, 1996). Previously, inositol phosphate P was considered to be fully available to ruminants because of the microbial phytase activity in the rumen (Clark et al., 1986; Morse et al., 1992a). However, studies have shown that ruminal degradation of $InsP_6$ is reduced by the processing of feedstuffs by means of, for example, heat or formaldehyde treatment (Konishi et al., 1999; Park et al., 1999; Bravo et al., 2000). Furthermore, recent studies have indicated that the ruminal degradation of $InsP_6$ is not complete and is increased by supplementation with exogenous phytase (Kincaid et al., 2005; Sehested and Lund, 2007). Other recent studies verify the potential to reduce the excretion or improve the digestibility of P in goats, dry cows, and lactating cows using exogenous phytase (Bravo et al., 2002; Knowlton et al., 2005, 2007).

The use of feeds rich in inositol phosphate such as grains and oilseed meals is substantial in cattle produc-

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tion, and exogenous phytase might play a significant role in securing the supply of available P to ruminal microbes and animals. Improved availability of feed P would make it possible to meet the needs of the microbes and the animal with reduced P intake, which in turn can decrease the P content of livestock manure. The un-utilized inositol phosphate P and inorganic P are excreted with manure and contribute to environmental problems by eutrophication of water resources (Sharpley and Winthers, 1994). In several studies, intake and excretion of P in cattle have been found to be closely related (Morse et al., 1992b; Wu et al., 2000; Dou et al., 2003), and therefore even small improvements in the availability of feed P will improve the whole-farm P balance and decrease the potential for P run-offs from farms (Knowlton et al., 2004).

Exogenous phytase is extensively used as a feed additive in monogastric animals, but to our knowledge, no commercial phytase has been developed specifically for ruminant application. An initial in vitro study simulating ruminal conditions was therefore performed to find a suitable phytase for ruminants (Brask-Pedersen et al., 2011), and the most efficient phytase was used in the present study.

The objective of the present experiment was to investigate the effect of exogenous phytase on the degradation of inositol phosphate in dairy cows. A second objective was to determine the relationship between dose and effect, to form a basis for future recommendations for the application of phytase for dairy cows.

MATERIALS AND METHODS

Animals and Feeding

The present experiment complied with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study.

Four lactating Danish Holstein dairy cows varying in weight from 501 to 787 kg were used in the study. Two cows were in second lactation, 1 was in fourth lactation, and 1 was in its first lactation. Cows were 221 ± 90 d postpartum at the beginning of the experiment and were fitted with ruminal (#1C; Bar Diamond Inc., Parma, ID), duodenal (open T-piece placed 60 cm caudal to the pylorus), and ileal (open T-piece placed 20 cm cranial to the cecum) cannulas. Chromic oxide (Cr_2O_3) was used as the intestinal digesta flow marker by administering 10 g via the rumen cannula at each feeding (0830 and 1730 h). Cows were housed in tie stalls with rubber mats, had free access to water, and were milked twice daily at 0500 and 1700 h.

The experiment was based on a 4 × 4 Latin square design with 4 cows, 4 treatments, and 4 periods. The 4 treatments consisted of 4 levels of microbial phytase added to the TMR (none, low, medium, or high, which aimed to correspond to 0, 1,200, 2,400, and 3,600 phytase units (**FTU**)/kg of feed or equal to 0, 2,122, 4,245, and 6,367 FTU/kg of DM). Based on the phytase content in the individual feeds and the addition of microbial phytase, the obtained (analyzed) phytase contents in the 4 treatments were 23, 2,023, 3,982, and 6,015 FTU/kg of DM for the treatments none, low, medium, and high, respectively. One FTU is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphate per min from a 0.0051 *M* Na-phytate solution at pH 5.5 and 37°C (Engelen et al., 1994). The microbial 6-phytase used in the present study was a histidine acid phosphatase phytase expressed by a recombinant strain of *Aspergillus oryzae* supplied by Novozymes A/S (Bagsvaerd, Denmark). The phytase was selected among 4 candidates on the basis of in vitro studies (Brask-Pedersen et al., 2011).

Cows were fed ad libitum with a TMR. The phytase was mixed with the rapeseed cake before it was added to the rations. The TMR (g/kg of DM) was composed of corn silage (167), clover grass silage (263), rapeseed cake (201), dried beet pulp (297), corn feed meal (24), and cane molasses (48). The chemical composition of the feedstuffs and the TMR is listed in Table 1.

Sampling

After an adaptation period of 14 d, 12 samples of ruminal fluid, duodenal and ileal contents, feces, and urine were obtained at 6- or 8-h intervals over 5 consecutive days. The collection procedure was arranged to give representative samples of the diurnal flow (i.e., every second hour of the 24-h day). Samples from the duodenum and ileum were collected in tube-formed plastic bags attached to the cannula with a plastic knee. Feces and urine were collected in cups. Duodenal and ileal digesta, feces, and urine were stored at −20°C and all were pooled within cow and period. Ruminal fluid was obtained from the ventral rumen compartment and strained through 2 layers of cheesecloth. The pH was determined immediately using a PHC-2412 combination electrode (Radiometer, Brønshøj, Denmark) connected to a RHM-220 pH measuring device (Radiometer), which was autocalibrated using standard buffers of pH 4 and 7 before each sampling. Afterward, the rumen fluid was stored at −20°C until analysis. The amounts of feed offered and refused were recorded and sampled at each sampling day and then pooled within treatment and period. Individual feeds were sampled